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biology

VCE UNITS 3 + 4

4E

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Fig. 6.1p.203: PNAS March 30, 2004 101 (13) 4620-4624; https://doi.org/10.1073/pnas.0308001101 Human and avian influenza viruses target different cell types in cultures of human airway epithelium Mikhail N. Matrosovich, Tatyana Y. Matrosovich, Thomas Gray, Noel A. Roberts, and Hans-Dieter Klenk Figures 1&2 Copyright (2004) National Academy of Sciences, U.S.A.
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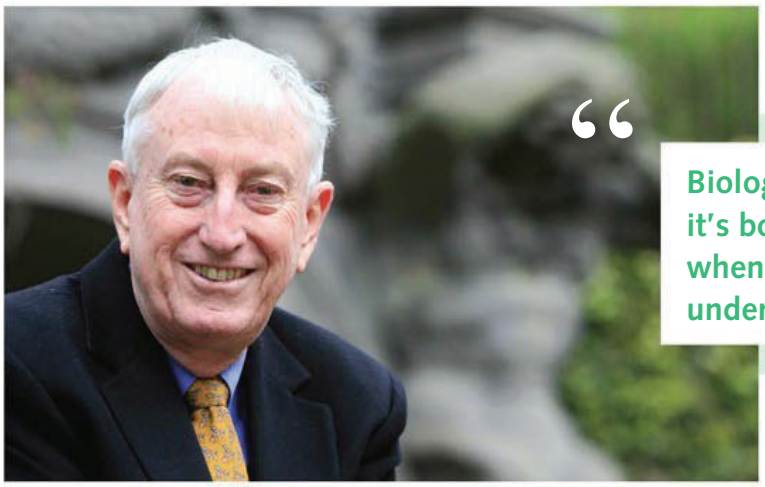
Foreword



Biology is fantastic because it's both complicated and, when you finally get to some understanding, beautiful!

Starting out as a veterinary scientist trying to understand how viruses kill, and how we might prevent that, I made a chance discovery with my Swiss colleague, Rolf Zinkernagel, that led to us sharing the 1996 Nobel Prize for Physiology or Medicine. Rolf trained as a medical doctor but, so far, I'm the only vet to win a Nobel. We found that the so-called transplant or *surveillance* molecules focus the *assassins* of immunity, the *killer* T cells, on cells that are damaged by infection or oncogenic (cancerous) changes. The following year I had the honour of being named the Australian of the Year. When asked to write this foreword my response was: are they thinking of Pete Doherty the musician? In a world where science communication has rapidly declined and fake news (or gossip over the back fence) is too often the norm, I felt it was important to add my opinion to the study of Science, specifically Biology, and say a little bit about how it can change thinking and lives.

I am passionate about promoting an evidence-based view of reality: my most recent book, *The Knowledge Wars*, describe the 'warts and all' view of science for non-scientists, even for people who don't like science. That's the great thing about biological science. It may be a surprise to some arty types, but science does teach you how to write clearly and concisely, though we're no good at fiction! So far I've published a diverse range of general books about science and the scientific life: *A Light History of Hot Air, The Beginners Guide to Winning the Nobel Prize : a Life in Science, Sentinel Chickens: What Birds Tell Us About our Health and our World and Pandemics: What Everyone Needs to Know*, and *The Incidental Tourist*.

Biology is fantastic because it's both complicated and, when you finally get some understanding, beautiful! Of course, it is important to pass exams if you want to get into medical or vet school, or become a researcher like me. Studying biology teaches all of us about ourselves, while strengthening important life skills such as critical thinking, problem solving, collaboration, scientific literacy and the importance of working together. And if you have a good basic grasp of biology, you'll understand why this discovery or that is important. It will also help you to tell the difference between reality, hype and downright lies. The *VICscience Biology* series tackles some of the big ethical issues and teaches students how to think scientifically and question ideas.

Now, having been involved in infectious disease research (especially immunity) for more than 55 years, I've handed over my research lab (plus whatever grant money I bring in) to my younger colleagues. I've still got stuff to say, and my focus now is on writing more books. Part of the delight of being a senior researcher is to see those who've worked with you mature and become great scientists. And it starts for them, as it did for me, at one place: with learning the basics and being excited by biology. Apart from allowing me to live and work in different countries, and opening doors, in terms of social and economic mobility, being part of the unravelling of the story of life has been immensely gratifying. There is still an enormous amount to be discovered and even if you are not intending to be part of that, understanding the basics of biology can only serve to position you better for any future you might be contemplating.

Dr Peter Charles Doherty AC FRS FMedSci
Laureate Professor, The University of Melbourne.
5 December 2019

Author team

Dr Sarah Jones – consultant

Dr Sarah Jones is a medical research scientist who leads a team of scientists and clinicians in the School of Clinical Sciences at Monash University in Melbourne. The goal of Sarah's research is to develop a safe and effective new treatment for autoimmune and inflammatory conditions. Before this, Sarah held research positions at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Harvard Medical School, US, and Trinity College, Ireland. Sarah was a medal-winning member of the Australian International Biology Olympiad Team before becoming a tutor then acting director of the program, designing theoretical and practical learning material and exams for Australia's highly successful Olympiad teams. Sarah was lead author for the 2016 edition of *Nelson Biology VCE Units 3 & 4* and was scientific consultant and editor for *Nelson Biology VCE Units 1&2* (2016) and *Nelson Biology Units 3 & 4 for the Australian Curriculum* (2015).

Ann Cathcart

Ann Cathcart (MEdAdmin, BSc, DipEd) has extensive experience in the development of scientific curriculum content of a biological and scientific nature. She also has vast experience in the publication of materials for learning. Ann brings specific skills to an authoring role. She is a current and practising secondary school teacher who has taught Years 2–12 in many Australian school systems, predominantly in senior Biology and Chemistry, for more than 40 years. She has also managed school science departments in positions of Head of Science and Head of Biology. Ann has worked in science-related industries, such as medicine, agriculture and mining, and at the tertiary level in medical education. Ann understands secondary students. She has addressed stakeholders' needs in each part of her career. She has written educational materials and loves doing this. While applying attention to detail based on a strong technical background, she demonstrates a passion for enabling others, including her students, to achieve an outcome.

Taylah Bennett

Taylah Bennett attained a BSc (Hons) and is currently completing her PhD at the Biomedicine Discovery Institute at Monash University in Melbourne. Taylah received the Nairn Prize in 2017, which recognises the top Honours student in the Department of Immunology at Monash University. She has held positions as a teaching associate in the Department of Immunology and has previously worked at the Australian Regenerative Medicine Institute and School of Clinical Sciences. Taylah is a member of the Australian and New Zealand Society for Immunology, and the Immunology Group of Victoria and has been president of the Monash University Microbiology Postgraduate Society. Her research aims to understand how T cells are controlled to fight viruses and cancer but can also become pathogenic in situations of autoimmune disease.

Dr Tony Chiovitti

Tony Chiovitti attained a BSc (Hons) at the University of Melbourne in 1992. He completed his PhD at the School of Botany, University of Melbourne in 1997 investigating cell wall biochemistry of Australian red algae and algal evolution using gene sequences. He has eight years of postdoctoral research experience in Australia and overseas with biochemical studies of bacteria and microalgae, including collaboration in the first phytoplankton genomes to be sequenced. He obtained a Dip. Ed (2004) and joined the education team at the Gene Technology Access Centre (GTAC), Parkville, Victoria becoming Deputy Director in 2012. Tony has developed and delivered educational programs for students and professional learning programs for teachers on the themes of cell and molecular biology, bioinformatics, immunology, ecology and evolution. He has also led educational programs that enable secondary school students to contribute as citizen scientists to biological research projects.

Dr Amanda Clarke

Amanda Clarke's interest in biology started when she was a child. Initially, she wanted to be a veterinarian, but at university she became fascinated with genetics, microbiology and immunology. She was granted a PhD in Immunology from the University of Melbourne for her studies into house dust mite allergy. While studying, she also taught practical classes at several universities and thoroughly enjoyed opening her students, minds to the wonders of medical research. Amanda then decided to become a Biology and Chemistry teacher. She developed a special interest in the biomedical applications of nanoscience and nanotechnology. She was part of a team at St Helena Secondary College who won a Victorian Government Education Innovation Award for the development of a nanotechnology curriculum at the school. Amanda is still teaching Nanotechnology and Biology and thoroughly enjoys it. She is currently employed as a learning specialist at Balwyn High School.

Xenia Pappas

Xenia Pappas is a Biology teacher with more than 30 years' experience. She has taught across all sectors of the Victorian education system, including time with the Zoo Education Service and Museum Victoria. She has held leadership roles within the Department of Education's Gifted Education Unit as well as Head of Year and Head of Biology for many years in schools. Xenia has always worked to engage her students by offering alternative approaches that taps into a range of learning modalities. As a long-time author of Biology and General Science resources, Xenia has developed a well-rounded knowledge and understanding of the curriculum from Year 7 to VCE and works to deliver the curriculum in a manner that addresses the individual needs of students.

Adrianne Harrowfield

Adrianne studied Genetics and Microbiology at La Trobe University gaining her BSc and subsequently received an Honours degree in Genetics. She began her scientific career as a research assistant at the Walter and Eliza Hall Institute of Medical Research within the Genetics Department. After two years working in research, she completed her DipEd at the University of Melbourne and has been teaching VCE Biology for 20 years. She has been a VCE Biology examination assessor and is currently a passionate teacher of Biology.

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- Natalie Cookson, Sacred Heart College, Geelong
- Tegan Tinsley, Lighthouse School
- Julie Gould, Brunswick Secondary College
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- Nicole Henry, consultant

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- Rebecca Famlonga, who reviewed the Aboriginal and Torres Strait Islander content. Rebecca is a proud Wadawurrung woman and Traditional Owner. She has taught and led in secondary schools for more than 20 years and is passionate about Aboriginal and Torres Strait Islander Education.

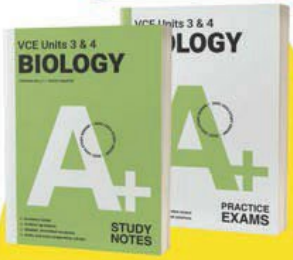
VICscience VCE Biology Learning Ecosystem

exam+

examplus is a comprehensive and innovative assessment platform that simulates real exam practice. **examplus** provides an extensive bank of filterable and difficulty-graded past VCAA and unseen exam questions, all in the one place.

Textbook

Students learn through stimulating, engaging and scaffolded content, activities and investigations. All content can be directly mapped to the VCAA VCE Biology Study Design.



A+ Biology Study Notes includes detailed summaries and revision questions, as well as study tips and information about the exam. **A+ Biology Practice Exams** contains 14 topic tests and two perforated practice exams.

Logbook

Students record all their investigation materials in one place for assessment and authentication purposes as required by the VCAA VCE Biology Study Design.

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Teachers expand their teaching and students reinforce their learning through the rich variety of extra resources provided on the NelsonNet student and teacher portals.

Workbook

Students develop, use and demonstrate key science skills through engaging activities; they practise exam skills by completing exam-style questions.

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cengage.com.au/secondary/vce-biology

To the student

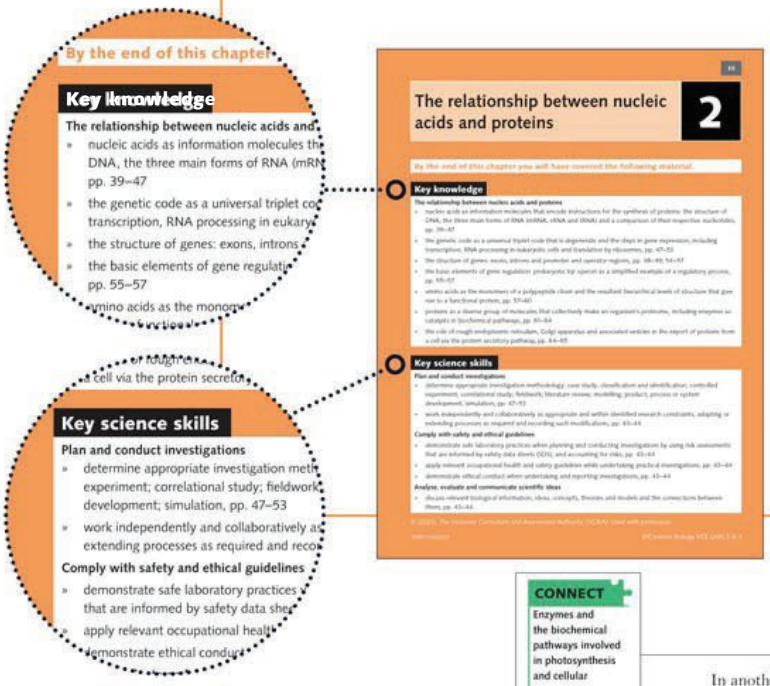
The VCE Biology course comprises both key knowledge and key science skills components, which will be assessed throughout your studies. We understand that undertaking VCE Units 3 & 4 can be an exciting but sometimes overwhelming time. You will learn a lot of content and develop scientific skills throughout a very busy year that will culminate in an external assessment. We have taken these stressors into account when designing the *VICscience Biology* suite of products. You will not need to go beyond these learning materials to study VCE Biology; they have been designed to work in unison so you can achieve at your best.

10 steps to study success

Ensure you take time to read the 10 ways we have organised your VCE biology study journey. You will see that at various stages in your studies, different aspects of this textbook will be more useful. Whether you are learning new concepts for the first time, reviewing what you have learnt or preparing for tests and exams, spending a little time now getting to know your textbook will help you reach your learning potential for VCE Biology.

1 Focus on the Study Design

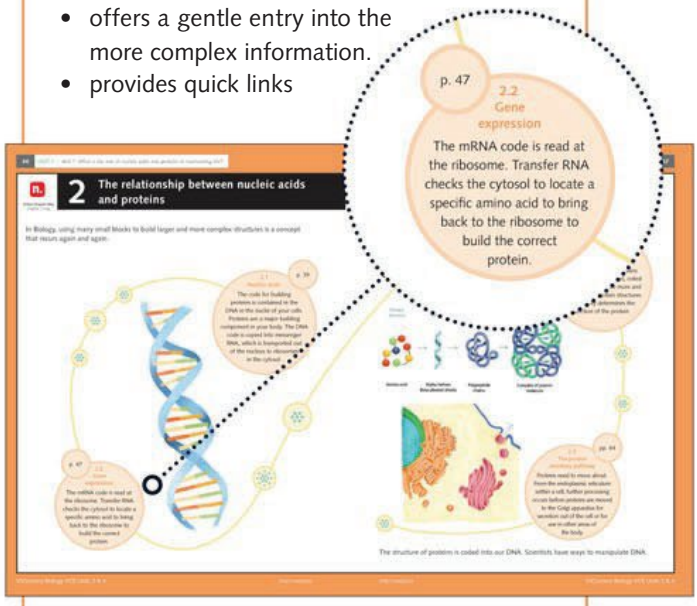
Each chapter starts with a chapter opening page that will guide you through the **key knowledge** and **key science skills** that are covered in the chapter with page reference numbers to help find all the content that you need quickly.

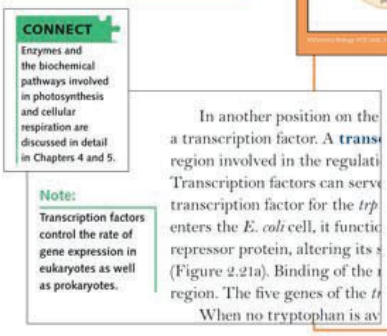


2 Look for connections

Each chapter begins with a **chapter map** that:

- are easy-to-use
- use shorthand familiarisation
- is a navigational tool to guide you through each chapter
- offers a gentle entry into the more complex information.
- provides quick links





We have made further connections throughout each chapter using margin **Notes**, which offer further explanation and **Connect** boxes, which link content to other relevant chapters where more information is provided.

3 Rehearse key terms

We have listed all the key terms at the beginning of each chapter.

You can use the **flashcards** study tool to learn and review key terms with their definitions, and assist with pronunciation and spelling of key vocabulary.

4 Test your memory

At the beginning of each chapter, use the **Remember** statements under the key terms list to bring previously learnt concepts to the front of your mind. Stronger foundations of knowledge make learning more difficult concepts easier.

The *VICscience Biology Skills Workbook* provides you with stepped questions to help you to engage your past learnings.

5 Develop your skills

Key science skills are examinable in the external assessment and therefore are a significant and important part of the course. To further develop and refine all the key science skills set out in the course, complete the activities in the accompanying *VICscience Biology Skills Workbook*. Signposts to workbook activities are found throughout the textbook. Look for the **Workbook icon**.

REMEMBER PAGE 22

6 Understand the concepts

Pictures tell a thousand words and are key in strengthening understanding, so ensure you look carefully at each **figure** and read the **labels** and **captions** so that you can understand what it is telling you.

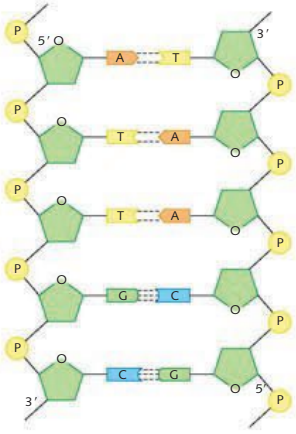
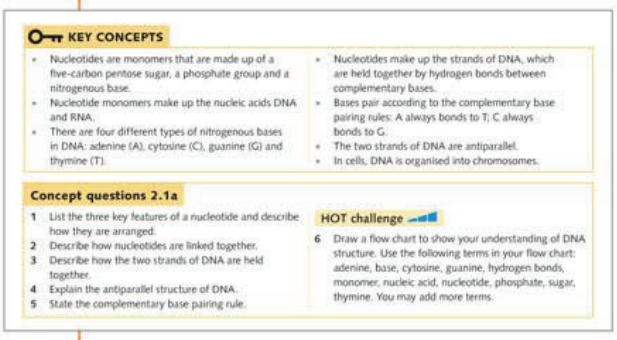


Figure 2.7 The antiparallel nature of DNA: the two strands of DNA run in opposite directions.

Sometimes words and pictures are not enough. Some of the key knowledge has been explained in videos. Look for the **video icons** throughout the chapters as these animated videos will help you to understand and make connections between content.

Important ideas, concepts and theories are summarised in **Key concept** boxes.



KEY CONCEPTS

- Nucleotides are monomers that are made up of a five-carbon pentose sugar, a phosphate group and a nitrogenous base.
- Nucleotide monomers make up the nucleic acids DNA and RNA.
- There are four different types of nitrogenous bases in DNA: adenine (A), cytosine (C), guanine (G) and thymine (T).
- Nucleotides make up the strands of DNA, which are held together by hydrogen bonds between complementary bases.
- Bases pair according to the complementary base pairing rules: A always bonds to T; C always bonds to G.
- The two strands of DNA are antiparallel.
- In cells, DNA is organised into chromosomes.

Concept questions 2.1a

1 List the three key features of a nucleotide and describe how they are arranged.
2 Describe how nucleotides are linked together.
3 Describe how the two strands of DNA are held together.
4 Explain the antiparallel structure of DNA.
5 State the complementary base pairing rule.

HOT challenge

6 Draw a flow chart to show your understanding of DNA structure. Use the following terms in your flow chart: adenine, base, cytosine, guanine, hydrogen bonds, monomer, nucleic acid, nucleotide, phosphate, sugar, thymine. You may add more terms.

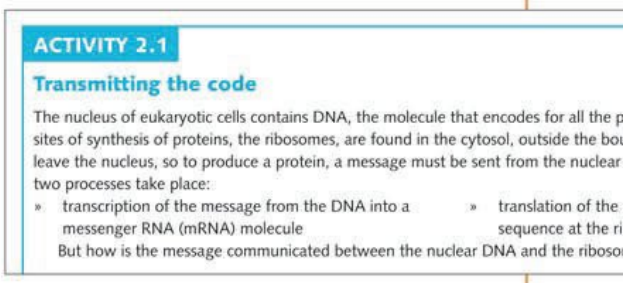
Concept questions follow each key concept box. These questions will help you to determine whether you have fully understood the content before you progress further in the chapter.

If you are feeling confident with the concepts you can give the **HOT Challenge** a go! This question is more difficult and may need further research. It will extend your understanding to a higher level.

7 Explore and learn

You will collaborate, explore and discover the living world through practical activities and investigations and also come to appreciate the collegial nature of Biology.

Complete short, hands-on tasks designed to clarify or reinforce a concept through the **Activity** boxes.



ACTIVITY 2.1

Transmitting the code

The nucleus of eukaryotic cells contains DNA, the molecule that encodes for all the p
sites of synthesis of proteins, the ribosomes, are found in the cytosol, outside the bou
leave the nucleus, so to produce a protein, a message must be sent from the nuclear
two processes take place:

- transcription of the message from the DNA into a messenger RNA (mRNA) molecule
- translation of the sequence at the ri

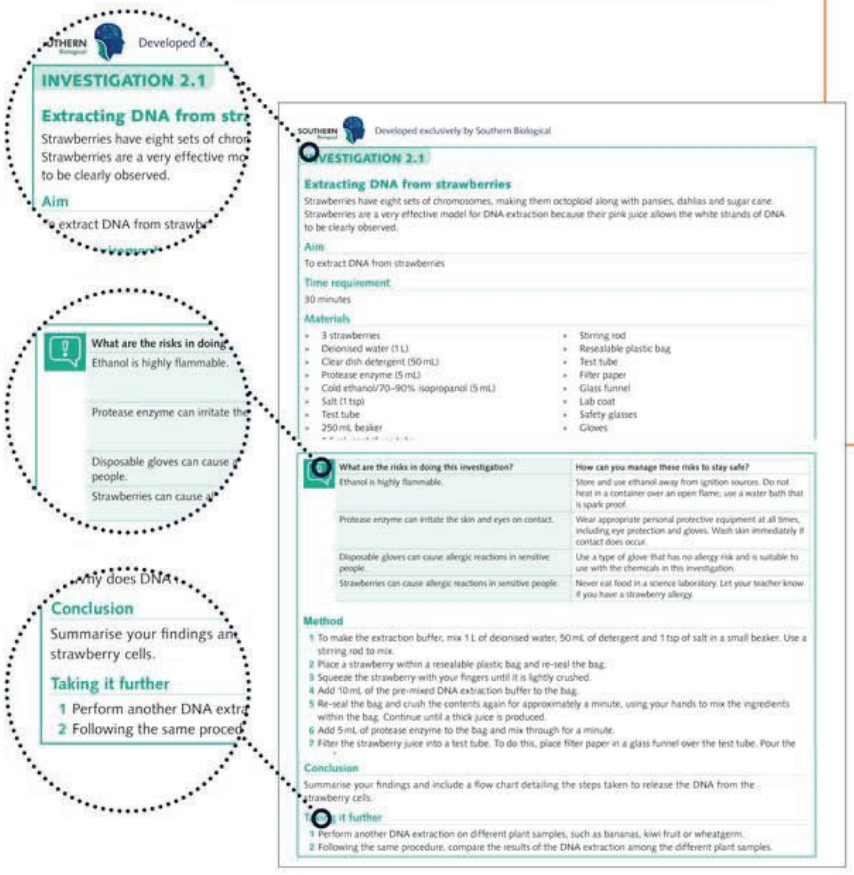
But how is the message communicated between the nuclear DNA and the riboso

Explore key knowledge and develop, use and demonstrate the key science skills through the **Investigations**. Investigations provide:

- guided instruction on the materials
- method
- collection
- analysis of results
- discussion.

Investigations are not without risks and part of learning to work like a scientist is learning to work safely. **Risk assessment** tables highlight the risks of the investigation and provides suggestions on how you can minimise risks.

Taking it further questions found at the end of some investigations provide you with ideas on how you could extend or adapt the investigation for further study.



Developed exclusively by Southern Biological

INVESTIGATION 2.1

Extracting DNA from strawberries

Strawberries have eight sets of chromosomes, making them octoploid along with pansies, dahlias and sugar cane. Strawberries are a very effective model for DNA extraction because their pink juice allows the white strands of DNA to be clearly observed.

Aim

To extract DNA from strawberries

Time requirement

30 minutes

Materials

- 3 strawberries
- Deionised water (1 L)
- Clear dish detergent (50 mL)
- Protease enzyme (5 mL)
- Cold ethanol/70–90% isopropanol (5 mL)
- Salt (1 tsp)
- Test tube
- 250 mL beaker
- Stirring rod
- Resealable plastic bag
- Test tube
- Filter paper
- Glass funnel
- Lab coat
- Safety glasses
- Gloves

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Ethanol is highly flammable.	Store and use ethanol away from ignition sources. Do not heat in a container over an open flame; use a water bath that is spark proof.
Protease enzyme can irritate the skin and eyes on contact.	Wear appropriate personal protective equipment at all times, including eye protection and gloves. Wash skin immediately if contact does occur.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.
Strawberries can cause allergic reactions in sensitive people.	Never eat food in a science laboratory. Let your teacher know if you have a strawberry allergy.

Method

1 To make the extraction buffer, mix 1 L of deionised water, 50 mL of detergent and 1 tsp of salt in a small beaker. Use a stirring rod to mix.
2 Place a strawberry within a resealable plastic bag and re-seal the bag.
3 Squeeze the strawberry with your fingers until it is lightly crushed.
4 Add 10 mL of the pre-mixed DNA extraction buffer to the bag.
5 Re-seal the bag and crush the contents again for approximately a minute, using your hands to mix the ingredients within the bag. Continue until a thick juice is produced.
6 Add 5 mL of protease enzyme to the bag and mix through for a minute.
7 Filter the strawberry juice into a test tube. To do this, place filter paper in a glass funnel over the test tube. Pour the

Conclusion

Summarise your findings and include a flow chart detailing the steps taken to release the DNA from the strawberry cells.

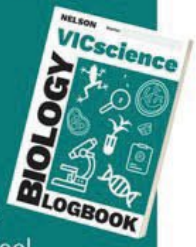
Taking it further

1 Perform another DNA extraction on different plant samples, such as bananas, kiwi fruit or wheatgerm.
2 Following the same procedure, compare the results of the DNA extraction among the different plant samples.

Remember that investigations work hand-in-hand with your logbook, which is where you record all your:

- investigation observations
- ideas
- data
- analysis
- discussion and conclusions.

Your logbook is an important assessment and authentication tool.



8 Prepare for tests and exams

The best way to prepare for exams is to use past exam questions. **Area of Study reviews** at the end of each Area of Study allow you to check your knowledge by completing difficulty-graded exam-style questions. The answers to these are at the back of the book. Look for **tips** in the margin that point out things to be aware of when answering exam questions.

EXAM TIP
Ensure you know the differences and similarities (compare) between DNA and RNA, their structure and functions.

9 Extend yourself

At the end of each chapter you will find a **Branching out** activity. This material is extension and non-examinable. It examines possible careers and future applications of what you have just learnt.

BRANCHING OUT

Testing drugs on cells

We are made up of a community of cells that work together to maintain life. At times cells may be damaged or start misbehaving. This causes disease, as body function is impaired. To treat diseases, we need drugs that target damaged cells and fix the problem. Medical research helps us to understand cell systems better and to discover new drugs to treat disease. Drug discoveries improve the health of our community. They increase our lifespan and that is good for the economy as we can work longer. They also reduce the cost burdens of healthcare.

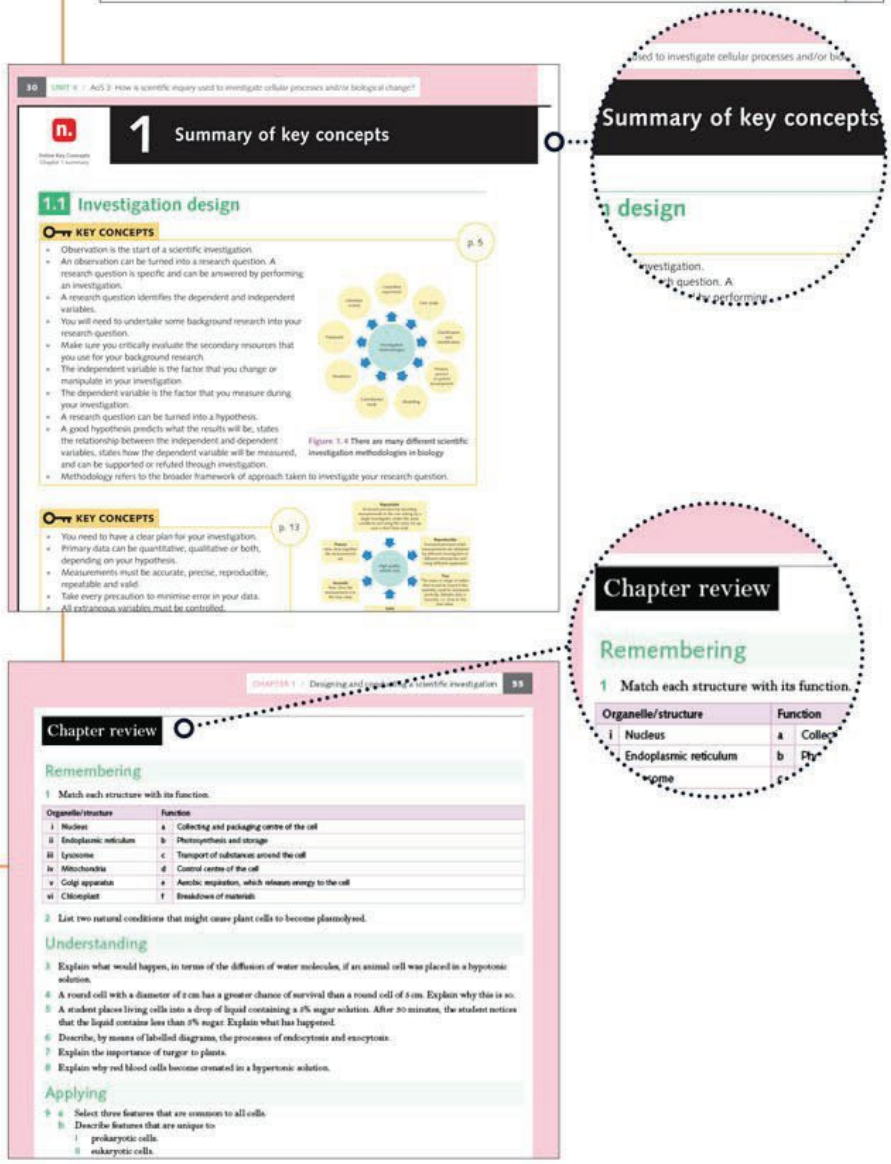
Discovering medical drugs is costly. Drug development takes a long time and often ends in failure. Matthew Herper is an investigative science journalist with Forbes. His research reveals that approximately 95% of drugs trialled are found to be ineffective or not safe when tested in humans (2013). He calculates that the cost of getting a drug to market ranges from $350 million to $5 billion.

Current methods for drug development involve testing them on cells grown in culture and on cells in animal models. While these methods provide us with valuable information, they do not always reveal how the drug will work on cells in the human body.

10 Consolidate your learning

At the end of every chapter you can consolidate your knowledge. Here you will find:

- **summary of key concepts** that you have met throughout the chapter. You can download a copy of the key concepts by accessing the worksheet icon on NelsonNet. Use this to assist you in revising and studying for internal and external assessments
- a **chapter glossary** of all the key terms for the chapter plus their definitions
- **chapter review** questions that will help you to recall, revise, understand and apply the concepts from the chapter. The questions are grouped under headings (**Remembering**, **Understanding**, **Applying**, **Analysing**, **Evaluating** and **Creating**) and reflect the level of thinking required to answer each question. These questions provide you with the practice needed to analyse and answer exam questions.



Online Resources

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FOR THE STUDENT

The NelsonNet website provides you with material that will help you understand, explore, engage and organise the key knowledge and key science skills you have learnt about in your textbook. You will find it at **www.nelsonnet.com.au** and once your registration is complete, you can access digital resources for each chapter to further reinforce your learning. Look on the back cover of this book to find out how to complete your registration.

You will find:

- **NelsonNet ebook** with annotation capability
- **Weblinks** to more information, online interactives and videos
- **Worksheets** for some online videos
- **Downloadable versions** of each chapter map and summary of key concepts
- **Videos** explaining significant concepts
- **Play and say** to help you to pronounce and spell words in the key terms list
- **Flash cards** to assist with remembering and revising key terms and their definitions.

FOR THE TEACHER

Please note that complimentary access to NelsonNet and the NelsonNet ebook is only available to teachers who use the accompanying student textbook as a core educational resource in their classroom.

Contact your sales representative for information about access codes and conditions.

To the teacher

The VCE Biology course comprises both key knowledge and key science skills. The *VICscience Biology* suite of products provides you with the perfect resource to teach all the key knowledge and key science skills in an integrated way and to prepare your students thoroughly for the school-based and external assessments.

1 Stick to the Study Design

This textbook has been written so all content closely aligns with the *VCAA VCE Biology Study Design* (2022–2026). It has been authored and reviewed by experienced biology teachers, academics and researchers to ensure up-to-date scientific and accurate content for students.

2 Access differentiated material

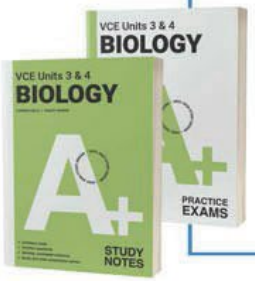
Differentiation is built into each chapter to assist you in helping those students that may struggle with content or skill development and extending those students who want to achieve at a higher level.

- **Chapter maps** provide students with a gentle and visual introduction to each chapter enabling students to engage with the chapter content prior to entering the chapter.
- **Key terms** at the beginning of each chapter present all the bolded key terms throughout the chapter in one place. Students can use the **flashcards** study tool to learn and review key terms with their definitions, and assist with pronunciation and spelling of key vocabulary.
- **Remember** provides students the opportunity to recall concepts previously learnt that will be revisited during the chapter.
- **Concept questions** are pitched to be lower-order questions to assist with learning consolidation but end with **HOT Challenge** questions for those students who would benefit from answering higher-order questions.
- **Investigations** end with a **Taking it further** section, which provides ideas on how the investigation can be extended.
- **Weblinks** to external, vetted websites provide extra information; worksheets are provided for some weblinks.
- Each chapter finishes with a **Branching out** activity. This activity provides an extension activity for students who are looking for more information on a particular topic.

3 Prepare for the exam

Students of VCE Biology are working toward external assessment at the end of Units 3 & 4. To fully prepare for this exam, students require access to a large number of quality exam-style questions with answers. *VICscience Biology* gives you the full complement including the following.

- **Area of study reviews** at the end of each Area of Study provide students with difficulty-graded multiple-choice and short-answer questions that have been adapted from VCAA exam questions with answers provided at the back of the book.
- You will also find a full practice **end-of-year exam with answers**.
- *VICscience Biology VCE Units 3 & 4 Skills Workbook* provides students with difficulty-graded multiple-choice and short-answer exam questions that have been adapted from VCAA exam questions.



- **examplus** simulates real exam-practice and comprises thousands of unseen exam-style and past VCAA exam questions with answers to use in your teaching. Simply select your questions for a quiz, topic test, or practice exam and **examplus** generates a practice test or exam.
- Consider bundling **A+ Study Notes** and **Practice Exams** with your VICscience Biology booklist for the most economical solution for students' exam preparation and readiness.

4 Support for the teacher

There is a wealth of teacher support materials on the teacher NelsonNet site that accompanies this product. These include:

- **Cognero Assess** comprising auto-correcting multiple-choice questions and short-answer questions with answers to be shared with your students for every chapter
- **answers** to all textbook questions, investigations and Branching out activities
- sample SACs with suggested marking schemes
- **practice end-of-year exam with answers**
- **teaching plans** for every chapter showing how all the components of the *VICscience Biology* suite are integrated to provide your students with a thorough and complete learning experience designed to prepare them for internal and external assessment
- support for the investigations provided through **Southern Biological**.

DEVELOPED EXCLUSIVELY BY SOUTHERN BIOLOGICAL

SOUTHERN Biological

ACCESS TO QUALITY INVESTIGATIONS

Practical work is a central component of the VCE Biology course and crucial in developing key science skills. The study design specifies the number of hours that students need to spend undertaking practical work. **Southern Biological and Cengage have partnered** to ensure that you and your students are provided with exciting and current practical investigations to introduce, reinforce and practise the key science skills listed in the *VCAA VCE Biology Study Design 2022–2026*, pages 7–8. Some of the investigations written by Southern Biological are exclusive to Cengage, and all investigations have been rigorously stress-tested by Southern Biological to ensure that they will work in your classroom.

Each investigation is accompanied by a risk assessment table that highlights risks to students or others posed by the materials or method. Teachers are expected to amend each table in the case of substitutions or in the case of any additional risks. This may mean obtaining and following Safety Data Sheets (SDS) for certain chemicals. All teachers are required to follow the safety guidelines of their specific school and associated government legislation when students are in their care.

Investigation support provided by Southern Biological include:

- **suggested answers** to investigation questions
- **videos to assist teachers** and laboratory technicians to prepare and dever the investigations to students providing them with optimal hands-on experience
- **videos aimed at students** to assist with undertaking the investigations including suggested answers and hints
- **risk assessments** for investigations where applicable
- resourcing, safety and investigation **preparation sheets.**

Study design

Unit	Area of study	Chapters										
		1	2	3	4	5	6	7	8	9	10	11
	1: What is the role of nucleic acids and proteins in maintaining life? Students explore the expression of the information encoded in a sequence of DNA to form a protein and outline the nature of the genetic code and the proteome. They apply their knowledge to the structure and function of the DNA molecule to examine how molecular tools and techniques can be used to manipulate the molecule for a particular purpose. Students compare gene technologies used to address human and agricultural issues and consider the ethical implications of their use. (p. 28 Study Design)		x	x								
	2: How are biochemical pathways regulated? Students focus on the structure and regulation of biochemical pathways. They examine how biochemical pathways, specifically photosynthesis and cellular respiration, involve many steps that are controlled by enzymes and assisted by coenzymes. Students investigate factors that affect the rate of cellular reactions and explore applications of biotechnology that focus on the regulation of biochemical pathways. (p. 30 Study Design)				x	x						
	1: How do organisms respond to pathogens? Students focus on the immune response of organisms to specific pathogens. Students examine unique molecules called antigens and how they elicit an immune response, the nature of immunity and the role of vaccinations in providing immunity. They explain how technological advances assist in managing immune system disorders and how immunotherapies can be applied to the treatment of other diseases. (p. 33 Study Design)						x	x	x			
	2: How are species related over time? Students focus on changes to genetic material over time and the evidence for biological evolution. They consider how the field of evolutionary biology is based upon the accumulation of evidence over time and develop an understanding of how interpretations of evidence can change in the light of new evidence as a result of technological advances, particularly in molecular biology. Students consider the biological consequences of changes in allele frequencies and how isolation and divergence are required elements for speciation. They consider the evidence for determining the relatedness between species and examine the evidence for major trends in hominin evolution, including the migration of modern human populations around the world. (p. 35 Study Design)									x	x	x
	3: How is scientific inquiry used to investigate cellular processes and/or biological change? Students undertake a student-designed scientific investigation in either Unit 3 or Unit 4, or across both Units 3 and 4. The investigation involves the generation of primary data relating to cellular processes and/or how life changes and responds to challenges. The investigation draws on knowledge and related key science skills developed across Units 3 and 4 and is undertaken by students in the laboratory and/or in the field. (p. 36 Study Design)	x										

Designing and conducting a scientific investigation

1

By the end of this chapter you will have covered the following material.

Key knowledge

Investigation design

- biological concepts specific to the selected scientific investigation and their significance, including definitions of key terms, pp. 4, 32–33
- characteristics of the selected scientific methodology and method, and appropriateness of the use of independent, dependent and controlled variables in the selected scientific investigation, pp. 8–11; 14–15; 23–24
- techniques of primary quantitative data generation relevant to the selected scientific investigation, pp. 12–13; 23–24
- the accuracy, precision, reproducibility, repeatability and validity of measurements, pp. 12–14
- the health, safety and ethical guidelines relevant to the selected scientific investigation, pp. 16–18; 23–24

Scientific evidence

- the nature of evidence that supports or refutes a hypothesis, model or theory, pp. 22; 23–24
- ways of organising, analysing and evaluating primary data to identify patterns and relationships including sources of error and uncertainty, pp. 19–21; 23–24
- authentication of generated primary data through the use of a logbook, pp. 18–19; 23–24
- assumptions and limitations of investigation methodology and/or data generation and/or analysis methods, pp. 9–14; 18–21

Science communication

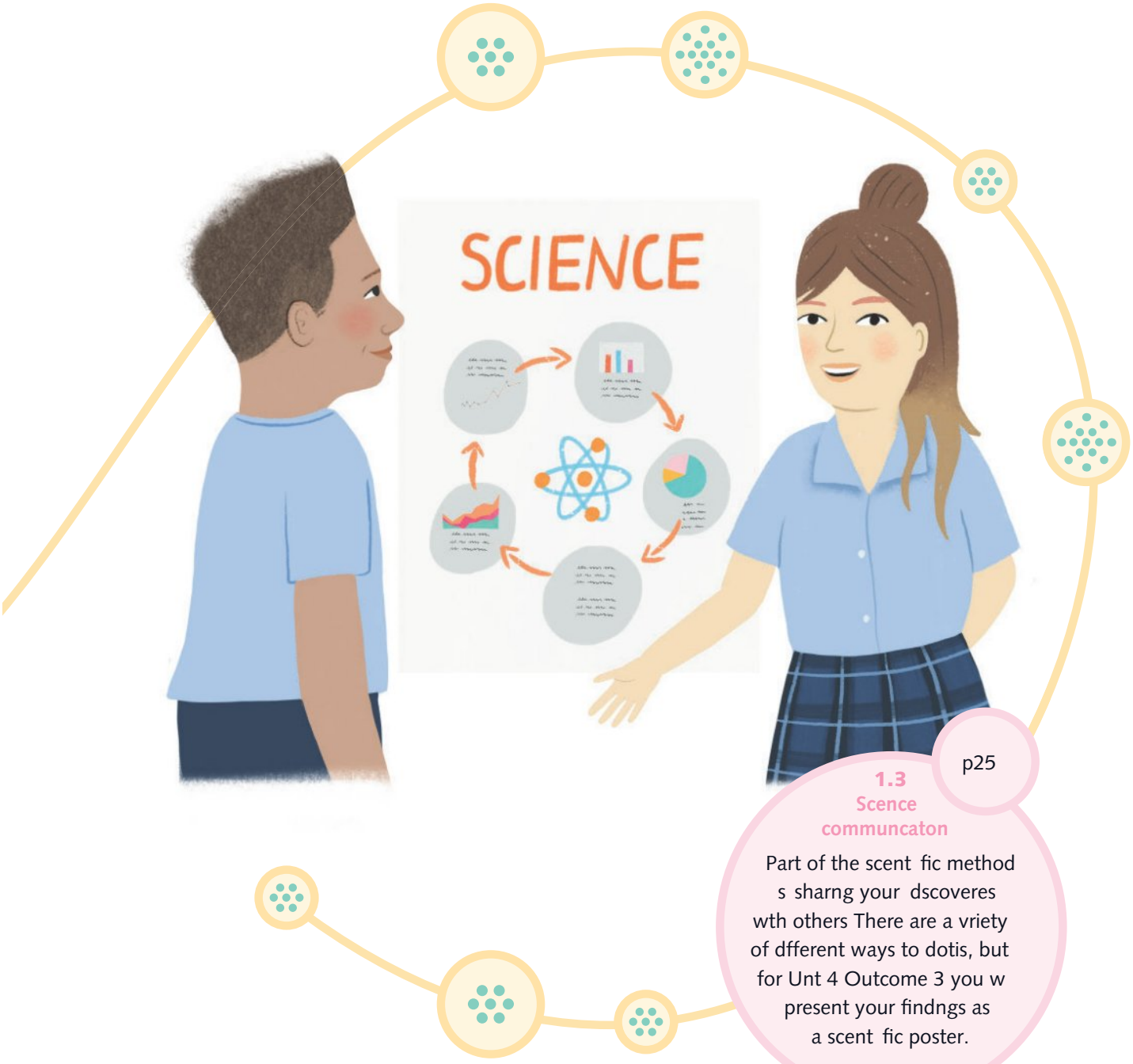
- conventions of science communication: scientific terminology and representations, symbols, formulas, standard abbreviations and units of measurement, pp. 18–20; 23–24
- conventions of scientific poster presentation, including succinct communication of the selected scientific investigation and acknowledgements and references, pp. 25–29
- the key findings and implications of the selected scientific investigation, p. 28

Online Chapter Map
Chapter 1 map

1 Designing and conducting a scientific investigation

Scientists look curiously at the world around them and ask 'why?'. The answer to this question leads to other questions such as 'how?', 'what?' and 'when?'. Science involves investigation, asking questions and designing ways to find answers. For Unit 4 Outcome 3, you will be working in this way to find answers.

9780170452533



p25

1.3
Scence communcaton

Part of the scent fic method s sharng your dscoveres wth others There are a vriety of dfferent ways to dotis, but for Unt 4 Outcome 3 you w present your findngs as a scent fic poster.

Using the scientific method is integral not only in science but also in other aspects of your life. It provides you with a well-tested process to design investigations, collect data and draw evidence-based conclusions.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 1 map (p. 2)

Online Key Terms:
- Chapter 1 flashcards (p. 4)

Weblinks:
- Howard Florey (p. 5)
- APA style guide (p. 7)
- Harvard system (p. 7)
- CSIRO (p. 7)
- Australian Academy of Science (p. 7)
- CRAAP test (p. 7)
- Sterile technique (p. 17)

Online Key Concepts:
- Chapter 1: Summary of key concepts (p. 30)

Online Key Terms
Chapter 1 flashcards

Know your key terms

accurate	independent variable	precise	research question
authentication	integrity	primary data	respect
beneficence	justice	primary source	risk assessment
bias	logbook	qualitative data	secondary data
control group	method	quantitative data	secondary source
controlled variable	methodology	random error	systematic error
dependent variable	model	references	theory
ethics	non-maleficence	reliable	true value
extraneous variable	observation	repeatable	uncertainty
gradient	outlier	replicates	valid
hypothesis	personal error	reproducible	variable

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

WB
REMEMBER
PAGE 2

1. The scientific method is a procedure by which scientists can examine a theory and test a hypothesis.
2. Scientists perform investigations, take careful observations or use models and simulations to gain evidence to either support or refute their hypothesis.
3. When designing a scientific investigation, you should always consider safety and the ethical implications of your work.
4. A well-designed scientific investigation includes relevant controls and clearly defined variables.
5. Scientists keep carefully detailed records of their observations, their ideas and their data in a logbook.

VCE Biology Unit 4 Area of study 3 Outcome 3 requires students to use the key science skills to plan, design, undertake and communicate an investigation that investigates cellular processes and/or biological change. This investigation can be undertaken during studies of Unit 3 or Unit 4 or could be completed across both Units 3 and 4. To complete this outcome, you need to have a good understanding of the key science skills. You will find these listed in the *VCE Biology Study Design* (pp. 7–8).

This chapter will explain and illustrate these key science skills, and you will be able to use this knowledge to help you plan, design, undertake and communicate your investigation. You should always refer to the *VCE Biology Study Design* Unit 4 Area of study 3 (pp. 36–37) when planning your investigation to make sure you cover all the requirements set out in the Study Design.

1.1 Investigation design

Weblink
Howard Florey

The most important question you should ask when you begin this outcome is 'What am I going to investigate?' Remember that you can draw the idea for the investigation from Unit 3 or Unit 4 so be aware of this as you work through these units in class or at home. If you find something particularly interesting, then you might be able to develop it into an investigation that satisfies all the VCAA criteria.

As part of your assessment, you will need to maintain a **logbook**. (See p. 10 of the *VCE Biology Study Design*.) The logbook will be discussed in more detail on pages 18–19, but at this point it would be a good idea to set aside some pages in your logbook to devote to your investigation. Start with writing down ideas that could form part or all of your investigation.

1.1.1
OBSERVATION
PAGE 3

Observation

Observation plays a critical part in any scientific investigation (Figure 1.1). It is usually the start of most investigations (Figure 1.2). You can observe through any of your senses – sight, smell, touch, hearing or taste.

If you observe something unusual or unexpected, you might wonder why and want to investigate it. This is exactly what Scottish scientist Alexander Fleming did in 1928. He was working at St Mary's Hospital in London, where he set up culture plates of *Staphylococcus* bacteria, which cause serious infections, particularly in people with weakened immune systems (such as hospital patients). He then went on a two-week holiday and, on his return, noticed that a mould had grown on the plates. Fleming observed that this mould was

Figure 1.1 Observation plays a critical part in any scientific investigation.

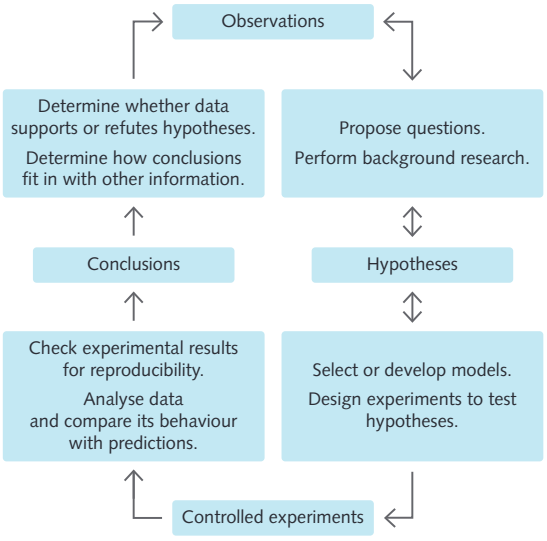


Figure 1.2 Steps in a scientific investigation

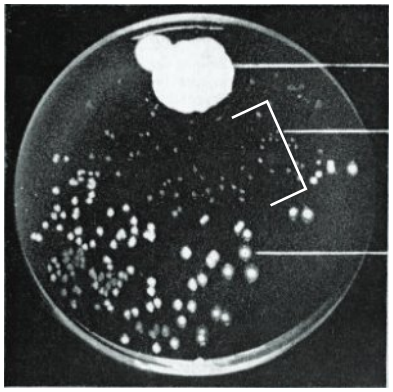


Figure 1.3 Alexander Fleming observed that bacterial colonies did not grow near the *Pencum* colony. He investigated further to find out why, which led to the discovery of the first antibiotic – penicillin.

inhibiting the growth of the *Staphylococcus* bacteria (Figure 1.3). This observation led to the development of the world's first antibiotic – penicillin. Imagine if he had just consigned those culture plates to the bin without observing them first. Millions of lives have been saved as a result of that one observation.

Another way to decide upon a topic is to pinpoint something that you find interesting in your course content. You could design a practical investigation to undertake further research on that topic.

Research question

Once you have decided on a topic for your investigation, or a list of topics that you can narrow down, you will need to come up with a research question.

An effective **research question** is specific and can be answered by performing your investigation and taking measurements with the resources and equipment that are available to you. A research question could be in the form 'What effect does a new fertiliser have on root growth?'. The aim of your research is to answer the question. It is important that you develop and frame the research question carefully.

Your research question needs to be specific enough that it guides the design of the investigation. Asking 'Does a new fertiliser increase root growth more than a standard fertiliser?' tells you what you will be varying (fertiliser) and what you will be measuring (root growth). It also gives a criterion for judging whether your results have answered the research question. If there is more root growth with the new fertiliser than with the standard fertiliser, then the results answer your research question positively. If there is not more root growth with the new fertiliser than with the standard fertiliser, then your results answer your research question negatively. This does not mean that your results are wrong. It just means the results showed that there was *not* more root growth with the new fertiliser.

Asking 'How can we optimise root growth?' is not a good question. This question does not say what will be varied, nor does it tell you when you have answered the question. The term 'optimise' is too vague and subjective.

Finally, a good research question should be feasible: you should be able to answer it with the time and equipment available.

A good research question identifies the independent and dependent **variables** that will be investigated. It asks what effect your proposed independent variable will have on your proposed dependent variable. Variables are factors or features that you vary on purpose or measure as part of your investigation and are discussed in more detail later in this chapter (p. 8).

Background research

Before you can turn your research question into a hypothesis, you need to undertake some background research on your topic and research question. This background research will inform you about what is already known about this topic and will probably lead you to change or refine your research question.

Primary sources

Primary sources of information contain original research; for example, findings within articles in scientific journals. The advantages of these sources are that they contain data from investigations for a specific purpose. They provide the background, method, results and discussion of the investigations. The disadvantage of these sources is that they tend to use a lot of highly technical language that can be difficult to understand.

Secondary sources

Secondary sources of information summarise, review or analyse primary sources. Secondary sources include reviews of the work of other scientists, and some periodicals and even textbooks. In general, secondary sources are written in a more accessible way. You may also be able to get an overview and understanding of a whole field of study through the one article.

Professional scientists consult both types of sources but prefer to read primary sources when planning their investigations. This helps them to develop a deep understanding of exactly what was done in the investigation, the **methodology** that was used and the method that was followed.

Table 1.1 lists the variety of sources of information that a scientist can use when undertaking background research.

Table 1.1 Types of information sources

Primary sources	Secondary sources
Scientific journals or periodicals Research reports Sessions presented at scientific conferences Patents Masters and PhD theses	Review ournals or periodicals that summarise recent research Reliable websites, e.g. Nature.com Textbooks

Referencing source material

Keep a list in your logbook of all the primary and secondary source reference material that you use to research your research question. It is better to do this as you go than to have to go back later on and try to find out where you sourced the material.

There are several ways to reference material but the ones usually used in scientific research are the American Psychological Society (APA) style or the Harvard system. (See weblinks.) Remember that **references** refer to sources that you cite in the write-up of your scientific investigation. A bibliography is different because it collates a list of references that you may have consulted but not necessarily sources that you cited in the write-up. Make sure you check with your teacher about the preferred style of referencing.

Weblinks
APA style guide
Harvard system
CSIRO
Australian Academy of Science
CRAAP test

Evaluating source material

Be critical of what you read. Do not assume that everything you read online or even in books is true. Try to find **reliable** sources of information (Table 1.2, p. 8). Textbooks, websites from universities and government research agencies are usually very reliable. Publications and web pages from professional organisations such as the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australian Academy of Science (see weblinks), and equivalent international organisations are also good sources.

Websites containing student research such as science fair projects are not always reliable, although they can be useful for getting ideas. Online sources that try to sell you something or promote a particular point of view should be treated sceptically. If there is clear **bias** or a limited perspective contained in the information, then you should avoid using that information.

Talk to your teacher or librarian about sources of information. Your library may also have access to databases containing scientific journal articles. Your teacher or librarian will be able to help you to assess whether a website is reliable and suggest suitable sites. The CRAAP (Currency, Relevance, Authority, Accuracy and Purpose) test will provide you with a list of questions to help evaluate the resources that you are using. (See weblink.)

Table 1.2 Features of reliable and unreliable information sources

Reliable sources	Unreliable sources
Contain current information and seek to inform the reader Contain information that is relevant to your project or inquiry Are from a reputable source such as a university or scientific research institution Contain information that is likely to be accurate, e.g. a peer-reviewed journal article Avoid bias	Are not from reputable sources Present obvious bias Do not contain references for their claims, or they provide links to unscientific references Have not been updated regularly Contain unrelated advertising content Use vague terminology

WB
1.1.2 DESIGNING AN INVESTIGATION PAGE 5

Variables

Once you have decided on your research question and done your background research, it is time to consider how to design an investigation to answer your research question. Before you do this, you need to understand variables. There are different types of variables.

Independent variable

The **independent variable** is the factor that you change or manipulate in your investigation. For example, if your research question was 'Does a new fertiliser increase root growth more than a standard fertiliser?', then your independent variable would be *type of fertiliser*. You would be using two different types of fertiliser and measuring their effect on root growth.

Dependent variable

The **dependent variable** is the factor that you measure during an investigation. For the above research question, the factor that you would be measuring is *root growth*, so this is your dependent variable.

Another example is a scientist testing the effect of water temperature on seed germination in *Acacia* seeds. The research question would be 'Do different temperatures of water affect the germination of *Acacia* seeds?'. The independent variable would be the temperature of the water poured onto the *Acacia* seeds and the dependent variable would be the number of *Acacia* seeds that germinate.

Usually your investigation will have one dependent variable and one independent variable. The investigator will change the independent variable and measure the influence on the dependent variable.

Hypothesis

If your research question requires a controlled experiment methodology, then your next step is to turn your research question into a hypothesis. A **hypothesis** is a tentative prediction, or a tentative explanation of an observation, based on an existing **model** or **theory**. A hypothesis should give you a prediction that you can test by performing an investigation. This means it should at least be falsifiable; that is, it should be able to be refuted (shown to be wrong). However, you will not generally be able to claim that you have proved your hypothesis. Rather, you may be able to say at the end of the study that your results support your hypothesis. Hence, an aim for an investigation should not start 'To prove …' because it is not possible to prove a hypothesis, only to refute it. If your investigations agree with predictions based on your hypothesis, then you can claim that they support your hypothesis. This increases your confidence in your model, but it does not prove that it is true.

A well-designed research question and hypothesis will guide your inquiry and help you to understand whether or not you have met the aims of your investigation (Table 1.3).

Table 1.3 Features of an effective research question and hypothesis

	Effective research question	Effective hypothesis
Phrasing	Framed as a question Specific and includes scientific terminology	Framed as a prediction based on your background research Specific and includes scientific terminology
Use of variables	Includes mention of the independent and dependent variables, e.g. 'How does the independent variable effect the dependent variable?'	Includes mention of the independent and dependent variables, e.g. 'If there is a change in independent variable, then this will result in a predicted trend in the dependent variable.'
Predicts results	No	Yes
Outcome	Can be answered by an investigation	Can be supported or refuted by an investigation

For example, using the research question 'Does a new fertiliser increase root growth more than a standard fertiliser?', the hypothesis could be: '*If* the new fertiliser is used rather than the standard fertiliser, *then* root growth will increase in length.' You will note that this hypothesis meets all the requirements set out in Table 1.3. This hypothesis:

- predicts what the result will be (root growth will increase)
- is specific in that it states: if … *the independent variable is changed* … then … *something will happen to the dependent variable* …
- mentions the independent (fertiliser) and dependent (root growth) variables
- states how the dependent variable will be measured (root growth measured in length)
- can be supported or refuted by an investigation.

Methodologies

WB
1.1.3
METHODOLOGIES
PAGE 6

Methodology refers to the broader framework of approach taken in the investigation to test your research question. If you wanted to test the effect of temperature on enzyme action, then you would choose a controlled experiment methodology. If you wanted to identify and name all the plant species in a particular area, then you would choose a classification and identification methodology. For the list of methodologies that are considered in VCE Biology, see pages 9–10 of the *VCE Biology Study Design*. These are summarised in Figure 1.4. Some of these methodologies can be carried out in the school science laboratory, others in the field (called fieldwork) and others in the computer lab using secondary sources or databases. The methodology that you choose depends on your research question. Table 1.4 gives you an idea of different methodologies that would be suitable for investigating different research ideas.

Figure 1.4 There are many different scientific investigation methodologies in biology.

Table 1.4 Types of methodologies and investigations (Not all methodologies mentioned in the *VCE Biology Study Design* have been included. Refer to the *VCE Biology Study Design* pages 9–10 for a full explanation of each methodology.)

Methodology	Where you would carry out your investigation		
	In the science laboratory	In the field	In the computer lab
Case study: research into a real or hypothetical situation that mirrors real life	Investigating the causes of recent environmental damage in an ecosystem		
Classification and identification: arranging and identifying individuals within a group	Sorting DNA from different species by gel electrophoresis Characterising DNA samples by restriction enzymes and gel electrophoresis	Surveying, classifying and identifying organisms in a particular habitat	Examining characteristics of fossils – e.g. hominin fossils from a museum database Comparing three-dimensional models (including ribbon diagrams) and/or amino acid sequences from protein databases
Controlled experiment: an experimental investigation that looks at the relationship between the independent and dependent variables, making sure all other extraneous variables are controlled	Enzymatic activity – e.g. examining the effects of pH, temperature and substrate concentrations on the rate of reaction; comparing commercial enzymes with those extracted from nature; comparing enzyme activity across different species (e.g. catalase in animal tissues and plant tissues) Cellular respiration – e.g. examining factors that affect the rate of cellular respiration (reactant concentration, enzyme activity, pH, age or subspecies/variety, such as comparing the rate of anaerobic respiration in baker's and brewer's yeast varieties) Photosynthesis – e.g. examining factors that affect the rate of photosynthesis (reactants, enzymes, pH); examining photosynthesis in different organisms; chromatography of different photosynthetic pigments		Determining evolutionary relationships between species by phylogenetic analysis of protein sequences Comparing genomes or chromosome maps from populations of organisms
Correlational study: a study to understand the relationship between two or more factors	Collecting leaves from different areas to discover if the number of chloroplasts in plant cells differs with environmental conditions		
Fieldwork: observing and recording observations beyond the classroom		Bioprospecting for useful antimicrobial substances (e.g. in native or indigenous flora) and their effectiveness against a range of bacteria	
Literature review: collation and analysis of **secondary data**			Surveying global distribution patterns of populations over time Analysing global health statistics – e.g. antibiotic resistance rates for particular microbial species and infection outcomes Conducting an epidemiological study of vaccination rates and infectious disease rates Systematically reviewing published studies – e.g. whether vitamin C cures the common cold

Methodology	Where you would carry out your investigation		
	In the science laboratory	In the field	In the computer lab
Modelling: constructing a physical, conceptual or mathematical model to represent a biological object, system or variables	Making a model to show how materials move through the plasma membrane		Making a computer model to show how proteins move through the protein secretory pathway Making a model to predict the impacts that an environmental policy has on an ecosystem
Simulation: using a model to study a biological system			Creating a simulation to show enzyme action in a number of different conditions, such as increased substrate or amount of enzyme

KEY CONCEPTS

- Observation is the start of a scienti fic nvestiation.
- An obsevation can e turned into rsearch question.
- A research uestion is speci fic and can be answered by performing a ivestigation.
- A research uestion identi fies the dependent and ndependent vaiables.
- Youll need to undertake some background research nto your researchquestion.
- Make sure y citically evaluate the secondary resources that you use for your background research.
- Th independentvriable is the factor that you change or maiplat in your invesigation.
- The dependen vaiable is the factor that you measure during your nvetigation.
- A research question can be turned into a hypothesis.
- A good hypotesis predictsatthe results will be, states he relationship between the independent and dependentvriables, states how the dependent varae will be easured, and can be supported or refuted thrughinestigation.
- Methdology refers to the broader framework of approach take to ivestigate you rsearch question.

Concept questions 1.1a

1 Define:
- **a** research question
- **b** ndependentvriable
- **c** dependentvriable
- **d** hypot esis.

2 What deermines the type of methodology that you would use in yur ivstigation?

HOT chaenge

3 What are the hree main features of a good hypothesis?

Designing your investigation to test your hypothesis

WB
1.1.4 DESIGNING YOUR INVESTIGATION TO TEST YOUR HYPOTHESIS PAGE 7

Once you have decided on a specific research question, hypothesis and methodology, you can begin to design your investigation. This information should be recorded in your logbook. (See the information about maintaining a logbook on p. 18.) Having a plan ensures that you take the measurements that you need. The longer the investigation, the more important it is that you have a clear plan. There are several things to consider.

- What data will you need to collect?
- When and where will you collect the data?
- How will you analyse the data?
- What materials and equipment will you need?
- What are the independent and dependent variables?
- Have you identified all the variables that could influence your results (p. 8)?

Note:

Unit 4 Area of Study 3 Outcome 3 specifies that your scientific investigation must generate primary quantitative data.

Figure 1.5 Plan exactly what you will measure to collect your data. Do you want to measure the length of roots or the mass of roots? Where do the roots end? Will you use fresh weight or dry weight?

Primary data

Primary data is data that you collect in an investigation. Generating primary data involves collecting quantitative and/or qualitative data.

Quantitative data is data that is a quantity and is recorded numerically. You measure the numerical value in the appropriate units. For example, you may measure root length in centimetres or mass of roots in grams.

Qualitative data is non-numerical and can be directly observed; it is a quality. For example, you may observe that when you add chemical X to chemical Y, a colour change of red to green occurs.

Sometimes you use a combination of qualitative and quantitative data. For example, you may describe the length of roots as reaching a maximum in centimetres (quantitative) but growing in a particular direction or pattern (qualitative).

As discussed earlier, your hypothesis should specify your dependent variable – what you are quantitatively measuring. In the case of the hypothesis '*If* the new fertiliser is used rather than the standard fertiliser, *then* root growth will increase', you will be measuring root growth. You need to determine how you are going to measure root growth (Figure 1.5). Will it be by length or by mass? If it is by length, then which unit will you use (millimetres or centimetres), and where does the root start and where does it end? Are you measuring all the roots, or only the longest? If you are measuring by mass, you will also need to decide which unit you are going to use (milligrams or grams), where the root starts, and if you are going to use wet or dry mass (all the water removed). You need to make all these decisions before you carry out your investigation.

Keep a record of all of your planning in your logbook. This is useful information as your research project evolves and is needed for authentication purposes. Writing down what you plan to do, and why, will also help you to stay focused during the investigation.

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1.1.5 PRIMARY DATA PAGE 9

Quality of primary data

Plan your investigation to generate the highest quality data. A well-designed investigation allows you to collect data that is accurate, precise, repeatable, reproducible and valid (Table 1.5 and Figure 1.6). This means you can rely on the data to draw conclusions, and be confident that a difference between one measurement and another reflects a real change in what is being measured.

Measurements are **valid** if they measure what is supposed to be measured. Validity of measurement is important because in a well-designed investigation, results are affected only by one single independent variable. Only investigations in which all extraneous variables have been controlled will produce valid results (p. 14). If the results are similar each time, then your results are more likely to be both valid and reliable.

A measurement is **accurate** if it is close to the **true value** of the parameter being measured. You will also aim to collect **precise** measurements; that is, repeated measurements that are close to each other. For example, you might measure the length of plant roots of five plants under the same conditions

and record the following measurements: 15.2 cm, 16.1 cm, 127.9 cm, 14.9 cm and 16.7 cm. The 127.9 cm measurement stands out as not being close to the other measurements and you would have to ask whether the 127.9 cm measurement is precise.

If a result is not repeatable by you or reproducible by others, it is probably not a valid result. A result is **repeatable** if the same measurement (within the limits of experimental uncertainty) is made more than once by the same investigator using the same equipment under the same conditions. A result is **reproducible** if another investigator, following your method, obtains data that replicates the effect you observed and leads them to the same conclusion as yours, even if there are some small differences between your results and theirs (e.g. due to the different equipment used to take the measurements). If a result is not repeatable or reproducible, then a variable other than the one you are controlling is affecting its value. If this is the case, you need to identify the other variable and control it if possible.

Table 1.5 Quality of data

Valid	Does it measure what it is supposed to measure?
Precise	How closely do individual measurements agree with each other?
Accurate	Has the data been measured and recorded correctly?
Repeatable	Can the same investigator use the same investigation and equipment and get the same result?
Reproducible	Can another investigator use the same method and equipment and get a similar result?

Sometimes investigations simply do not work or cannot be done for some reason, such as equipment failure or unforeseen variables. For example, root growth will be affected if the plants contract a disease during the investigation. Try to think of all the things that could go wrong. If possible, come up with back-up plans. Allowing plenty of time helps as does starting your investigations as early as possible.

Think about how you can minimise **uncertainty**. Experimental uncertainty is the doubt associated with the value derived from measuring a variable, usually affected by the equipment used to take that measurement. For example, are your scales working properly, or has your tape measure stretched?

Make sure you have allowed time for analysis. Do as much analysis as you can while you collect results. If you plot graphs as you take measurements, then you will be able to identify outliers early. An **outlier** is a data point that does not fit the pattern of the rest of the data and may distort the data, acting as a source of error. If you identify an outlier while you still have access to equipment and space, then you can check the measurement and make sure that you did not make a mistake or that the investigation has not been compromised by an **extraneous variable** (p. 14).

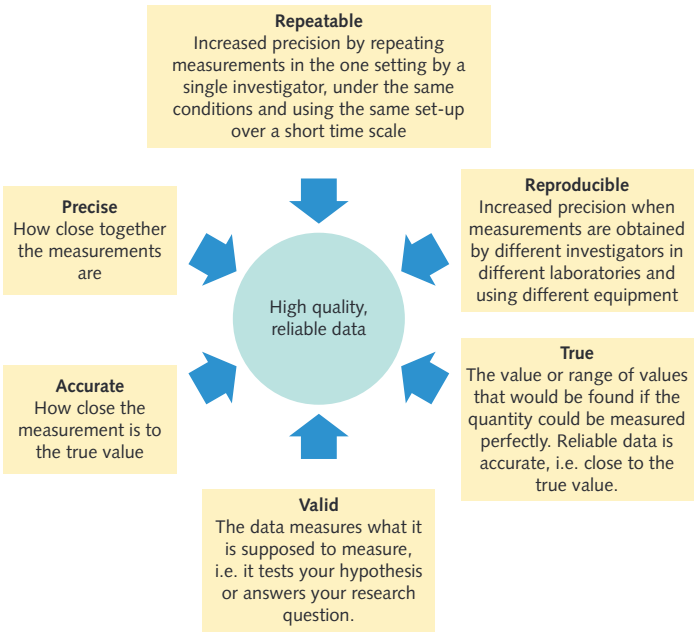


Figure 1.6 Features of data from a well-designed investigation

With these things in mind, you may need to consider the number of **replicates** to include in your investigations. These are independent samples that allow you to take multiple measurements, increasing the reliability of your data. In the root growth example, growing 10 plants in each experimental condition allows you to calculate an average value as well as the variation between values in your sample set. If the variation is small, it is likely that only one independent variable is acting in your investigation and your results are more likely to be reliable.

WB
1.1.6 MINIMISING ERROR PAGE 10

Minimising error

There are various causes of errors in investigations. For example, errors can be due to investigators' mistakes or desire to get particular results, equipment errors or if experimental subjects are not randomly assigned to experimental groups.

Personal errors arise as a result of investigators' mistakes or miscalculations.

Systematic errors are predictable errors that arise through imperfections in the equipment used to take the measurements. They cause measurements to differ from the true value by a consistent amount each time a measurement is made. To minimise systematic errors, you first need to make sure that all your equipment has been calibrated and tested. Calibration ensures that the equipment gives the correct readings with known standards. For example, scales can be calibrated by using known weights. The equipment should at least be calibrated at the top and the bottom of its range. Instructions for calibrating equipment correctly can be obtained from your teacher, laboratory technician or the user manual for the equipment.

Random assignment of subjects into experimental and control groups is an important part of the design of an investigation. For example, in a clinical trial of a new drug, patients should be randomly assigned to the **control group** or the drug (experimental) group with equal representation in both groups of age, gender, ethnicity and other variables. Clinical trials are also designed as double-blind studies in which neither the patient nor the nurse or doctor treating the patient knows which group they have been assigned to. These steps are essential for reducing bias.

Even in a well-planned investigation, **random errors** can occur. These are variations in the data and result in less precise measurements. The influence of random errors can be reduced by using multiple trials or samples (replicates) and ensuring that your investigation is repeatable.

When the person conducting the investigation and making the measurements has particular expectations about the results, this can introduce bias in the study. An example of bias in an investigation is an investigator choosing the tallest or healthiest plants to treat with the new fertiliser; these plants might give the biggest growth measurements at the end of the study. When an investigation is biased, the results are not valid and no conclusions can be made from the investigation.

For every step of your investigation, try to identify possible sources of error and come up with ways of eliminating systematic, random and personal errors, and incorporate these into your investigation design. If you make a mistake during your investigation, then you should repeat the investigation. For example, it is good planning to make sure that you have germinated enough seeds so that you have extra plants in case you need to repeat the investigation.

Figure 1.7 Keeping conditions as consistent as possible helps to control the extraneous variables.

Controlled variables

Consider the hypothesis: '*If* the new fertiliser is used rather than the standard fertiliser, *then* root growth will increase in length'. You may already have realised that there are a few more things that can influence root growth other than type of fertiliser. For example, root growth could also be influenced by the species of plant, amount of water the plants receive, the amount of sunlight, the type of soil they are growing in and how much wind they are exposed to. These other things that could affect your results are extraneous variables and they need to be controlled or kept constant (Figure 1.7). By controlling all other variables, you are able to obtain baseline data that shows that only your independent variable is influencing the results, not any other extraneous variable (Figure 1.8).

An extraneous variable must become a **controlled variable** by identifying it and keeping it constant during the investigation. If this happens, then it does not affect the

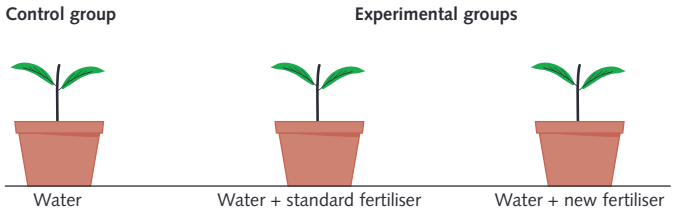


Figure 1.8 **It is important to control all the extraneous variables so that only the independent variable influences the dependent variable. The following should be the same in the two groups: size of plants, potting mix, exposure to sunlight and other environmental factors (e.g. atmosphere, wind).**

interpretation of the relationship between the dependent and independent variables. In the root growth investigation example, controlled variables would include plant type, plant health, growing medium, planting depth, light exposure, water available to plants, air circulation and the temperature of the growing medium. These are all potential confounding factors that would need to be controlled; that is, kept constant.

Table 1.6 summarises the different types of variables in an investigation.

Table 1.6 **Types of variables**

Type of variable	Definition
Independent variable	The variable that is changed or manipulated by the investigator. It is assumed to have an effect on the dependent variable.
Dependent variable	The variable that is measured. Its value depends on the independent variable, i.e. it responds to the independent variable.
Extraneous variable	A variable, other than the independent variable, that may affect the outcome of an investigation. These variables need to be controlled (kept constant).
Controlled variable	A variable that is kept constant during the investigation so that the investigator can determine the relationship between the independent and dependent variable.

Resources to carry out your investigation

When planning your investigation, you will need to consider several things (Table 1.7). As you are going to collect primary data for your investigation, you will need to make a list in your logbook of all the equipment that you will require. This will also help you later when you write your experimental **method**. An experimental method is the steps you take to carry out your investigation. You will need to consider how precise your measurements must be. If your hypothesis predicts a temperature change of 0.1°C but you can only measure to a precision of 0.5°C, then you will not be able to test your hypothesis. You may need to think carefully about how you measure some things. For example, in an investigation into root growth, you may need to measure the dry weight of the roots, which means finding a consistent way to dry them. Are you going to measure in grams or milligrams? If you measure in milligrams, you will need measuring scales that measure to that level of precision.

Table 1.7 **Considerations when planning an investigation**

Consideration	Questions to ask
Data and measurements	What kind of data needs to be collected, how often and by whom? Does data need to be collected outside class hours?
Materials and equipment	Can your equipment enable you to collect data that is precise enough? How can you best collect the data and minimise uncertainty?
Safety	Do you have access to all the required safety equipment, including a fume cupboard or specialised methods for disposal of waste?

The equipment you use in your investigation must be safe. Consider whether you will need personal protective equipment such as lab coats, safety glasses or gloves. (See Risk assessment below.) Include any safety equipment needed in your equipment list.

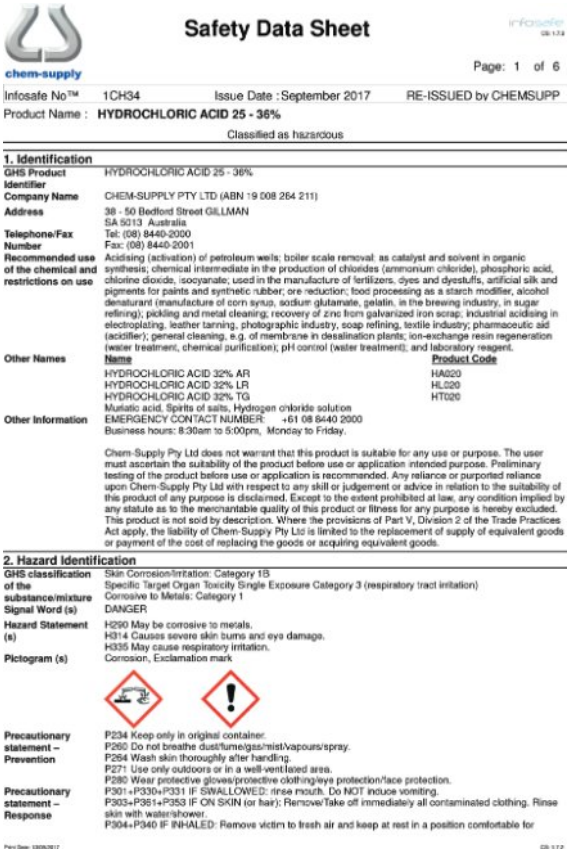
When you have made your list, discuss with your teacher the equipment that is available. You might need to modify your research question or hypothesis at this stage if the equipment that you require is not available. Consider where you will perform your investigations or observations. Can you use the normal classroom space, or do you need to be outside? If you are outside, how can you make sure that you can work without interference? Will you need to consider the convenience or safety of others? Talk to your teacher about what space is available.

Risk assessment

Risk assessment is the process of evaluating the potential risks of an investigation. Even if this is not a requirement for your own investigation, you should consider the following.

- What are the possible risks to you, other people, the environment or property?
- How likely is it that there will be an injury or damage?
- If there is an injury or damage to property or the environment, how serious are the consequences likely to be?

If you intend to use hazardous chemicals, you will need the relevant safety data sheet (SDS). This provides information on how the chemical affects health and safety (Figure 1.9). An SDS gives guidance on the safe handling, storage and disposal of the chemical, as well as emergency procedures for exposure. The SDS for a chemical can usually be found by an Internet search or by looking on the manufacturer's website. It is important to read the SDS when assessing the risk associated with using a chemical and the precautions you should take in your investigations. Table 1.8 shows a matrix for assessing the likelihoods and consequences of risks in investigations.



chem-supply

Safety Data Sheet

Page: 1 of 6

Infosafe No™ 1CH34 Issue Date : September 2017 RE-ISSUED by CHEMSUPP

Product Name : **HYDROCHLORIC ACID 25 - 36%**

Classified as hazardous

1. Identification

GHS Product Identifier	HYDROCHLORIC ACID 25 - 36%
Company Name	CHEM-SUPPLY PTY LTD (ABN 19 008 264 211)
Address	38 - 50 Bedford Street GILLMAN SA 5013 Australia
Telephone/Fax Number	Tel: (08) 8440-2000 Fax: (08) 8440-2001
Recommended use of the chemical and restrictions on use	Acidising (activation) of petroleum wells; boiler scale removal; as catalyst and solvent in organic synthesis; chemical intermediate in the production of chlorides (ammonium chloride), phosphoric acid, chlorine dioxide, isocyanate; used in the manufacture of fertilizers, dyes and dyestuffs, artificial silk and pigments for paints and synthetic rubber; ore reduction; food processing as a starch modifier, alcohol denaturant (manufacture of corn syrup, sodium glutamate, gelatin, in the brewing industry, in sugar refining); pickling and metal cleaning; recovery of zinc from galvanized iron scrap; industrial acidising in electroplating, leather tanning, photographic industry, soap refining, textile industry; pharmaceutic aid (acidifier); general cleaning, e.g. of membrane in desalination plants; ion-exchange resin regeneration (water treatment, chemical purification); pH control (water treatment); and laboratory reagent.
Other Names	Name: HYDROCHLORIC ACID 32% AR; HYDROCHLORIC ACID 32% LR; HYDROCHLORIC ACID 32% TG; Muriatic acid, Spirits of salts, Hydrogen chloride solution. Product Code: HA020, HL020, HT020
Other Information	EMERGENCY CONTACT NUMBER: +61 08 8440 2000 Business hours: 8:30am to 5:00pm, Monday to Friday.

Chem-Supply Pty Ltd does not warrant that this product is suitable for any use or purpose. The user must ascertain the suitability of the product before use or application intended purpose. Preliminary testing of the product before use or application is recommended. Any reliance or purported reliance upon Chem-Supply Pty Ltd with respect to any skill or judgement or advice in relation to the suitability of this product of any purpose is disclaimed. Except to the extent prohibited at law, any condition implied by any statute as to the merchantable quality of this product or fitness for any purpose is hereby excluded. This product is not sold by description. Where the provisions of Part V, Division 2 of the Trade Practices Act apply, the liability of Chem-Supply Pty Ltd is limited to the replacement of supply of equivalent goods or payment of the cost of replacing the goods or acquiring equivalent goods.

2. Hazard Identification

GHS classification of the substance/mixture	Skin Corrosion/Irritation: Category 1B; Specific Target Organ Toxicity Single Exposure Category 3 (respiratory tract irritation); Corrosive to Metals: Category 1
Signal Word (s)	DANGER
Hazard Statement (s)	H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage. H335 May cause respiratory irritation.
Pictogram (s)	Corrosion, Exclamation mark
Precautionary statement – Prevention	P234 Keep only in original container. P260 Do not breathe dust/fume/gas/mist/vapours/spray. P264 Wash skin thoroughly after handling. P271 Use only outdoors or in a well-ventilated area. P280 Wear protective gloves/protective clothing/eye protection/face protection.
Precautionary statement – Response	P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting. P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. P304+P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for

Figure 1.9 A safety data sheet (SDS) for hydrochloric acid

Table 1.8 A matrix for assessing severity of risk

Likelihood	Consequences			
	Negligible	Marginal	Severe	Catastrophic
Rare	Low risk	Low risk	Moderate risk	High risk
Unlikely	Low risk	Low risk	High risk	Extreme risk
Possible	Low risk	Moderate risk	Extreme risk	Extreme risk
Likely	Moderate risk	High risk	Extreme risk	Extreme risk
Certain	Moderate risk	High risk	Extreme risk	Extreme risk

Once you have considered possible risks, you need to plan how to address them. What will you do to minimise them, and how will you deal with the consequences if something does happen? This may be as

Table 1.9 Risk assessment table

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
The fertiliser might be spilled on clothes or skin during application.	Wear a lab coat, gloves and safety glasses. Clean up spills immediately.

simple as using personal protective equipment such as a lab coat, gloves and safety glasses. Table 1.9 is an example of a risk assessment for an investigation.

Safe use and disposal of biological material

It is important to know how to safely handle and dispose of biological materials. For example, when growing known or unknown microbes on agar plates, you must use safe sterile techniques (see weblink) and wear lab coats, safety glasses, gloves and, if required, face masks. Treat all microbes on agar plates as potentially pathogenic and kill the bacteria by autoclaving (heating with pressurised steam) used plates before disposing of them. If you are uncertain about how to dispose of material used in your investigation, ask your teacher or laboratory technician.

Weblink
Sterile technique

Ethical guidelines

WB
1.1.7 ETHICAL GUIDELINES PAGE 11

Ethics is a system of moral principles that considers what is good and bad for society. Put simply, it considers what is right and wrong. Bioethics is ethics in the context of biological research. You need to consider ethical guidelines relevant to your selected investigation during the planning stages of your investigation.

The following approaches can help guide you through ethical considerations relating to your investigation.

- Consequences-based approaches aim to maximise the positive effects (benefits) and minimise the negative effect (harms) of a particular action. The *end results* are key in this approach.
- Duty- and/or rule-based approaches state that people have the duty to act in a certain way, and obey certain rules, regardless of the outcome. The *actions*, or *means*, are key in this approach.
- Virtues-based approaches consider the moral character or virtue of the person conducting the action: are they seeking to exhibit 'good' characteristics and behaviours? The *person conducting the action* is key in this approach.

There are several concepts relating to acting ethically in your research.

- **Integrity** is about being honest as a scientist. This means recording data accurately and not ignoring, hiding or changing any data that does not support your hypothesis. It means acknowledging and referencing sources of information, including books, websites, articles and people who have helped you. It means not using other people's ideas or data without their knowledge or permission. It also means allowing others to fully scrutinise your work to further public knowledge and understanding. Put simply, showing integrity is 'doing the right thing'. A good rule is that if you would not want someone to know what you were doing, you probably should not be doing it. It is no different from behaving ethically in any other area of your life.
- **Justice** is the moral obligation to consider competing claims, not place unfair burden on a particular group and fairly distribute or allow access to the benefits of an action.
- **Respect** means giving intrinsic or instrumental value to living things, and being considerate of their welfare, freedom, autonomy, beliefs, perceptions, customs and cultural heritage. It also means considering that living things can make their own decisions and empowering and protecting those who have diminished capacity to do so.

When planning your research, you should also be guided by the following ethical principles.

- **Beneficence** is a commitment to maximise the benefits and minimise the risks and harms involved in taking a particular course of action.
- **Non-maleficence** is the commitment to avoid causing harm and ensuring that any harm caused is proportional to the benefit gained from taking that course of action.

Refer to the *VCE Biology Study Design* for further information about the ethical conduct of scientific investigations (p. 5) and ethical approaches and concepts (pp. 15–16).

KEY CONCEPTS

- You need to hæ aclear plan or our investigation.
- Primary data can bequatitatiqualitative or both, depeding on your hypothesis.
- Measurements must be ccuae, precise, reproducible, repeatable nd valid.
- Take every pecauo to minimise errr in your data.
- ll extraneos variablemut be controlled.
- The metho details the sts that you will undertake to perform ourinestgatio, including a risk assessent.
- Check whether you need to coside any bioethical ssue in yur ivstigation.

Concept questions 1.1b

1 Wich data type recods numerical measurements?
2 List five types of inputs ou could us for qualitative data.
3 Define vldi' in termsof cienti fic research.
4 Define prison' in terms ofata collected.
5 Wh is caefulplanning importat when conducting an inestigation?
6 Wha is a cotro andis it different fom a controlled variable?
7 What is uncrtainty and how can you take uncertainty into account when planning your investigation?

HOT chaenge

8 Ethial onsidration and risk assessment are two aspectsof scienti fic methodology. How do these two mportant features inerset inexprimental design? Is there poetial scope for con flct between the two?

1.2 Scientific evidence

Scientific evidence is primary data that comes from scientific investigations. It is only valid if the investigation has been designed carefully, with errors minimised and bias eliminated, has been properly experimentally controlled, and has been found to be repeatable and reproducible.

Record keeping

Students studying VCE Biology are required to keep a record of their practical activities in a logbook. This is for recording, **authentication** and assessment purposes. You can keep your logbook in hard copy form or soft copy form (electronic), although hard copy is preferred.

Logbooks include details of investigations such as methods and results. They include comments and ideas, thoughts about the investigations, and analysis. They frequently include printouts of data, photocopies of relevant information, photos and other items (Figure 1.10).

Discuss with your teacher about the form of logbook records you are required keep and any specific formatting required for your logbook. Make an entry in the logbook every time you work on your investigation.

Each entry in your logbook needs to be dated. Write down what you do as you do it. It is easy to forget what you did if you do not write it down immediately. An accurate record is important if you need to repeat any measurements or if you get unexpected results. The more detail you include, the easier it will be to prepare your report or poster at the end of this study. Include large, clear diagrams of any experimental set-up and details of equipment used. You can also include photos of investigations.

Record the results of all measurements immediately and directly in your logbook. Never record data on bits of scrap paper. Use a pen, not a pencil, to record your results. Never use liquid paper or scribble over anything in your logbook. If you want to cross something out, put a line through it. It is also a good idea to make a note explaining why it was crossed out.

Collecting raw data

To determine a relationship between your variables, you need to have enough data points and the range of your data points should be as large as possible. A minimum of six data points (therefore six replicates) is generally considered adequate, but collect as many as you reasonably can in the available time.

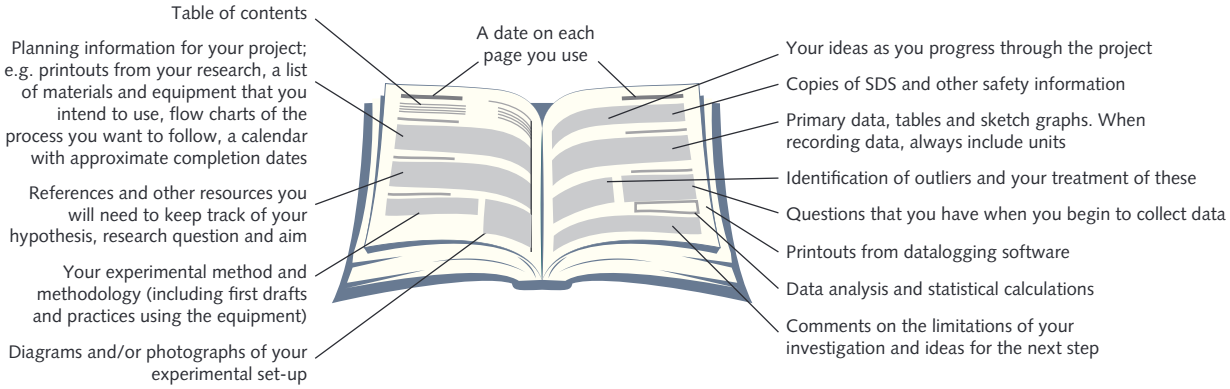


Figure 1.10 Features of an effective logbook

Always record the raw data directly in your logbook unless you are using a data logger connected to a computer to record the data. In this case, attach a printout of the data to your logbook and record the file name and location. Make sure that you measure and record everything you will need for your analysis. For example, if you were investigating root growth, you would record the amount of fertiliser used, the temperature and the starting length of the roots. It is much better to measure something that you discover later that you do not need than to start your analysis and realise that you did not measure something that you need.

Use appropriate units; for example, millimetres (mm) for length and grams (g) for mass. If you collect multiple data points, it is a good idea to record them in a table. Label the columns in the table with the name and units of the variables. Do not put the units in the table cells. The instruments that you use will often restrict the precision of your measurements. For example, a ruler may only have markings down to 0.1 cm. Make a note of these restrictions because they can also affect the accuracy of your final results, especially if the changes measured are small.

Analysing your data

WB
1.2.1 ANALYSING YOUR DATA
PAGE 15

Having gathered your data, there are usually a number of steps you need to take to analyse it. This allows you to draw meaningful conclusions from your investigation, leading you to either support or refute your hypothesis. Usually, you will use descriptive statistics to describe the data (such as calculating the mean), plot a graph of the data, and try to determine whether any trends or patterns emerge in the data.

Record all your analysis in your logbook. If you use a computer for your analysis, then record the file name and location and attach a printout of the analysis to your logbook. Many scientists have logbooks that are bulging with printouts of their analysis.

Diagrams

You may need to include scientific diagrams in your results. You can include drawings of your experimental equipment and how it is set up when collecting data, drawings of structures under the microscope, or drawings of organisms that you are studying in your investigation. Like other kinds of data, diagrams require a figure number and a clear title.

There are other important rules to follow when constructing scientific drawings (Figure 1.11).

» Use pencil.
» Create a large drawing that fills at least half a page.
» If using a microscope, include the magnification at which the image was viewed.

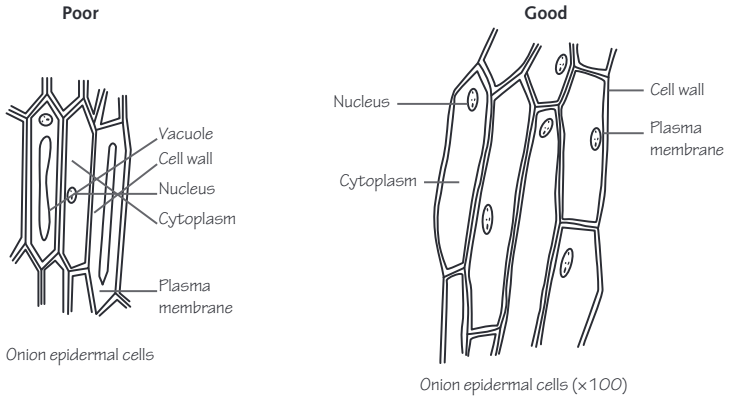


Figure 1.11 Effective scientific drawings: include the magnification (where appropriate) and make sure label lines do not cross.

» If the drawing is of an organism, include this information.
» If drawing your experiment, draw all equipment in cross-section.
» Label all the parts of your diagram and rule a line to each label. Ensure that the lines do not cross.

Tables

When recording data from your investigation, you will probably write it in a table. The advantage of this is that you can organise your data as you record it and begin to identify trends. A well-organised table saves you significant time later. Your table should have a clear title and be organised so that you can easily record and compare your independent and dependent variables. If you have completed any calculations on your data, you should record this information alongside your primary data. An example is shown in Figure 1.12.

Table 1: The effect of fertiliser on root growth ← Table number and a descriptive title

Dependent variable and units

Heading clearly identifies the independent variable

	Standard fertiliser							New fertiliser						
	Pant 1		lant Plant 3											
	Root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Root length (cm)	Change in root length (cm)	Change in root length (cm)
Day 0														
Day 1														
Day 2														

Multiple tests are completed

Control or baseline value is included

Mean result is calculated

Figure 1.12 An effective table to record primary data and simple statistics

Note:

The acronym DRY MIX can help you remember how to plot variables on a graph.
D = dependent variable
R = responding variable
Y = *y*-axis
M = manipulated variable
I = independent variable
X = *x*-axis
Plot the dependent (responding) variable on the *y*-axis. Plot the independent (manipulated) variable on the *x*-axis.

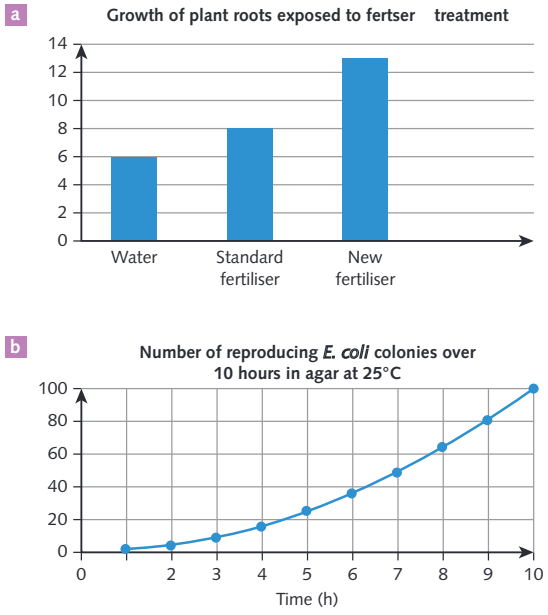


Figure 1.13 A correctly drawn **a** bar graph and **b** line graph

Graphs

You may be able to see a pattern simply by looking at the list of numbers in your table. However, the easiest way to identify a pattern in data or a relationship between variables is to plot a graph.

A graph should be large and clear. The axes should be labelled with the names of the variables and their units (Figure 1.13). Put the variable that you are measuring (dependent) on the vertical axis (y) and the variable that you are altering (independent) on the horizontal axis (x). Choose a scale so that your data takes up most of the plot area. You don't always need to show the origin in a graph but, by including it, you will provide an honest representation of the data without any exaggeration.

Interpreting your results

Once you have visualised your data by plotting it as a graph, you can begin to consider what trends, patterns or relationships the results are showing, and determine whether the trends are likely to reflect true relationships between the factors in your study. You can then relate your findings to your research question and hypothesis.

Determining relationships

When you have created a graph of your data, provided you have carefully considered the scale of your graph, and you have considered any outliers, you should be able to see any relationships. When writing your results section for your poster, you should describe any relationship that you notice between the independent and dependent variables, noting the **gradient** or slope of the graph. You should then try to interpret whether the relationship between the independent and dependent variables is a causative one (i.e. your data is a result of the treatments or test groups in your investigation). Your interpretation of the data, and what it means to the field of science that you are investigating, should be explained. Figure 1.14 shows the types of relationships that you may observe in your data.

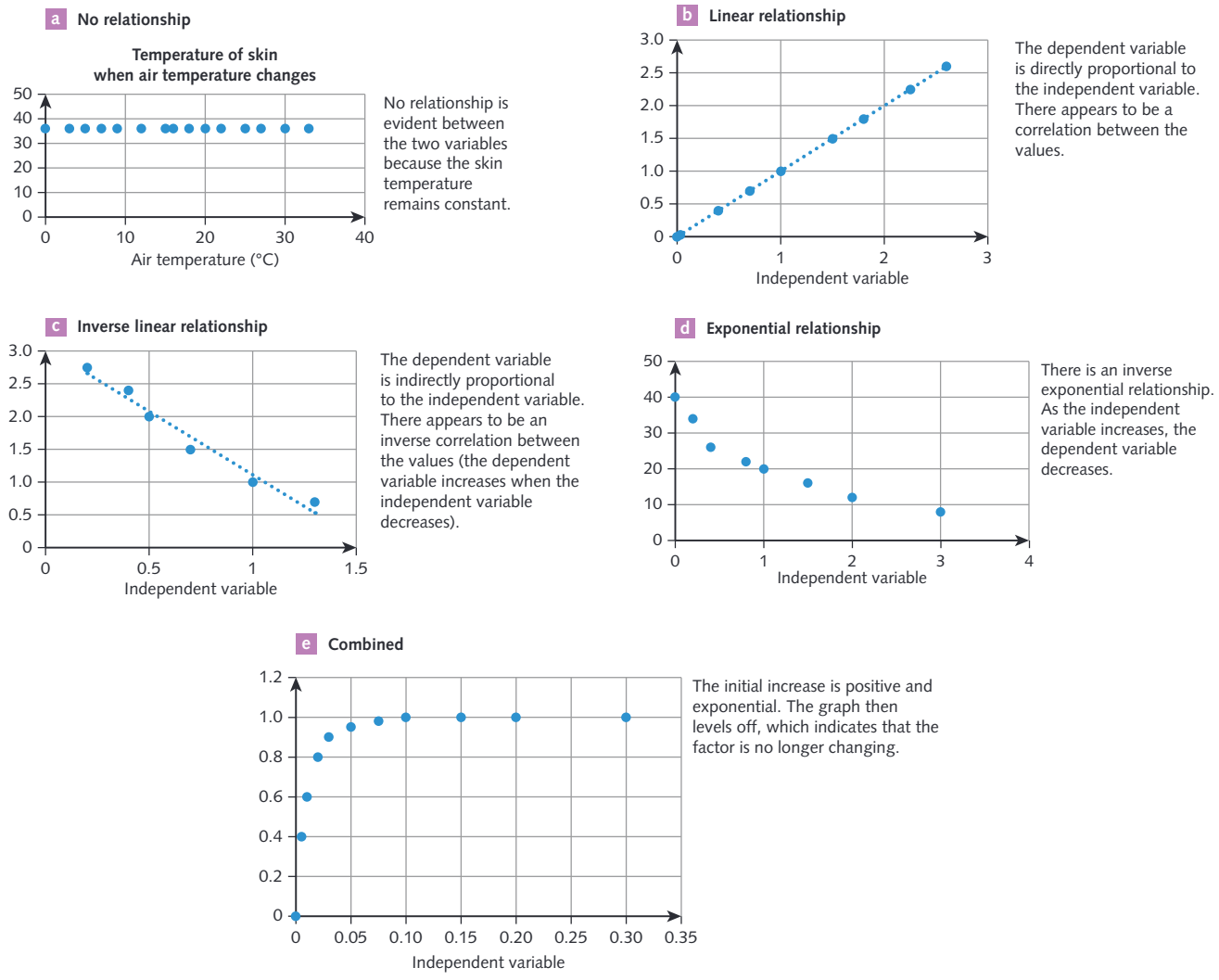


Figure 1.14 Different types of relationships that may be evident in data. There may be a no mathematical relationship, b & c a clear linear relationship or d & e an exponential relationship.

Relating your results to your hypothesis

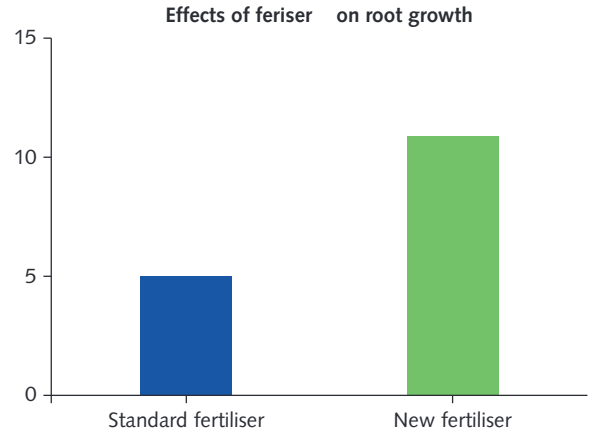


Figure 1.15 **This bar graph shows that there is a difference between the independent variables, which supports the hypothesis.**

Once you have analysed your results, you need to interpret them. This means being able to either answer your research question or state whether your results support your hypothesis. For example, if the new fertiliser induces greater root growth than the standard fertiliser, with all other variables being equal, this would support the hypothesis that 'The new fertiliser increases the rate of root growth in two weeks compared to standard fertiliser' (Figure 1.15). If there was no difference between the two, or if the new fertiliser induced significantly less root growth than the standard fertiliser, this would not support the hypothesis.

If your hypothesis is not supported

If there is not a significant difference in your data, your hypothesis may not be supported by the data. This may occur if the investigation could not show the effect posed by the hypothesis; for example, by not having enough replicates to reduce variability and produce a significant difference. Alternatively, the hypothesis may be wrong. However, it is not enough to simply say 'our hypothesis is wrong'. If the hypothesis is wrong, what is wrong with it?

Your method may have been too simple or may not have taken into account all of the other variables. For example, in the root growth investigations, the fertiliser may work best at a particular temperature, or over a longer time, or in conjunction with certain soil conditions. Or it may not work with the chosen type of plant. The investigation may have been too limited to fully test the hypothesis. You might conclude that further investigations are required to test these other variables.

However, before you decide that the method is at fault, check carefully that you have not made mistakes or ignored any variables. Think about factors that you did not consider, but which might have affected your investigation. Go through your method, results and analysis. Check that your equipment was correctly calibrated and that you were using it correctly. Check that you used the correct units for your data and that the units are correctly carried through all calculations during analysis. Check your analysis carefully. If you are working in a group, ask another person to repeat your calculations.

If you are certain your investigation results are real, and they still refute your hypothesis, do not be disappointed. The process of scientific enquiry is often propelled forward when old hypotheses are tested and refuted, and new observations pave the way to discoveries that change our understanding of biological systems.

INVESTIGATION 1.1

The effect of temperature on trypsin activity

Caein is a commonprtein imamianmik. asein is di gested bytrypsin, an enzye that hydrolyses proteins nto pepids which other enzymes further break down to am no acids for us in the ody Trypsin wors in the small ntestne, after acid nd pepsin in the stomach have starte d breaing down the potins.C seinisrelatively hydrophobic, main it pory oluble n wate; however, when trypsin s added to a lute olutionf milkpowder, he casein is dgested andte soluio goes clear.

Am

To detemine the ptimal temperatur for ypsin activity

Time reuirement

45minutes

Materas

- 1% trypsi solution
- 3% olution fkim milk powder
- Bufferslution (pH 7)
- Water bath
- 6 test tubes and a test-tube rack
- Bungs or cork for test tubes
- Stopwatch
- Marker
- latc pipettes
- Thermometer
- Lab coats
- Safety glasses
- ispsal gloves

What are the rsks n dong th is investigation?	How can you manage these rsks to stay safe?
Trypsin can cause allergic reactions in sensitive peope	Make sure your teache r is aware of any allergis.
Trypsin can irritate the skn and eyes on contactWear	appropriate personal protective equpment at a tmes including eye protection and gloves Wash skin immediately if contact does occur.
Disposable gloves can cause allergic reactions in sensitive people	Use a type of glove that has no aergyrisk an is uitble to use with the chemicas n ths nvestgaton
Hot water can scad.	Be carful when wo rking with water hotter than 50°C Do not touch the outside of the glass beaker.

Method

1. For the control test, set the watr bath to 20°C.
2. Mark three tes tub wih an 'X' halfwy dow eah tube (Figure 1.16).
3. Usng a iptte, add 10 mL of th milk power solution to each of thethree test tubes.
4. Cllect another three test tubes and add 3 mL pH 7 buffr olution and 3 mL of trysin oluion.
5. Pace alsix test tbes in the water bath for 10minuts. Ensure he six tubes are uprigt.
6. Pour the trypsin and bfer solution from ne testtube into the milk powder sltin in another test tube.
7. Tomix thouhly, place a cork in th test tueand invert 4–6 times.
8. Pace the test ube in the testtube rak with the Xplaced at the back; mmediatelybegi te timer.
9. Record th tme it takefor he milk solution to becoe clear by measuring the time it takes for the X t come visibl though the solution.
10. Repeat steps 6–8 for the remaining test tubesand record the time for each of the three exerimentsin your logbook.

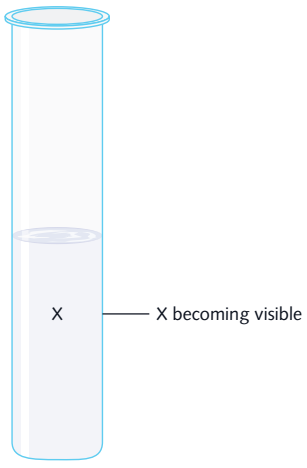


Figure 1.16 Measure the time it takes for the X to become visible.

11 Clulate the men eaction time adrecordthe esult in your logbook.
12 Your teacheill now ssign you one of four temperatures to test: 0°, 40°C, 0°C o 6°C. a class, you will testall four tempertues, pool your data and compare yor esults. Set the wat er bath to you assigned tmperature. Once your water bath has reaced the desied temperature, repeat steps 2–10 to test how the reaction time is affecte by different temperatures.
13 Record your data inyourlobok, calculate the mean reacio time and shareyor results wth the class.
14 Record te class ean reaction time or each of te assigned teperaues in cmbined results table. (See below.) f one of the temperaure variables was testemore than oce – i.e the 20°C control test – find the mean among them.
15 Draw a grph inyour lobook usig the ata in the table. Be sure to clerly abel both axes and use an appropriate scae.

Resuts

Sampe cass resuts for five temperatures

Temperature (°C)	Mean tim for ilksolution to clear (s)
20	
30	
40	
50	
60	

Dscusson

1 What is your hpothesis?
2 What is he independntvariable?
3 What is the rangeof the indepedent variable?
4 What is the depenen variable?
5 What are the extraeous variables ad how were they controlled?
6 Why ar all the tet ubes left in the water baths for 10minutes before the tryps n andilk are added together?
7 What are the advanage of calculatin a mean of test samples as opposed to just one?
8 What type fdistribution does the data show?
9 What is the ptimal temperatur for ypsin activity?
10 s the hypthesis supported by the data?

Concuson

Summaise the findngsusing the daaprovided. Relate the resul to the hypothesis.

KEY CONCEPTS

- Maintain a logbook thrughut our investigation for authetiation and asessment.
- Raw dtais analysed andsummaised as diagrams, tables or raphs.
- Detemine an reationshipsor trendsin your data.
- Relate your results to you hptesis, which is either supported or rfuted.

Concept questions 1.2

1 How doeskeeing a ogbook aidthescientist in their research?
2 Lst three reasons why a ypothesis may not be supported.
3 Reltinships and trends ar nmcalto cienti fic presenttion ofata.' Wat does this mean?
4 Lst at least four featuresf well-designed table.

HOT Chaenge

5 Ther is a genea rule about wich axis of a graph the ndependent vaiablis aplied to. State a general rule an itmise some exceptions.

1.3 Science communication

Once you have finished your investigation, you must be able to communicate what you have found to other people. This is important in science because often ideas from other people will spark new ideas and prompt further investigation. You are also adding to the body of scientific knowledge that you would have tapped into when you did your background research when you were planning your investigation.

There are a number of ways to present your findings, including journal articles and conference presentations. However, here you are required to present your findings as a scientific poster.

Presenting your work as a scientific poster

WB
1.3.1 PRESENTING YOUR WORK AS A SCIENTIFIC POSTER PAGE 18

You need to format your scientific investigation into a poster of no more than 600 words. This word count is limiting, so you will need to think very carefully about what you put in each section of the poster (Table 1.10).

Table 1.10 Sections in a scientific poster

Section	Description
Title	This should be the same as your research question.
Introduction	Make sure that you explain the reason for your research and link it to the underlying biological theory. You should also include an aim, hypothesis and/or prediction in this section.
Methodology and methods	Briefly outline your methodology here. Summarise the data generation and data analysis methods that you used.
Results	Your analysed data should go here. Your results may include graphs, perhaps with statistical analysis such as a line of best fit. You may also include tables of mean data. Remember that your raw data will be available in your logbook.
Discussion	In this section, you will need to show that you understand what your data means and be able to make links to the underlying scientific concepts that you were investigating. You need to analyse your data and explain the trends. You should also state whether you have answered your research question and/or whether the data supports your hypothesis or not. You may also have identified some limitations to your investigation and you may have ideas for further research.
Conclusion	This is a brief summary of your main findings. You should state whether or not your investigation answered the research question. Do not provide new information.
References and acknowledgements	You will need to include your references and acknowledge anyone who helped you or provided you with information. References and acknowledgements are not included in the poster word count.

Title

The title for your poster is the research question that you are investigating.

Introduction

The introduction to your poster could also be called the background research that you undertook in the planning stages of your investigation. The introduction outlines the existing knowledge about the research topic. This is where you summarise any existing theories, models, concept and similar studies, all of which should be correctly referenced (p. 28).

The introduction contains a clear aim and a stated hypothesis for the investigation with (or without) a prediction of whether you think the hypothesis will be supported or refuted.

Methodology and methods

The methodology is the framework of the approach that you have adopted to investigate your hypothesis.

You should briefly but clearly describe the method used in your investigation. In a poster, it is not necessary to have the complete sequence for someone else to follow. Instead, you can summarise it as a series of explained photographs or diagrams. You are not instructing anyone to do anything. You are telling people what you did. For example, you would write 'root length was measured' not 'measure the root length'.

If your study has potential safety issues or ethical considerations, identify them in this section and briefly describe the ways in which you handled them.

Results

The results section is a summary of your results together with schematic diagrams, flow charts, bar charts, tables or line graphs showing trends in the data. Do not interpret your results in this section. Make sure you label your graph axes, including units. Choose an appropriate scale so that the data takes up most of the plot area. Do not include tables of raw data in your poster.

When stating the findings of your study in the main text of the results section, refer immediately afterwards to the figure in which the finding is shown; for example, 'The vertical growth of *Arabidopsis* seedlings was significantly greater following two weeks of new fertiliser treatment than with a standard fertiliser or water alone (Figure 2).'

Figures

There are several types of figures that you may include in a scientific poster. The most informative figures are quantitative, such as graphs (Figures 1.17 and 1.18) or tables, although it is often useful to include qualitative data such as cross-sections or photographs. Regardless of the type of figure used, it should be chosen for its ability to best communicate the findings of the study. Sometimes a figure will have multiple panels (labelled A, B etc.). For example, a graph and a photograph showing the same pattern could be presented in two panels of the same figure.

Each diagram should have a figure number, and you should refer to it in the text of your poster. Position the diagram close to where it is referred to in the text. Figure captions are essential. They are usually below the figure and begin with the figure number followed by a brief description of the figure.

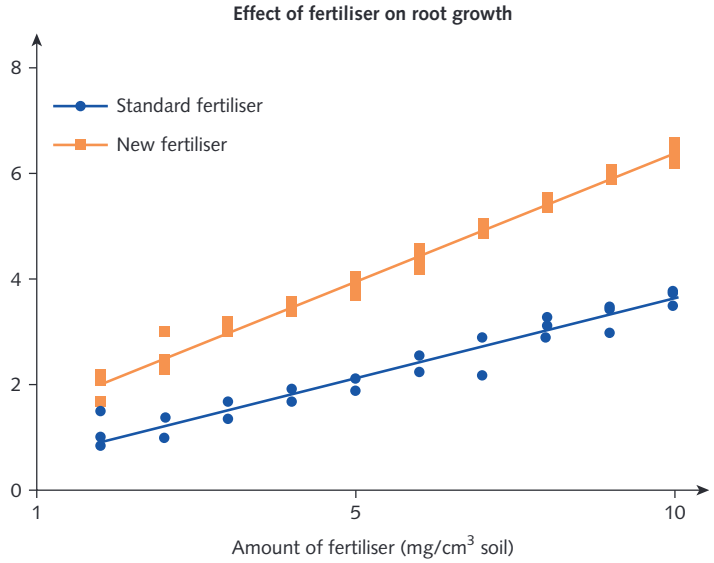


Figure 1.17 An example of a graph that demonstrates a mathematical relationship that could be used in the results section of a scientific poster

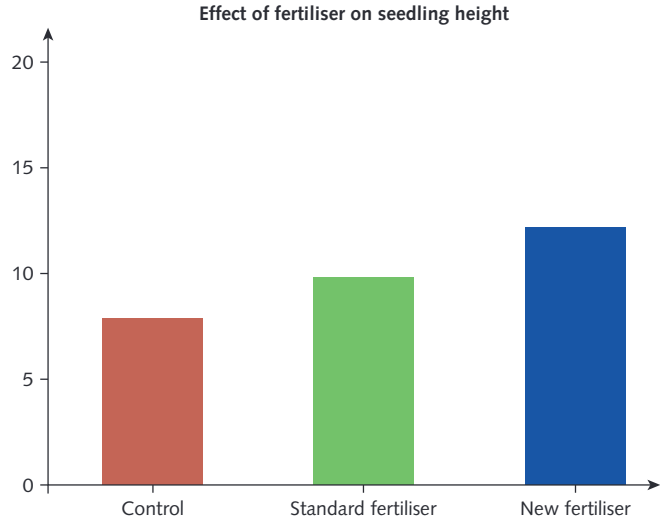


Figure 1.18 Seedlings were grown under standard conditions to a height of 2 cm, then separated and treated daily for two weeks with 50 mL of 2% new feriser (lue) or 2% standard fertiliser in tap water (green). The control group was given water alone (brown).

Discussion

In the discussion section, you need to use the evidence that you have produced from your investigation to construct a scientific argument about how well your investigation answered your research question and achieved the intended aim(s). This is probably the most difficult part of the report that you have to write.

Some questions you should consider when writing your discussion section are as follows.

- What relationships did you observe in your data?
- Did your results support or refute your hypothesis?
- How could you improve your investigation design to more accurately address your hypothesis?
- What do your results add to the current scientific knowledge of biological concepts?
- Do they agree with or contradict models or classification keys based on other published findings?
- What were the implications of your study, and how could you address these in future real or hypothetical studies?
- How might your findings affect the scientific community, industry, medical practice or the community at large?

It will be difficult to address all these questions in the limited space you have available. Start by writing down all the key points and then read through them several times, cutting down unnecessary words each time. Do not remove your own connections or ideas; this type of critical thinking is often a significant part of what is assessed in scientific writing. Remember that concise, coherent writing is an important scientific skill to practise.

Conclusion

This short section allows you to draw conclusions from the evidence you have gathered during your study. It is a very brief summary of the results and their implications. It should provide a response to your research question and directly address the hypothesis you proposed in your introduction. The conclusion should also state the extent to which the analysis answered the research question, without introducing new information. A conclusion should only be a few sentences long.

References

A reference list details all the sources of information you used to write the text and figures for the poster. Whenever you use a piece of information or quotation in your poster, you must reference it at that point. This is typically done by placing a number in brackets [2], or the author and the year of publication (Smith 2019), depending on which referencing style you use. The complete reference list is provided in a single, alphabetical list at the bottom of the poster. You must reference in a consistent style (p. 7). Check with your teacher about the preferred style. References are not included in the poster word count.

Acknowledgements

You should thank anyone who helped you in your investigation. This includes people who supplied equipment or funding, as well as people who gave you good ideas or helped you with the analysis. Acknowledgements are not included in the poster word count.

<table>
<tr><td colspan="3">Title
Student name</td></tr>
<tr><td>Introduction

Methodology and methods

Results</td><td>Communication statement reporting the key finding of the investigation as a one-sentence summary (20–25% of poster space)</td><td>Discussion

Conclusion</td></tr>
<tr><td colspan="3">References and acknowledgements</td></tr>
</table>

Source: adapted from the *VCE Biology Study Design* (2022–2026) p. 11; © VCAA, by permission

Figure 1.19 Template for scientific poster

KEY CONCEPTS

- Once you have finshed your invesigation, you have to communcate what you have found to her people.
- A s ci fic pt sterinclud itle,introduction, methodology nd etod rsults, discussion, concluio, references and cknowlegements.

Concept questions 1.3

1. Wh i i importat o include th appopriate units of measuremnt in qantitative data?
2. Discuss th speci fic reqirements ofthe inroduction setion of a cienti fic pos er.
3. Draw up atable that summarises the main features of tables and figures an iclude sumarised examples to demonstrate your udestanding.
4. Why does he iscusion section of a report require you to consider thereults in terms of the current theory?
5. denify the three mai piecs f information included n the conclusion of a eport.

HOT Chaenge

6. What is th speci fic mannerin wich you answer your researchquestion?

Online Key Concepts
Chapter 1 summary

1 Summary of key concepts

1.1 Investigation design

KEY CONCEPTS

p5

- Observation is the start of a scienti fic nvestiation.
- An observation canbe turned intoa esearch question.
- A research uestion is speci fic and can be answered by performing a nvestigation.
- A research uestion identi fies the dependen and independent varia les.
- Yoill need to undertake some backround research into your researchqustion.
- Make sure y citically evaluate the secondary resources that you use for your backgroundresearch.
- Th independen vaiable is the factor that you change or maiplat in your invesigation.
- The dependet ariable is the factor hat you measure during you invetigation.
- A research question can be turned into a hypothesis.
- A good hypotesis predics tthe results will be, states the eat onship between th e ndependent and dependent varables, states how th dpedent varable will be measured, and can be supported or reftedthrogh investigation.
- Methdology refers to the broader framework of appoach taken to invesigate your research question.

Figure 1.4 There are many different scientific investigation methodologies in biology

KEY CONCEPTS

p11

- You need to hæ aclear plan or our investigation.
- Primary data can bequatitativ, qualitative or both, depeding on your hypothesis.
- Measurements must beaccurate, pree, reproducible, repeatable nd valid.
- Take every pecautio to minimise errr in your data.
- ll extraneos variablemut be controlled.
- The metho details the sts that you will undertake to perform your nveigtion, includin a risk assessment.
- Check whether you need to coside any bioethical issues n you investgation.

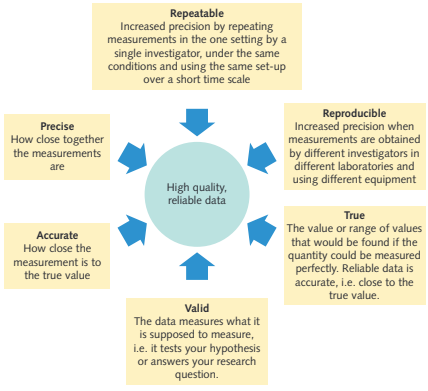


Figure 1.6 Features of data from a well-designed investigation

1.2 Scientific evidence

KEY CONCEPTS

p18

- Maintain a logbook thrughut our investigation for authenication and assessment.
- Raw dtais analysed andsummaised as diagrams, tables or raphs.
- Detemine an reationship or trends in your data.
- Relate you results to you yohesis, which is either supported r refuted.

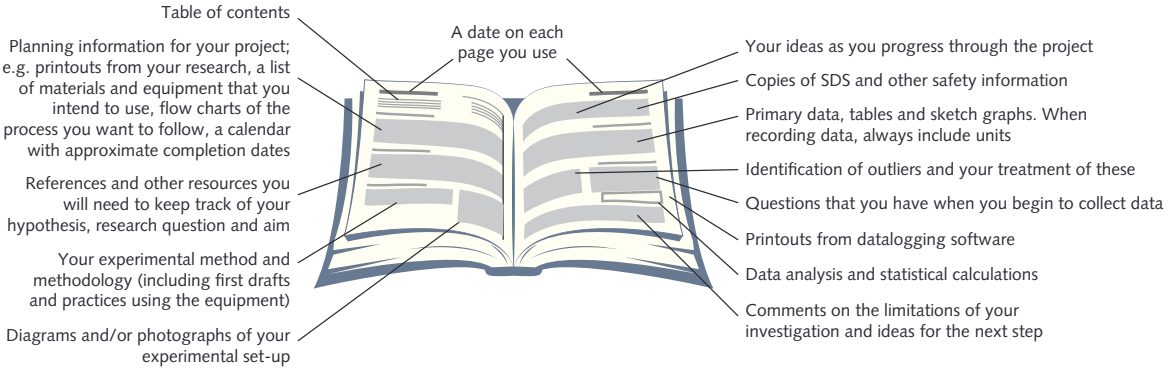


Figure 1.10 Features of an effective logbook

1.3 Science communication

KEY CONCEPTS

p25

- Once you have finshed your nvestiation, you have to communcate what you have found to other people.
- A centi fic posterincludes a title, intrducton, methodology and meho,result, iscussion, concluio, references and acknowledgemnts.

Title Student name		
Introduction		**Discussion**
Methodology and methods	**Communication statement reporting the key finding of the investigation as a one-sentence summary (20–25% of poster space)**	
Results		**Conclusion**
References and acknowledgements		

Figure 1.19 Template for a scientific poster

WB
1.4.1 KEY TERMS PAGE 19
1.4.2 EXAM PRACTICE PAGE 20

1 Chapter glossary

accurate without any mistakes

authentication confirming that the submitted assessment has been completed by the student

beneficence an ethical concept that involves taking positive action that maximises the benefit or 'good', and minimises the risks and potential harms

bias an error that occurs when an investigation is not randomised, particularly if the investigator is affected by their expectations of the outcome

control group a group in an investigation that receives no treatment (independent variable) so a baseline value can be established

controlled variable the variable that is kept constant during an investigation in order to determine the relationship between the independent and dependent variables

dependent variable the variable that is measured and whose value depends on the independent variable, i.e. it responds to the independent variable

ethics a system of moral principles that considers what is good and bad for society

extraneous variable a variable, other than the independent variable, that can influence the dependent variable

gradient the slope of a graph

hypothesis a tentative prediction, or explanation of an observation, based on an existing model or theory

independent variable the variable changed or manipulated by the scientist and assumed to have an effect on the dependent variable

integrity an ethical concept that means being honest about one's actions; in science it means fully reporting data (even if it doesn't fit your hypothesis) and acknowledging all sources of information

justice a moral obligation to give fair consideration to competing claims, not place unfair burden on a particular group, and ensure fair access and distribution of benefits of an action

logbook a record of experimental investigations kept by scientists performing the investigations; it is a legal record of the investigations and their results

method the steps taken to carry out a scientific investigation

methodology the broader framework of approach taken in the investigation to test your research question

model a representation of a system or phenomenon that explains the system or phenomenon; a model may be mathematical equations, a computer simulation, a physical object, words or some other form

non-maleficence an ethical concept that involves avoiding harm or ensuring that harm caused by action is proportionate to the benefit gained from the action

observation acquisition of information through your senses

outlier a data point that does not fit the pattern shown by the other measured data points

personal error a mistake or miscalculation due to human error

precise how closely together measurements are to one another

primary data data that you have measured or collected yourself

primary source an original source of information, created by the author and usually including primary data

qualitative data a measurement with descriptive or non-numerical results

quantitative data a measurement with numerical values

random error an unpredictable variation in measurement; can be improved by taking multiple measurements and calculating an average

references a list of all the sources that have been used in the write-up of a scientific investigation

reliable highly likely to be a trustworthy source of information or reproducible data

repeatable an investigation that can be conducted again by the same investigator under the same conditions to generate similar results

replicates independent samples that allow you to take multiple measurements, increasing the reliability of your data

reproducible giving the same result within uncertainty limits; when repeated measurements are made by a different investigator

research question a specific question that a particular investigation or investigator is attempting to answer

respect an ethical concept that considers the rights of an individual or a group, e.g. respect for animals considers their welfare

risk assessment a process of evaluating potential risks of an investigation

secondary data data that has been measured and collected by someone other than you

secondary source a source of information that has been obtained from another source and/or summarised, e.g. a popular science magazine

systematic error a predictable deviation in data, e.g. as a result of the equipment used

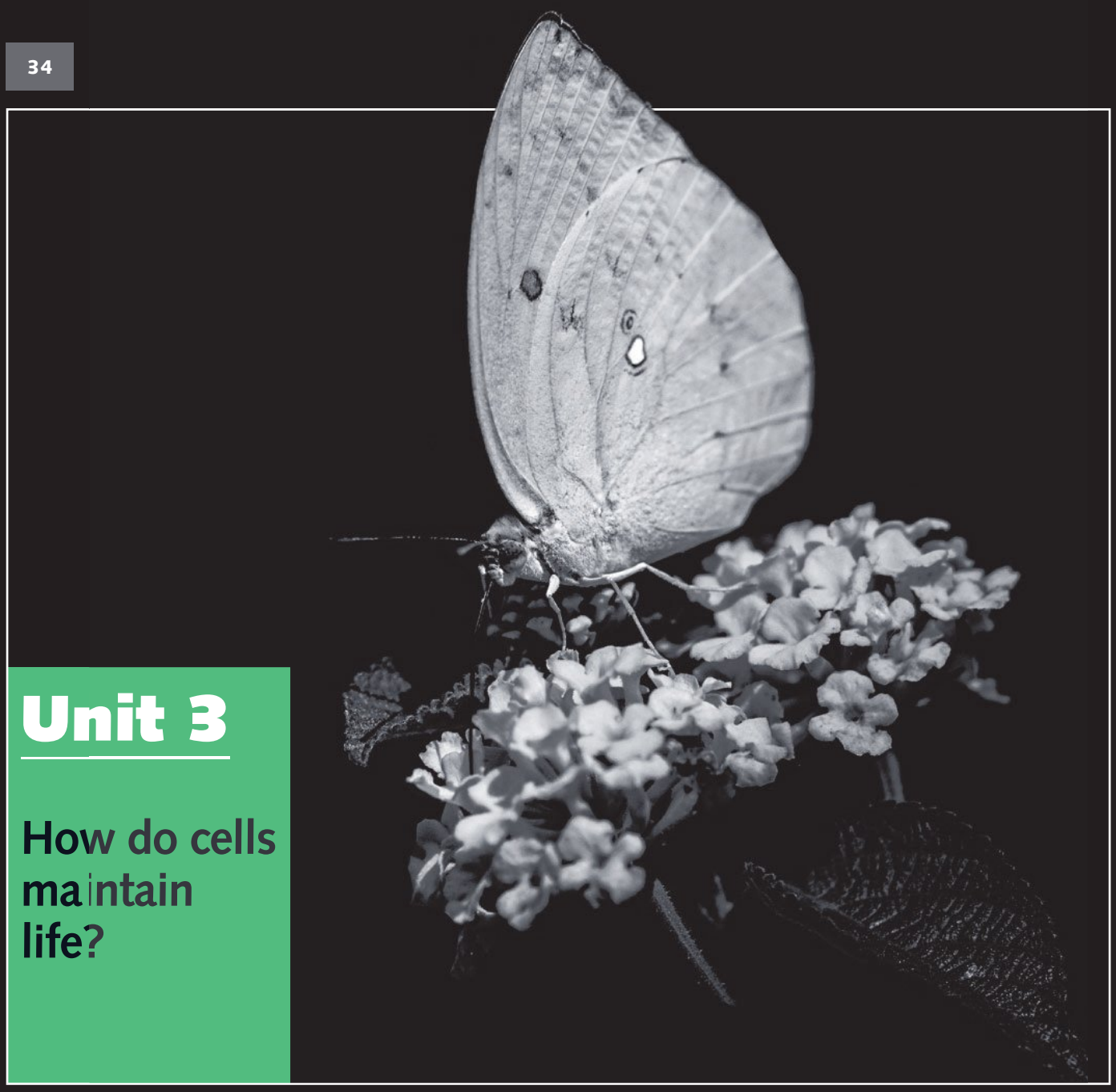
theory a collection of models and concepts that explains specific systems or phenomena; scientific theories allow predictions to be made and hence are falsifiable

true value a value obtained in an ideal measurement

uncertainty a range of values that the true value falls within

valid describes results that are affected by only a single independent variable and hence are reproducible

variable something that can change or be changed, as distinct from a constant, which does not change



Unit 3

How do cells maintain life?

Area of Study 1: What is the role of nucleic acids and proteins in maintaining life?

Area of Study 2: How are biochemical pathways regulated?

9780170452533

The relationship between nucleic acids and proteins

2

By the end of this chapter you will have covered the following material.

Key knowledge

The relationship between nucleic acids and proteins

- nucleic acids as information molecules that encode instructions for the synthesis of proteins: the structure of DNA, the three main forms of RNA (mRNA, rRNA and tRNA) and a comparison of their respective nucleotides, pp. 39–47
- the genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes, pp. 47–53
- the structure of genes: exons, introns and promoter and operator regions, pp. 48–49; 54–57
- the basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of a regulatory process, pp. 55–57
- amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein, pp. 57–60
- proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways, pp. 61–64
- the role of rough endoplasmic reticulum, Golgi apparatus and associated vesicles in the export of proteins from a cell via the protein secretory pathway, pp. 64–65

Key science skills

Plan and conduct investigations

- determine appropriate investigation methodology: case study; classification and identification; controlled experiment; correlational study; fieldwork; literature review; modelling; product, process or system development; simulation, pp. 47–53
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications, pp. 43–44

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks, pp. 43–44
- apply relevant occupational health and safety guidelines while undertaking practical investigations, pp. 43–44
- demonstrate ethical conduct when undertaking and reporting investigations, pp. 43–44

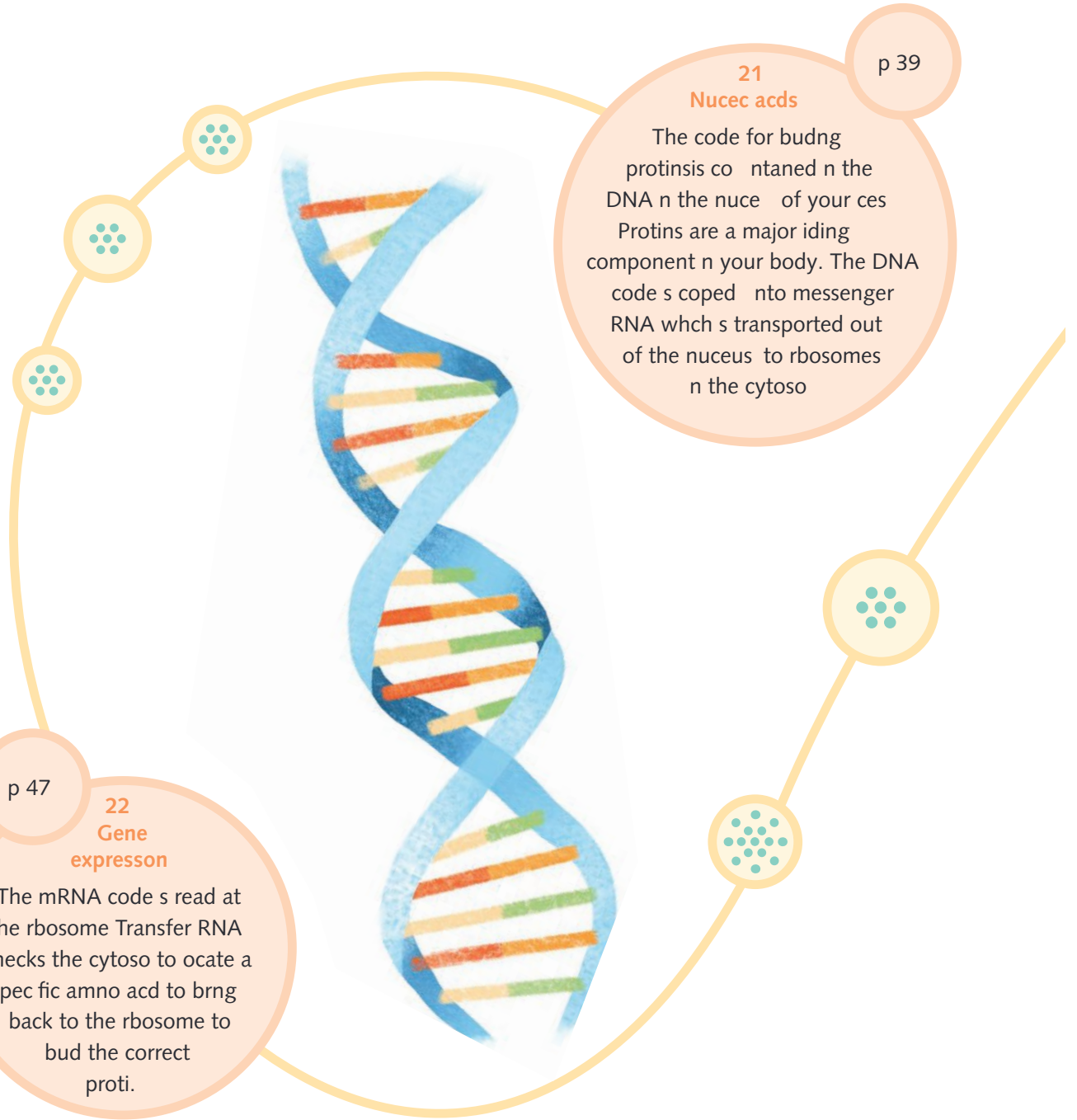
Analyse, evaluate and communicate scientific ideas

- discuss relevant biological information, ideas, concepts, theories and models and the connections between them, pp. 43–44

Online Chapter Map
Chapter 2 map

2 The relationship between nucleic acids and proteins

n Boogy, uing many sml b ocks to bldlarger and more compex structur es s a concept that recurs aain and aan.



p 39

21 Nucec acds

The code for budng protinsis co ntaned n the DNA n the nuce of your ces Protins are a major iding component n your body. The DNA code s coped nto messenger RNA whch s transported out of the nuceus to rbosomes n the cytoso

p 47

22 Gene expresson

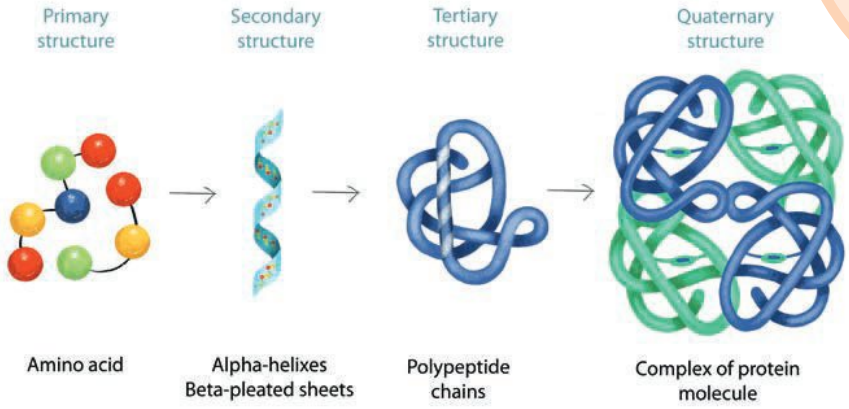
The mRNA code s read at the rbosome Transfer RNA checks the cytoso to ocate a spec fic amno acd to brng back to the rbosome to bud the correct proti.

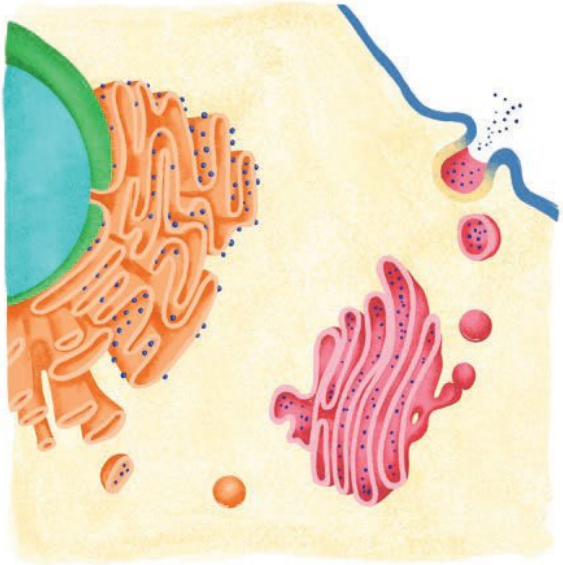
23 Gene reguaton p 54

Not a protins are ilt n every ce Musce ces and bood ces are made from dfferent protins.Regions of codng DNA can be swtched on or off depening on the needs of the ce

24 Protens p 57

The sequences of amno acds determne the type of protins producd. Cains of amno acds are flde, cled and bonded to create more and more coplex prtein structues. Compexty determnes the functon of the proti.





25 The proten secretory pathway pp 64

Protins need to move abou. From the endolasic rtculum wthn a ce further procesing occurs before prteins are moved to the Gog apparatus for secreion out of the cl or for use n other areas of the body.

The structure of prtein s s coded nto our DN A Scentsts have wa ys to manpuate DNA

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 2 map (p. 36)

Online Key Terms:
- Chapter 2 flashcards (p. 38)

Weblinks:
- Nucleic acids (p. 46)
- From DNA to protein (p. 51)

Online Worksheets:
- Nucleic acids (p. 46)
- From DNA to protein (p. 51)

Video:
- Gene expression (p. 47)
- The *trp* operon (p. 55)

Online Key Concepts:
- Chapter 2: Summary of key concepts (p. 68)

Online Key Terms
Chapter 2 flashcards

Know your key terms

α-helix
allele
alternative splicing
amino acid
anticodon
antiparallel
β-pleated sheet
base pair
biological functionality
catalyse
chromosome
cisterna
codon
complementary base pairing
condensation polymerisation
conformation
degenerate
denature
deoxyribonucleic acid (DNA)
DNA polymerase
endoplasmic reticulum
enzyme
exon
functional proteomics
gene
gene expression
gene regulation
genetic code
genome
Golgi apparatus
histone
hydrogen bond
hydrophilic
hydrophobic
inducer
intron
messenger RNA (mRNA)
non-coding region
non-template strand
nucleic acid
nucleosome
nucleotide
operator
operon
peptide bond
phosphodiester bond
plasmid
poly-A tail
polypeptide
polyribosome
pre-mRNA
primary structure
product
promoter region
protein
protein secretory pathway
proteome
proteomics
quaternary structure
random coil
regulatory gene
repressor protein
ribonucleic acid (RNA)
ribosomal RNA (rRNA)
ribosome
RNA polymerase
rough endoplasmic reticulum
secondary structure
semi-conservative replication
structural gene
substrate
subunit
template
template strand
tertiary structure
transcribe
transcription
transcription factor
transfer RNA (tRNA)
translation
transport vesicle
triplet

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 DNA contains the coded instructions to produce proteins, the 'workhorses' of a cell.
2 Organelles involved in protein synthesis and secretion include the rough endoplasmic reticulum, ribosomes and Golgi apparatus.
3 Proteins are building blocks for cellular structures and carry out many cellular processes.

WB
REMEMBER
PAGE 22

Origami is the ancient Japanese art of paper folding, where sheets of paper are transformed into sculptures through precise folding techniques. Several sheets of coloured paper can be used to produce complex sculptures such as those seen in Figure 2.1. Paper is the basic material of origami and complex folding of the paper produces the different sculptures.

Proteins are large complex molecules made from monomers called **amino acids**. Chains of amino acids form **polypeptide** chains, which can then be folded, in a similar way to folding paper in origami, to produce different types of proteins. Proteins are the building blocks for most of the structural and functional components of a cell. The complexity and diversity of the biological world around us can be explained by proteins.

This chapter will unfold the story of proteins from their beginning as a code stored in the **nucleotides** that make up the nucleic acids of the DNA molecule to how the cell deciphers that code to produce a complex protein molecule.

Figure 2.1 Sheets of paper folded in different ways produce many different types of sculptures, just as chains of amino acids folded into different shapes can produce many different types of proteins.

2.1 Nucleic acids

Many years of work and investigation by scientists contributed to the body of knowledge that eventually led molecular biologists James Watson and Francis Crick to report the famous model of the double helix in 1953 (Figure 2.2). Watson and Crick used X-ray images of the DNA molecule that were made by X-ray

Figure 2.2 Watson and Crick with their DNA model

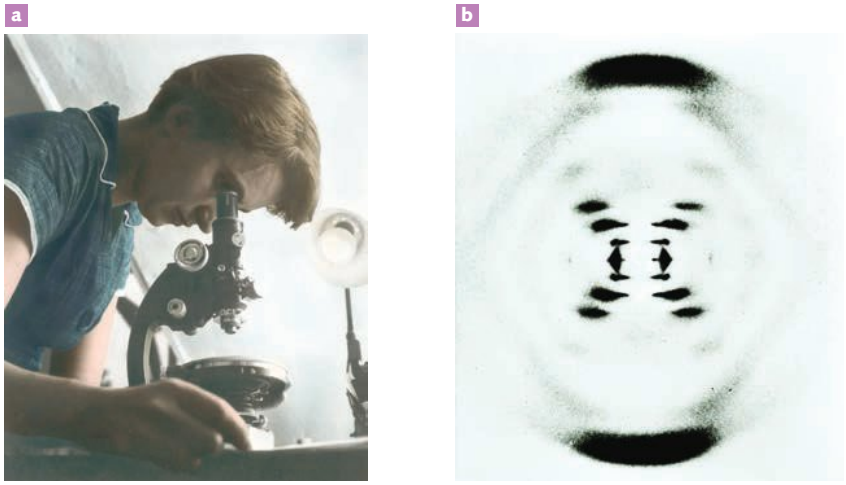


Figure 2.3 a Rosalind Franklin and **b** her X-ray diffraction picture of DNA, which was crucial for the discovery that DNA is a double helix.

crystallographer Rosalind Franklin, which were critical to determining its structure (Figure 2.3). DNA is an example of a nucleic acid. **Nucleic acids** store information in a chemical code that directs the cell to produce proteins. The genetic code of every living organism is contained in its DNA, the molecule of life. DNA is necessary for the survival of every living organism, and ultimately the evolution of life over time.

The nucleic acids **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)** are polymers that form when the monomers (nucleotides) bond together.

WB
2.1.1 STRUCTURE OF NUCLEIC ACIDS PAGE 23

Structure of nucleic acids

Nucleotides are repeating units, or monomers, which form polymers called nucleic acids. DNA and RNA differ slightly in the structure of their nucleotides. Each nucleotide has three distinct chemical components:

- a five-carbon pentose sugar (either ribose or deoxyribose)
- a negatively charged phosphate group
- an organic nitrogen-containing compound called a base (Figure 2.4).

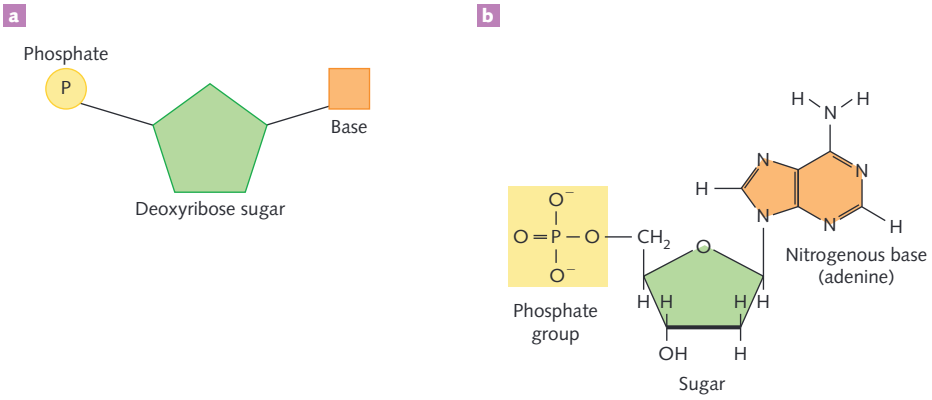


Figure 2.4 a A general representation of the structure of a DNA nucleotide. **b** A chemical representation of a nucleotide with the nitrogenous base adenine.

Structure of DNA

DNA is made up of nucleotides in which the pentose sugar has one fewer oxygen atom (hence: *de-oxy-ribose*) than the ribose sugar (p. 45). DNA is a stable molecule that carries genetic information of organisms through generations.

There are four kinds of nitrogenous (nitrogen-containing) bases in DNA:

» adenine (A)
» thymine (T)
» guanine (G)
» cytosine (C).

Thymine and cytosine are the smallest bases because they consist of one six-membered ring containing carbon and nitrogen. They are known as pyrimidines. Adenine and guanine are larger and they consist of two rings containing carbon and nitrogen. They are known as purines (Figure 2.5).

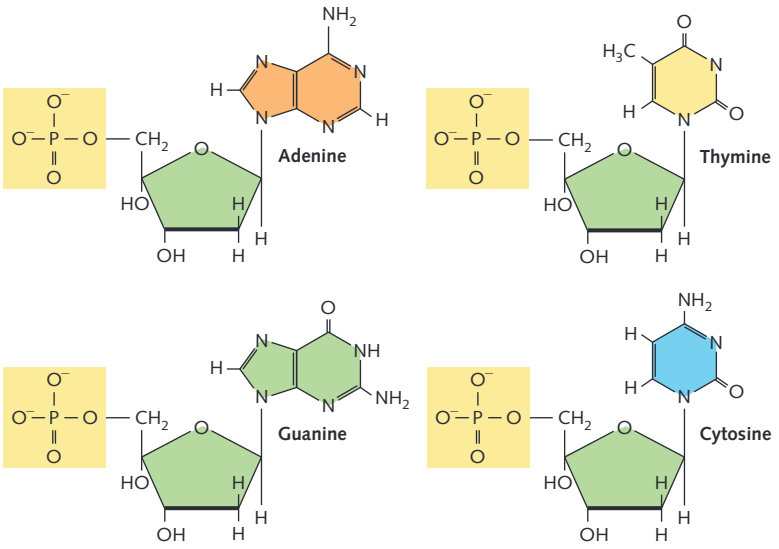


Figure 2.5 The four nucleotides that make up DNA. Each nucleotide contains a different base: adenine, guanine, thymine or cytosine.

DNA molecules are very long and consist of two strands of nucleotide polymers wound around each other to form a double helix. In each strand, the sugar–phosphate groups that make up the backbone of each strand are linked by **phosphodiester bonds**. The two strands are tightly bonded to each other. They are like a zip that can be unzipped when the genetic information is 'read'. **Hydrogen bonds** between the adjoining pairs of nitrogenous bases hold the two chains of the double helix together, much like the rungs of a ladder.

WB
2.1.2 STRUCTURE OF DNA, BUILDING A MODEL PAGE 24

The nitrogen bases do not bond randomly. They bond according to strict **complementary base pairing** rules: adenine (A) always bonds with thymine (T), and guanine (G) always bonds with cytosine (C). A smaller base (T or C) is always bonded to a larger base (A or G), which ensures that the two strands remain a fixed distance from each other (Figure 2.6).

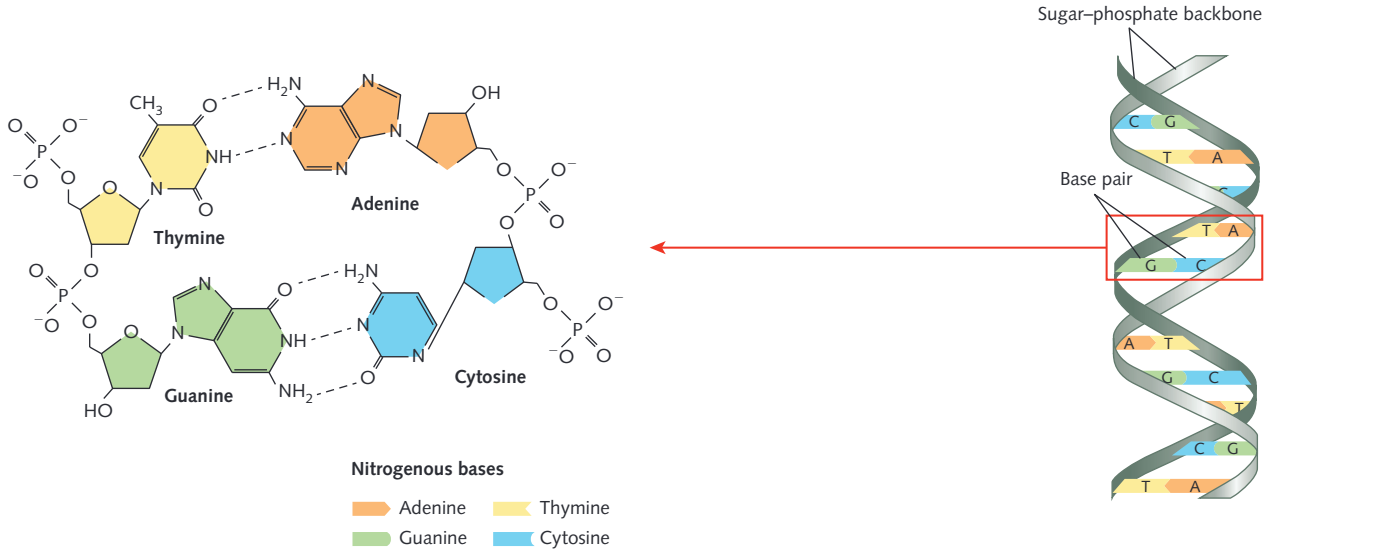


Figure 2.6 The DNA helix is a double-stranded molecule. Two strands are held together by hydrogen bonding between complementary nitrogen bases. An A–T pair is held together by two hydrogen bonds, whereas a G–C pair is held together by three hydrogen bonds.

Figure 2.7 shows that at the top left of the strand of DNA the 5′ (5 prime) carbon is facing upwards and this nucleotide (A) is bonded to a nucleotide (T) with the 3′ carbon facing upwards. This continues down the two strands of DNA so that they are running in opposite directions, with the top of one strand always laid against the bottom of the other strand. This is known as **antiparallel**.

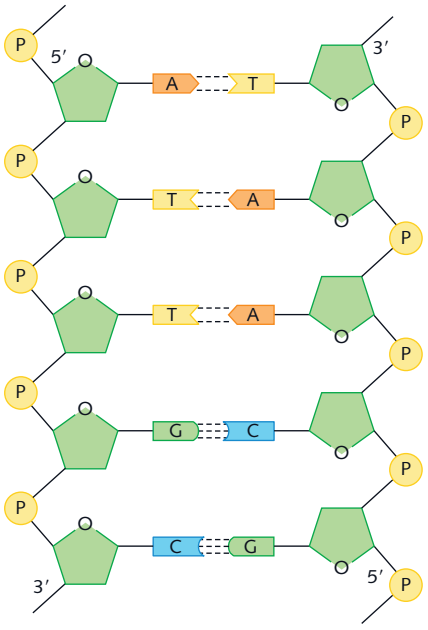


Figure 2.7 The antiparallel nature of DNA: the two strands of DNA run in opposite directions.

In prokaryotic cells, the DNA is in the form of a single, circular **chromosome**. Some DNA is also found as small circular pieces of DNA called **plasmids**. The chromosome and plasmids are in the cytosol. In eukaryotic cells, the DNA is in the form of linear chromosomes contained within the nucleus. Proteins called **histones** are bound to the DNA in eukaryotic chromosomes to form **nucleosomes**. Like winding cotton around many reels, histones help pack the large DNA threads into the confined space of the nucleus (Figure 2.8). Nucleosomes are then coiled and condensed into a fibre, which are supercoiled to form chromosomes during cell division. Some DNA is also found in the form of circular chromosomes in the mitochondria and chloroplasts.

The complete base sequence of DNA in a single (haploid) set of an organism's chromosomes is called its **genome**. Segments of the DNA are called **genes** and certain sequences of nucleotides in the DNA of the gene code are responsible for making polypeptides. The sequence of the coding nucleotides in a segment of DNA ultimately determines the sequence of amino acids in the polypeptide. In turn, the sequence of amino acids determines which protein is formed.

Each cell of the human body contains more than a metre of DNA, twisted and coiled into 46 chromosomes that have more than three billion base pairs (bp). However, not all the DNA codes for polypeptides. Genes account for only about 1% of the human genome. Although the other 99% of the human genome is not protein coding, this does not mean that it is not important. In fact, **non-coding regions** of the genome contain many important regulatory regions that are involved in switching genes on or off. This is discussed in more detail on page 54.

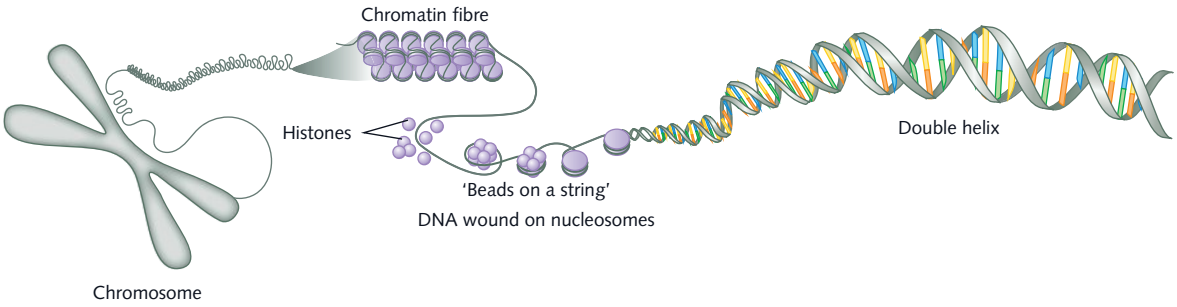


Figure 2.8 DNA is tightly wound around histones to package it inside the nucleus.

Most genes have small differences in the nucleotide sequences from one individual to another, except for identical twins. This means there may be differences in the polypeptides that are encoded by any given gene. These different versions of the same gene are called **alleles**. Alleles account for much of the variation between individuals in a population.

As already discussed, each DNA molecule consists of two complementary strands held together by hydrogen bonds. If the nucleotide sequence is known for one strand, it is possible to determine the sequence of the other strand because of the base-pairing rules (p. 41). When DNA is copied, the two strands are first separated by the action of **enzymes**. Each single strand serves as a **template** for the production of a new complementary strand (Figure 2.9). The new strand is built towards its 3′ end. The enzyme **DNA polymerase** moves along the template strand, adding complementary nucleotides. This is described as **semi-conservative replication** because each new daughter chromosome consists of one new strand and one conserved or original strand.

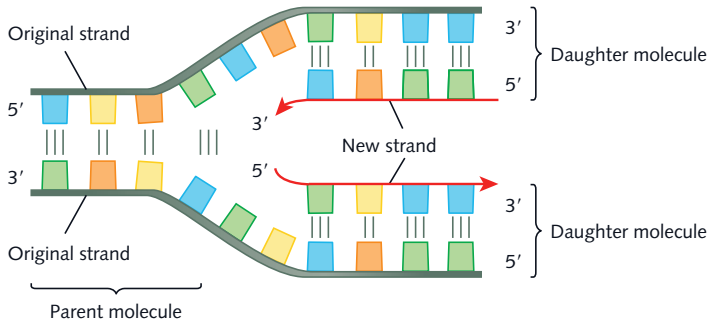


Figure 2.9 **A model for semi-conservative replication of DNA. Each daughter molecule contains one strand from the parent DNA molecule and one new strand.**

Double-stranded DNA is a persistent, long-lived molecule that carries the codes for protein synthesis, determining structure and functioning in cells. Because of its unique ability to replicate with high accuracy, the genetic information encoded in the nucleotides of DNA is passed from one generation of cells during cell division to the next and from one generation of organisms to the next through the process of reproduction. DNA is the master code that determines the very nature of cells and therefore of living things.

SOUTHERN Biological Developed exclusively by Southern Biological

INVESTIGATION 2.1

Extracting DNA from strawberries

Strawberrie have eight sesof chromosomes,wich mean theyre octoploid, as are pansies, dahlias and sugar cane. Strawberries are a eryeffective mdel for DN extrcton becase their pink juice allows the white strands of DNA to beclearly oserved.

Am

To extract DNA fromstrawberries

Time reuirement

30minutes

Materas

- 3 Straberries
- 10 mL DNA exraction buffer
- 5 mL Protease enzyme
- 5 mL ice-cold 95 ethanol
- 2Plastic pipettes
- 1.5 mL cenrifuge tube
- 1 tirring rod
- 1 Inoultion loop
- 1 Reealable plastic bag
- 1 Test tube
- ilter paper
- 1Glass unnel
- Lab coat
- Safety glasses
- ispsabe gloves

What are the rsks n dong th is investigation?	How can you manage these rsks to stay safe?
Ethano s hghy flammabe.	Store and use etanol away fromigiion sourcs. Do not heat n a contaner over an open flame use a water bath that is spark proo.
Protease enzyme can irritate the skn and eyes on contactWear approp riate personal protective equpment at a tmes including eye protection and gloves Wash ki immdiael if contact does occur.	
Disposable gloves can cause aleric rectios in snitive people.	Use a type of gove that has no aergyrisk an is uitble to use with the chemicals n ths nvestgaton
Strawberries can cause aeric rectios in sen stve peopeNever eat foo in a cie nce laboratory. Let your teacher know f you have a strawberry allergy.	

Method

1 Pace three strawbrries i a esalable plstic bag and re-seal.
2 Squeeze the s rawberries in the bag with your fingers ul lghtly rushed.
3 Open bag and add 10 mL of the DNAextraction buffer.
4 Re-sal the bag and crush the cntets again using your han ds to mix te ingredents witin t e bag Continue until a tick uie is prodced.
5 Usng a lsic ippete, add 5 mL of protease enzyme to th bag and mi for 1 minute.
6 Fter the strawber juice into a est tu.To do this, place the fiter pape in aglassfunnel over te test tube. Pour the straberries out of the bag and over the fiter paper an a lar, pup-e juice will fiter nto the testtube.
7 Remove the fitering apparatus and se a piette to sloly ad approximtely 5L of cold ethanol into the test tube to cover the strawerryj ice solution. Do *not* aitate th solution. he thnol shoud sit separately on top of the strawbery slution.
8 Wite strands of strawbry DNAwl become visible in the ethaol layer as the DNA is extractd from the nucleus as thce walls brek down. Thetran will look like very fine spider webs.
9 Use the incuatiolo to 'spool' strands of DNA andosrvete mre closely. Alternatively, hold the test tube at eyelevel and use a pipette to draw up the DNA srands in the top layer flud.
10 Transfer the DNA t a centrifuge tube or urther examination.

Resuts

Describe wha you see.

Dscusson

1 DNA extaction buffr contains detergnt andsalt. What rle did the deergent, protese enzyme, ethanol and salt hav in the process of NA extraction?
2 Many of the foods we cnsume conain NA Explain wh ingestingDNA from other plants and organisms does not cause us harmor alter ur DNA.
3 What is thefunction of DNA?
4 Wher is DA loctd witin a cell?
5 Drawa diagram NA. Include five sets fnucleotide ases and label the hydroge bonds between these bases.
6 Wh is thbility to remove N from cellsimportant to scientists?
7 Why does NA rise towards the surfac hen the alcohol is added?

Concuson

Summaise your findngs an include a flow chart ailing the steps aken to release the DNA from the strawbery cells.

Takin it further

1 Perform another DNA extactio on difernt plant sampls, uch as baanas, kiwi fruit or wheatgerm.
2 Fllwing the sameprocedure, ompare the resuts of theDNA exracto among the different plant samples.

KEY CONCEPTS

- Nclotides are monomers that are made up of a five-carbon pentse sugar, a phosphate group and a ntrogenous bse.
- Nclotide monomers make up he nucleic acids DNA and RA.
- There are for different types of nitrogenous bases n DNA: denine(A) cyosin (C), guanine (G) and thmine T).
- Nclotides make up the strnds of DNA, which are hld together by hydrogen bonds between complementary ases.
- Bases pairaccording to he complementary base paiingrles: A alwaysbods to T; C always bonds toG.
- The two strands of DN ar ntiparallel.
- In cls, DA is ogaised into chromosomes.

Concept questions 2.1a

1 Lst the three key feature of a nucleotide and describe how they are rranged.
2 Describehownucletides ar linked together.
3 Describe how the two strand of DNA are held togethe.
4 Explain the niparallel sructure of DNA.
5 State th complemenly base pairing rule.

HOT chaenge

6 Draw a flow chart to show your undestanding of DNA structure. Uste folloing terms in your flow chart: adenne, base, ytosn, guanine,hydrogen bonds, monomernuceic aid, ucleotide,phosphate, sugar, thmne. You may add mre terms.

Structure of RNA

WB 2.1.3 STRUCTURE OF RNA PAGE 29

RNA is composed of nucleotides that are linked together by phosphodiester bonds. However, whereas DNA nucleotides contain a deoxyribose sugar, RNA nucleotides contain a ribose sugar, which contains one more oxygen atom (Figure 2.10).

RNA contains the base uracil (U) instead of thymine (T) found in DNA. The bases T and U are very similar, both being pyrimidines (Figure 2.11).

Another difference between RNA and DNA is that RNA is a single-stranded molecule and has a variety of folding patterns. DNA is a double-stranded molecule and coils into a double helix. RNA molecules are much shorter than DNA molecules. RNA ranges in size from a few dozen up to several thousand nucleotides, whereas DNA molecules range from tens of thousands up to hundreds of millions of **base pairs** in one molecule.

Table 2.1 summarises the differences between DNA and RNA.

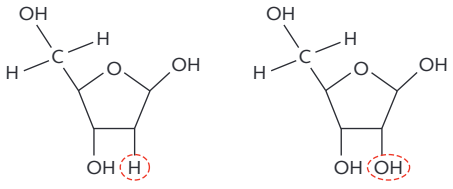


Figure 2.10 The pentose sugars of DNA (deoxyribose) and RNA (ribose)

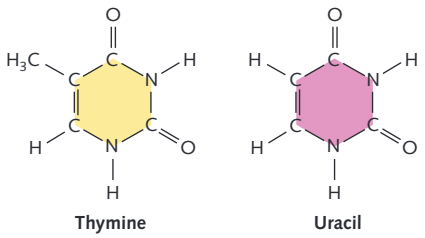


Figure 2.11 RNA contains the nitrogenous base uracil instead of thymine, which is found in DNA.

Table 2.1 Differences between DNA and RNA

	DNA	RNA
Nucleotide structure		
Sugar–phosphate backbone	Deoxyribose sugar	Ribose sugar
Nitrogen bases	Adenine, cytosine, guanine, thymine	Adenine, cytosine, guanine, uracil
Longevity	Highly stable; usually exists as a double helix that can form coils and supercoils	Unstable; exists as a single chain that often folds into secondary structures
Forms	Linear or circular chromosomes Plasmids	mRNA – linear shape tRNA – clover leaf shape rRNA – 2 subunits of ribosome (others that are beyond the scope of this course)
Enzyme responsible for synthesis	DNA polymerase	RNA polymerase
Location in eukaryotic cells	Nucleus, mitochondria and chloroplasts	Nucleus and cytosol

Function of RNA

The differences between the structure of DNA and RNA determine major differences in the longevity and functioning of the two types of molecules.

RNA is a single-stranded molecule that is relatively short-lived; it is made and degraded rapidly by cells. RNA is a versatile molecule with a variety of functions that facilitate and regulate protein production. The three main types of RNA are:

» **messenger RNA (mRNA)**
» **transfer RNA (tRNA)**
» **ribosomal RNA (rRNA)**.

The different forms of RNA are described in more detail in Table 2.2. Their roles in **gene expression** are discussed on page 47. Table 2.3 compares where DNA and RNA are found in cells.

Weblink
Nucleic acids
Watch the video on nucleic acids to revise the importance and structure of DNA and RNA.

Worksheet
Nucleic acids

Table 2.2 A comparison of the different forms of RNA in eukaryotic cells

	mRNA	tRNA	rRNA
Function	Carries the DNA code from the nucleus to the ribosome containing instructions for protein synthesis	Delivers amino acids to ribosomes for protein synthesis	A component of the ribosome: associates with proteins to form ribosomes
Location in which it functions	Nucleus and cytosol	Cytosol	Cytosol
Shape	Linear	Clover leaf	Three-dimensional fold

EXAM TIP
Ensure you know the differences and similarities (compare) between DNA and RNA, their structure and functions.

Table 2.3 Locations of DNA and RNA in prokaryotic and eukaryotic cells

Nucleic acid		Location in prokaryotic cell	Location in eukaryotic cell
DNA		A single circular chromosome in the cytosol Small circular plasmids in the cytosol	Linear chromosomes in the nucleus In mitochondria (circular DNA) In chloroplasts of plant cells (circular DNA)
RNA	mRNA	Cytosol	Nucleus and cytosol
	tRNA	Cytosol	Cytosol
	rRNA	Within ribosomes in the cytosol	Within ribosomes free in the cytosol, or attached to endoplasmic reticulum

KEY CONCEPTS

- RNA s ingle-stranded.
- RNAnucleotide containribose sugar.
- RNA cntains the seuracil (U) instead of the DNA base thymie (T).
- RNA strands are shorter tha DNA strands.
- RN plays a mjr role in gen epression.
- There are thee main tyes f RNA: mRNA, tRNA and rNA.

Concept questions 2.1b

1 dentify three fnamental differences between the structures of DNA and RNA.
2 Lst the three major types ofRNA and describe the function of each.
3 Explain tefollowingstatement. 'RNA plays a major rolein gene expresi.'
4 What are he main constituent o an RNA nucleotide?
5 Why are RNA strands shorter than DNA strands?

HOT chaenge

6 n a table, consruct diagam hat discriminate between th general strctures of mRNA, tRNA and rRNA.

2.2 Gene expression

Gene expression is the transfer of the DNA code in a gene, by transcription, to **ribosomes** in the cytosol to produce a functional gene product, through translation (Figure 2.12).

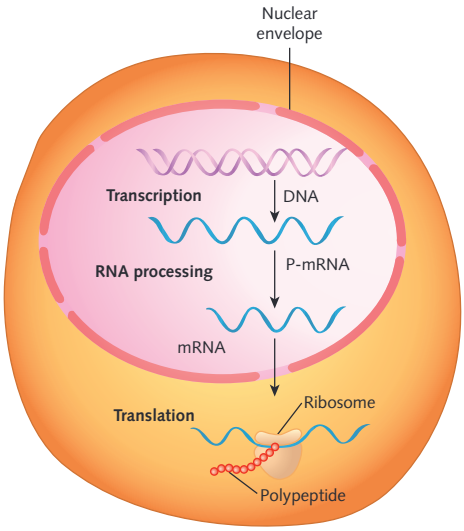


Figure 2.12 Gene expression relies on the processes of transcription and translation. During transcription, DNA is copied into pre-mRNA, which is processed into mature mRNA for export from the nucleus. During translation, the ribosome 'reads' the nucleotide sequence in the mRNA and forms the amino acid sequence of a polypeptide.

Video
Gene expression

2.2.1 TRANSCRIPTION PAGE 30

Transcription

DNA is too large a molecule to leave the nucleus through a nuclear pore. The smaller molecule mRNA serves as the messenger that carries the information coded on the DNA out of the nucleus. The process of copying DNA to produce a complementary RNA molecule is called **transcription**. The DNA in the region of the gene first unwinds and then unzips with the aid of the enzyme helicase, exposing the nucleotide bases of both DNA strands. One strand of the DNA has the sequence that codes for the polypeptide and is called the **non-template strand**. The sequence of bases on the non-template strand is the same as the sequence of bases on the mRNA transcript, except thymine is replaced by uracil. The other strand is called the **template strand**. Due to the complementary base pairing of DNA, the template strand is read and the sequence is transcribed to produce the mRNA transcript.

A sequence of DNA, called the **promoter region**, signals the start of the gene. The promoter region does not code for a gene but contains the information that determines where (in which cell type) and when (at what stage of development or activation) a gene is transcribed. Transcription begins when proteins position the enzyme **RNA polymerase** onto the DNA to bind with the promoter region. RNA polymerase then proceeds along the DNA, progressively building a strand of **pre-mRNA** from RNA nucleotides, that is complementary to the template strand (Figure 2.13).

Note:
The two strands of DNA have different names. In this resource they will be called the template strand and the non-template strand. In other resources you might also see them called the non-coding (template) and coding (non-template) strand or the anti-sense (template) and sense (non-template) strands.

Although both DNA strands are exposed when the DNA separates in the region of te gene, the antiparallel structure of DNA determines which strand will be used as the template strand for trascription. Only the DNA template strand (from 3′ to 5′) will be **transcribed** into pre-mRNA (from 5′ to 3′). The nucleotide sequence of the mRNA is complementary to the non-template strnd of DNA, with uracil added instead of thymine (Figure 2.13).

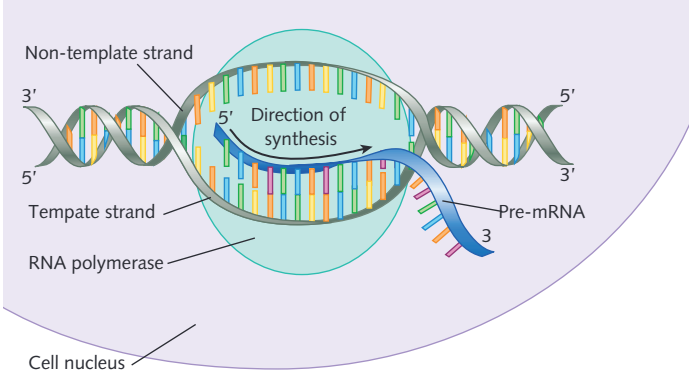


Figure 2.13 During transcription, the pre-mRNA strand is synthesised in the 5 to 3 direction from the template strand of DNA.

A sequence of nucleotides downstream of the gene serves as a signal to stop transcription. The RNA is released as a single strand of pre-mRNA. Once the pre-mRNA has peeled off, the DNA zips up and coils back into a double helix.

RNA processing

WB
2.2.2 RNA PROCESSING PAGE 35

Pre-mRNA is not yet in its mature mRNA form. It contains **introns**, which are non-coding regions, and **exons**, which contain the codes for the amino acids for polypeptide formation. Both the exons and introns are transcribed into pre-mRNA. The introns are then cut out, while exons rejoin by RNA splicing to form a shorter strand of mature mRNA that moves out of the nucleus to the cytosol.

As it is being transcribed, the pre-mRNA is modified by the addition of a methyl group (CH_3) at the 5′ end. Before it leaves the nucleus, the pre-mRNA is modified at the 3′ end by polyadenylation (the addition of 100–200 adenine bases) called the poly-A tail (Figure 2.14). The addition of these structures protects the RNA from degradation. When the methylated cap and **poly-A tail** are later removed, the RNA is rapidly digested and the nucleotides are recycled for further RNA synthesis.

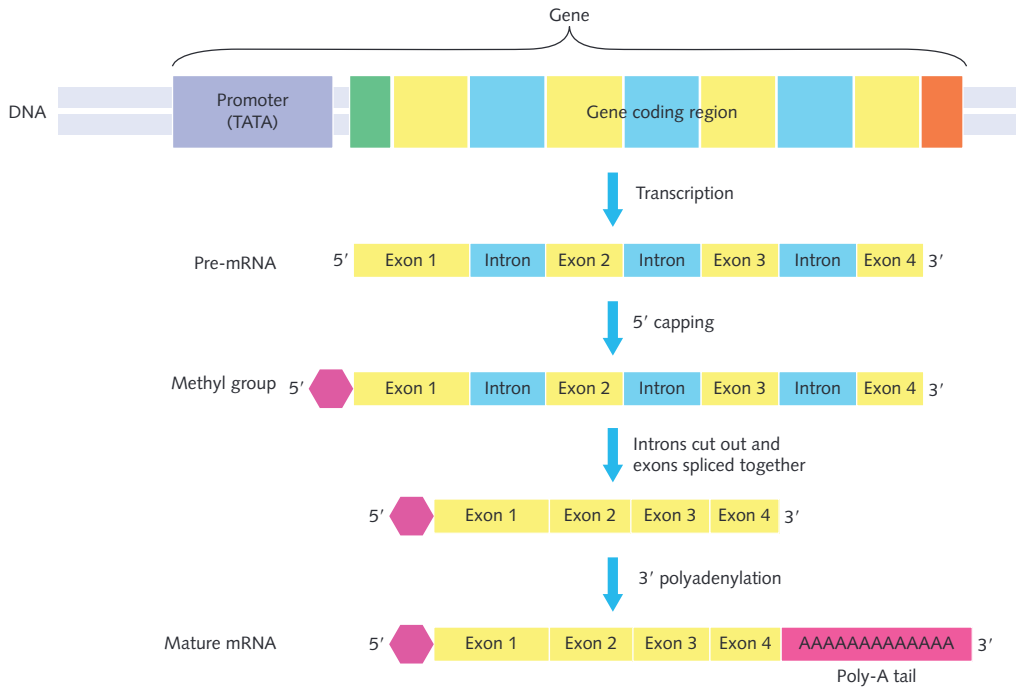


Figure 2.14 Transcription and RNA processing generates mature mRNA from pre-mRNA in the nucleus of a cell.

After processing, the mature mRNA is ready to leave the nucleus and move into the cytosol, where ribosomes translate the nuclear code into polypeptides. The average mRNA strand is 1000–2000 bases long, including the methylated cap and 100–200 adenine bases in the poly-A tail.

Alternative splicing

Before the human genome ws sequenced, scientists predicted that it would contain more than 100 000 genes, based on the estimated number of proteins in the human body. Surprisingly, after sequencing, scientists found that there are only 20000–25000 genes. This is because a single gene can code for several different polypeptides.

During pre-mRNA processing, different exons may be removed along with the introns to produce mRNA molecules of different length and sequence from the same pre-mRNA molecule (Figure 2.15). This process is referred to as **alternative splicing**. The polypeptides translated from the alternative mRNA molecules are of different sizes, have different sequences, and have their own unique functions.

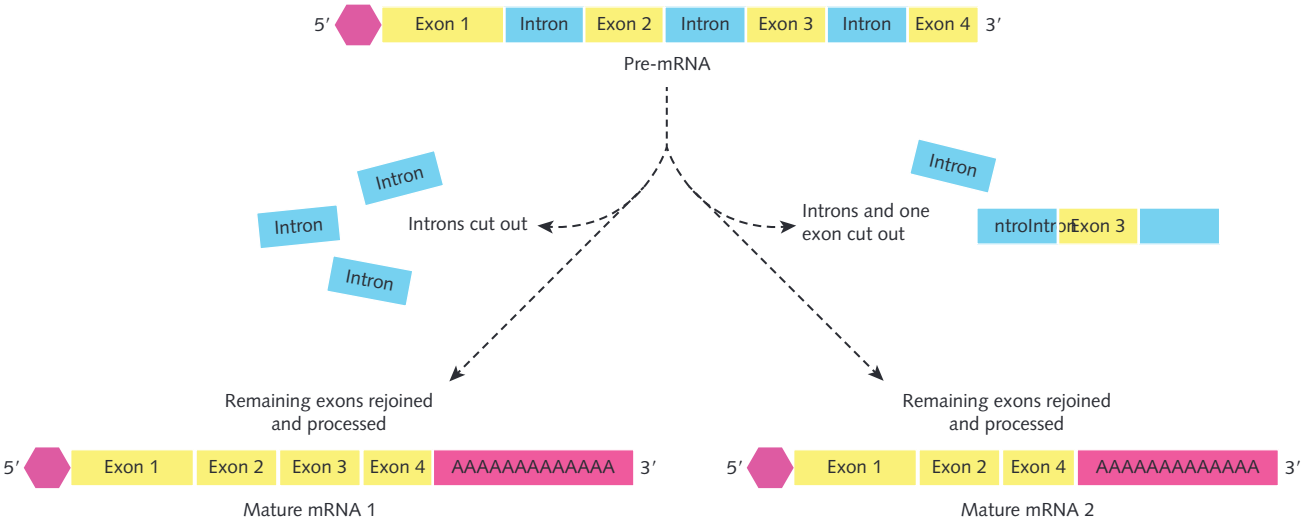


Figure 2.15 Alternative splicing allows the production of different mRNA molecules, and therefore different polypeptides, from the same gene. The more exons a gene has, the more options there are for alternatively spliced mRNA transcripts. The average number of exons per gene is nine, interspersed with eight introns.

How a cell 'knows' which exons to keep and which to remove during alternative splicing is an area of intensive research. The fundamental mechanism involves interactions between specific mRNA sequences and nuclear proteins found in specific cell and tissue types. The same transcript expressed in two different tissues may be bound by different nuclear proteins present in each tissue. In one, the proteins may protect exons from removal. In the other, the proteins may loop introns together so that the exon in between is 'ignored' and cut out.

Ribosomes

In eukaryotic cells, ribosomal RNA (rRNA) combines with special proteins to form ribosomes in the nucleolus. Ribosomes are made up of two **subunits**: a smaller one called the 40 S subunit and a larger one called the 60 S subunit (S is a unit of size). The subunits move from the nucleolus in the nucleus into the cytosol. Both subunits contain many protein molecules together with rRNA. The subunits combine to form the functional units, the ribosomes, for translation.

Ribosomes are also found in prokaryotes, and in the mitochondria and chloroplasts of eukaryotes. These ribosomes are smaller than the ribosomes in the cytoplasm of eukaryotic cells, although they also consist of two subunits and are involved in protein synthesis. In eukaryotic cells, ribosomes are found free throughout the cytosol. Ribosomes are also bound to the **endoplasmic reticulum**, forming **rough endoplasmic reticulum**, an organelle consisting of a series of flattened sacs (Figure 2.16).

EXAM TIP
Make sure you write rough endoplasmic reticulum in full when answering exam questions. The abbreviation RER or rER is not an accepted abbreviation.

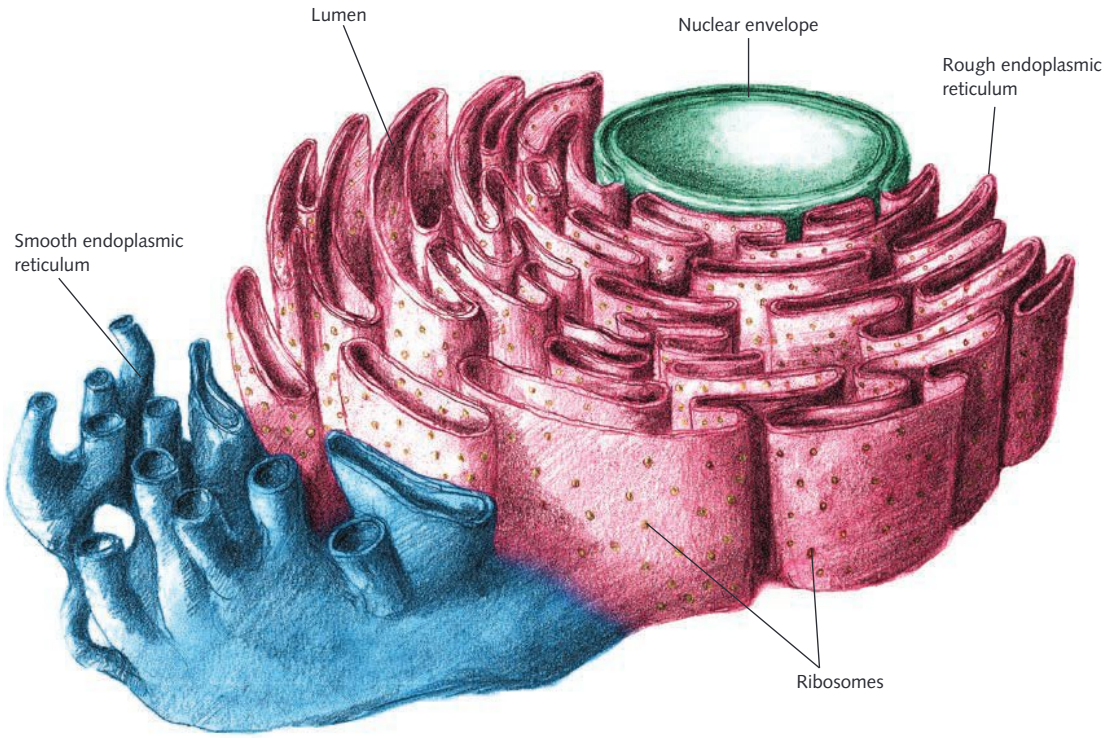


Figure 2.16 A ribosome attached to the endoplasmic reticulum is the site of synthesis of a polypeptide. The polypeptide is transferred to the lumen (inside) of the endoplasmic reticulum.

WB
2.2.3 TRANSLATION PAGE 36

Generally, proteins that are to be used for structure and functioning in the cell are made on free ribosomes. In contrast, proteins that are secreted from the cell or expressed on the cell's surface are made on ribosomes bound to the rough endoplasmic reticulum.

Ribosomes can form chains, called **polyribosomes** or polysomes, which all bind to a single mRNA strand. The advantage of polyribosomes is that they allow many polypeptides to be made from a single mRNA strand in a comparatively short time. This greatly increases the rate of polypeptide synthesis. In bacterial cells, polypeptide synthesis happens even more rapidly. This is because prokaryotes lack a nucleus, they do not have introns, and protein synthesis can begin even before mRNA synthesis is complete.

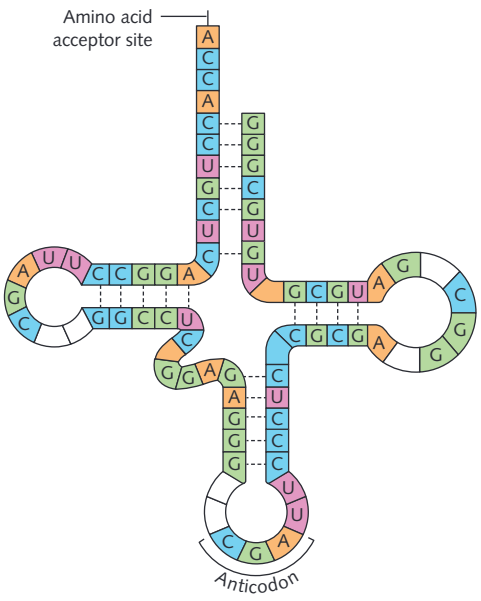


Figure 2.17 A tRNA molecule is a clover leaf shape, with three unpaired bases called the anticodon and a specific amino acid acceptor site.

Translation

When mRNA passes through a nuclear pore into the cytosol, it then moves to and binds with a ribosome. A small ribosome subunit loaded with an initiator Met-tRNA (one that can start translation) recognises the mRNA strand and binds to the methylated cap on the mRNA. It moves along it, 'scanning' for an AUG start **codon** (a set of three nucleotides). Once the start codon is found, a large ribosomal subunit joins with the small one. The ribosome then moves along the mRNA strand 'reading' the mRNA nucleotides in codons. This process is known as **translation**. The ribosome serves as the workbench for protein synthesis, while tRNA molecules provide the raw materials.

Transfer RNA (tRNA) molecules each carry one specific amino acid of the 20 amino acids. They exist as free-floating molecules within the cytosol. Unlike mRNA, tRNA molecules are folded into loops with a distinctive clover shape (Figure 2.17). Three unpaired nucleotides in the

central loop of the tRNA molecule are called the **anticodon** and will bind to a complementary mRNA codon, following the base-pairing rules. The amino acid is attached to an amino acid acceptor site on the stem of the tRNA molecule.

As the ribosome moves along the mRNA strand, a tRNA molecule moves into the correct position by binding its anticodon with a complementary mRNA codon and delivers a specific amino acid. As the ribosome moves on to the next codon of the mRNA strand, another tRNA molecule with a complementary anticodon binds to the next codon and another amino acid is placed into position, and so on. (How the specific amino acid is determined from the mRNA code is explained below.) As the ribosome moves further along the mRNA strand, more and more amino acids are delivered and joined by peptide bonds to produce the growing polypeptide chain. In this way, the amino acids are linked in an order corresponding to the sequence of codons in the mRNA. As this is determined by the sequence of base triplets in the original DNA, it follows that the base sequence in the DNA determines the order in which the amino acids link up. The process is shown in Figure 2.18. Several ribosomes can move along one mRNA strand simultaneously carrying out translation, each synthesising a polypeptide chain as they go. On reaching a stop codon, the ribosome releases the mRNA strand and the newly synthesised polypeptide chain.

A protein molecule is made up of one or more polypeptide chains joined to make a three-dimensional structure. The diversity of proteins can be explained by how their building blocks, the 20 amino acids, are arranged in various combinations. It is rather like arranging 20 kinds of beads in unique ways to make different necklaces of different lengths. The necklace chains can then be arranged variously in loops and folds to give each its characteristic features.

EXAM TIP
Could you create a flow diagram to explain the steps involved in gene expression and could you show each step with a labelled diagram? Make sure you understand how the expression of a single gene can lead to the production of several different proteins.

The genetic code

DNA is the molecule of life – it is essential in all living organisms to code for the proteins produced for the structure and functioning of the cells. It functions primarily as an information molecule by determining the sequence of the 20 different types of amino acids joined in a polypeptide.

A three-letter DNA code (A, C, G or T) can specify for each of 20 different amino acids in polypeptides. The nucleotides are read in groups of three, called **triplets** in DNA and codons in mRNA. If one of four nucleotides can occur in each of the three positions in a codon, then there are $4 \times 4 \times 4 = 64$ possible combinations. One codon in mRNA (AUG) codes for start, three codons (UAA, UAG and UGA) code for stop (no amino acid will be added and polypeptide synthesis is terminated), and the other 60 codons code for the 20 amino acids.

WB
2.2.4 THE GENETIC CODE PAGE 37

Weblink
From DNA to protein

Online Worksheet
From DNA to protein

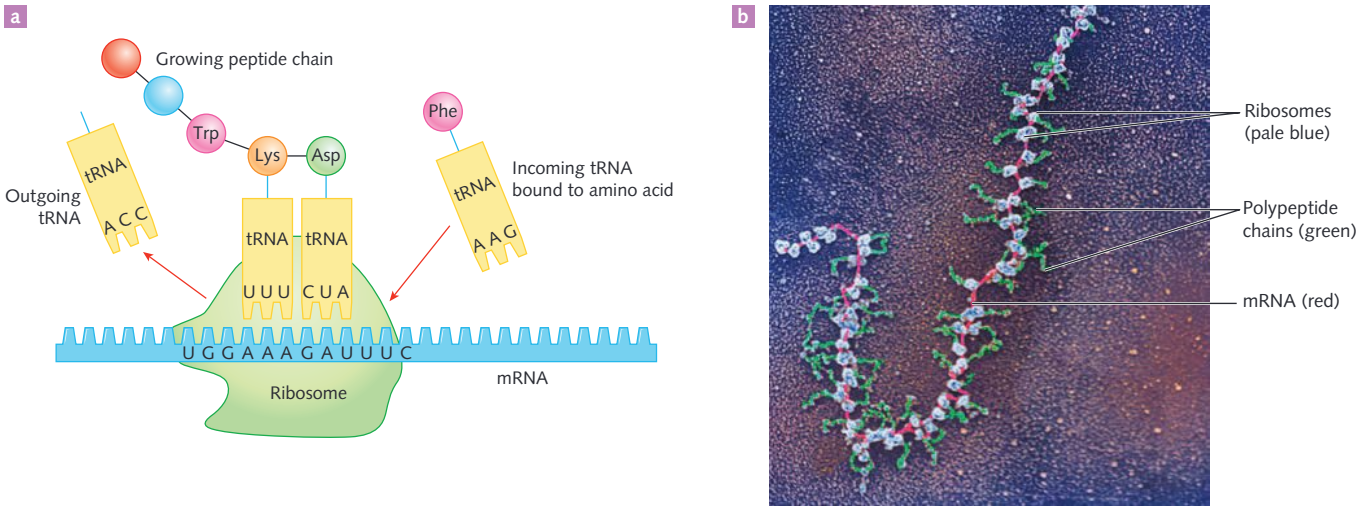


Figure 2.18 a The mRNA sequence of codons determines the order in which amino acids link up to form the polypeptide chain. The three-base anticodon of each tRNA molecule is complementary to the three-base codon in the mRNA. **b** An electron micrograph of ribosomes and growing amino acid chains of the proteins being made.

Eighteen amino acids are coded for by several different codons. Amino acids such as serine (Ser) and arginine (Arg) are specified by six codons, while threonine (Thr) and leucine (Leu) are specified by two codons (Figure 2.19). These observations show that there is a level of redundancy within the genetic code. Therefore, the genetic code can be described as **degenerate** because most amino acids can be encoded by two or more codons. This means that if a mutation occurs in the DNA, there is less chance that it will result in an amino acid change when the codons are read, and this means that the resulting protein can still be functional.

WB 2.2.5 SCIENTIFIC LITERACY PAGE 43

When using the genetic code table (Figure 2.19), it is important to remember that it is usually presented as a table of mRNA codons. (If the information given is for DNA, the template strand of the DNA must first be transcribed into mRNA, and then the genetic code table can be used to determine the amino acid sequence.) To use the table, read the first letter of the mRNA codon in the left-hand column of the table, then read the second letter of the codon in the row across the top of the table, and finally read the third letter in the column on the right side of the table. The point of intersection of these three will be the name of the amino acid coded for by the mRNA codon. For example, if the mRNA codon is GCU, according to the genetic code, the GCU codon specifies the amino acid alanine (Ala). Similarly, an mRNA codon ACU specifies the amino acid threonine (Thr). Figure 2.19 shows the mRNA codon sequences for each of the 20 amino acids needed to produce all the proteins required by cells.

As already stated, the order of the codons in the mRNA specifies the order of the amino acids in the polypeptide. The fundamental rules of base-pairing are the foundation of the **genetic code**. With very few exceptions, the genetic code is universal to all organisms because they all have the same 20 amino acids specified by the same codons.

Ala = alanine
Arg = arginine
Asn = asparagine
Asp = aspartic acid
Cys = cysteine
Gln = glutamine
Glu = glutamic acid
Gly = glycine
His = histidine
Ile = isoleucine
Leu = leucine
Lys = lysine
Met = methionine
Phe = phenylalanine
Pro = proline
Ser = serine
Thr = threonine
Trp = tryptophan
Tyr = tyrosine
Val = valine

First base	Second base U	Second base C	Second base A	Second base G	Third base
U	UUU, UUC: Phe UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr UAA, UAG: Stop	UGU, UGC: Cys UGA: Stop UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile AUG: Met/Start	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn AAA, AAG: Lys	AGU, AGC: Ser AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

Figure 2.19 **The genetic code. Sixty of the mRNA codons correspond to the 20 amino acids; three codons act as stop codons; and the codon AUG initiates protein synthesis.**

ACTIVITY 2.1

Transmitting the code

The nuceus of eukaryotc ce s contans DNA the moecue that encodes for a the prot ens produced by the c. The stes of synthess of protins the rbosomes are foun d n the cytoso outsde the bounda ry of the nuceus DNA cannot eave the nuceus so to produc e a proti, a message must be sent from th e nucear DNA to the rb osome To do hs, two processes take lac:

» trancription of the messag from the DNA into a messenger RNA (RA) molecule
» transation of the mRN into a speci fic aino ci d sequence atthe ribosome.

But how s the message co mmuncated between the nucear DNA and th e rbosome n the c ytopasm of a ce?

Am

Tosiulate how the gentic code s transcrbed from DNA an d transated nto a poypeptde

Youll need

Each par of students reuires:

» paper
» coloured encils
» s

What to do

The foowng sequence of nuceotdes s from the *lol* gene of a fungus
DNA 5′ A T G G A A A C T T G T A T A T A A 3′
DNA 3′ T A C C T T T G AA C A T A T A T T 5′

1 On two separate stips of pa per, rite each sequence of nuceotdes and abe each strand DNA. Ensure the base pars agn when the two strps are brought togethr.
2 On a sheet of pape, draw a ce wth a nuceus Ensure th e nuceus and cytop asm are bothlarge enough so the strps with the DNA sequences w fit nsde them Pace the st rps wth the DNA sequences nsde the nuceus

Transcipion flows base parng rues except that th ymne n DNA s replacedwith urac n RNA The compementary sequences n RNA are adeine– urac and guanne–cytosne

3 Labl a tird srip of pape r wth mRNA′ at one ed. Separate the tw o nuceotde sequences and poston the thrd strp of paper n between them Usng the tempate strand of DNA wrte the mRNA sequ ence on ths strp gong from the 5′ end to the 3′ end

Tranlaion of mRNA occurs at ib osomes n the cytop asm The sequence of ntrogen ba ses n mRNA s rea in codons – groups of thre. Transfer RN A (tRNA) moecues contan an antcodon whch s compe mentary to the codon of mRNA and each tRNA carres a spec fic amno acd The tRNA moecu es brng these amn o acds to be bo nded together and form a ong chan of amno acds n a spec fic sequence

4 Move the mRNA transcipt to a ibos ome n the cytoso of your mode ce Pace a fourth strp of paper aongsde t and abe t Poypeptde Use the genetc code tabe n F gure2.19 to tran sate the mRNA codons nto amno acds
5 Comlete Tbl 2.4 to show the sequences

Table 2.4 Transrition and trasltion of the *o* gene

DNA tempate sequence						
mRNA sequence						
mRNA codons						
tRNA antcodons						
Amno acd sequence						

What dd you dscover?

1 a dentfy from the modl wich DNA st rand was the non-tempate strand and whch was the tempate strand for transcrpton
   b Expan how you decded whch stran d to use as the tempate strand
   c Descrbe the rltionhip of the ncl eotde sequence n th e mRNA wth the nuceo tde sequences of the non-tempate and tempate strands
2 Exlin how you deid ed from wich end of the mRNA to start transatng
3 Whatis the first amno acd n the poypeptde sequence? s t possbe for any other amno acd to appear first n any poypeptde? Expan
4 a How many nuceotdes were n the mRNA sequence?
   b How many aino aids were coded for?
   c What rlaionsipis there between th e number of nuceotdes n the mRNA transcrpt and the number of amno acdsin the trans ated poypeptde?
5 Has tis modl of transcipio n and transaton made the pr ocesses easer to understand?
6 Idenify anyimprovements you ould make tothis mo de to make the processe s easer to understand

KEY CONCEPTS

» Inforation i DNA i coded into groups of three nucleotides r triplets, and each tplet corresponds to a specific aino cid.
» Dring trascripion mRNA iscopied from template DNA.
» Pre-mNA is processe b utting, splicing and caping to become mature mRN before it leaves the nucleu.
» Pre-mRNA cn be altenatively spliced to generate dfferent mRNA tascripts.
» mRA is raslated ino a polypeptide by ribosomes n the cyosol or ribosomes bun to the endoplasmic reticulm.
» Transation is complished with the assistance of tRNA moleculesthat brin amin cids into position for ncorporton into te p lpeptide.

Concept questions 2.2

1 Define two key processes in gen epresin. Explain how they are diffrent.
2 Explain wy only one of the DNA strands can serve as the teplate strand duringtanscription.
3 Define exns'nd 'ntrons nd explain how they reate to the proessing of the pre-mRNA.
4 Desribe your understandng of the relationship between codns, anticodons and the different forms of RNA.
5 Explain hat is meant by a *degenerate* cod.
6 Translate h followng mRA ito a polypeptide using the gnetic coe shon i Table 2.19.
   AUGUCCUACCGGGCCUAG
7 A partcular mRA contin 12 nuleotides, excluding the 5′ and 3′ cap.
   a How many codons oes this mRNA have?
   b How man amino ids will e tranlated from this mRNA?
8 s a tple different from a codon?
9 What is a STOP codn? Ue Fiure .19 to identify two STOP odons.

HOT chaenge

10 The lowing statement describe crrent theoretical understnig. 'Spice sites in pre-mRNA are marked by tw univrsally conserved sequences contained at the ends ofthe trons' Unpack this statement by eplainig what it means. You may use annotated dagras.

2.3 Gene regulation

The information encoded in DNA determines which proteins are made in particular cells or tissues, and under what environmental conditions gene transcription occurs. Proteins and enzymes (expressed in particular cells at particular times) can bind to these regulatory regions of DNA to turn gene expression on or off. The process of switching gene expression on and off is referred to as **gene regulation**. Genes

are regulated during cell differentiation, development, or in response to physiological or environmental cues. Consider how a brain cell differs from a skin cell. These cell types carry out vastly different functions, yet they are packaged with the same DNA. A gene critically important for the functioning of a brain cell might never be expressed in a skin cell.

Cells use several mechanisms to regulate gene expression. For example, the promoter region of DNA where RNA polymerase binds to begin transcription of the gene determines when and in what tissue a gene is transcribed. As scientists sequence and study more genomes, and compare them with the proteomes of the same cells, more methods of gene regulation may be discovered.

Regulatory genes and structural genes

One way of broadly classifying genes is based on whether or not they control the expression of other genes. **Regulatory genes** are involved in controlling the expression of one or more other genes. The products of these genes may be functional pieces of RNA or proteins. The proteins may be enzymes, signalling molecules, receptor molecules or DNA-binding proteins. The key feature of a regulatory gene is that its product alters the expression of other genes.

Structural genes are any genes that are not regulatory genes. Structural genes include proteins that form the cytoskeleton, keratin in hair and nails, tRNAs and rRNAs, enzymes and signalling proteins that control cellular processes other than the expression of genes.

Structural genes are regulated by regulatory genes. This is shown in the example of the *trp* operon in the bacterium *Escherichia coli*, in which the genes were first described.

The *trp* operon: an example of gene regulation

WB
2.3.1 THE *TRP* OPERON: AN EXAMPLE OF GENE REGULATION PART A PAGE 45

The bacterium *E coli* inhabits the mammalian intestine, living on sugars and other nutrients. As in all living organisms, *E. coli* requires amino acids to build proteins. One of the amino acids needed by *E coli* is tryptophan, which *E coli* takes up and uses from the environment. However, if tryptophan is unavailable, *E coli* living in a mammalian gut produces the necessary protein enzymes for tryptophan synthesis. When tryptophan is present in the environment, these proteins are not produced because it would be a waste of the bacterium's resources and energy to do so.

The enzymatic proteins required to produce tryptophan are encoded by five structural genes: *trpE*, *trpD*, *trpC*, *trpB* and *trpA*. The coding regions for these five genes appear next to each other on the *E. coli* chromosome and are transcribed as a single mRNA strand. They are under the control of their promoter and operator regions plus the repressor *trpR*. A group of genes transcribed as a single unit from one promoter is known as an **operon**. The *trp* operon is shown in Figure 2.20.

n.
Video
The *trp* operon

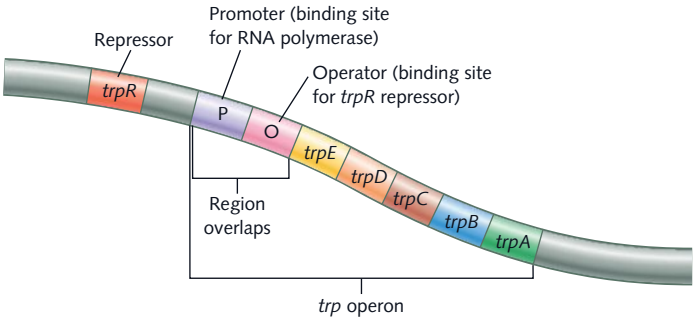


Figure 2.20 The structure of the *trp* operon, including the operator (the binding site for the repressor protein), the promoter (the binding site for RNA polymerase) and the five structural genes that code for proteins involved in tryptophan synthesis. The regulator gene trpR encodes the repressor protein and is located at another position on the chromosome of *E.coli*.

In another position on the circular chromosome of *E coli* is the regulatory gene *trpR*, which codes for a transcription factor. A **transcription factor** is any protein that binds to DNA, at a promoter or other region involved in the regulation of gene expression, to control the rate of transcription from a gene. Transcription factors can serve to initiate or enhance transcription, or they may act to prevent it. The transcription factor for the *trp* operon is a **repressor protein**. When tryptophan from the environment enters the *E coli* cell, it functions as an **inducer**, a kind of signalling molecule. Tryptophan binds to the repressor protein, altering its shape so that it can bind to a region of non-coding DNA, called an **operator** (Figure 2.21a). Binding of the repressor protein blocks RNA polymerase from attaching to the promoter region. The five genes of the *trp* operon cannot be transcribed and gene expression is switched off.

Note:
Transcription factors control the rate of gene expression in eukaryotes as well as prokaryotes.

When no tryptophan is available to bind to the repressor, the repressor is inactive and cannot bind to the operator. The promoter is now exposed for RNA polymerase binding, and the five structural genes are transcribed into pre-mRNA. Gene expression is switched on (Figure 2.21b).

The binding of the tryptophan and the repressor is reversible. When tryptophan concentrations are sufficiently high, tryptophan binds to the repressor and keeps the *trp* operon repressed. When tryptophan levels decrease, the repressor no longer binds to the operator. RNA polymerase can now bind to the promoter and the five structural genes can be transcribed.

WB
2.3.2 THE *TRP* OPERON: AN EXAMPLE OF GENE REGULATION PART B PAGE 46

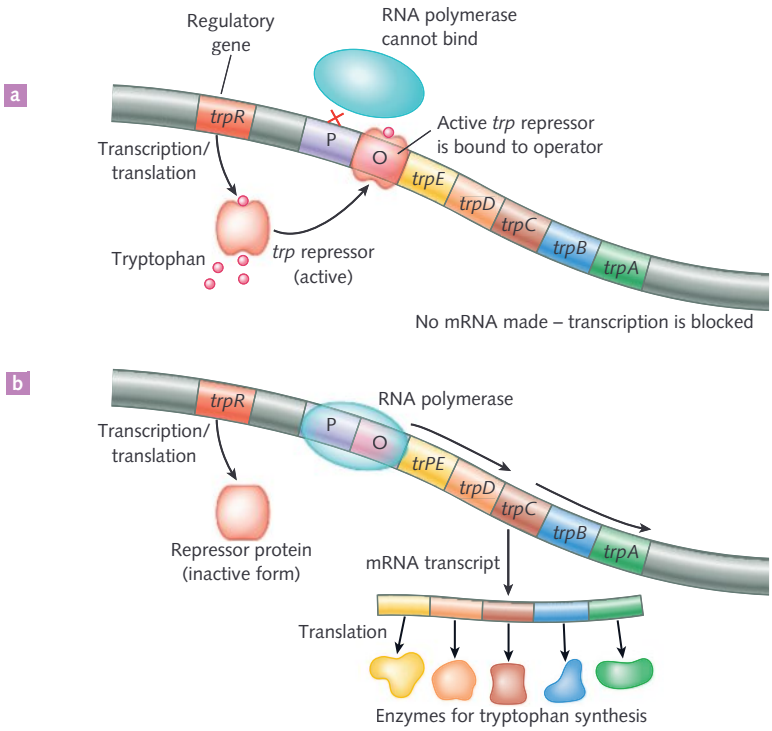


Figure 2.21 a When tryptophan is present, it binds to the repressor protein and alters its shape, so that the repressor can bind to the operator. The repressor then covers part of the promoter, preventing RNA polymerase from binding to the promoter, and blocking transcription of the structural genes in the operon. b When tryptophan is absent, the repressor protein is inactive, and it cannot bind to the operator. RNA polymerase can bind to the promoter and transcribe the five genes into a single mRNA transcript.

KEY CONCEPTS

» Gene rguation is the procs of switching gene expresson on or off. The *trp* operonis an exmple of gene reglation in prokaryotes.

»In the *trp* opero, a rgulatory gene codes for a repressor proein that bloks RNA polymerase from transcribing the *trp* opero.

» Tryptophan act as an indcer that activates the repressor and consquntly switches off expression of the *trp* operon gens.

»If there relowlevels of typtophn in the cell, the repressor l notbind to the operator, so the RNA plymere will bind to the promoter and the five structuralnes will b expressed.

Concept questions 2.3

1 What is the promoter of a gene?
2 What is meant by an operon?
3 What is the difference between a regulatory gene and a structural gene?
4 Wha might happen if genes are not regulated?
5 Tryptophan is coded for by five structural genes found n the *trp* opero. ist these.

HOT chaenge

6 Gene egulation enabls gene expression to be contlled. The gene can be switched on or off accoring to the vailing evirmental conditions or cel ialling. Order by numerth steps involved in reulating te production of tryptophan in an *E coli* ce. nclude two pathways that demonstrate what happens when ther arehigh and low levels of tryptophan.

2.4 Proteins

Virtually everything a cell is or does depends on the **proteins** it contains. Proteins are large complex molecules that are the building blocks for many different structures and are produced by the activities of cells. For example, keratin is a strong fibrous protein found in your hair, the feathers of birds, the scales of snakes and the spines of echidnas. Haemoglobin is a protein in the blood that carries oxygen from the lungs to the rest of the body (Figure 2.22).

Proteins are large molecules, consisting of polymers called polypeptides. The monomers making up the polymers are amino acids.

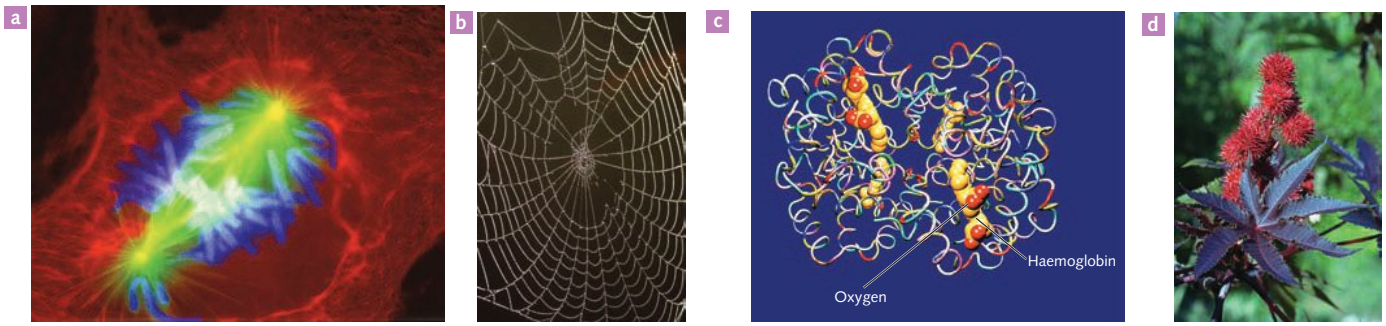


Figure 2.22 Proteins have a diverse range of functions. a Spindle fibres attach to chromosomes in cell division. b A spider web is composed of fibroin. c Haemoglobin carries oxygen to the cells in the body. d The castor oil plant produces the deadly toxin ricin.

Structure of amino acids

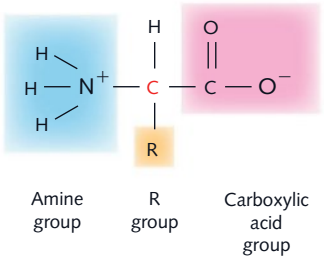


Figure 2.23 A generalised structural formula for an amino acid. Each amino acid has a different R group, which affects the structure and properties of the amino acid.

Amino acids are small molecules that have the same basic structure. Each of the 20 amino acids has a central carbon atom that has attached a hydrogen atom, an amine group (NH_2) and a carboxylic acid group (COOH). Also attached is an R group, which is a different group in each amino acid. The amine and acid groups react with water to become charged NH_3^+ and COO^- groups, respectively (Figure 2.23). The different R groups distinguish one amino acid from another and give the amino acids their particular chemical properties.

Some R groups make regions of the protein molecule polar (have a positive end and a negative end). Polar amino acids are **hydrophilic** ('water loving'). These R groups tend to be on the surface of proteins because of their affinity for the polar water molecules in their environment (Figure 2.24). There are 10 polar amino acids, including glutamine, tyrosine and serine. Other R groups make regions of the protein non-polar. Non-polar **hydrophobic** ('water hating') regions are generally tucked within the protein molecule, away from the water molecules in the aqueous environment. Hydrophobic amino acids can also be on the outside of sections of a protein that are embedded inside the hydrophobic centre of the plasma membrane. Non-polar amino acids include lycine, alanine and proline.

Plants synthesise all of their amino acids, but animals only synthesise some and must obtain the rest from their diet. More than 100 kinds of amino acids can be found in cells but only 20 are used to make up proteins.

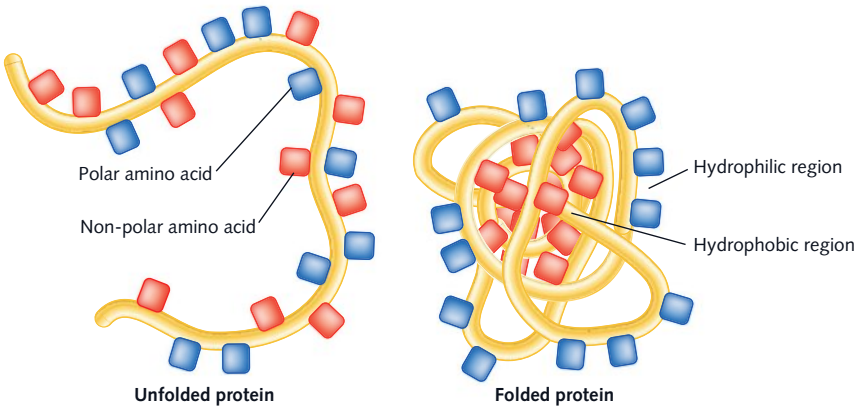


Figure 2.24 In an aqueous environment, hydrophobic amino acids (red) associate in the centre of the protein and the hydrophilic (blue) amino acids tend to interact with the surrounding environment.

WB 2.4.1 PROTEINS: BUILDING A MODEL PAGE 46

Hierarchy of protein structure

The shape of a protein is very important to its function. To understand how a protein gets its final shape or conformation, it is necessary to understand the hierarchical classification of the structure of proteins. There are four different levels that give rise to the final structure.

Primary structure

The genetic code stored in the form of DNA determines the linear sequence of amino acids in the polypeptide. This is the **primary structure** of the protein. Amino acids bond together in the process of

condensation polymerisation, releasing a water molecule. The bond between two adjacent amino acids is called a **peptide bond** (Figure 2.25).

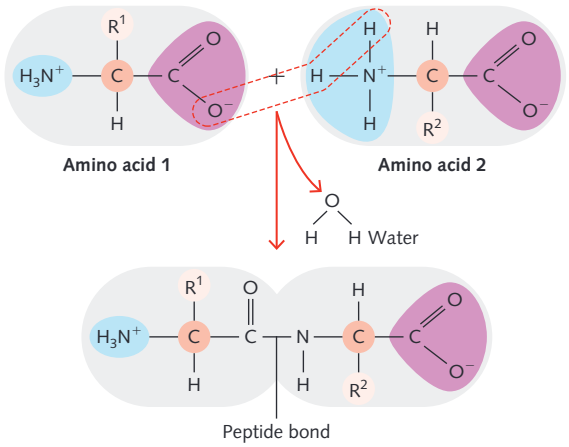


Figure 2.25 A condensation polymerisation reaction forms the peptide bond between two amino acids. Water is released during the reaction.

Secondary structure

Once the polypeptide chain is formed, various parts undergo coiling and folding due to hydrogen bonding between the peptide bonds of neighbouring amino acids. Hydrogen bonds are weak chemical bonds that form between a partially charged hydrogen atom on one amino acid and a partially charged oxygen or nitrogen atom on another amino acid. These coiled and folded portions of the polypeptide chain form the **secondary structure** of the protein (Figure 2.26). Tight coils are called alpha-helices (α-helices; singular, **α-helix**) and flattened folding forms are called beta-pleated sheets (**β-pleated sheets**). Other parts of the polypeptide chain do not fold into defined arrangements and are called **random coils**. This nomenclature (naming system) is perhaps misleading because the random loops are usually highly functional.

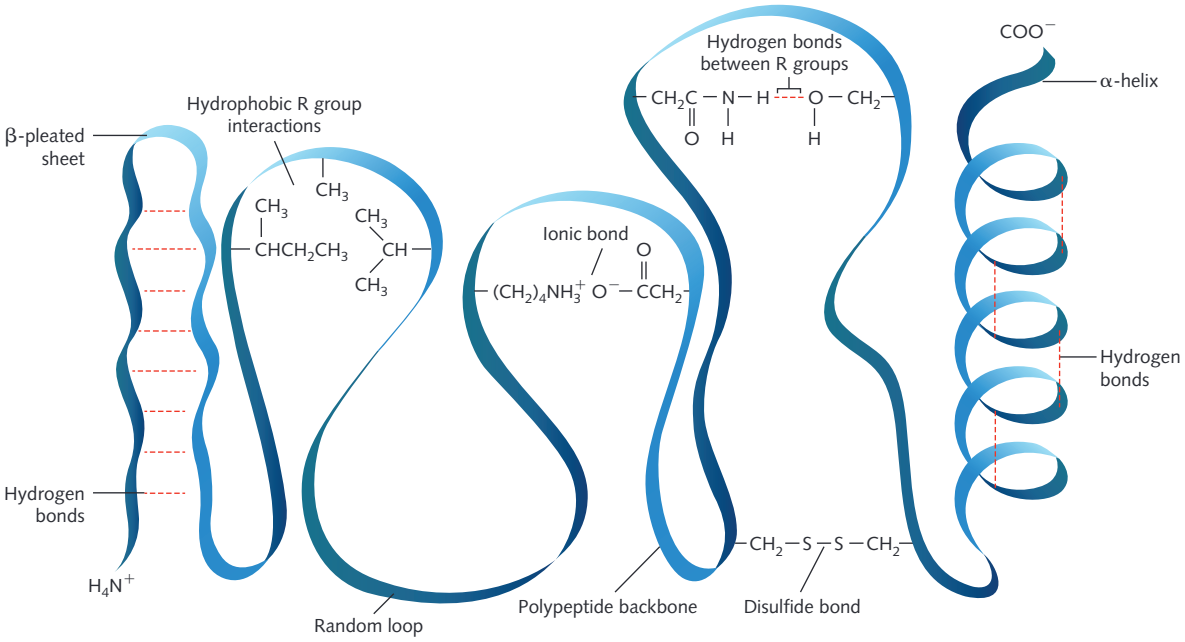


Figure 2.26 Secondary structures, such as α-helices and β-pleated sheets, form by hydrogen bonding within localised regions of the polypeptide. The polypeptide chain becomes folded, coiled or twisted in the protein.

Tertiary structure

Hydrophilic R groups of individual amino acids within the polypeptide chain attract hydrophilic R groups of other amino acids in other parts of the chain. Hydrophobic R groups attract other hydrophobic R groups, according to the chemical principle 'like attracts like'. These interactions between the R groups of the amino acids cause the polypeptide chains to become folded, coiled or twisted into the protein's functional shape or **conformation**, described as the **tertiary structure** of the protein (Figure 2.27c).

The tertiary structure determines the **biological functionality** of a protein. Some proteins form long, closely packed fibres that are insoluble in water and form structural components of cells. For example, collagen is the fibrous protein that provides structure in connective tissue. Collagen is made up of three polypeptide chains that coil around one another to form a triple helix. Many collagen molecules pack together side by side to form fibrils that are arranged in different ways in different tissues to form collagen fibres.

Most proteins form spherical or globular molecules that are soluble in water and perform a variety of functional tasks. Enzymes are proteins that are mainly globular and the tertiary structure results in a spherical or ball-shaped structure. Depending on the way the protein folds into its tertiary structure, it has hollows on its surface called active sites. The substrates in a reaction fit into these active sites, thereby speeding up the reaction. This binding determines the specificity of an enzyme, as each enzyme can only speed up one particular reaction.

Receptor proteins on the surface or in the cytosol of target cells also have a tertiary structure with specific binding sites. This feature can be used in the development of highly specific drugs that will either stimulate the activity of the target or block its activity.

Quaternary structure

Many large, complex protein molecules consist of two or more polypeptide chains. The **quaternary structure** of a protein is formed when two or more polypeptides associate into the mature protein. A variety of hydrogen bonds, ionic bonds and covalent bonds hold the polypeptide chains together and give the overall shape to the molecule. Haemoglobin consists of four polypeptide chains – α_1, α_2, β_1 and β_2 (Figure 2.27d). Antibodies or immunoglobulins, involved in the immune system, consist of two heavy polypeptide chains and two light chains. (See Chapter 7 for more detail.)

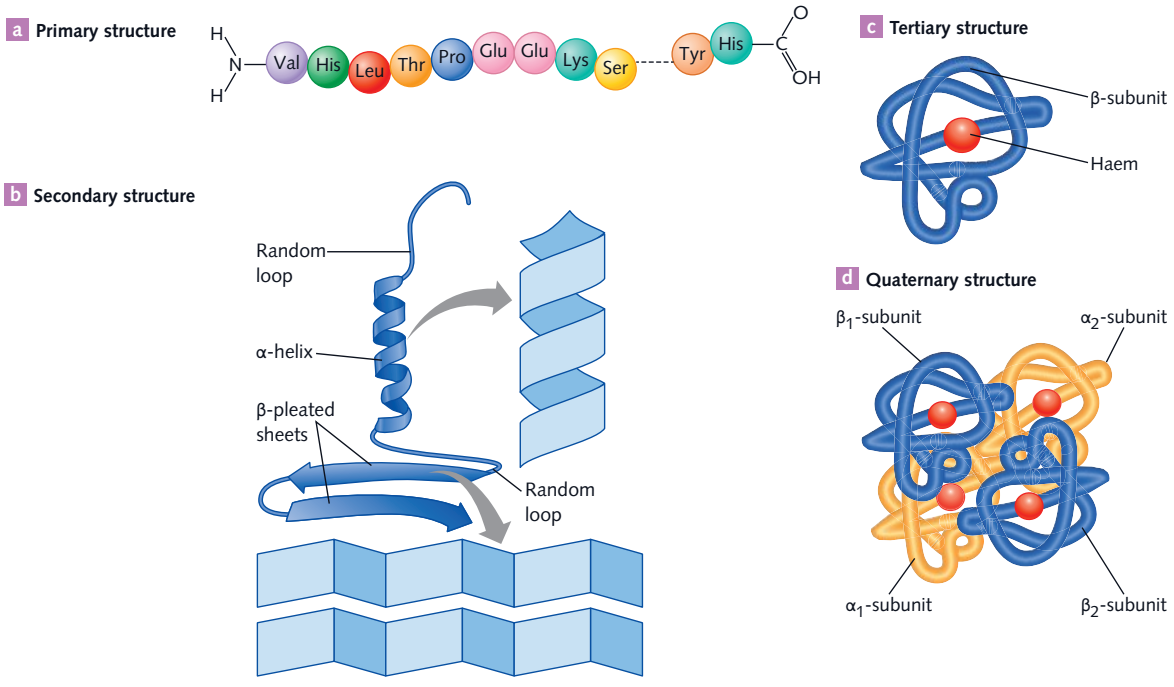


Figure 2.27 The four levels of organisation that give rise to the final protein structure of haemoglobin. **a** The primary structure consists of four chains of amino acids (two chains of 141 amino acids and two chains of 146 amino acids); **b** the secondary structure results from folding and coiling of the chains; **c** the tertiary structure is the conformation of the protein and **d** the quaternary structure consists of four polypeptide chains.

Changing the nature of proteins

A change to just one amino acid can alter the shape of the protein molecule so that it no longer functions properly. For instance, haemoglobin is made up of 574 amino acids. Some people with a different form of the gene, called an allele, produce haemoglobin with one different amino acid in one of the chains. This alters the shape of the haemoglobin molecule, leading to crescent-shaped rather than smooth disc-shaped red blood cells (Figure 2.28). These 'sickle cells' get stuck in blood vessels, obstructing blood flow and leading to the symptoms associated with sickle cell anaemia. Symptoms include episodes of severe pain, swelling of hands and feet and stroke (obstructed blood flow to the brain). Interestingly, the sickle shape protects red blood cells from infection by the malaria parasite, and this is probably why the allele persists throughout generations in populations that are exposed to malaria.

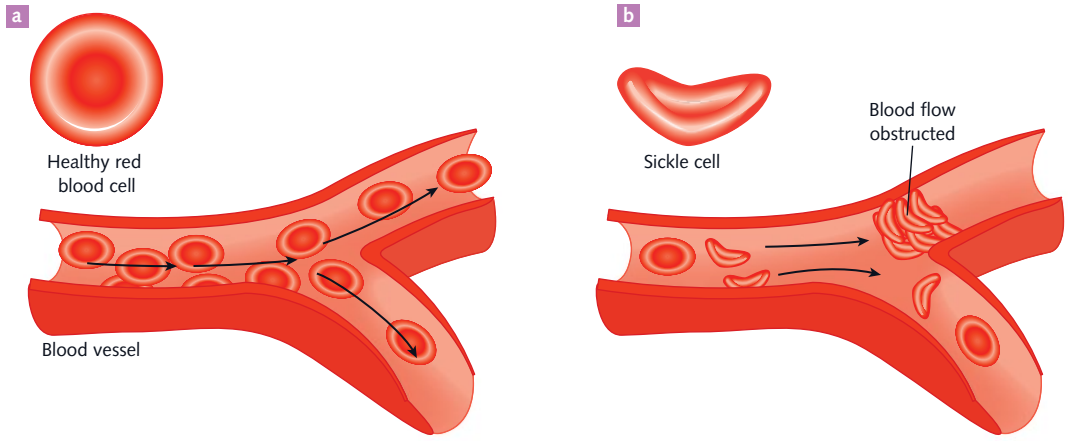


Figure 2.28 Changing one amino acid alters the structure of the haemoglobin protein. a Correctly folded haemoglobin in healthy smooth disc-shaped red blood cells means that blood flows normally through vessels. b Crescent-shaped red blood cells called sickle cells get stuck in small blood vessels, obstructing blood flow.

As well the sickle cell mutation, other mutations in the DNA code may result in amino acid changes that prevent polypeptides from folding correctly. Proteins may also lose their functional shape if they are exposed to high temperatures, concentrated salt solutions, or very acidic or alkaline conditions. These conditions can **denature** the protein molecules.

Cooking an egg causes the egg white to change from clear to white (Figure 2.29). Egg whites contain a protein called albumin. Heating breaks the bonds between different amino acids that give the protein

Figure 2.29 a Raw and b cooked egg. Albumin, the colourless protein of the egg 'white', is changed by heat.

EXAM TIP
Often questions appear to have a lot of information that you don't know the first time you read it. Sometimes a question will describe a scenario to frame the real question. Think critically about how the scenario might apply to what you know about protein structure.

its 3D shape. The hydrophobic amino acids that are usually inside the protein become exposed to water molecules surrounding them in the egg white. The hydrophobic amino acids move to avoid the water molecules and clump together with other hydrophobic amino acids. This produces a solid protein network that turns the egg white from clear to white.

Types of proteins

The number of different proteins and their various modified versions in the human body is estimated to reach millions. This number will be refined with advancements in the technologies used to detect rare proteins. Proteins carry out many functions (Table 2.5). Proteins can:

- promote cellular motility, or movement
- provide structural support to cells and whole organisms
- transport molecules in and out of cells
- transmit signals within and between cells and organisms
- receive cellular signals and activate cellular responses to the signals
- help protect against attack by other organisms
- control the thousands of chemical reactions that maintain life processes (enzymes).

Table 2.5 The functional diversity of proteins

Type of protein	Function	Examples
Motility	Allow movement of cells and their organelles	Tubulin forms microtubules to move flagella, cilia, chromosomes and organelles. Actin and myosin work together to move muscles.
Structural	Provide support, strength and protection	Collagen supports body tissues. Fibroin makes a spider web stronger, weight for weight, than steel. Keratin forms nails and hair.
Transport	Carry molecules from one location to another or across plasma membranes	Haemoglobin carries oxygen to body cells. Porin forms a hydrophilic pore in the outer membrane of mitochondria for the passage of molecules.
Cellular signalling	Signal between different cell types; stimulation or inhibition	Insulin travels in the blood and binds to cell receptors to trigger the uptake of glucose. Endorphins activate nerve receptors to alleviate pain or stress. Cytokines signal between cells of the immune system to coordinate immune responses.
Cell-surface receptors	Receive signals such as hormones and growth factors, transmission of nerve impulse	Insulin receptors bind insulin to trigger the uptake of glucose by the cell. Rhodopsin in the retina membrane of the eye is a light-sensitive receptor that allows us to see in dim light.
Defence	Recognise and protect against foreign organisms	Antibodies cause foreign material to clump and be ingested by large white blood cells (macrophages). The castor oil plant produces ricin, a deadly toxin.
Enzymes	Caalyse, or actively assist, biochemical reactions	Catalase removes toxic hydrogen peroxide from cells by breaking it down into water and oxygen. DNA polymerase duplicates genetic information (DNA).

Enzymes

Enzymes are vital for life. They are proteins that **catalyse** or speed up the rate of every chemical reaction of the body, without themselves being changed in the process.

To catalyse biochemical reactions (chemical reactions in cells), enzymes locate molecules in the enzyme's active site – a pocket that fits the specific target molecule. The target molecule is called the **substrate** (Figure 4.5, p. 143.) Within the active site, the chemical and electrical properties of the enzyme apply pressure and tension to bend and twist the substrate, so that chemical bonds within the substrate are more easily broken down. Each enzyme has a specific substrate (either a single molecule or a small range of molecules) and produces specific **products** from the reaction. Importantly, the enzyme is not altered by the reaction, so it releases the products and is free to carry out further reactions. Enzymes are carefully controlled by regulatory processes to ensure that their functions in carrying out biochemical reactions occur only when they are needed.

In plants, enzymes are involved in building simple carbohydrates from inorganic materials in the process of photosynthesis. In all living cells, enzymes are essential in transforming energy in the process of cellular respiration. Without enzymes, all of the chemical reactions that sustain life would proceed at a very slow rate, so life as we know it would not be possible.

CONNECT

Enzymes and the biochemical pathways involved in photosynthesis and cellular respiration are discussed in detail in Chapters 4 and 5.

Proteome

The whole set of proteins produced by a cell, a tissue or an organism is called its **proteome**. The proteome of one cell type varies from that of another cell type because different genes are expressed in different cells depending on the function of the cell. Collectively, the proteins (including enzymes) that make up each cell's proteome are responsible for all of the functions and activities of that cell.

It is estimated that there are more than 100 000 different proteins in the human proteome. These proteins can be identified by a common laboratory technique called mass spectrometry. Humans can produce approximately 100 000 different proteins throughout their bodies, but each cell makes only a proportion of these. A sperm cell's proteome contains 2000–2500 different proteins. This is the set of proteins that are necessary for the specific function of sperm, which is to deliver the male's genetic information to the oocyte (egg cell). Intestinal epithelial cells, which are the cells that line the gut, have a similar number of proteins in their proteome. However, the proteins they express are quite different from those of sperm cells and give them the capacity to absorb nutrients and keep harmful invaders out of the body.

Proteomics

Scientists often study single proteins of interest, one by one. However, in the last 20 years, new technologies and knowledge have led to a new field of study: **proteomics**. Proteomics is the study of proteomes – all the proteins in a cell, tissue or organ all at once. Proteomics is a dynamic field of research that is concerned with investigating the collection of proteins, their modifications and features, their subcellular locations, and the ways they interact with each other, in a particular cell type or tissue.

Functional proteomics particularly refers to the study of what proteins do in different cells or tissues. It can involve studying how proteins interact with each other in a specific tissue type, or how the collection of proteins in a tissue changes in response to particular conditions, such as during disease.

The combined expertise of computational biologists, mathematicians and molecular biologists has resulted in the development of powerful tools, techniques and databases for studying proteins, and this field of study is likely to expand greatly as better technologies are developed.

KEY CONCEPTS

- Proteins are a class of biological molecules with a diverse range of functions vital to cell structure, organisation and operation.
- Proteins consist of linear polypeptide chains of amino acids.
- There are 20 different amino acids that can be grouped according to their properties.
- Proteins fold into shapes that are defined by their amino acid sequence, and they exhibit four levels of structure in the course of folding into their proper shape.
- A protein's function depends on its shape.
- Heating proteins can cause them to unfold irreversibly.
- A proteome is the complete set of proteins in a cell, an organ or a tissue.

Concept questions 2.4

1 State the name of the monomers that make up polypeptides. What holds these monomers together?
2 List at least five types of proteins, state their functions and give an example of each.
3 Distinguish between hydrophilic and hydrophobic amino acids. Suggest where each is likely to be found in a folded protein and explain why.
4 Describe the basic structure of an amino acid molecule.
5 Describe how amino acids are linked together. Use the correct term for the process and name the small molecule that is released during the reaction.
6 Describe the four levels of protein structure.
7 What three types of folds are associated with the secondary structure of a polypeptide?
8 Define 'functional proteomics'.
9 Describe some potential benefits or uses of proteomics in medical research.

HOT challenge

10 Differentiate between genomics and proteomics.

WB 2.5.1 TELLING THE STORY OF THE PROTEIN SECRETORY PATHWAY PAGE 51

2.5 The protein secretory pathway

Proteins destined for secretion by cells (such as hormones) or expression in the endoplasmic reticulum, **Golgi apparatus** (or Golgi bodies), lysosomes or other membrane-bound organelles, can enter the **protein secretory pathway**. In the first step of this pathway, polypeptides that are made on endoplasmic reticulum-bound ribosomes are transferred through the endoplasmic reticulum membrane as they are synthesised and enter the lumen (interior) of the rough endoplasmic reticulum (Figure 2.30). Here, the polypeptides are assisted to fold in the correct way, then are sorted and transported to the Golgi apparatus in membrane-bound vesicles called **transport vesicles**. The Golgi apparatus is an organelle consisting of stacks of flattened pockets called **cisternae** (singular, cisterna) usually 4–8 cisternae per Golgi in a multicellular organism), held together by matrix proteins and microtubules of the cell's cytoskeleton. As proteins progress through the cisternae of the Golgi apparatus, they are modified by enzymes that may add or remove components until the protein is in its mature, functional form. In the last stage of progression through the Golgi apparatus, the proteins are concentrated and further packaged into secretory vesicles, which are then shuttled to the plasma membrane of the cell. Here, the secretory vesicles fuse with the plasma membrane to export their protein cargo into the extracellular environment (Figure 2.30).

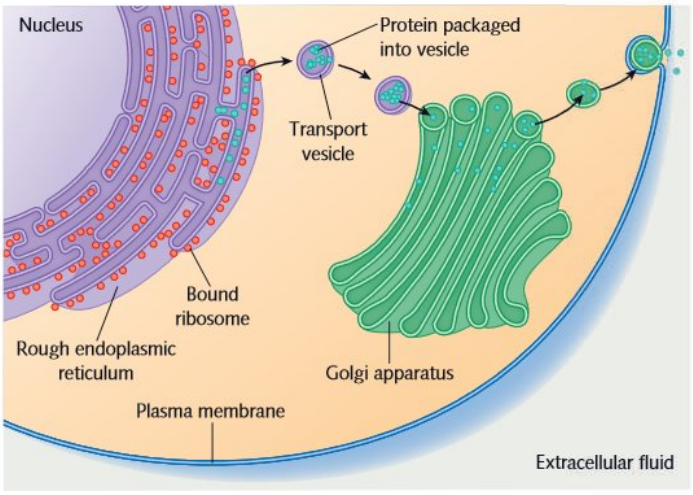


Figure 2.30 The protein secretory pathway packages proteins to be exported from the cell.

Proteins that have transmembrane domains or chemical attachments that allow them to be joined to plasma membranes stay membrane-bound through the process and end up tethered to the plasma membrane at the cell's surface. Some specialised cells secrete high concentrations of certain proteins. For example, plasma cells produce and secrete large numbers of antibody molecules that help to destroy pathogens. Plasma cells have abundant rough endoplasmic reticulum, which allow them to shuttle large amounts of antibodies through the protein secretory pathway.

Proteins are tagged with molecular labels so that they get to the right place in the cell to be secreted. This is analagous to how a letter is labelled with a postal address that is recognised by the post office so it gets sent to the right place.

CONNECT

Plasma cells and antibodies will be further discussed in Chapter 7.

KEY CONCEPTS

» Protins destined forsecretion can eter the protein secretory pahway.

» The rough edolami retilum, the Golgi apparatus and secreto veicles modify, sort and package proteins for secretion fromhe cell and expression on the ouside surface of e cell.

Concept questions 2.5

1 Stating at the ribosome construct a flow chart to show the steps of a potein that s secreted from tcell.

2 Describe how h enolasm reticulum and Golgi apparatu differ fntionally.

HOT chaenge

3 Secretorvesicles fuse with the plasma membrane to enale prtein scretion as par of a protein secretory pathy. Interpre wat this fusion' entails.

BRANCHING OUT

Biological knowledge and society: the speed gene

a

b

Figure 2.31 Studies reveal genetic differences between a elite sprinters and b marathon runners.

The *ACTN3* gene on chromosome 11 habeen alled the 'speed gene becaueit is crrelted wi thlite atlet ability n spint events. A controversy has arien around genetic scre enng for the speed gene and cocern that it may lead to dscrimnation and t potential for dsigne athletes' adgene doping.

The genetics

The proein product of the *ACTN3* gene isalpha-actnin-3. It s expressed in ast-twitch muscle fibres where t connects acti proein chais to coordinate fa s, repetitive and powerfl muscle contractions

CONNECT

For more information on energy pathways used by cells, go to Chapter 5.

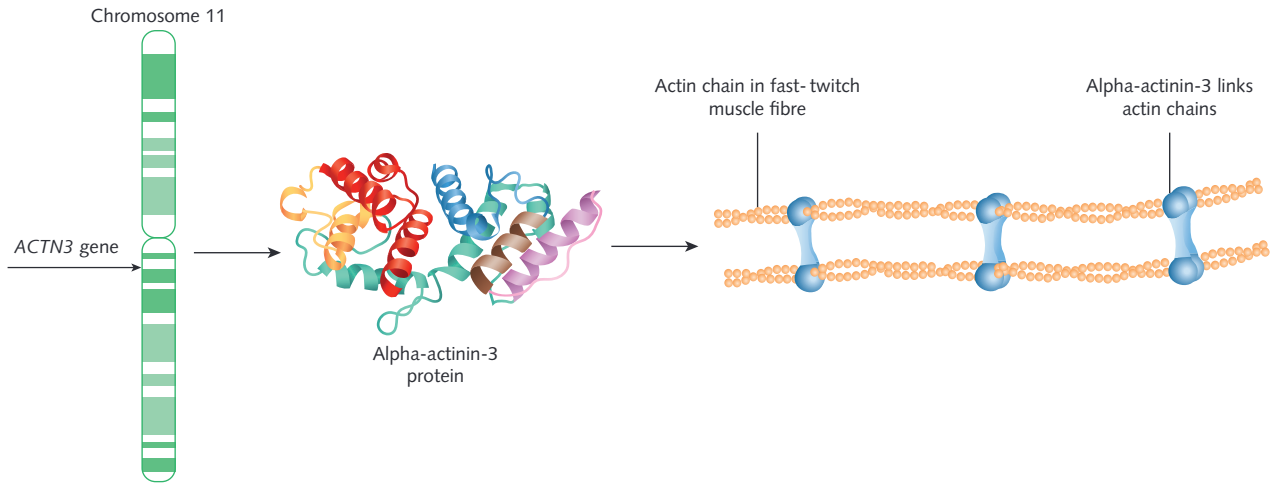


Figure 2.32 Expresson of the *ACTN3* gene n fast-twtch musce fibres

(Fgure2.2). These fast-twitch muscle fibres are poweed by glucose nd usanaerobic lycolysis pathways to provide energy in the form of ATP.

n 199, Autralan geneticist Professor Katrn North and a team o scientists discovered a common mutation in the *ACTN3* gene. Twalleles for the *ACTN3* gene xist in theppuain: allele 577R coesfo funconl alpha-actinin-3, but a secondele 577X, has a mutaton hat results in a pr emature stop codon so he protein is truncted (shortened). Tis mutan alp-actni-3 proten inot functional.

Haing just one R allele means you can poduc untionl protein. Thi R variant codes for the dominant phenotpe while te X variant codes for the recessive phen oty. neriting two copies of the X variant means you cannot makefucionaalpha-actinin-3, as seen in 20% of the Austalia poplatio. Ther is no obvous effect if you do not mak alpha-actinin-3; it oes not cause disease.

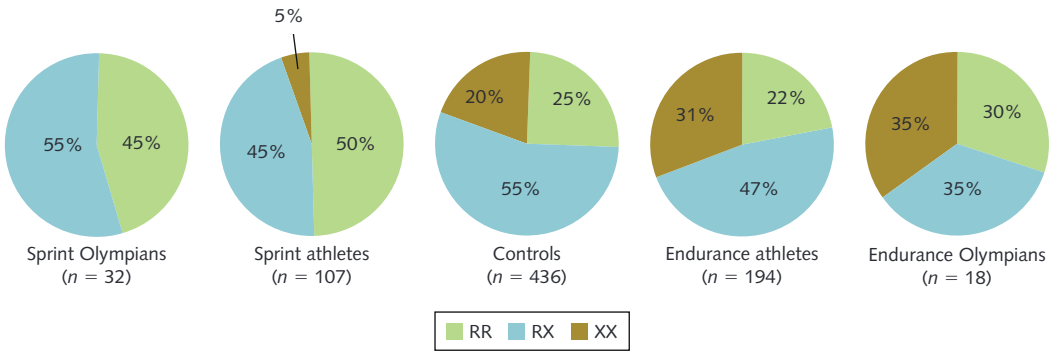


Figure 2.33 The *ACTN3* allele status of Olympian sprint and endurance athletes, non-Olympian sprint and endurance athletes, and the general population (controls)

A genetic test can e carriedout to determine the *ACTN3* aele statusof niidual. In 2003, Professor North colaborated on a tudy with heAustralian Instit ute of Sprt. Th sudy involed determining the *ACTN3* aele status for 436 pepe in the gnerlpopulatioas a ontrol, 32 sprint Olymins, 107 sprintathletes, 194 endurance athletes and 18 enduranc Oymians. The re suts are shon i Figue .33.

Alpha-actinin-3 influence on athletic performance

To study the effecto apaactinin-3,scientists generated a strain of *ACTN3* knock-out mice hat could not produce the proein. The knock-out mice produced higher amounts of th e proein alpha-acii-2 This cosely related protein is

producedto replaeth mis an p a-actinin-3. Table 2.6 sho ws reslts of further tets comparin the knock-out strain wth contrls.

Table 2.6 An overvew of resuts comparng knock-out m ce that cannot produce apha-actn n-3 wth contro mce that do produce ths proten (sum marsed from a paper by Berman and North 2010)

Test	Knock-out mouse strain (no alpha-actinin-3) compared with control mouse strain
Weight	Slightly reduced
Muscle mass	Significantly reduced
Muscle fibre compoiion	hift from fast mscle fibres towards having more slow oxidative fibres
Grip strength	Reduced by an average 6%
Endurance running	Run an average 33% further before exhaustion
Glycolysis enzymes	Elevated
Converting pyruvate to lactate through activity of lactate dehydrogenase (anaerobic pathway)	Decreased
Mitochondrial enzymes associated with aerobc repirtioElevated	
Oxidise fats for energy	Increased capacity

Many contries,includin utralia, invest large amounts of mone in programsto ientifyandtr in athletes. The Austalia Institute ofSport provides rograms to adolecnts basedonphyscal and psychological tests.

Many parentsareso illin to nvstin their children's sporting fues. Similar resuls o thse seen in Figure 2.33 have beerepliated in a nmber of indepndent studies around the world, so they are nsdred reliable. Companies such as 23andM and AtlasSports Genetics offergentic tests to consumers. Atlas first recommends parents test cildren aged 0–8 yeas in order to rovid erly iformation on gene tic prdissition for sucess in speed/power or endurance evnt Critics are concerned tat tese copaniesar misrepresenting he science. Professor North cautions that the *ACTN3* gene i iolation is a por redicor. The traits of succesfu athletes ar complex, with hundreds of genes nvolved andthe enironet alo playig an important role.

The Ausralian Law Rfom Commissin and he Natoal Healt ad Medical Research Council released advice on the use of enetic iformation in sort (203). They advised th at 'there are concerns about the efectof genetic testing on ndidul thetes,specally when this nvolve children or yongpeople. Ina ppropiate interpretation of test results coud at best led to incorrct advice abou placeent insporg activities,and at wost could be detrimental to the phscal or scholgica halt of an individual.'

Questions

1 Idenify hebetical issues.
2 Identifythe biology that relate to these issues.
3 How can the results of the North (20) study shown in Fig ure 233 be xplained by the results of the Berman and North (2010) study hownin able 2.6?
4 Refer to the rsuts i Tle 2.6. Di scuss the altered muscle prformance in knock-ot mice and then formulate a hypohesis as to why anabsene o alpha-actinin-3 leads to ncreased endurance performance anddecreased sprint and power perormance.
5 How tcally acet ble i it t select althletes based on genetc infomation? Frame our response in terms of one or more of thfollowing thical concept intgrity ustice, bene ficence, non-ale ficenc, resect.

References

Berman Y, and Nort, K. (2010). A gene for speed: The emerging role of alpha-actinin-3 in muscle metabolism. Physiology. 25(4): 250–259.

Online Key Concepts
Chapter 2: Summary of key concepts

2 Summary of key concepts

2.1 Nucleic acids

KEY CONCEPTS

p 39

- Nclotides are monomers that are made up of a five-carbon pentose sgar, a phosphate goup and a nirogenous base.
- Nclotide monomers makup the nuclei acids DNA and RNA.
- There are for different types of nitroenousbases in DNA: adenne (A), cytosne (), guanine G) and thymine (T).
- Nclotides make up the strnds of DNA, which are held together by hydrogen bonds between compleentary bases.
- Bases pairaccording to he complemenry ae pairing rules: A always bond to ; C alway bonds to G.
- The two strands of DA aentiparallel.
- In cls, DA is ogaised into chromosomes.
- RNA s ingle-stranded.
- RNAnucleotide containribose sugar.
- RNA cntains the seuracil (U) instead of the DNA base thmine T).
- RNA strands are shorter tha DNA strands.
- RN plays a mjr role in gen epression.
- There are thee main tyes f RNA: mRNA, tRNA and rNA.

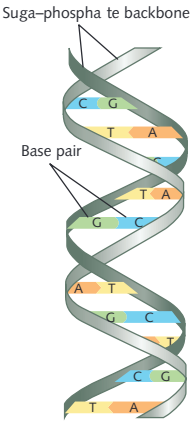


Figure 2.6 The DNA helix is a double-stranded molecule. Two strands are held together by hydrogen bonding between complementary nitrogen bases. An A–T pair is held together by two hydrogen bonds, whereas a G–C pair is held together by three hydrogen bonds

2.2 Gene expression

KEY CONCEPTS

p 47

- Inforation i DNA i coded into groups of three nucleotides r triplets, an d each tplet corresponds to a specific aino cid.
- Dring tasritin, mRNis copied from template DNA.
- Pre-mNA is processe b utting, splicing and capping to become mature mRNA before t eaves th nuleus.
- Pre-mRNA cn be altenatively spliced to generate dfferent mRNA tascripts.
- mRA is raslated ino a polypeptde by ribosomes in the cytosol or ribosomes bond o the ndplasmic reticulum.
- Transation is complished with the assistance of tRNA mlecules tatbring amino ads into position for ncorporton into the poypeptide.

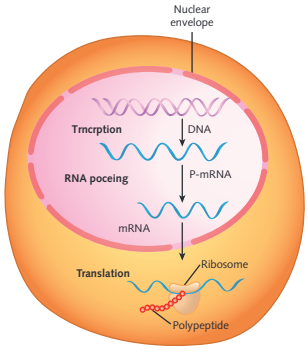


Figure 2.12 Gene expression relies on the processes of transcription and translation. During transcription, DNA is copied into pre-mRNA, which is processed into mature mRNA for export from the nucleus. During translation, the ribosome 'reads' the nucleotide sequence in the mRNA and forms the amino acid sequence of a polypeptide

2.3 Gene regulation

KEY CONCEPTS

p. 54

- Gene regulation is the process of switching gene expression on or off. The *trp* operon is an example of gene regulation in prokaryotes.
- In the *trp* operon, a regulatory gene codes for a repressor protein that blocks RNA polymerase from transcribing the *trp* operon.
- Tryptophan acts as an inducer that activates the repressor and consequently switches off expression of the *trp* operon genes.
- If there are low levels of tryptophan in the cell, the repressor will not bind to the operator, so the RNA polymerase will bind to the promoter and the five structural genes will be expressed.

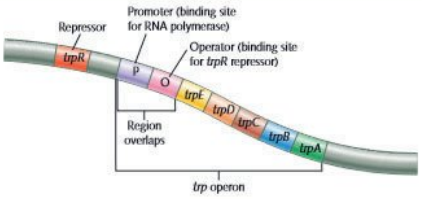


Figure 2.20 The structure of the *trp* operon, including the operator (the binding site for the repressor protein), the promoter (the binding site for RNA polymerase) and the five structural genes that code for proteins involved in tryptophan synthesis. The regulator gene trpR encodes the repressor protein and is located at another position on the chromosome of *E. coli*

2.4 Proteins

KEY CONCEPTS

p. 57

- Proteins are a class of biological molecules with a diverse range of functions vital to cell structure, organisation and operation.
- Proteins consist of linear polypeptide chains of amino acids.
- There are 20 different amino acids that can be grouped according to their properties.
- Proteins fold into shapes that are defined by their amino acid sequence, and they exhibit four levels of structure in the course of folding into their proper shape.
- A protein's function depends on its shape.
- Heating proteins can cause them to unfold irreversibly.
- A proteome is the complete set of proteins in a cell, an organ or a tissue.

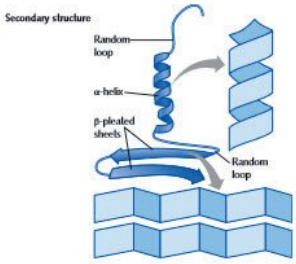


Figure 2.27 The four levels of organisation that give rise to the final protein structure of haemoglobin. **a** The primary structure consists of four chains of amino acids (two chains of 141 amino acids and two chains of 146 amino acids); **b** the secondary structure results from folding and coiling of the chains; **c** the tertiary structure is the conformation of the protein and **d** the quaternary structure consists of four polypeptide chains

2.5 The protein secretory pathway

KEY CONCEPTS

p. 64

- Proteins destined for secretion can enter the protein secretory pathway.
- The rough endoplasmic reticulum, the Golgi apparatus and secretory vesicles modify, sort and package proteins for secretion from the cell and expression on the outside surface of the cell.

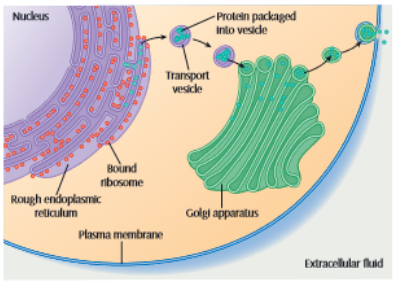


Figure 2.30 The protein secretory pathway packages proteins to be exported from the cell

WB
2.6.2 KEY TERMS PAGE 53
2.6.1 SPOT THE ERRORS PAGE 53

2 Chapter glossary

α-helix a type of secondary protein structure in which the polypeptide chain folds into a tight coil

allele a different version of the same gene (at the same locus) determined by small differences in the DNA sequence of the gene

alternative splicing a process in which one or more exons are removed with the introns to produce mRNA molecules of different length and sequence

amino acid a nitrogen-containing compound that is the monomer from which proteins are built

anticodon the three nucleotides in tRNA that bind to the complementary codon in mRNA according to base-pairing rules, resulting in the addition of a specific amino acid to the polypeptide chain

antiparallel parallel but orientated in the opposite direction

β-pleated sheet a type of secondary protein structure in which segments of the polypeptide chain bond side by side into a flattened assembly

base pair two complementary nitrogen bases linked by hydrogen bonding

biological functionality the function of a protein

catalyse to speed up a biochemical reaction by using an enzyme

chromosome a thread-like structure made of nucleic acids and proteins that encode genetic information

cisterna a flattened membrane disc that makes up the Golgi apparatus and endoplasmic reticulum

codon a group of three nucleotides in mRNA that specifies an amino acid

complementary base pairing the linking together of complementary nitrogen bases by hydrogen bonding; A pairs with T (or U in RNA) and C pairs with G

condensation polymerisation a reaction in which monomers are linked together into a polymer with the release of a small molecule, such as water, as a by-product

conformation the proper or functional shape of a protein

degenerate a property of the genetic code in which most amino acids are encoded by two or more codons

denature to permanently change the molecular structure of a protein or DNA

deoxyribonucleic acid (DNA) the information molecule that is the basis of an organism's genetic material

DNA polymerase the enzyme that catalyses the bonding of nucleotides to form new strands of DNA

endoplasmic reticulum an organelle made up of a network of membranous tubules involved in protein synthesis and folding for secretion

enzyme a specific protein catalyst that increases the rate of a biochemical reaction within the cell by lowering the amount of energy required for the reaction to proceed

exon a segment of DNA or RNA containing information that codes for a polypeptide or part of a polypeptide

functional proteomics the study of how proteins work together in different cells or tissues, or under different circumstances

gene a segment of DNA in a chromosome that codes for a polypeptide; comprises the promoter, exons and introns

gene expression the process by which the information in a gene is turned into a polypeptide

gene regulation the process by which gene expression is switched on or off

genetic code the complete set of mRNA codons and the corresponding amino acids they specify

genome the complete sequence of DNA in a single (haploid) set of an organism's chromosomes, including nuclear, mitochondrial and chloroplast DNA

Golgi apparatus a collection of membranes that package and store substances into vesicles in preparation for their release from the cell

histone a protein that binds and packages DNA in eukaryotic chromosomes

hydrogen bond a weak chemical bond between a hydrogen atom on one molecule and a more electronegative element, usually an oxygen or nitrogen atom, on another molecule

hydrophilic describes substances such as polar molecules and ionic compounds that dissolve readily in water

hydrophobic describes substances such as non-polar molecules that are insoluble in water

inducer a signalling molecule that switches on expression of a gene

intron a segment of DNA within a gene or pre-mRNA that does not code for a polypeptide and interrupts the sequence of a gene

messenger RNA (mRNA) RNA copied from DNA that conveys the instructions needed for polypeptide synthesis from the nucleus to the cytoplasm

non-coding region DNA that does not encode a protein sequence

non-template strand the DNA strand that has the same sequence of nucleotides as the mRNA (except it has T instead of U)

nucleic acid a large, linear polymer built from nucleotide monomers bonded together; includes DNA and RNA

nucleosome a histone with a length of DNA coiled around it

nucleotide the monomer, or building block, of DNA and RNA, consisting of sugar, phosphate and a nitrogen base

operator a segment of DNA to which a protein binds, usually to switch off gene expression

operon a group of genes that are expressed as a single unit

peptide bond a chemical bond that links two amino acids in a chain

phosphodiester bond a chemical bond that links two nucleotides in a growing chain

plasmid a small, circular DNA structure independent of the chromosome in prokaryotic cells

poly-A tail a chain of 100–200 adenine nucleotides added at the 3′ end of an mRNA strand

polypeptide a linear polymer built from amino acid monomers

polyribosome a chain of ribosomes formed by attaching to and translating from a single mRNA strand

pre-mRNA an unprocessed RNA strand that is transcribed directly from the DNA

primary structure the linear sequence of amino acids that makes up a polypeptide chain

product the outputs of a chemical reaction that are formed from the reactants or inputs

promoter region a segment of DNA to which RNA polymerase binds to begin transcription

protein a polymer made up of amino acid monomers; may consist of a single polymer chain or many polymers bonded together into a functional molecule

protein secretory pathway the pathway through which cells package proteins into vesicles for release into the extracellular environment

proteome the complete set of proteins produced by a cell, a tissue, or an organism

proteomics the study of proteomes

quaternary structure the structure formed when two or more polypeptides associate into a mature protein

random coil a secondary protein structure in which the polypeptide chain does not fold into a specified arrangement

regulatory gene a gene whose product switches on or switches off expression of one or more other genes

repressor protein a protein that binds DNA to prevent RNA polymerase attaching or transcribing; essentially shuts off gene expression

ribonucleic acid (RNA) a type of nucleic acid consisting of a single strand of nucleotides; has essential roles in protein synthesis

ribosomal RNA (rRNA) an RNA strand that serves as a structural component of a ribosome

ribosome a small structure consisting of RNA and proteins where amino acids are joined to form polypeptides

RNA polymerase the enzyme that catalyses the synthesis of RNA

rough endoplasmic reticulum endoplasmic reticulum with ribosomes attached

secondary structure the localised folding of a polypeptide chain when neighbouring amino acids bond to each other to form α-helices, β-pleated sheets or random loops

semi-conservative replication the replication of DNA in which the product contains one original and one newly made strand

structural gene a gene that codes for tRNA, rRNA or a polypeptide other than a regulatory molecule

substrate the substance that an enzyme acts on

subunit a distinct component of a biological particle; in proteins, it refers to each polypeptide that contributes to the quaternary structure

template a pattern that can be used for making many more copies

template strand a strand of DNA that is copied during DNA or RNA synthesis

tertiary structure the overall three-dimensional shape of a completely folded polypeptide

transcribe to copy DNA into mRNA

transcription the process by which DNA is copied into mRNA

transcription factor a protein that binds to DNA to control the rate of transcription from a gene

transfer RNA (tRNA) an RNA molecule that transports an amino acid to the ribosome for assembly into a polypeptide

translation the process of turning the nucleotide sequence of mRNA into the amino acid sequence of a polypeptide

transport vesicle a small membrane-bound sac containing protein that is transported from the Golgi apparatus to the plasma membrane for release into the extracellular environment

triplet a set of three nucleotide codes

2 Chapter review

Remembering

1 Why is nitrogen (N) considered to be an essential element for all living things?

2 List two ways that different tRNAs are the same, and two ways they are different.

3 On an A3 sheet of paper, outline the main steps in protein synthesis. Include transcription, translation, gene regulation, primary and secondary structures of α-helices, β-pleated sheets, or random coils, tertiary and quaternary formation of proteins and final examples of product.

4 Structural genes and regulatory genes have different functions.
- a Define the main functions of the different types of genes.
- b Explain how the genes operate to affect the processes that they are involved in.

5 What are the 5′ and 3′ caps, and what do they achieve for the mature mRNA?

6 How are DNA and RNA related?

7 How does the antiparallel structure of DNA determine the template strand?

8 Do all codons code for an amino acid? Explain your answer.

Understanding

9 Explain the relationship in the sequence between the triplets in the non-template strand of DNA, the mRNA codons and the tRNA anticodons.

10 All amino acids contain the same two functional groups. How do the 20 amino acids differ from one another?

11 How many different types of polypeptide can be constructed from just five amino acids?

12 Polymers result when bonds between monomers are formed with the removal of water. Suggest a way that the bonds between monomers could be broken. Justify your answer.

13 Explain how some proteins are located in the cytosol, whereas others are secreted by the cell.

14 Explain why the ability to control which genes are expressed is important:
- a during cell differentiation
- b in mature cells.

Applying

15 Egg white is rich in the globular protein albumin. When heated, this liquid becomes a white opaque solid. Use your knowledge of protein structure to explain this observation.

16 Antibodies are proteins of the immune system. Antibodies contain many disulfide bridges, which are strong bonds between two sulfur atoms within a protein. Suggest why this feature of antibodies might be beneficial during a fever.

17 Histones are predominantly made of positively charged amino acids.
- a Would these proteins be soluble or insoluble in water? Explain why.
- b Describe the properties of histone proteins that enables them to package DNA into chromosomes.

18 The *ara* operon of *E coli* contains three genes that import and digest the five-carbon sugar arabinose (Figure 2.34).

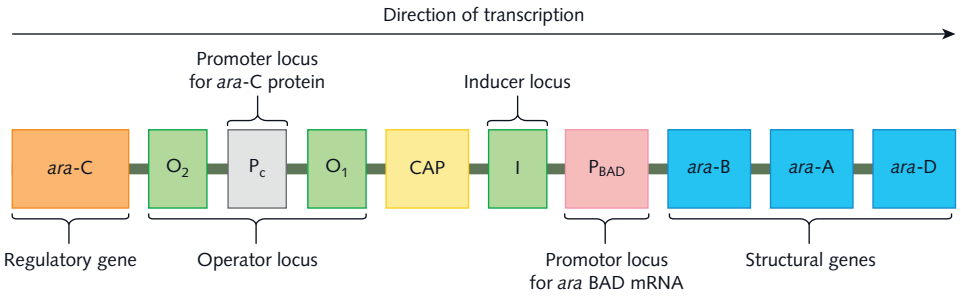


Figure 2.34 Structural elements of the *ara* operon

The *ara* operon is under the control of a protein called *ara*-C. Gene expression from the operon is normally switched off. If arabinose is present, gene expression from the operon is switched on.

- **a** Use your understanding of the *trp* operon (pp. 55–56) to describe the mechanisms regulating gene expression at the *ara* operon.
- **b** Use annotated diagrams to demonstrate the *ara* operon of *E coli* and this system of gene regulation in the absence and presence of arabinose.

19 Figure 2.35 shows diagrams of two mammalian cells: a spermatid (a sperm in the process of development) and a mature sperm cell.

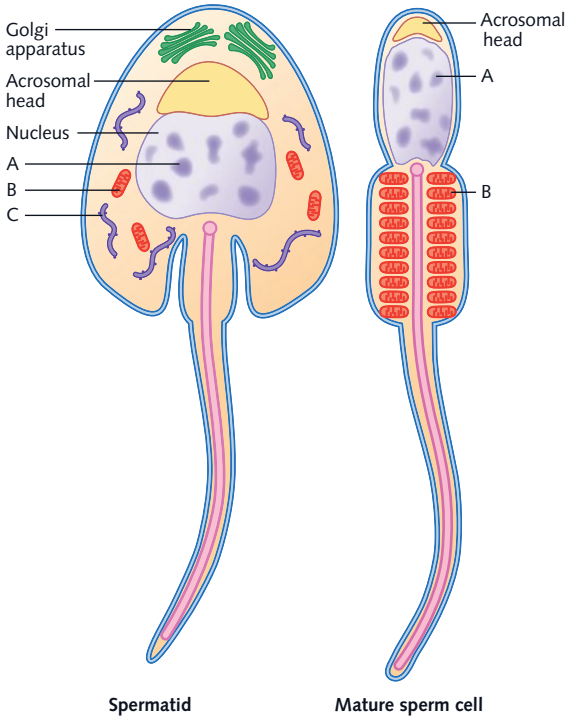


Figure 2.35 Immature and mature sperm cells

- **a** Name the organelles labelled A, B and C.
- **b** The Golgi apparatus and the ribosomes no longer exist in the mature sperm cell. Explain why.
- **c** **i** What is the function of the ribosomes in this process of development?
  - **ii** What is the function of the Golgi apparatus in this process of development?
  - **iii** What is the function of structure C in this process of development?

Analysing

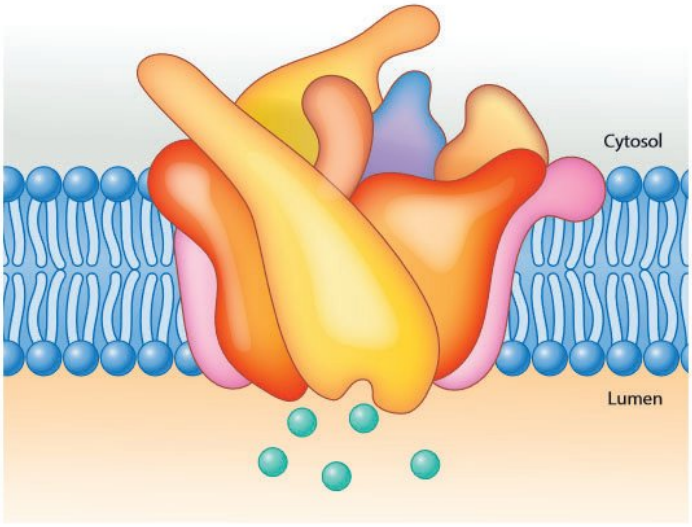


Figure 2.36 A calcium channel protein

20 Figure 2.36 shows a calcium channel protein embedded in the membrane of the endoplasmic reticulum. The protein transports calcium ions from the cytosol side of the membrane to inside the lumen of the endoplasmic reticulum.
- a What sort of technique might have been used to determine the structure of the protein?
- b What type of secondary structure dominates the protein?
- c What properties might the amino acids have that are on the face of the protein embedded in the membrane?
- d What properties might the amino acids have that line the inside of the channel?
- e Each polypeptide chain in the protein is coloured differently. How many polypeptides make up the mature protein, and what level of protein structure is this?

Evaluating

21 Rats have two forms of the muscle protein troponin T. One form comprises four exons, called W, X, Alpha and Z. The other also comprises four exons, called W, X, Beta and Z. However, rats have only one copy of the troponin T gene with five exons. What might explain these observations? Draw an annotated diagram to support your explanation.

22 Figures 2.37 refers to a type of protein called an enzyme. Evaluate the graphs in Figure 2.38 in terms of what is being depicted by Figure 2.37.

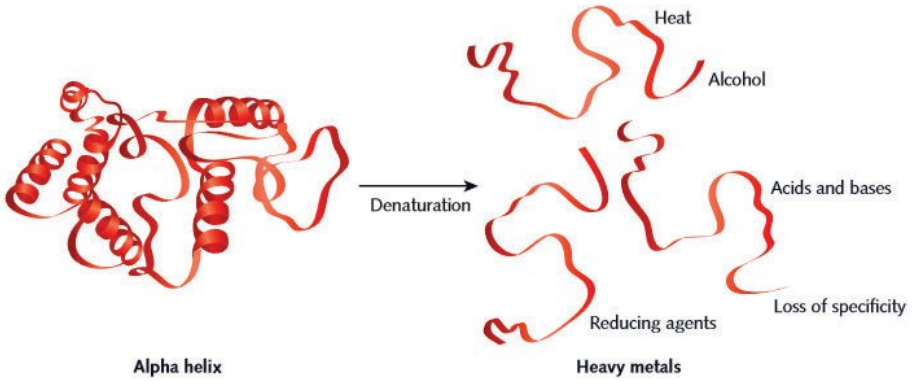


Figure 2.37

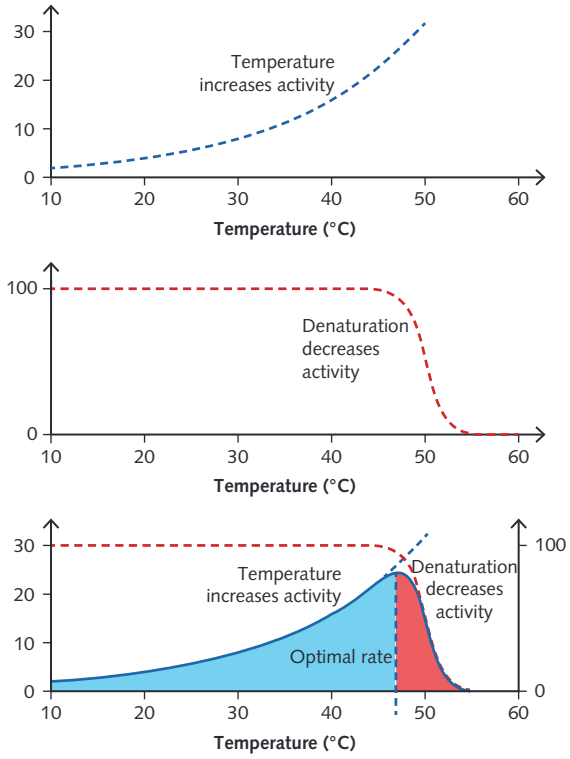


Figure 2.38

Creating

23 Scientists are concerned with identifying proteins involved in the progression of liver cancer. What sort of methodology could they use, and how might the results inform their research?

24 The nucleotide base sequence of a strand of DNA that codes for a specific amino acid is GGAATGCTCGACATC.

- **a** What is the base sequence of the complementary strand of mRNA?
- **b** How many amino acids are coded for by the strand of mRNA? List them.
- **c** What does the last codon code for and what does this mean?

25 Splicing joins two exon sequences. The following are steps in the production of a mature mRNA. Place them in the correct order and put them in a flow chart.

- » Addition of 5′ cap
- » Transport to cytoplasm
- » Initiation of transcription
- » Splicing
- » Addition of poly-A tail

3 DNA manipulation techniques and applications

By the end of this chapter you will have covered the following material.

Key knowledge

DNA manipulation techniques and applications

- the use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA and endonucleases to cut DNA, pp. 84–86
- the function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome, pp. 87–92
- amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling, pp. 92–104
- the use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin, pp. 105–15
- the use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease, pp. 115–118

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify, research and construct aims and questions for investigation, pp. 96–98; 109–111
- predict possible outcomes, pp. 96–98; 109–111

Plan and conduct investigations

- determine appropriate investigation methodology: case study; classification and identification; controlled experiment; correlational study; fieldwork; literature review; modelling; product, process or system development; simulation, pp. 96–98
- design and conduct investigations; select and use methods appropriate to the investigation, including consideration of sampling technique and size, equipment and procedures, taking into account potential sources of error and uncertainty; determine the type and amount of qualitative and/or quantitative data to be generated or collated, pp. 96–98; 109–111
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications, pp. 96–98; 109–111

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks, pp. 96–98; 109–111

- apply relevant occupational health and safety guidelines while undertaking practical investigations, pp. 96–98; 109–111
- demonstrate ethical conduct when undertaking and reporting investigations, pp. 96–98; 109–111

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources, pp. 96–98
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs, pp. 96–98; 109–111
- plot graphs involving two variables that show linear and non-linear relationships, pp. 96–98

Analyse and evaluate data and investigation methods

- evaluate investigation methods and possible sources of personal errors/mistakes or bias, and suggest improvements to increase accuracy and precision, and to reduce the likelihood of errors, pp. 96–98

Construct evidence-based arguments and draw conclusions

- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation, pp. 102–104; 117

Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement, pp. 96–98; 109–111
- discuss relevant biological information, ideas, concepts theories and models and the connections between them, pp. 109–111
- analyse and evaluate bioethical issues using relevant approaches to bioethics and ethical concepts, including the influence of social, economic, legal and political factors relevant to the selected issue, pp. 102–104; 117

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- Chapter 3 map (p. 78)

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- Chapter 3 flashcards (p. 80)

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- CRISPR: The hacking tool that modifies DNA (p. 88)
- Polymerase chain reaction (p. 94)

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- CRISPR: The hacking tool that modifies DNA (p. 88)
- Polymerase chain reaction (p. 94)

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- CRISPR: (p. 87)

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- Chapter 3: Summary of key concepts (p. 120)

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Chapter 3 map

3 DNA manipulation techniques and applications

The structure of DNA and the processes of transcription and translation have led to DNA manipulation techniques to make new combinations of genes within a genome.

31 Genetcay mod fied organsms p81

Genetcay mod fied orgaisms have had ther genomes mod fied by genetc engne erng Transgeic or knock-n orgaisms have had genes from other speies added to ther genomes and knock-out orgaisms have had genes removed o inativatd.

32 Enzymes for modfyng DNA p84

Endonucease cuts DNA at known stes to make stcky or bunt end s gase gues DNA back together and poymerase makes new copes of DNA

33 CRSPR-Cas9 p87

The CRSPR-Cas9 enzyme makes preise cuts n DNA so segments or snge bases can be replacd. RISPR s a technque wdey used n cancer treatmen, gene therapy and agrcutur.

34 Ampfyng DNA p92

DNA poymerase s a bactera enzyme that can make many copes to ampfy DNA The poymerase chan reation ncreases the amount of DNA for anayss n technques such as ge eectrophorss.

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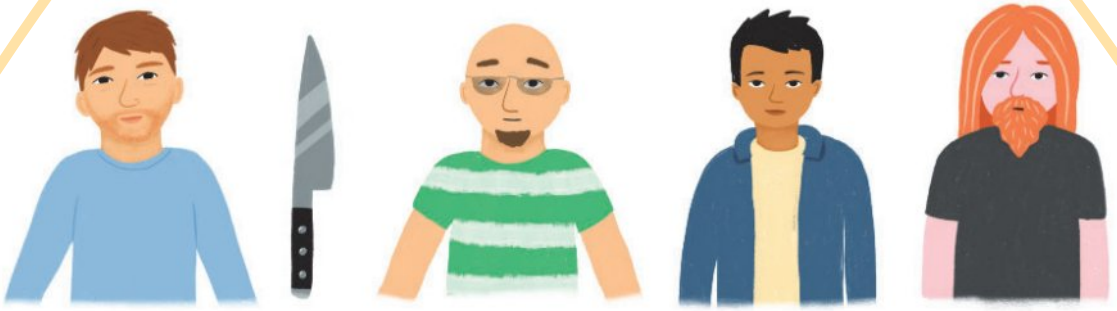
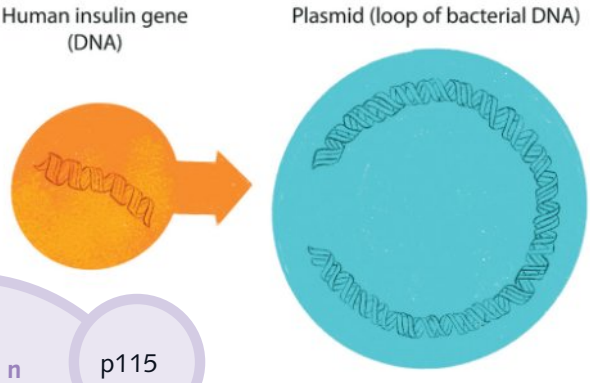
35 Ge eectrophoress p95

Ge eectrophorsis sorts DNA nto fragments accoring to sze Unque patterns emerge by runnng DNA through a charged g. Tis DNA pro fie s unque to ev ery ndvdua except dentca twns and trpets

36 DNA profi ng p98

DNA profi ng s used to dentfy peop e by ther DNA Ths s used n crmna nvestgatons dentfyng human reains anddisastervitims and estabshng parentage of chdren. DNA does not e





37 Recombnant pasmds and human nsun p105

Recombnant DNA technoogy s used to nsert the human nsun gene nto the *E coli* bacteria genome So we can use bactera rather than anmas to produceinsin for human use

38 Genetc engneerng n agrcuture p115

Genetc engneerng can ncreaseyeld anddisease resistanc in crops tohelp the word prepare for an ncresinggloal popuaton

Do humans have the right to alter the genomes of other organisms? If genetic modification of other organisms aids human survival, does it make it acceptable? These are some of the bioethical questions you need to consider.

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Chapter 3 Flashcards

Know your key terms

agarose gel
annealing
antibiotic selection
bacteriophage
bioethics
biotechnology
blunt end
Cas9 protein
CRISPR-Cas9
crRNA (CRISPR RNA)
DNA ligase
DNA profiling
DNA sequencing
frameshift mutation
gel electrophoresis
gene cloning
genetic engineering
genetically modified organism (GMO)
guide RNA
knock-in organism
knock-out organism
molecular size marker
polymerase chain reaction (PCR)
polymorphism
primer
recombinant DNA technology
recombinant plasmid
reporter gene
restriction digest reaction
restriction endonuclease
restriction enzyme
restriction fragment
restriction site
short tandem repeat (STR)
sticky end
transformation
transgenic organism
vector
wild type

Remember

WB
REMEMBER
PAGE 58

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 DNA is an information molecule that is the basis of an organism's genetic material.
2 DNA is made up of two strands of nucleotides linked together through complementary base pairing.
3 Genes are segments of DNA that code for proteins.
4 DNA is copied into two identical copies in the process of DNA replication.

Less than a century after the discovery of the structure of DNA by Watson and Crick in 1953, the 'gene revolution' has completely changed agriculture, medicine, global health and scientific research. Researchers now have an extensive toolkit of technologies to modify DNA. The current and potential applications of these technologies are vast and hold great promise. For example, rice has been nutritionally enhanced to reduce malnourishment in developing countries (Figure 3.1), insulin is now made in bulk so that type 1 diabetes is no longer a fatal disease, and scientists now have hundreds of animal models to study diseases and their potential treatments. In this chapter, you will explore basic and more recently developed techniques such as CRISPR-Cas9, which have been borrowed from Biology for use in a wide range of technological and medical applications, such as changing the genetic sequence of organisms.

Figure 3.1 Genetically modified rice (left) compared to normal rice (right) has been developed to aid the digestion of its nutrients for humans and animals, as well as reduce the amount of phosphate run-off on the surrounding land, reducing the negative effects on the environment.

3.1 Genetically modified organisms

The term **genetic engineering** means changing the genetic sequence of an organism through human use of modern **biotechnology** techniques. Such genetically engineered organisms are called **genetically modified organisms (GMOs)**.

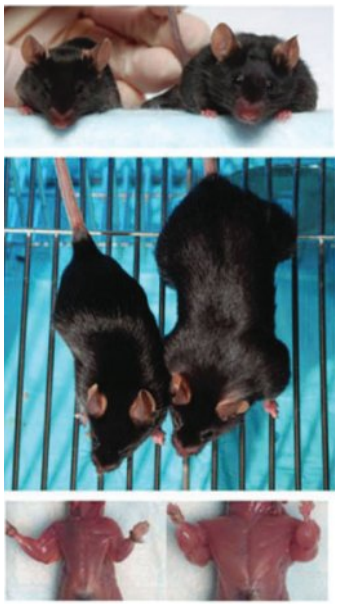


Figure 3.2 A comparison of body size between normal mice (left), and mice genetically engineered (right) so that the gene myostatin is deleted and the gene follistatin is overproduced. These genes control muscle growth.

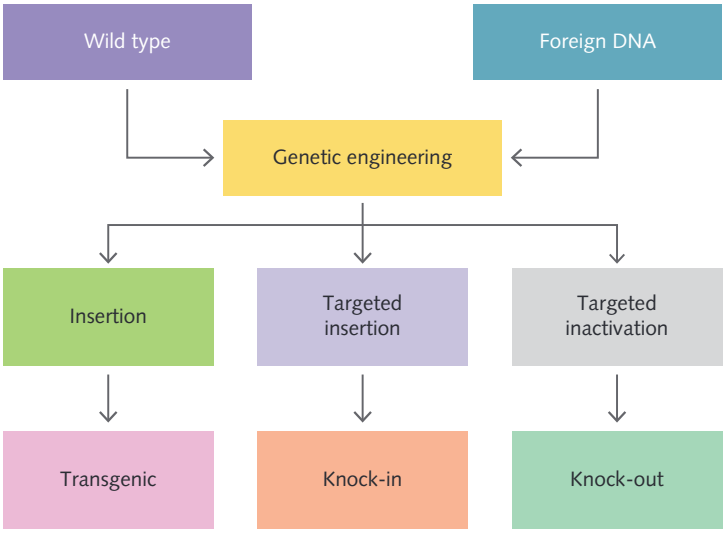
The term 'genetic engineering' applies to a range of techniques and processes for investigating and modifying DNA, genes and genomes of species. Scientists can use genetic engineering to switch genes on or off, remove genes and introduce genes from one species into another (Figure 3.2). Common genetic modifications include knock-out, knock-in and transgenic organisms.

Transgenic organisms

Inserting DNA from one organism into the genome of another unrelated organism produces a **transgenic organism**.

Genes inserted into transgenic organisms are inserted into a locus that is known to be available for transcription all the time, allowing strong, constant expression across different tissues and not disrupting other genes. Scientists may also insert genes randomly, although by chance this may sometimes affect tumour suppressor genes, proto-oncogenes (genes involved in cell growth that promote cancer when mutated) or other genes that are important for normal development or function. The genes may be inserted with a promoter that drives strong expression, or a promoter or extra domain that allows gene expression to be manipulated (switched on or off); for example, by treatment with a drug.

Figure 3.3 summarises transgenic, knock-out and knock-in organisms.



WB
3.1 GENETICALLY MODIFIED ORGANISMS PAGE 59

Figure 3.3 Genetically modified organisms include knock-out, knock-in and transgenic organisms.

Knock-out organisms

Knock-out organisms are produced by cutting out genes or gene segments to prevent their expression or the proper functioning of particular gene products.

This technique is commonly used in research laboratories to study the functions of the knocked-out gene in animal models, especially mice. Scientists can study biological processes or disease models in the knock-out animals and infer the functions of the gene from the differences between the knock-out animals and control (called **wild type**) animals. Studies of this type can provide information about the activities of homologous genes in humans that cannot usually be gathered from *in vitro* tissue culture techniques, but instead can only be studied accurately when the gene is acting in the context of the whole animal.

Knock-in organisms

Knock-in organisms are produced by inserting genes into a specific locus so that they are controlled by a particular promoter in the organism's genome.

Scientists use this technique to insert a **reporter gene**, such as that for green fluorescent protein, into the locus of a gene whose pattern of expression is unknown. The reporter gene will be expressed under the control of the promoter of the gene of interest and its characteristics are easily identified in the organism. Organisms that express green fluorescent protein show bright green fluorescence (Figure 3.4) when exposed to ultraviolet light. The green fluorescence can be measured in cells and tissues to give information about the location and timing of expression of the gene of interest. Inserting the reporter gene can sometimes inhibit the activity of the gene of interest, in which case the knock-in organism is also a knock-out organism for the gene.

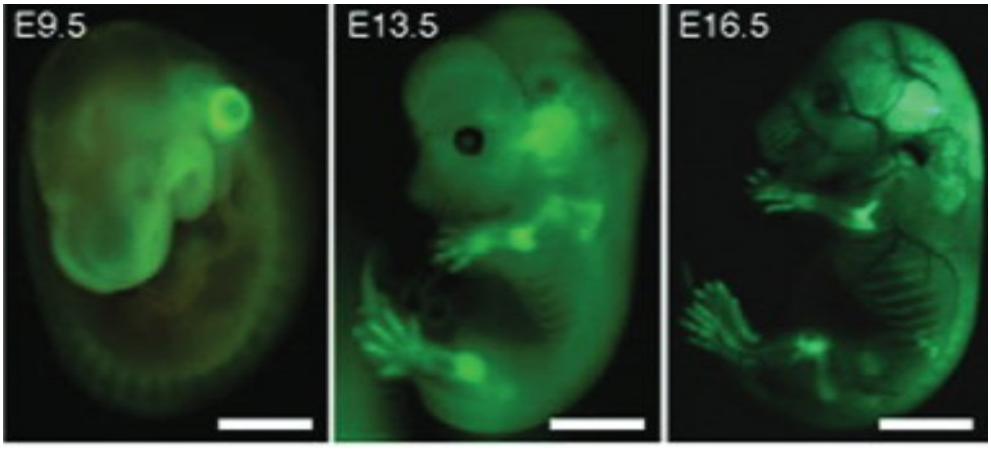


Figure 3.4 **Expression of Sox9, which is important for normal skeletal development, in mouse embryos at different days of embryonic development (for example, E9.5 = embryonic day 9.5), measured by knocking green fluorescent protein into the Sox9 locus. The expression of green fluorescent protein is therefore under the control of the Sox9 promoter.**

KEY CONCEPTS

- Geneticengineering refers toe use of living things to make new products or systems by switching genes on or off, rmoving gnes o introducing genes from one species into another.
- Oranisms tht are altered or prduced by genetic enineringtechniques are kowas genetically modified oranisms (MOs).
- Genetcally modi fied oranimsinclude knock-out organiss, knck-i organisms and transgenic organisms.
- Knock-ou organisms have ad a gene deleted or its function interrupted.
- Knok-in rganisms have had a gene or fragment of DN inserted int apaticular locus in the genome.
- Transenic organisms have had a gene or fragment of DN inserted into a on-speci fic ocs.

Concept questions 3.1

1 Lst three typesof enetic modi ficatin.
2 How has genome sequencing ade genetic modification osible?
3 What type fgenetically modi fied oransm is a knoc-in mousean is this the sameas a transgenic organism?
4 What dosswitching genes on or off mean?
5 Define knock-outoraism'. Givethree examples.
6 What is the difference between a specific ocus and a non-specific ocus whendiscussing t ansgenic organisms?
7 Define:
   a genetic engieering
   b genetic modification
   c trangenic oganism
   d gene ocus
   e promoter gene
   f reporter ene.

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8 Figure 3.4 demonstræs expression of the Sox9 ocusin embryonicskletal develpmnt of mice. Why have the researchers used a reporter gene that codes for green fluorescence prtein and how does tis elate to the promote gene inoperation at thi locus?

WB
3.2 ENZYMES FOR MODIFYING DNA PAGE 59

3.2 Enzymes for modifying DNA

Genetic engineers use different tools for specific purposes. Genetic engineering, also called biotechnology, has its own set of specialised tools, which are mostly derived from other organisms. Some of the fundamental tools in biotechnology are enzymes for synthesising, cutting and pasting DNA.

Polymerase to synthesise DNA

All life forms must be able to produce identical copies of DNA. The class of enzymes that is responsible for this is the DNA polymerases, which catalyse the formation of new DNA molecules from free nucleotides and a template DNA strand. In biotechnology, polymerases are used to:

- make copies of DNA
- read the sequence of DNA fragments
- detect single nucleotide differences
- amplify whole genomes
- diagnose medical conditions
- artificially synthesise new DNA fragments without a template strand.

CONNECT
The polymerase chain reaction is discussed in detail on page 93.

The **polymerase chain reaction (PCR)** is a common biotechnology technique that has been in use since 1985. The key enzyme used in PCR is polymerase. PCR produces billions of copies of a section of DNA, which can subsequently be manipulated by other enzymes such as endonucleases for cutting and ligases for joining.

Endonucleases to cut DNA

One of the essential requirements in genetic engineering is the ability to cut segments of DNA at known sequences. The cutting tools used are enzymes known as **restriction endonucleases** ('endo' within, 'nuclease' an enzyme that cleaves or cuts nucleic acids) or **restriction enzymes**. These are like molecular scissors, cutting DNA molecules into smaller pieces, called **restriction fragments**, in a controlled way. DNA cut with restriction enzymes is often said to be 'digested' by the enzymes. Restriction enzymes only cut specific sequences of DNA, known as **restriction sites** or recognition sequences. Different restriction endonucleases have different restriction sites, although some endonucleases do share restriction sites with other endonucleases. Most recognition sequences are palindromes of their complementary sequence; that is, the sequence of the non-template strand in the 5′–3′ direction is the same as the sequence from 5′ to 3′ on the complementary strand.

Endonucleases occur naturally in bacteria, where they cleave 'foreign' DNA that enters from invading viruses, thus destroying any potential threat. They are an important mechanism of immunity for bacteria. Endonucleases are named according to the bacterial strain from which they are derived. The first endonuclease isolated from *Escherichia coli* strain RY13 was thus named EcoRI. Table 3.1 identifies a number of common endonucleases and their bacterial sources.

Table 3.1 Common endonucleases and their restriction sites

Enzyme	Bacterial source	Restriction site	After cutting
EcoRI	*Escherichia coli*	5′GAATTC3′ 3′CTTAAG5′	5′G AATTC3′ 3′CTTAA G5′
HindIII	*Haemophilus influenzae*	5′AAGCTT3′ 3′TTCGAA5′	5′A AGCTT3′ 3′TTCGA A5′
AluI	*Arthrobacter luteus*	5′AGCT3′ 3′TCGA5′	5′AG CT3′ 3′TC GA5′
BamHI	*Bacillus amyloliquefaciens H*	5′GGATCC3′ 3′CCTAGG5′	5′G GATCC3′ 3′CCTAG G5′

To date, about 3000 different endonucleases have been identified. Although each endonuclease recognises a specific sequence of 4–8 nucleotide base pairs (bp) of the double-stranded DNA, multiple enzymes isolated from different organisms can recognise the same sequence. Endonucleases bind to their restriction site and cut the double-stranded DNA at that point. The cuts may form either overhanging steps, called **sticky ends**, which leave some nucleotides exposed (Figure 3.5a), or **blunt ends** (Figure 3.5b), in which the cut has occurred at the same position in each strand of the DNA and there are no overlapping strands. Spontaneous hydrogen bonding between overhanging nucleotides helps restriction fragments with sticky ends bind to other DNA fragments with complementary sticky ends.

In molecular biology, endonucleases are used in **restriction digest reactions**, in which the substrate may be a plasmid, genomic DNA or a PCR product.

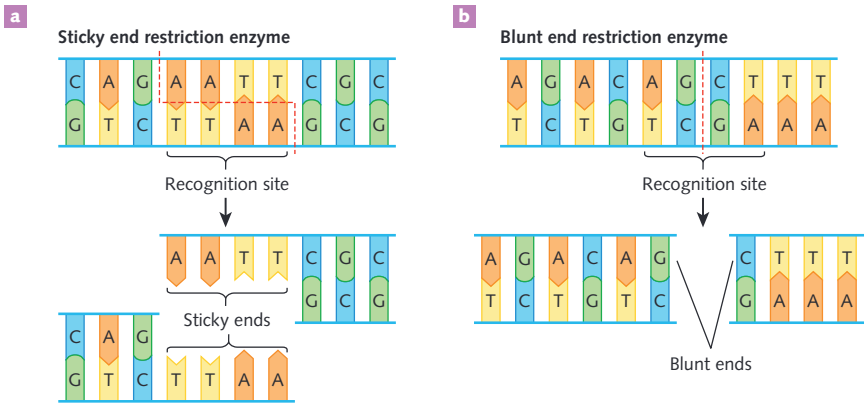

Figure 3.5 a Sticky ends produced by cutting DNA with the endonuclease EcoRI. **b** Blunt ends produced by cutting DNA with the endonuclease AluI.

Ligase to join DNA

Sometimes, molecular biologists want to combine two samples of DNA; for example, they might want to insert a piece of DNA into a plasmid (a small circular piece of bacterial DNA). **DNA ligase** is an enzyme used to join different pieces of DNA together. DNA ligase acts by forming a phosphodiester bond between the two fragments of DNA. It joins the 3′ end of one nucleotide with the 5′ phosphate end of another nucleotide. DNA ligase requires magnesium ions and ATP for its activity, so DNA ligation is an energy-consuming reaction.

The process of joining two strands of DNA together using DNA ligase is more successful if the two strands can be brought together. If the restriction enzymes used to cut the DNA generate sticky ends, two DNA fragments that have been cut with the same enzyme will have identical sticky ends and thus the complementary bases will be exposed. This means that if the ends of the two strands come into contact with each other by chance, their nucleotides will form hydrogen bonds at the sticky ends and remain in place, leaving just a nick in the DNA backbone to be ligated. DNA ligase can then be used to recombine these two fragments by creating a covalent phosphodiester bond between them, even if they are from two unrelated organisms. For example, EcoRI can be used to cut both human DNA and bacterial plasmid DNA, leaving sticky ends that are complementary and able to bond to each other (Figure 3.6).

CONNECT

The application of these techniques to make insulin is discussed on page 105.

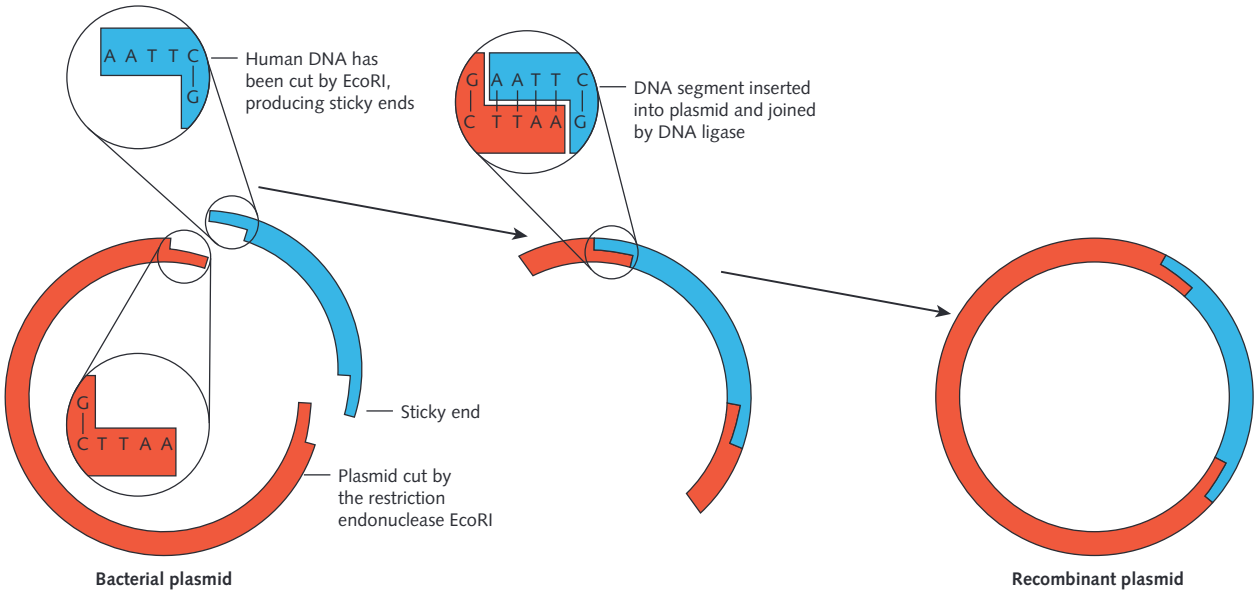

Figure 3.6 DNA ligases join DNA inserted from a foreign source that has complementary sticky ends.

Fragments with blunt ends can also be joined by DNA ligase, but this process is much less efficient. Sticky end ligation also ensures the joined DNA fragments are the right orientation when joined. The technology that recombines DNA from different sources to modify the DNA sequence is called **recombinant DNA technology**.

KEY CONCEPTS

- » DNA polymeraes catayse the formation of new DNA mlecules from fe nucleotdes and a template DNA strand.
- » The technology that recombies DNA from different sources to odify the DNAseence is called recominant DNA echnology.
- » The cutin tools used are enzymes known as restrction edonuleases,or restiction enzymes.
- » Endoncleases can gnerat blunt r sticky ends.
- » DN liase is an enzym used to jin different pieces of DNA together.
- » Coplementry sticky ends helpDNA strands bind to each othe via hydroge bonding.

Concept questions 3.2

1 State thefuntion of:
   - **a** DNA olymerase
   - **b** restrction ndouclease.
2 What is the difference between ticky and blunt ends created by etriction enzymes?
3 What proces completes he formtion of restriction fragments?
4 Wich rstricti enzmes litd n Table 3.1 ‘cut’ fragments ith stick edsand which ‘cut’ fragments wthblunt ends?
5 DNligases act y formig phosphodiester bonds between fragmntsof DNA. xlain how the following terms are conneced ligase, phoshodiester bond, 5′ phosphate end, 3′ hydrxyl en,nuleotide, DNA fragment and hydrgen bonds.

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6 Resrictio endonucleases were firs solated from bac ria.What is heir speci fic usein bacteria? Why are they pplicabefor use indifeent species of living organisms?

3.3 CRISPR-Cas9

WB
3.3
CRISPR-CAS9
PAGE 60

New biotechnological tools are continually being developed as our understanding of biological mechanisms grows. An example is the recent development of the CRISPR (clustered regularly interspaced short palindromic repeats) (pronounced 'crisper') genome editing system, which is based on a bacterial defence mechanism.

n.
Wken
CRISPR

Figure 3.7 Professors Emmanuelle Charpentier (left) and Jennifer Doudna (right) demonstrated how the bacterial immune defence mechanism CRISPR-Cas9 could be applied to precisely edit the genome.

In 2012, scientists Professor Jennifer Doudna and Professor Emmanuelle Charpentier (Figure 3.7) demonstrated for the first time that a bacterial antiviral immune defence mechanism could be harnessed to apply breaks in specific sites of double-stranded DNA to edit genomes. The resulting **CRISPR-Cas9** genome editing system has revolutionised the capacity to modify DNA. Charpentier and Doudna were awarded the Nobel Prize in Chemistry in 2020 for their work.

Function of CRISPR-Cas9 in bacteria

On infection by a virus (bacteriophage), bacteria collect small fragments of the viral DNA and integrate (insert) them into certain regions of their bacterial genome. These regions are called CRISPR loci. Throughout the CRISPR loci, viral sequences derived from previous exposure to viruses are interspersed with short repeats of host bacterial DNA. Every time the bacterial strain is exposed to a new viral infection, a new piece of viral DNA is integrated at the 5′ end of the CRISPR locus within the bacterium's genome. Therefore, if you read the CRISPR locus from the 5′ end to the 3′ end, you will get a chronological record of previous viral infections that the bacteria has encountered. Transcription of the CRISPR locus produces **crRNAs (CRISPR RNAs)** that are complementary to the integrated fragments of viral DNA. crRNAs form a complex with CRISPR-associated 9 proteins (**Cas9 proteins**), which can cleave DNA. The crRNA sequences in the complex act as molecular guides that direct the complex to bind to viral DNA within the bacterium, where the Cas9 proteins embedded in the complex can then cleave and destroy the complementary viral DNA. Much like receiving an immunisation, the CRISPR system provides the bacterial cell with a 'memory' of previous viral infections that it uses to defend itself against reinfection.

Biotechnology applications of CRISPR-Cas9

In biotechnology applications of CRISPR-Cas9, the bacteria-derived Cas9 enzyme (the molecular scissors) is directed by a short piece of RNA called a **guide RNA** to a complementary target site in genomic DNA, rather than the crRNAs generated from transcription of the CRISPR locus that occurs in the bacterial CRISPR-Cas9 system. Here, it creates a double-strand break (which would naturally target **bacteriophage** DNA for destruction inside infected bacterial cells). The eukaryotic host cell's machinery tries to repair the break. The repair mechanism is error prone and this often results in nucleotides being inserted or deleted, which causes a **frameshift mutation** that interferes with translation of the targeted gene and results in a knock-out organism. If a donor DNA fragment has been added to the reaction, the DNA repair machinery may incorporate it at the target site, resulting in a knock-in organism (Figure 3.8).

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CRISPR: The hacking tool that modifies DNA

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CRISPR: The hacking tool that modifies DNA

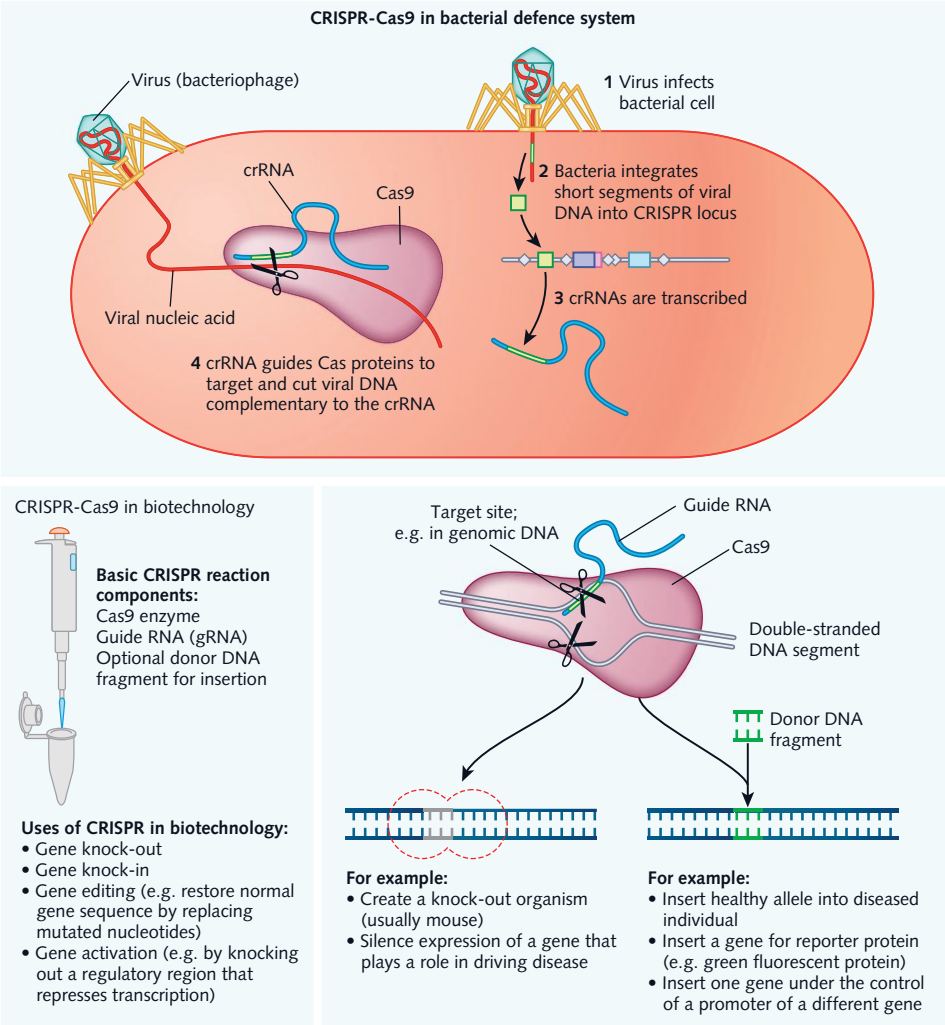

Figure 3.8 The CRISPR-Cas9 system in bacteria and its application to genetically modify DNA. A short molecule of RNA (guide RNA) binds to the target gene and guides the Cas9 enzyme to the gene. The Cas9 enzyme breaks the DNA and the bacterial cell tries to repair the break. This can result in a frameshift mutation – deletion or insertion of a gene – if a donor DNA fragment is incorporated.

The CRISPR-Cas9 system is a revolutionary biotechnological tool because:

» its use of specific guide RNA sequences (around 20 bases long) can direct the complex to exactly the desired location in the genome

» it can cut double-stranded DNA at any desired site.

This technique has greatly simplified the processes involved in genetically modifying organisms.

Applications of CRISPR technology

The CRISPR-Cas9 system has revolutionised the capacity for scientists to modify DNA of previously intractable organisms. Imagine being able to apply precise gene editing to awaken the immune cells of a patient with an aggressive form of cancer that switches the immune system off. This approach would transform cancer treatment and is an intense subject of scientific research using mouse models of cancer.

The CRISPR-Cas9 system can also be applied to create a knock-out mouse to decipher the function of a gene. Using CRISPR-Cas9, a new mouse strain takes around three months to make, instead of one or two years.

Current and potential applications of the CRISPR-Cas9 genome editing system are extensive. Some examples are described here.

Editing faulty alleles in genetic diseases

Sickle cell anaemia is a blood disease caused by a genetic mutation that produces an abnormal form of haemoglobin (the molecule that carries oxygen around the body, Figure 2.28, p. 61). The red blood cells are distorted. They are shaped like a sickle, rather than the smooth disc of healthy red blood cells. The rigid sickle cells clump together and obstruct blood flow in small blood vessels, causing organ damage and severe pain. Sickle cell anaemia can cause stroke, organ failure and sometimes death.

Gene therapy is a promising approach for treating the disease. Researchers have applied CRISPR-Cas9 using two different approaches to try to cure the underlying genetic cause. Both approaches are based on the same overall idea. First, blood from the patient is taken into the laboratory where the haemopoietic stem cells (the precursor cells of blood cells) are edited by using the CRISPR-Cas9 system. After careful tests to ensure the genetic modification is correct, the edited cells can then be transfused back into the patient's bloodstream.

The first approach was developed at Stanford University in the United States. CRISPR-Cas9 was used to induce a double-stranded break in the β-globulin gene of haemoglobin. A donor fragment of DNA containing the normal healthy version of the gene was added into the reaction and the DNA repair machinery incorporated it into the target site. The edited cells then transcribed the normal version of haemoglobin and had healthy disc-shaped red blood cells. This method of applying CRISPR-Cas9 to cure sickle cell anaemia is still in the preclinical stages of research.

The second approach uses CRISPR-Cas9 to introduce a mutation in a gene encoding a transcription factor called BCL11A. BCL11A represses haemoglobin F, a form of haemoglobin expressed in foetuses but repressed in adults. By silencing BCL11A, the 'molecular handbrake' is released and haemoglobin F is transcribed. Haemoglobin F interferes with the polymerisation of the sickle haemoglobin molecule, thus preventing the formation of sickle cells (Figure 3.9). This approach was inspired by the observation that carriers of the sickle cell mutation who also had a mutation in the BCL11A gene were resistant to sickle cell disease.

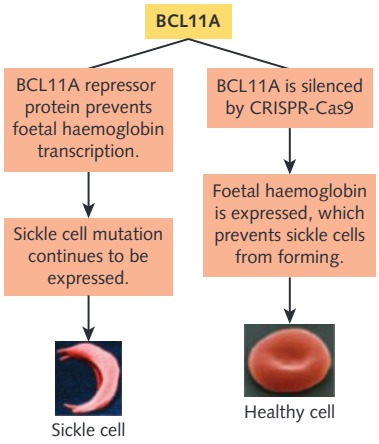

Figure 3.9 In adults, foetal haemoglobin is not expressed because it is repressed by the repressor protein BCL11A. Scientists used CRISPR-Cas9 to target BCL11A, silencing its expression and allowing foetal haemoglobin to be produced in adult cells.

In 2019, two companies, CRISPR Therapeutics and Vertex Pharmaceuticals, performed clinical trials together on this CRISPR-Cas9 therapy (called CTX001). The initial four-month trial assessed safety and efficacy in one patient with sickle cell anaemia. The preliminary results were encouraging. The patient did not experience any vaso-occlusive crises (when sickle cells get stuck in blood vessels) and expressed 46% foetal haemoglobin (25–30% foetal haemoglobin is considered to cure a patient with sickle cell disease). In the next stage of the clinical trial, more patients will be enrolled in the study and will be followed for a longer time after the treatment (two years) to assess safety and efficacy.

CRISPR in agriculture

CRISPR-Cas9 enables scientists to quickly and precisely insert or delete the desired traits to improve yield, tolerate environmental stress and resist disease. Since 2013, CRISPR-Cas9 has been applied to crops such as rice, wheat, corn, tomatoes and mushrooms. In the US, plant biologist Yinong Yang from Pennsylvania State University applied CRISPR-Cas9 to the white button mushroom genome, knocking out one of the genes that encodes polyphenol oxidase (PPO). PPO is an enzyme that causes browning. By deleting one of the genes that encodes PPO, the activity of the enzyme was reduced by 30%. Therefore, the CRISPR-edited white button mushrooms have a longer shelf life and are resistant to browning commonly caused by mechanical handling (Figure 3.10).

Figure 3.10 White *Agarcus bsporus* mushrooms that have been modified by CRISPR to reduce browning

In 2016, a letter addressed to Yinong Yang from the US Department of Agriculture (USDA) made headlines about genetically modified organisms. In the letter, the USDA confirmed that they did not require the genetically edited mushrooms to pass through the agency's regulatory process because the CRISPR-mushrooms did not contain any foreign DNA integrated into the mushroom genome. Therefore, the USDA did not consider it necessary to regulate the mushrooms, and gave permission for the mushrooms to be grown and sold in the US. This opened public debate and dialogue about newer technologies that make GMOs and prompted the US government to review the regulatory process to consider newer technologies such as CRISPR-Cas9.

Using CRISPR to make new mouse strains

The mouse genome is similar to the human genome and many symptoms of human disease can be replicated in mice. This makes the mouse a useful animal model in the laboratory. Scientists commonly knock out genes in mice to study the function of the deleted gene. Traditional methods of making a knock-out mouse were laborious and time consuming. Despite this, at the time, the technique of genetically modifying embryonic stem cells and injecting them into mouse embryos was groundbreaking. This technology led to the 2007 Nobel Prize in Physiology or Medicine being awarded to Mario Capecchi, Martin Evans and Oliver Smithies. In 2013, the discovery that the CRISPR-Cas9 system could be harnessed to edit the mammalian genome meant that efficient, targeted modifications of the mouse genome were now possible. What would take one or more years with traditional approaches now would take only a few months.

CONNECT
The steps involved in CRISPR technology are explained on page 88.

Keeping up with CRISPR

The sudden arrival of CRISPR technology, and its vast potential applications, has thrown up major ethical considerations about its use. Organisms, including humans, with heritable genome alterations can be produced relatively easily, but specific legal frameworks and a clear pathway towards translation of the technology have yet to be established.

Controversial use of CRISPR on human embryos

In November 2018, Chinese scientist He Jiankui announced to the international scientific community that he and his colleagues had applied CRISPR-Cas9 to edit the genome of twin baby girls born that month. The scientists used CRISPR-Cas9 to target the gene *CCR5* to inhibit its expression. Edited embryos were produced by *in vitro* fertilisation (IVF) and then implanted into a woman, who gave birth to the baby girls. CCR5 is a protein expressed on the surface of immune cells. The human immunodeficiency virus (HIV) uses CCR5 as a receptor to enter T cells (a type of white blood cell), where it replicates and then goes on to infect other T cells. Therefore, CCR5 can be considered a 'door handle' for HIV entry into T cells. He Jiankui targeted CCR5 because the father of the girls had HIV. Silencing CCR5 meant that HIV had no 'door handle' to allow the entry into the babies' T cells and they could not become infected with HIV. Effectively, the genome modification conferred resistance to HIV.

The announcement of the world's first 'CRISPR babies' made news headlines around the world. It sparked intense controversy and in many cases horror. Immediately following the announcement, a World Health Organization (WHO) expert Advisory Committee on Human Genome Editing examined the ethical and technical consequences of editing the human genome, particularly germline cells (eggs and sperm and their precursors, responsible for passing on genetic material to the next generation). The committee recommended that all countries inhibit any application of human genome modifications until they could vigorously consider the ethical and biological implications of such work.

In late 2019, He Jiankui was sentenced to three years in prison and a fine of 3 million yuan for practising medicine without a license, fabricating ethical review documents and violating Chinese law regarding assistive reproductive technology.

Policies and laws lag behind the pace of CRISPR technology

A US National Academies of Sciences, Engineering, and Medicine committee, which consisted of bioethicists, scientists, lawyers and patient advocates, suggested that strict regulations were needed to monitor CRISPR-Cas9 technology. However, both the WHO committee and the US committee made their recommendations after the announcement of the CRISPR babies. This highlights how policies and regulations lag behind the pace of CRISPR technology.

In agriculture, CRISPR-Cas9 modified plants, such as Yinong Yang's white button mushrooms, were not subject to the same regulatory process as plants genetically modified by traditional methods. Additionally, they did not have to be labelled 'transgenic' because foreign DNA had not been introduced into the mushroom genome. Should CRISPR-Cas9 modified plants be subject to regulation like other DNA manipulation approaches? Or is it reasonable that because foreign DNA is not inserted, they should be allowed to be grown and sold without approval? These are questions being considered by different regulatory bodies around the world. For instance, in 2018, the Court of Justice of the European Union ruled that CRISPR-edited crops should be subject to the same regulations as traditionally genetically modified organisms.

Bioethical considerations for CRISPR-Cas9 technology

CRISPR-Cas9 technology holds enormous promise and potential for developing new treatments for disease, improving crops in agriculture and developing new mouse strains and disease models for scientific research, but it also raises serious ethical questions. In the case of the CRISPR babies, the genetic change to the germline (sperm and egg cells) means that the edited genetic material will be passed down from generation to generation. In this situation, the genetic change was intended to benefit human health and prevent disease. Therefore, it could be considered beneficial to pass on a genetic change that will confer resistance to a disease such as HIV. By this logic, it might be possible in the future to cure human disease, if it is deemed safe. However, we do not know if it is possible to predict the full implications and perhaps unanticipated consequences of such a genetic change over a whole human lifespan, or several generations.

We need to consider whether editing the human genome is altering the human species. Consider the potential application of CRISPR-Cas9 gene editing to enhance a desired trait for a non-therapeutic purpose. We need to consider that genome editing might only be available to the wealthy and would

therefore increase the disparity in access to health care between the rich and poor. Could these issues be managed through policy and laws?

There is a long list of ethical considerations surrounding CRISPR-Cas9 technology. **Bioethics** is the study of such ethical issues that arise from advances in biology, such as CRISPR-Cas9. Bioethics committees examine bioethical issues that emerge because of advancements made in biological research. These committees are made up of experts and people from various disciplines, including ethics, philosophy, genetics, politics, psychiatry, law, medicine, scientific research and teaching. Together, they consider the ethical questions that emerge as a result of biological and medical research, aiming to inform policy and public debate.

KEY CONCEPTS

- The RISPR system i acterial immune defence mecanim agant via infection.
- CRISPR-Ca9 is applied to edt enomes to precisely create knock-out orknock-in enetic modi ficatins.
- Aliationsof CRISPR-Cas9 technology nclude editin falyallelesin diease, improving agrculture nd making newmouse strains for sieti fic research.
- The use o CRISPR-Cas9 technolgy raises serious biotica questions.

Concept questions 3.3

1. CRISP is an aconym. What oes each letter stand for?
2. Define plinromic rpeat'and provie an example.
3. What is te CISPR locus?
4. crRNAs ae complementary to the integrated fragments f vral DNAina bactriu. Where did this vral DNA come from?
5. crRNAs form a cmple with CISPR-associated 9 (Cas9)prteins, whch re able tcleave (cut) DNA.' nterpret his statemen explaining each of the terms and the verll meaning.

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6. CRISPR-Cas9techologyraises many issues that need to be resolved. Choose ne ofthe biotechnology aplcation of thisformof 'NA cissors' and discuss the reasons why te application is useful for humans but s etially oplex.

WB
3.4 AMPLIFYING DNA PAGE 63

3.4 Amplifying DNA

Each eukaryotic somatic cell has only two copies of a gene of interest and prokaryotic cells have only one copy. This small amount of DNA poses a problem for scientists wishing to work with it. Similarly, only a small sample of DNA may be available for analysis; for example, at a crime scene or in preserved bones. The first step in DNA analysis or in genetic engineering is to make enough copies of the DNA to be able to work effectively. Biotechnologists have an important tool to do this: the polymerase chain reaction (PCR). PCR is used to amplify, or make many copies of, a specific sequence of DNA. It uses DNA polymerase that is usually derived from *Thermus aquaticus*, a thermophilic species of bacteria that thrives in hot conditions such as the geothermal springs of the Yellowstone National Park, US. *T. aquaticus* has evolved to withstand these hot conditions, and so its DNA polymerase (called Taq polymerase) remains stable during the high temperature cycles of the PCR process. The US biochemist who invented PCR, Kary Mullis (Figure 3.11), was awarded the 1993 Nobel Prize in Chemistry for his work.

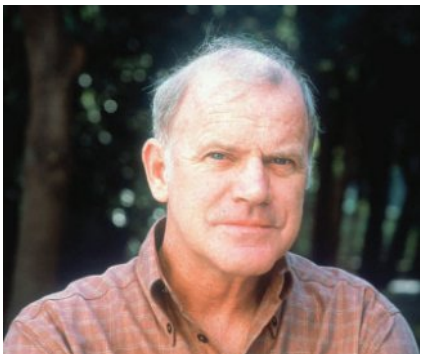


Figure 3.11 Kary Mullis was awarded the 1993 Nobel Prize in Chemistry for his work inventing the polymerase chain reaction.

Polymerase chain reaction

A number of components are required for PCR:

» the DNA that is to be copied (the template)
» DNA polymerase (the enzyme)
» a buffer solution that contains salts and other chemicals that help the polymerase to function (called cofactors)
» a supply of the four nucleotides (A, T, C, G) from which to build the new DNA molecules
» two **primers** – short sequences (about 20 nucleotides) of single-stranded DNA, complementary to the nucleotide sequences at either end of the DNA section that is to be copied.

The primers are necessary as a starting point from which the DNA polymerase can add new DNA nucleotides. DNA polymerase can only extend a DNA strand from an existing nucleotide; it cannot create a new complementary strand without primers to begin extending from. The nucleotide composition of the primer determines its annealing (joining) temperature because it determines the number of hydrogen bonds that form between the primer and template strand. Because G–C complementary nucleotides pair with three hydrogen bonds, they require more kinetic energy (a higher temperature) to separate than do A–T nucleotides, which pair with two hydrogen bonds.

PCR has three steps (Figure 3.12).

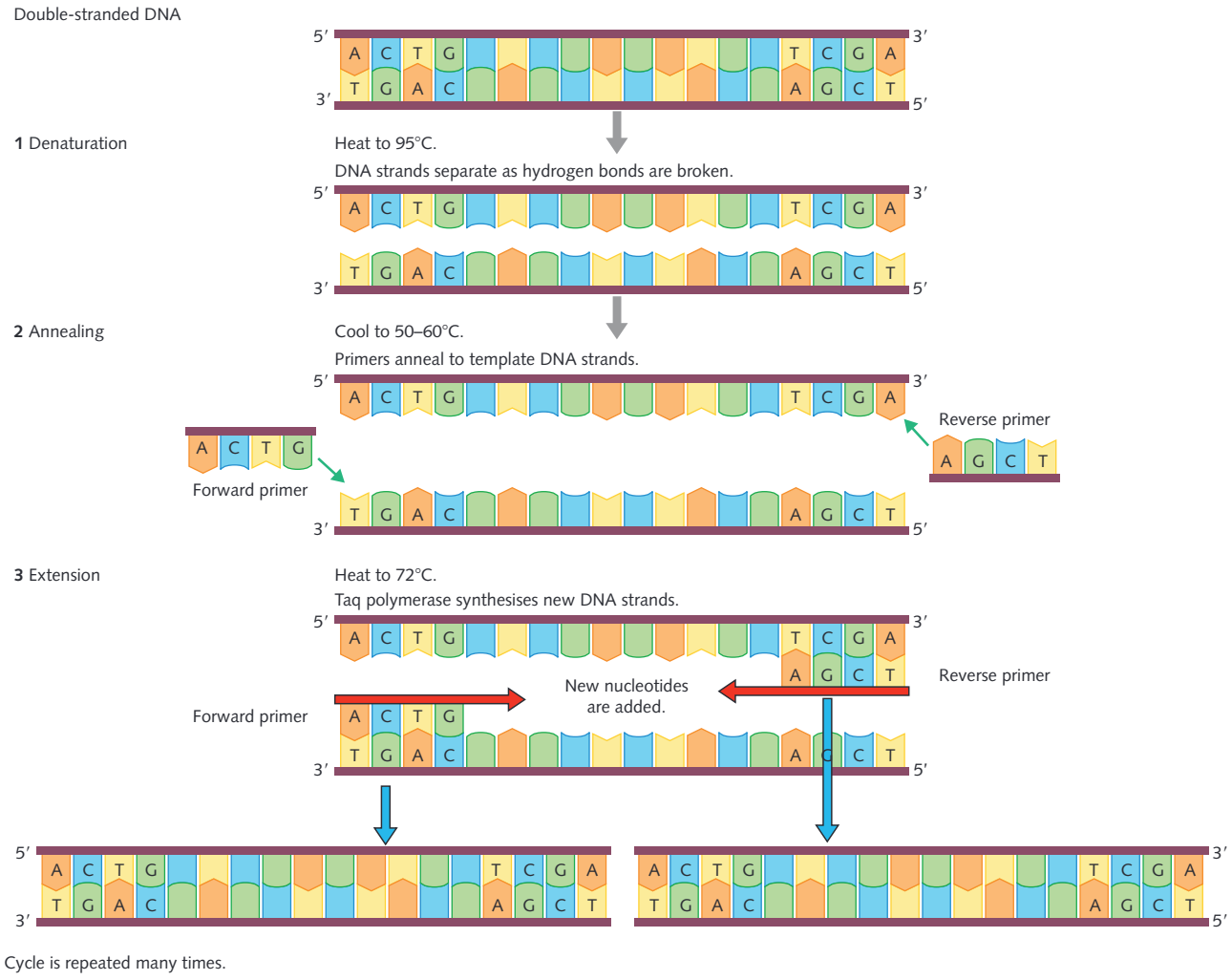


Figure 3.12 Amplifying DNA using PCR

epto uK
Polymerase Chain Reaction

wutaue : nHKCyeec
Polymerase Chain Reaction

1 Denaturation: the double-stranded DNA is heated to 95°C. This breaks the hydrogen bonds between the bases and causes the two strands to denature.
2 **Annealing**: the temperature is reduced to 50–60°C, allowing the primers to join to complementary sequences on opposite ends of each strand: either genomic DNA in the first cycle or PCR products generated during the previous cycle. The reduced temperature is necessary to allow base pairing and the formation of hydrogen bonds.
3 Extension: the temperature is raised to 72°C, the optimum temperature for the particular DNA polymerase used in PCR. Starting from the primers, new DNA strands are synthesised in the 5′ to 3′ direction, using DNA polymerase and the available nucleotides. At the end of this phase, there are two copies of the double-stranded DNA. Each copy consists of one 'parent' strand and one new strand.

EXAM TIP
Read the question carefully and answer what is being asked. Only discuss the specific aspect of PCR that the question is asking about. The number of marks will indicate the depth required in the answer.

Figure 3.13 A thermal cycler, in which the PCR is carried out as a programmable automated process. The thermal cycler raises and lowers the temperature of the samples, which allows denaturation, annealing and extension to take place in a controlled way.

This cycle is repeated until enough DNA has been produced. Each cycle doubles the number of DNA strands; therefore, in just 20 cycles more than one million copies of target DNA will be produced (2^{20} = 1 048 576 copies of DNA). There are usually 30–35 cycles in a PCR program (to produce 2^{35} = 3.44 × 10^{10} copies of DNA), which take place in instruments called thermal cyclers (Figure 3.13).

KEY CONCEPTS

» PC is a process tht ampli fies a specific DNA sequence for anysis.
» The sequence of the priers determines the DNA sequence to bempli fied.
» The stpsinvolved in PCR re denauration, annealing and extnsion. These steps are repeated many times to yeld large numbe of ienticlDA molecules.

Concept questions 3.4

1 What does NA ampli fication mean an why is it used n biotechnology?
2 State the components of PCR reaction.
3 Lst the thee main stps of PCR.
4 What specific tas in his processdo primers perform?
5 f you start with five cpies of DNA region, how many opis will be roducedif your sample goes through 3 cycles of PCR?

HOT Chaenge

6 At each stage of the CR process, the temperature is adjusted to enble ertain steps to occur.
  a Draw up atable that summarises the name of each step, the temperature at which the step occrs, and the actualrocess facilitated.
  b n your table, state where and ho the properties associted with hydroge bonding are vital and how the otmal temperature fooperation of the DNA olymerase is 72°C nd not 95°C.

3.5 Gel electrophoresis

WB
3.5 GEL ELECTROPHORESIS PAGE 67

DNA molecules are too small to see. One way to visualise them is to separate the fragments according to size by gel electrophoresis. Alternatively, scientists use a DNA probe to identify fragments, or analyse the nucleotide sequence by **DNA sequencing**.

Gel electrophoresis method

Gel electrophoresis separates fragments of DNA according to their size and charge. DNA has an overall negative charge due to the phosphate groups on its backbone. Gel electrophoresis makes use of this property to separate DNA fragments within an **agarose gel**. The agarose gel is melted and poured into a flat mould to cool. Wells are formed by placing a plastic comb into the gel as it sets, creating indentations into which DNA samples can be loaded.

The gel is placed in a tray filled with buffer solution (Figure 3.14), and positive and negative electrodes are attached at each end of the gel. When the electric current runs, the DNA fragments are repelled from the negative electrode and move towards the positive electrode at the other end. The gel acts as a large sponge through which the DNA strands move while under the influence of the electric current. Smaller strands can move faster than the larger strands through the gel matrix. Therefore, this method separates DNA strands based on their size.

DNA itself will not be visible in the gel. To view the separated DNA fragments, a fluorescent DNA-binding dye such as ethidium bromide is added to the agarose gel before it sets. The dye binds to DNA and fluoresces under ultraviolet light, showing a pattern of bands that can then be photographed. Each band on the gel contains millions of pieces of DNA of the same size. The bands can also be cut out and the DNA purified to yield a solution of DNA fragments of the required size.

The position of bands on an agarose gel depends on the size of DNA fragments in each band; the smaller the fragments, the further they move in a given time. To determine the size of a given piece of DNA, molecular biologists use standards called **molecular size markers**, or molecular weight ladders. These are pieces of DNA with a known number of base pairs. They are used to determine the size of the separated DNA fragments by comparing their location along the gel. Figure 3.15 shows four markers in the molecular marker (calibration) lane: 1700 bp, 1000 bp, 500 bp and 200 bp (bp = base pairs).

Figure 3.14 A researcher loads genetic material from coral into an agarose electrophoresis gel apparatus.

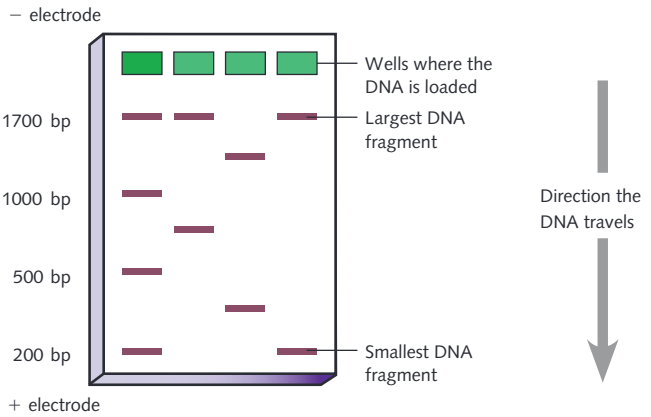


Figure 3.15 Standards are molecular markers of known size that are run alongside samples and allow estimation of the size of the DNA fragments migrating through the gel.

KEY CONCEPTS

- Ge electrophoresis seprates DAmolecules by size.
- Negtively charge DNA travels hrough a gel matrix towards a sitve eectrode.
- DNA s vualied with aDNA inding dye.
- Thesizes of DNA fragmets can be estimated by compaing heir movement trugh the gel with that of fragments ofknon sizes, referred to as molecular sze marers.

Concept questions 3.5

1 Wha givs DNAits negative charge?
2 How can you estimte the size of a DNA fragment by ge eectroporesis?
3 Wh is a standard used?
4 What does 700 bp mean?
5 Name twapplications of DNA sequencing.

HOT Chaenge

6 n ge eectropoesis, hat is he main property of the DNA fragments (notthe negative charge) that is used to separate the mixture ito the different fragments?

SOUTHERN Biological Developed exclusively by Southern Biological

INVESTIGATION 3.1

Effect of restriction digestion enzymes on lambda DNA

Restrction igstion is the proess o ctting DNA molecule s nto smlle pieces ih specal enzyes called restriction endonucleases (o rstricion enzymes). hes enzymes recognise speci fic sequencs in the N molcue (e.g. EcoRI reconises GAATTC) wherever thatsequence occurs in th e DNA. Lambda DNA is a common DNA substrate extracted from a baceriohage.

Ams

1 To use resriction enzyme to cut DA into respective fragments
2 To analyse yourrestr ctiondigestion by gel electrophoresis

Time reuirement

55minutes

Materas

Restrcton dgeston

- Lambda DNA (1 µg) (8 µL)
- Resticiondigestion buffer (20 µL)
- EcRI enzyme (1 µL)
- iII enzyme (1 µL)
- BamHI enzyme (1 µL)
- Stile uclease-free water (0.2 mL)
- 4 × 05 mL terile microtubes
- Vriable (550µL) micropipette
- icrotube rack
- Vriable (0.510µL) micropipette
- Stil pip tte tips
- Water bath
- icro cetrifue (optional)
- Lab coat
- Safety glasses
- G loves
- Ruler

Eectrophoress

- TBE buffer (25 mL)
- .8% agaoe gel wth 2 µL Midori Green safe tain (fo re-staining technique)
- Loding dye (50 µL)
- lectrophoresis chambr (blueGel ™)
- Power spply 100 V(if uing an alternative to bueGl ™)
- lulight tnsilluminao (optional)

Noe: the above measurements are base on using a blueGel ™ eectrophoresis aparats. I using an alternative electrohoresis hmber, incrase TBE quantiies based on chamber size.

What are the rsks n dong th is investigation?	How can you manage these rs ks to stay safe?
TBE buffer can irritate the skn on contact	Wear ap propriate personal protective equpment at a tmes including eye protection and gloves Wash skin immediately if contact does occur.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no aergyrisk an is uitble to use with the chemicals in this investigation

Method

Restrcton dgeston

1 Lael four 500 µL(0.5 L microtubes , E, B an C, as shown in Figure 3.16.

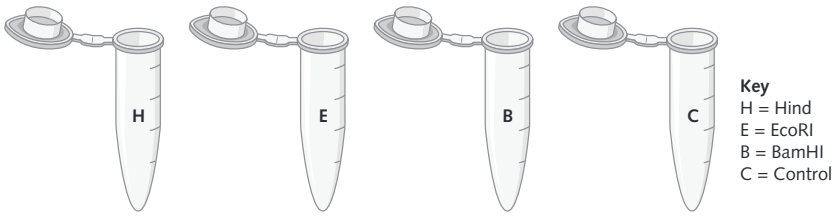


Figure 3.16 Labe your mcrotubes H (Hnd), E (EcoR), B (BamH) and C (contro)

2 Usng a vriablemicrpipette, add 42 µL of nuclease-free water to each of the microtubes.
3 Add 2 µL f lambda DNA to eachof the microtubes.
4 Usng a fres micropiptte tip, add 5 µL of bufferto eachof the microtubes.
5 Usng a fres micropiptte tip for ec sample, add 1 µL ofHdIII enzym to microtube H, 1 µL of EcRI enzyme to mcrotue E, 1 µL of BmHI enzye to microtube B and 1 µL of nclease-free ater tomicrotube C.
6 Mix the amples horoghly by pipetting up and down a few timesntil he solutions have an ven consistency. Use a freshmicopiptte tip for ech sale. T cllect the liquid a he bae of the tubs, close the lids and spin them with a mcrocntrifuge.
7 Pace themicrotues in a 37 °C water bath for10 inutes.

Anaysng your dgeston by ge eectrophoress

1 Remove the fur microtubes from the water bath n add 10 µL of oading dye to each sample.
2 Mix the amples horoughly by pipetting up and down a fe w timesntil he solutions have an ven consistency. Use a dfferent ipette for echsluti. To lect the liquid at th basef th tubes close the lids and spin them with a microcentrifuge.
3 Pace the prepared 0.8% arose e into the gel electrophor ess chamber. Make sure tat the wells are at the top or negaive electrode sectionof the chamber.
4 Pour TBE buffer into th gel electroporesis chamber Make sure you complete cover the surface of the gel.
5 Usng a strie pipette for ahsample, load 10 µL of each sa mpleinto theells near te ngative electrode and note the specific ane in hich he differnt samleswere loaded.
6 Careuly placthe lid n the el chmber turn t on and le th gel run for 0minte. Turn on the built-in blue light to vsuaise DNA band separation f usng a blueGel ™ eectroporesis chmber.

Noe: I usin agel electrophoresis chmber that requires an exteral power suppy, carefuly plug the positive and negaive elecrodes into he gelbox without dislodging th e ge. The neative nd should be connected to the end closest to the DN samles. Plug in the power ourc (set at 100 V) turn it on and let the gel run.

7 After 3 mintes, turn the power suplyff and viualis your esults b eher turning on the blue light or transferring a bluegt transilluminator.

Noe: If ou did not use the Mido Green sin, the DNwill not be visible ntil the gel has been soaked in methylee blue r quivalent for up o 24 hours.

Resuts

1 How many cts did eac rstriction enzyme make?
2 Copy th resut tabl into your logboo k Measure te disain millimetres and fi n the resultstable.
3 Graph your esults for he HindI dgest to detrmine he sizes of the coRI adBamHI digests.
4 Do those fragments add p to the size f lmbd DNAIf ot, provide possibe explanation(s) as to why not.

Anayss of restrct on dgests of DNA

HindIII		EcoRI			BamHI		
Distance mm)	Size (bp)	Distance (mm)	Calulated bp	Size (bp)	Distance (mm)	Calulated bp	Sze (bp)
	23 130			21 226			16 841
	9416						
	6557						
	4361						
	2322						
	2027						

Dscusson

1 Why was 1 µLof nuclease-free water aded to microtube C in step 5?
2 Whydid yu incubate terestriction digests at 37°C?
3 What is the purpose of the dye?
4 Whatwould ccur f he gel eectrophoresis chamber was fi ed ith dtilled ater instead of TBE buffer?
5 Explain why DA sampls must be loaded at the negaive end o gel eletrophoesis chamber.
6 Whatwould ccurif the lectodes i the electrophoresis chamber were reversed?

Concuson

Wite a conclusion for your nvetigation, ncluing a short discussion of our results.

Takin it further

nvestigate whrerestriction enzymes are sed adho theyassst in medical disease diagnosis.

WB
3.6
DNA PROFILING
PAGE 70

3.6 DNA profiling

Most DNA is identical from one person to the next apart from the different alleles for characteristics such as eye and hair colour. However, some regions in our genome show high variability – called **polymorphisms**. Each of us inherits a unique combination of polymorphisms from our parents, which can be examined to create a DNA profile. In **DNA profiling**, DNA is extracted from a sample of body fluid, skin cells, hair root or blood. DNA profiling is used to help solve crimes, determine family relationships and identify human remains and disaster victims.

Solving crimes

Television shows tend to oversimplify and exaggerate the scientific processes involved in forensic investigations. However, DNA profiling is a crucial scientific technique applied to solve crimes. We leave cells behind everywhere we go, so at a crime scene typical pieces of evidence include skin cells and hair.

DNA profiles from samples obtained from crime scenes, convicted offenders, items from missing people and unidentified bodies are added to DNA databases. DNA databases can help to solve crimes, including cold cases, and identify missing people, disaster victims or human remains. The first example of a DNA database was in 1986, after a young woman, Dawn Ashworth, was murdered in Leicestershire, UK. In the investigation, police collected blood samples from the males in the town (about 4000 men). At the time, this was an informal database, and the police applied DNA fingerprinting to try and solve the crime. No match was found. However, a man was overheard saying that he had been paid to provide false samples for another man, Colin Pitchfork. When Colin Pitchfork's DNA was collected and analysed, it matched that found at the crime scene. He was arrested in 1987.

Today, most developed countries have a DNA database. The Australian National Criminal Investigation DNA Database (NCIDD) managed by CrimTrac was established in 2001 and has more than 1.2 million DNA profiles. Although DNA databases are valuable, especially in solving crimes, their use raises ethical questions, especially about privacy. For example, do the benefits to society outweigh a person's right to privacy in having their DNA profile stored? Should everyone give a DNA sample? How long should information from a person's DNA profile be stored?

Determining family relationships

DNA profiling can be used to determine if people are related to each other. Paternity testing usually aims to determine if someone is the biological parent of an individual. In New York, there are buses similar to the one shown in Figure 3.17 – mobile units for collecting DNA samples from citizens wanting to establish relatedness. Demand for tests in the US to establish parentage is estimated at 500 000 per year. People using the service have discovered that they have half-sisters or half-brothers in other parts of the country. In other cases, men have discovered that they are, or are not, the fathers of their children.

There are no mobile clinics in Australia, but more people are having DNA tests to establish the parentage of their children. Men may want to confirm that they are the father of their children. Women may want to ensure they came home from hospital with the right baby or confirm the father of their child. More than 10 000 tests are ordered from laboratories registered in Australia every year. It is a simple process. A DNA kit is ordered online so that DNA samples of parents and children can be collected in the home and mailed to DNA testing laboratories. The results arrive in the mail a few weeks later.

There are concerns about using the tests, including the:

- possibility of contamination when collecting DNA
- interested party not knowing how to interpret the results of the test
- issue of consent if DNA is collected without a child's or parent's knowledge.

Occasionally the issue of paternity arises in cases where child support is being sought from a man who claims he is not the father of a child. In such cases, the court orders a DNA test to be conducted if paternity cannot be determined in any other way and if evidence places the paternity in question. Legal tests are required to comply with the Family Law Act, so samples must be collected and tracked from a registered collection centre and sent to specified testing laboratories. The results of non-legal tests are not admissible in a court of law.

Figure 3.17 A DNA bus in New York is popular with people who want to establish relatedness, including legal paternity.

Identifying human remains and disaster victims

After a disaster, such as an aeroplane crash, where there are unidentified victims, family members will voluntarily submit a DNA sample of their own to reduce the time taken to identify bodies by DNA profiling. This approach was used after the 2014 Malaysia Airlines plane crash in the Ukraine to identify the bodies of victims from countries all over the world.

Short tandem repeats construct DNA profiles

In Chapter 2, you learned that not all your DNA codes for polypeptides – 99% of the genome is non-coding. Non-coding regions of the genome contain inherited sequences of DNA that vary between individuals (polymorphisms). The polymorphisms used to construct a DNA profile for an individual are **short tandem repeats (STRs)**. STRs are segments of DNA that contain repeats of 2–6 nucleotides in tandem, such as AGATAGATAGATAGATAGAT. The human genome has many of these STRs at fixed locations on our chromosomes.

The number of repeats we have at each STR region is how we vary from one another. Each person has two copies (alleles) of each STR region, one inherited from their mother and one inherited from their father. Each can have a different number of repeats at the same region, increasing the specificity of a person's profile. The information can be entered into a forensic database, and used to indicate the number of repeats in two alleles. For example, in Figure 3.18, the numbers recorded would be 5, 10 and 12, 7. STR loci refers to the STR locations on a chromosome. What makes DNA profiling such a powerful tool is that multiple STR loci are examined simultaneously to obtain a profile, which is then compared with a reference sample. For example, a profile obtained from the DNA at a crime scene would be compared with samples from a database or a suspect. Similarly, a profile obtained from unidentified human remains could be compared with a sample obtained from a family member.

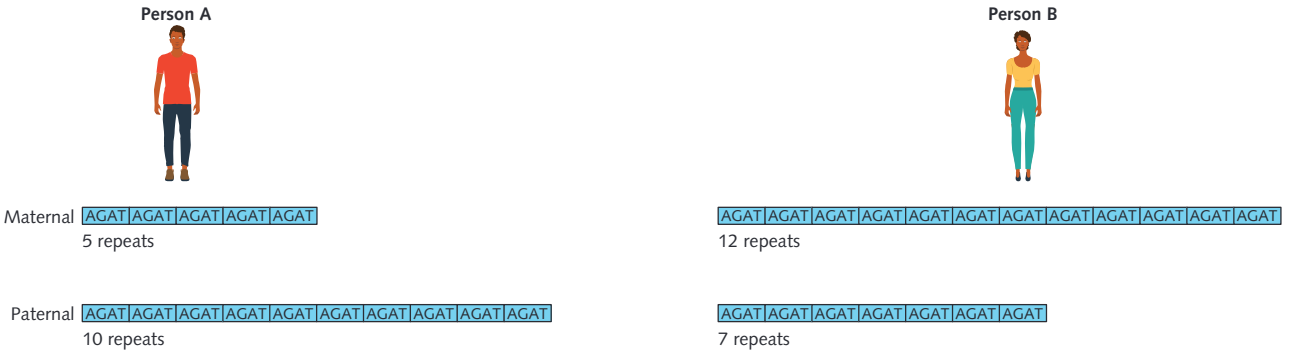


Figure 3.18 The number of repeats at each STR loci varies from person to person.

Different countries used different STR DNA profiling systems, based on the population size of that country. For example, the US originally used 13 loci, while Australia used nine. In 2013, Australia increased the number to 18. In 2017, the US added an additional seven STR loci for a total of 20 STR markers. In both countries, this includes the Amelogenin gene (although it is not an STR), which is located on the X and Y chromosomes and used as a gender marker. The gender of a person can be determined from this marker because on the X chromosome the Amelogenin gene is six base pairs shorter than on the Y chromosome. The STR loci used in Australia are shown in Figure 3.19.

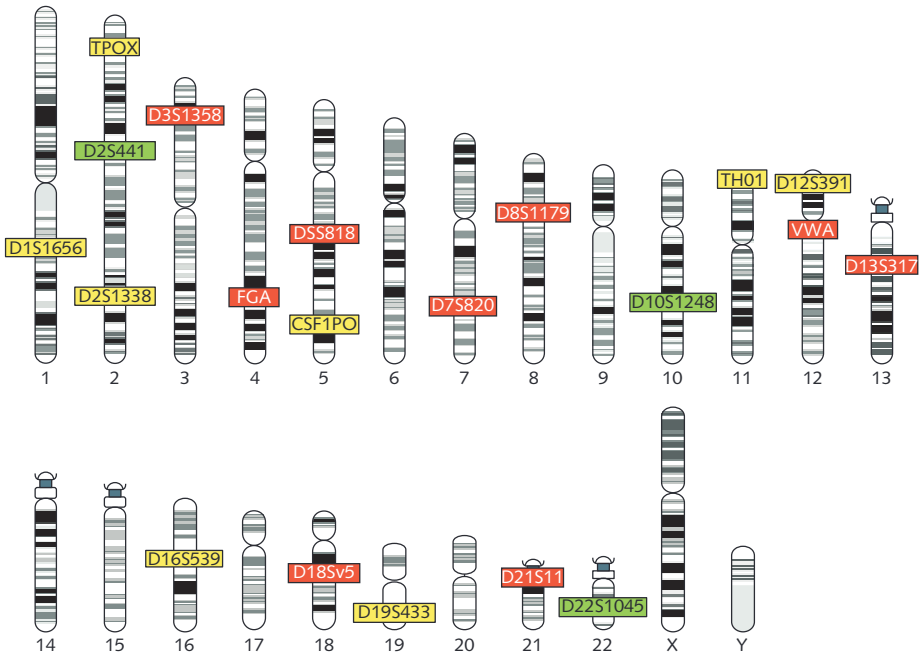


Figure 3.19 **Chromosomal location of the 17 STRs (not including Amelogenin gene) used to construct a DNA profile in Australia. Those shaded in pink are the original nine STR loci used in Australia. Those shaded in yellow are the eight STR loci added in 2013. Green shading represents the additional STR loci used in the US but not in Australia.**

How DNA profiles are produced today

Today, DNA profiling does not use restriction enzymes as in the original DNA fingerprinting method. Rather, it uses the PCR reaction to generate many copies of specific STR regions for analysis.

Type of DNA used to obtain profiles

First, DNA is extracted from the samples, usually skin cells, hair roots, cheek swabs (from the inner cheek) or blood. Two types of DNA can be used for DNA profiling. Commonly, genomic DNA is used (chromosomal DNA, making up most of a person's DNA). However, if there is not enough nuclear DNA available to create a profile, mitochondrial DNA (mtDNA), which can be more resistant to degradation, is used. Mitochondria are the cell organelles responsible for energy production. They contain a single circular chromosome inherited from the mother. There are several hundred mitochondria in each cell and so there might be hundreds or thousands of copies of mtDNA per cell. Because it is maternally inherited, mtDNA is identical between siblings and maternal relatives.

Amplification and visualisation of STRs

Once DNA has been extracted from a sample, PCR is used to amplify the specific STR sequences. PCR amplification is useful when analysing DNA from crime scenes, where only a small amount of DNA might be recovered.

As you learned on page 93, primers bind to complementary sequences of DNA and mark the starting point from which DNA polymerase will add new nucleotides. The primers used in the PCR reaction are designed to amplify the STR sequences of interest for profiling. Primers for each STR are tagged with a specific fluorescent molecule.

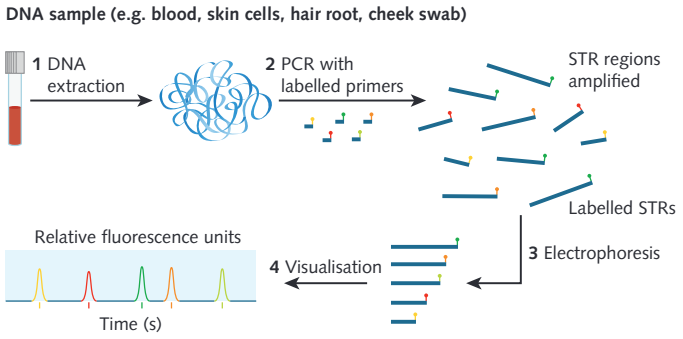


Figure 3.20 1–3: The steps involved in obtaining an electropherogram: DNA extraction is followed by PCR and electrophoresis. 4: The vertical axis (fluorescence) of the electropherogram is a measurement of the amount of DNA present. The horizontal axis (time) identifies the size of the STR alleles.

Once the STR sequences have been amplified, they are separated according to size by electrophoresis. Lasers are then used to excite the fluorescent molecules attached to the primers. This enables visualisation of the PCR-amplified DNA fragments that have fluorescent primers incorporated. Fluorescence measurements are collected by a detector and converted to a series of coloured peaks called an electropherogram. From this, the length of each STR sequence can be determined to give the DNA profile. This process is illustrated in Figure 3.20.

An example of an electropherogram is shown in Figure 3.21. Each peak represents an allele and, because there are two chromosomes, each individual has two alleles at each STR locus. For example, in this individual, at the STR locus D5S818, one allele has 11 repeats and the other allele has 12 repeats. This individual has two identical alleles of 11 repeats at D16S539. As the two alleles of 11 repeats at D16S539 are exactly the same length, they occur at exactly the same position on the electropherogram. The height of the peak (amount of DNA) is double the height as if only one allele was present because there is twice the amount of DNA present.

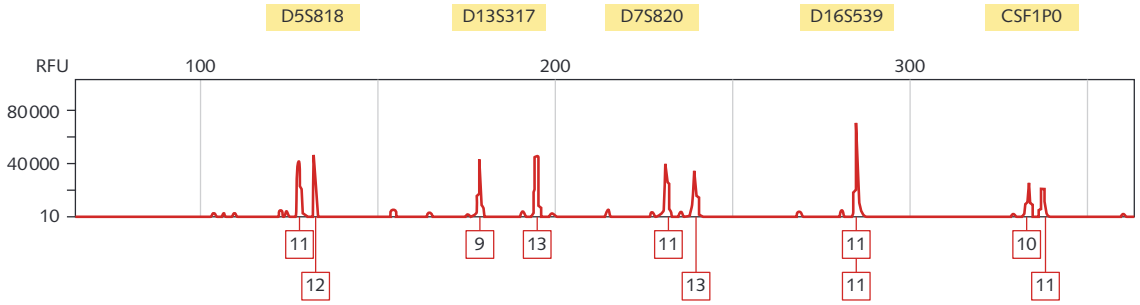


Figure 3.21 An electropherogram of five STRs

ACTIVITY 3.1

DNA profile analysis

Am

To aalyse DNA pro fies and consder ssues surrou ndng use of ths technoogy

Task : Paterity case study 1

A ega paternty test was ordered by a judge to determne whether a man shoud pay chd support A DNA sampe was coected from the mothe, the ild and the man Each DNA sampe was tr eated separatey to produce a DNA pro fie for each ndvdua DNA was extracted fr om ces and PCR was used to ampfy chosen STR regios, sing sequence-specific prmers These prmers are comlem entary to sequences of DNA that flank the STR regin.Figur 3.22 shows the THO1 aees nherted by an ndvdu a Each ndvdua has two aees for each STR one nherted from each parent. Ths ndvdua has nherte d the aees THO1-6 (THO1 aee wth 6 repe ats of TCAT) and THO1-5 (THO1lelewith five repeats of TCAT) Tisidivual's ge notype can be wrtten as THO1-56

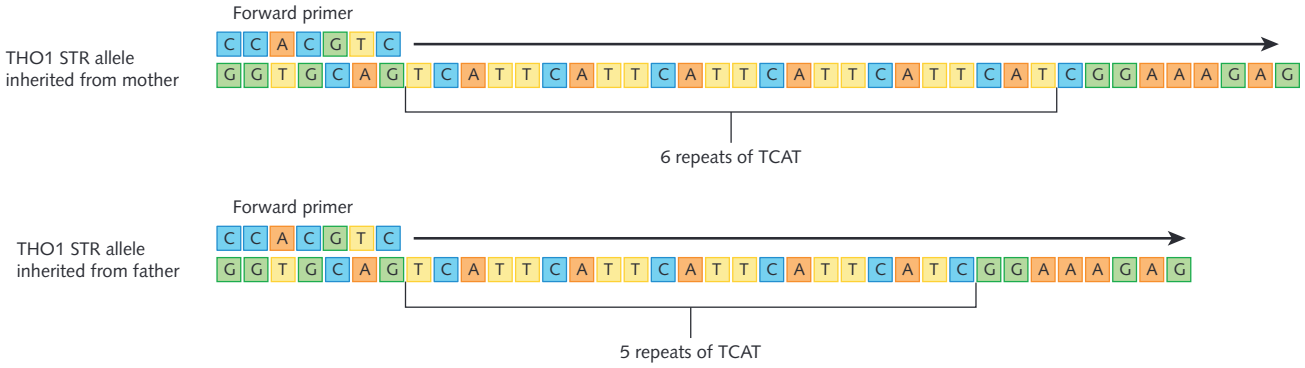


Figure 3.22 **The THO1 alleles for an individual can be amplified using polymerase chain reaction (PCR). Forward and reverse primers are designed to complement sequences flanking the STR region so the specific STR region is amplified. Forward primers are shown here.**

After PCR has been performed the DNA samplsare subjted o gel lectrophoresis. Table 3.3 provides results for the STRsinherited y the child, mother and poss ble fathe. Only thre STR regionhave been ampli fied for each ndidul. al 3.2 lists te STRs used in DNA pro fi ng and the kw alleles of tese STRs. This information can be compared with te STRs inhertd by the chd, mother ad possbe father to determine paternity.

Use the data n Tble 3.2ad 3.3to calculate the length of DNA fragments generated after PCR for eachiniidul. Then construt an image f theresultant el electrophoresis run for eachiniidul. Labe your el o ndicate which ndidul's DNA pro fie s shown n each lane.

Questons

1 What can you tl the judge prsding over his court case?
2 Can you be certin of the res uts of paternty usng ths test?
3 What ightincrease the certanty of these reults?

Table 3.2 **Vaiaions foundin ST R oc used n DNA pro fi ng**

STR name	Locus	Repeat	Number of known aees of ths STR	Vaiaionin number of repeats that can be found (known aees of ths STR)	Vaiaioninlength of DNA fragments (nuceotdes)
TPOX	2p25.3	GAAT	15	4–16	12–64
D5S818	5q23.2	AGAT	15	7–18	28–72
CSF1PO	5q33.1	TAGA	20	5–16	20–64
D7S820	7q21.11	GATA	30	5–16	20–64
THO1	11p15.5	TCAT	20	3–14	12–56
D13S317	13q31.1	TATC	17	5–16	20–64

Table 3.3 **The STR aees nhert ed by three ndvduas (resuts shown for th ree STR regons ony)**

Individual	STR al ees nherted		
Child	TPOX-4,12	D5S818-7,7	THO1-5,14
Mother	TPOX—12,16	D5S818-7,12	THO1-3,14
Man who the mother claims is the father	TPOX-4,9	D5S818-7,10	THO1-5,6

Task2: Patrnity case study 2

A man (mae 2) returned ho me to Austraa after workng overseas for 10 year. On hs return he became aware that the grfrend he had dated up unt eavng had a 10-year-od son He suspected that he mght be the boogca father of ths chd However, the mother was happy marred to another man (mae 1) who sh e camed was the boogca father of the chd Mae 2 or dered a DNA tetingkit and managed to secrely clect a DNA sampe fr om the chd mother and husband He sent these for testng aong wth hs own DNA A ge wth se ven STR region is show in Fgure .2. The STRsinheited by the son from is mother are igighte.

Questons

1 Use the gl results to de termne whch mae s the boogca father.
2 Isit eticly sound to or der DNA tests wthout the consent of a ndvduas concerned?
3 Can you tink ofissues th at may emerge iven the reults of his test?

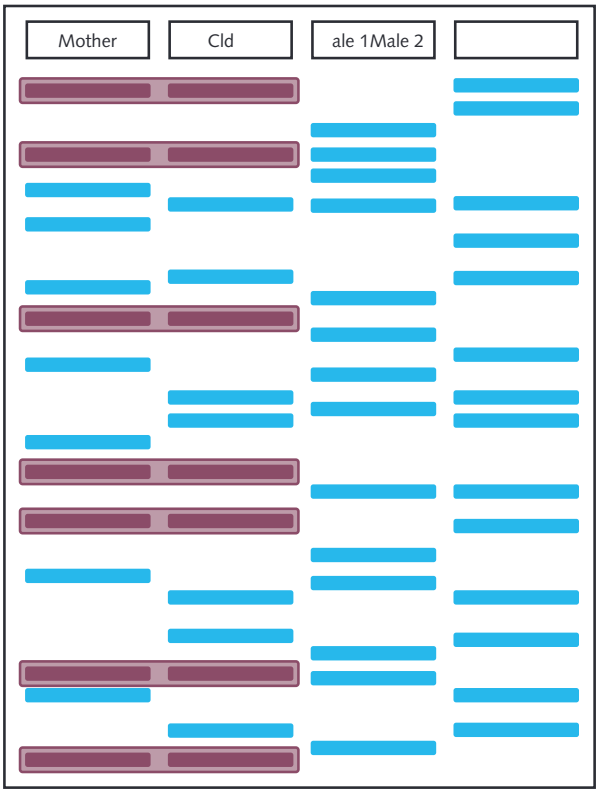


Figure 3.23 This gel electrophoresis run reveals the DNA profiles of the four individuals in case study 2. Primers for seven STR regions were used to generate these DNA profiles.

KEY CONCEPTS

- DNA profi ng is used o solv crims, dermine family reaioships and dentify uan samples.
- By exining TRs i non-coding rgions of DNA, a profie can be btained that can be compared to a database of samples iven by family members.

Concept questions 3.6

1 What amples of hman organis can be collected for DNA profiling?
2 Plymorphisms are egions in ou DNA of high varation. Wha does this mean?
3 ST is an aconym. Whdo the letters stand for and what do STRs mean?
4 How are STRs used to perform the process of DNA profiling?
5 There are standard STR markers used for DNA profi ng n various counries. How does this enable DNA profi ng to ocur if te wholegenome is not being examined?

HOT Chaenge

6 How easyis it t challenge DNA pro fi ing? List the reasons for nd against asto how one cold do it or not.

3.7 Recombinant plasmids and human insulin

Biotechnology applications are used to produce recombinant insulin to treat people with diabetes. Insulin is a peptide hormone that promotes uptake of sugar from the bloodstream and its storage in muscle or adipose tissue. Insulin is essential for normal metabolism; without insulin, the body relies on fat as an energy source. This can result in the build-up of dangerous substances in the blood, which can be life-threatening.

Type 1 diabetes is an autoimmune disease in which the insulin-producing cells of the pancreas come under attack from the body's own immune system and are unable to produce sufficient insulin (Figure 3.24). People with type 1 diabetes must inject insulin up to four times a day. Before insulin became available, people who had diabetes would likely die.

In 1889, German researchers Oskar Minkowski and Joseph Von Mering discovered that when they removed the pancreas from dogs, the dogs developed symptoms of diabetes. In the years that followed, the chemical responsible for diabetes, insulin, was described. In 1921, Canadian scientists Frederick Banting and Charles Best removed insulin from the pancreas of a dog, and used this insulin to keep another dog with diabetes alive. Soon after this striking observation, Frederick Banting and Charles Best worked together with another Canadian scientist, John Macleod, to purify insulin. Insulin was further purified by James Collip. In 1922, a 14-year-old boy in Toronto dying from diabetes became the first person to receive insulin injections, which was eventually successful. This work led to the 1923 Nobel Prize in Physiology or Medicine being awarded to Frederick Banting and John Macleod.

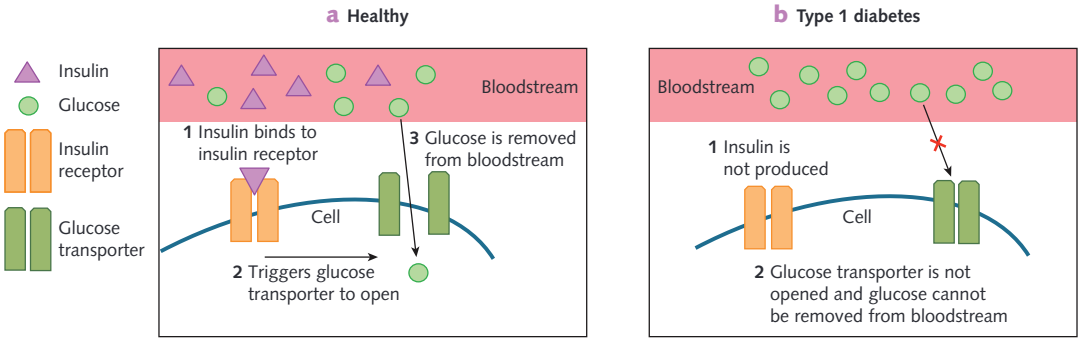


Figure 3.24 a Under healthy conditions, insulin binding to the insulin receptor activates glucose transporters to uptake glucose from the bloodstream into cells, maintaining healthy glucose levels. b In type 1 diabetes, insulin is not produced and glucose cannot be taken up by cells from the bloodstream (right).

Recombinant DNA technology: a game changer for insulin production

The first synthetic human insulin was produced in 1978 by using *E coli* bacteria. Bacteria can be grown quickly and in large batches in controlled environments. Bacterial cells can be lysed (ruptured) and proteins extracted from their cytosol. Genetic engineering of *E coli* has allowed the human insulin gene to be inserted into bacteria, replicated to vast numbers as the bacteria replicate, and expressed by the bacteria to produce the functional insulin protein. Insulin is then extracted, purified and distributed as the injectable therapy that is a lifesaver for many people worldwide. This process is summarised in Figure 3.25.

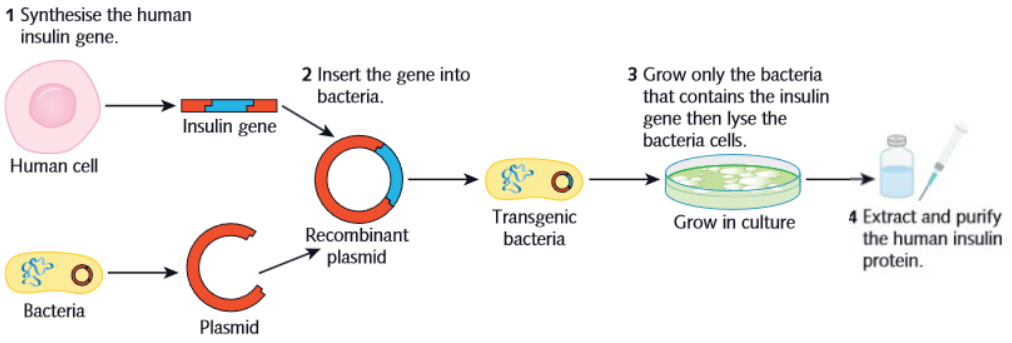
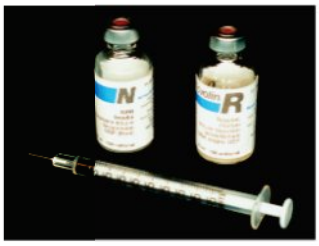


Figure 3.25 The steps involved in producing insulin in bacterial cells

In 1982, Humulin became the first approved product made by recombinant DNA technology. Today, three kinds of Humulin are used to manage diabetes (Figure 3.26). Each kind acts over a specific amount of time, which is affected by factors such as diet and exercise. Humulin R U-100 (100 units of insulin per millilitre) provides a short-acting insulin peak, which manages increased blood sugar levels that occur after a meal (within 30 minutes). Humulin N has a longer period of activity, and is used to manage blood sugar levels between large meals (e.g. breakfast and lunch) or overnight. Humulin 70 combines the short effects of Humulin R U-100 with the longer lasting 'intermediate' Humulin N to manage blood sugar levels after meals, throughout the day and during the night. People with diabetes work with their doctor to find the best type of insulin to manage their blood sugar levels.



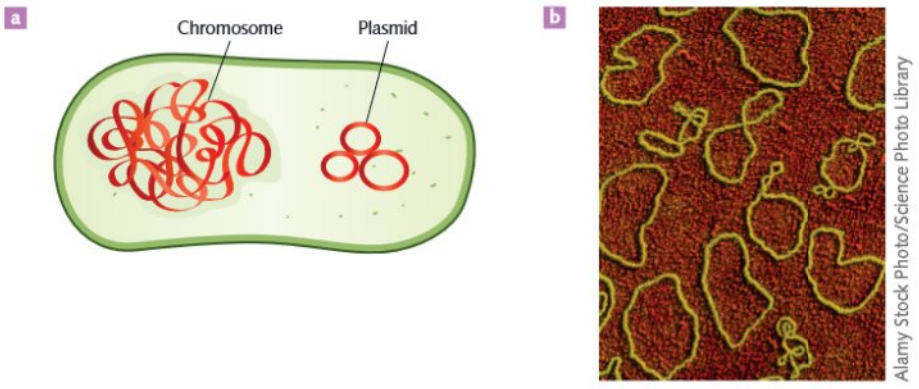
Science Photo Library/Sue Trainor/Scott Camazine

Figure 3.26 Humulin N and Humulin R U-100 are two of the three kinds of Humulin that are used to manage type 1 diabetes. The other kind is a mix of the two.

WB 3.7.1 RECOMBINANT PLASMIDS PAGE 73

Recombinant plasmids

Before the human insulin gene can be used in genetic engineering, it must usually be copied, or amplified, to produce enough to work with; for example, by PCR. Short linear DNA fragments produced by PCR or cut out from a chromosome by restriction endonucleases are unstable. They do not survive long in cells or a test tube and can lose base pairs from the ends through enzymatic or mechanical degradation. They are also often too small to manipulate in the laboratory. For this reason, scientists use bacterial plasmids – circular pieces of DNA that reproduce independently of the bacterial chromosome (Figure 3.27).



Alamy Stock Photo/Science Photo Library

Figure 3.27 a A bacterial cell containing bacterial DNA and plasmids; **b** a transmission electron micrograph of bacterial plasmids from *Escherichia coli*

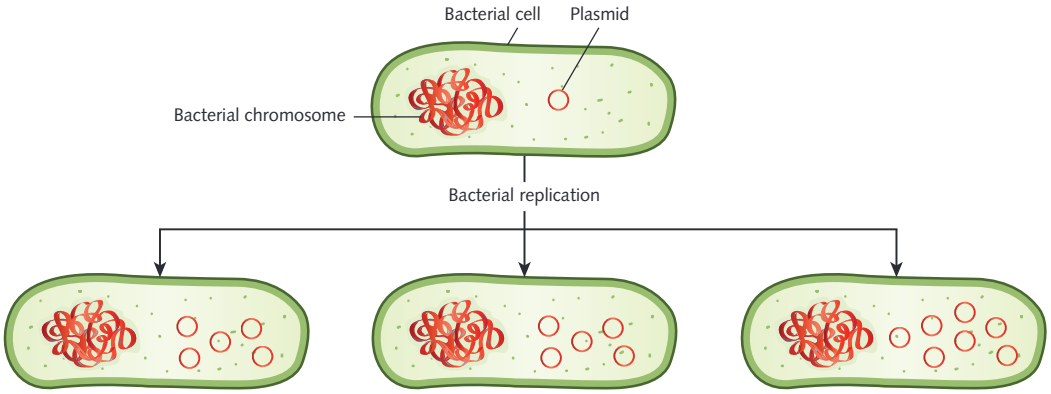


Figure 3.28 Plasmids are copied when bacterial cells replicate.

DNA fragments, such as PCR products amplified from a gene of interest, can be inserted into plasmids that have been cut open, and then the plasmid can be closed again by ligases, incorporating the DNA fragment. A plasmid that has incorporated a DNA fragment is called a **recombinant plasmid**.

In biotechnology, plasmids are ideal for DNA fragment **vectors** (the plasmid will carry the DNA or gene into a bacterium). Plasmids are copied many times within the bacterial cells and are copied when the bacteria replicate, and this also copies any DNA fragments inserted into them (Figure 3.28). Because they are circular, plasmids are much more stable than linear fragments. Their stability also allows them to survive the harsh conditions that are used to rupture the bacterial cells and purify the plasmid DNA. They are small enough to be distinguished from the main bacterial chromosome, but large enough to be extracted and manipulated in the laboratory. Plasmids can also be easily engineered to carry a number of different genes or DNA elements such as promoters and restriction sites, making them ideal tools for manipulation of DNA fragments. Promoter regions on plasmids can allow genes to be expressed in prokaryotes or eukaryotes depending on the application.

An alternative to using PCR to generate many copies of a DNA sequence is to insert the DNA into bacteria. This process is called **gene cloning** and it has many advantages. Gene cloning allows replication of larger segments of DNA, and it permits the analysis of any gene and associated proteins encoded in the DNA sequence in an environment where they are active.

Plasmids are used to insert DNA into the bacteria. The key to using plasmids as DNA copiers lies in our ability to incorporate foreign genes into plasmid DNA and in their ability to replicate in bacteria. A number of steps are involved in this process (Figure 3.29).

1. Plasmids are extracted from bacteria by rupturing the cell walls. A restriction enzyme is used to cut the plasmid DNA to produce sticky or blunt ends.
2. The same restriction enzyme is used to cut the DNA of the gene to be inserted so that both pieces of DNA have complementary sticky ends.
3. DNA ligase binds the 'foreign' DNA fragment into the plasmid DNA. After binding, the DNA fragment becomes a permanent part of the recombinant plasmid.
4. The recombinant plasmids are added to a bacterial culture. They are taken up by some bacteria, in which they replicate. In the normal process of growth and division, bacteria replicate the plasmid, and thus numerous copies of the incorporated foreign DNA are made.

Only a small percentage of the bacteria take up the recombinant plasmids; others simply seal up without taking up the plasmid. The process of bacteria taking up the plasmid is called **transformation**. After transformation, the bacterial cells that contain recombinant plasmids have to be isolated from most of the cells in the colony, which have not taken up plasmids.

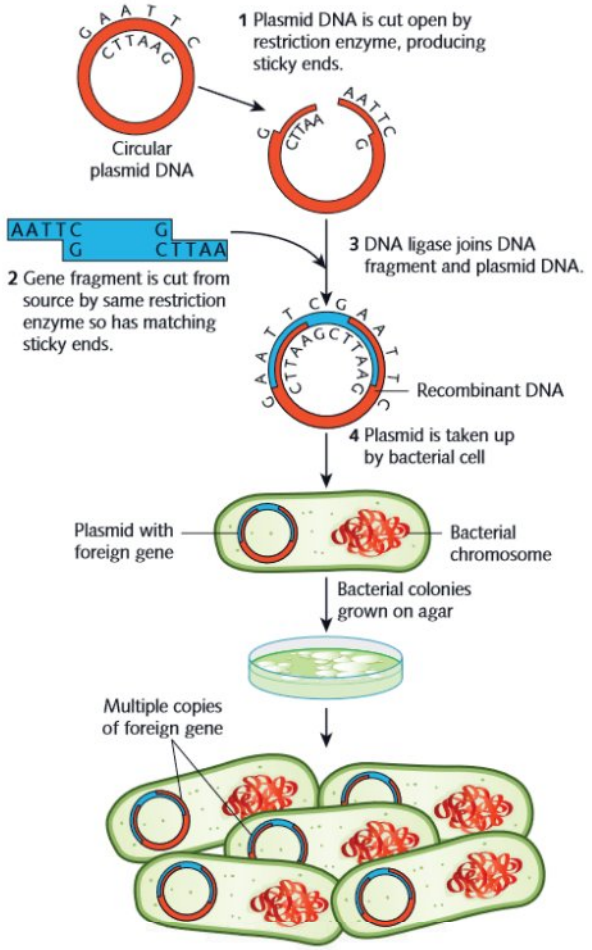


Figure 3.29 Transformation: a foreign gene is inserted into plasmid DNA to produce a recombinant plasmid. This is introduced into bacteria, where it can make multiple copies of itself. When bacteria take up the plasmid, they are transformed. Note: the plasmid is not normally as large as shown here. It has been magnified in this diagram.

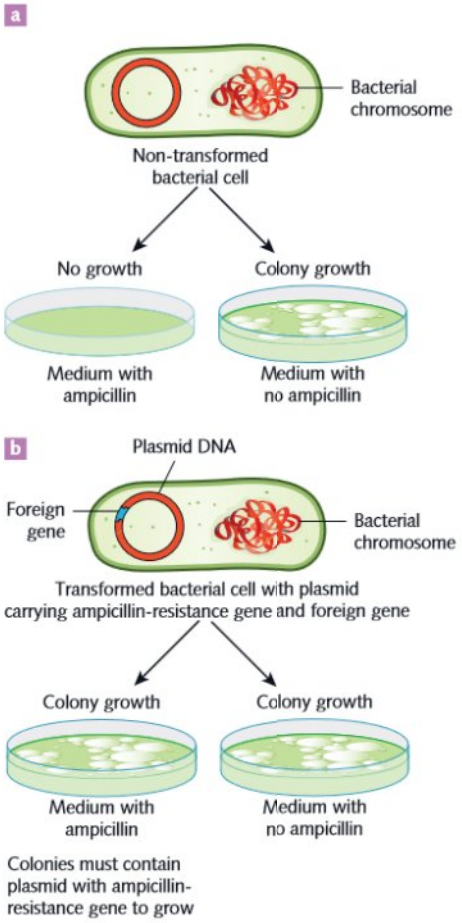


Figure 3.30 Antibiotic selection of transformed bacteria. **a** Non-transformed bacteria cannot grow on media supplemented with ampicillin, but grow well on normal media. **b** Transformed bacteria can grow equally well on either medium. The plate without ampicillin provides a positive control condition.

Plasmid DNA often contains genes for resistance to an antibiotic, such as ampicillin. Bacteria that have been transformed with the plasmid can grow and multiply on a medium that is supplemented with ampicillin because they are resistant to it. The bacteria without the plasmid do not grow because they are sensitive to the antibiotic ampicillin (Figure 3.30). This process is called **antibiotic selection** and is an important component of many biotechnology techniques.

Plasmids are very useful vectors in genetic engineering. Vectors in this context are agents that can deliver a piece of foreign DNA into a host cell. Other types of vectors include recombinant viruses and liposomes, which are synthetic spherical vesicles encased by a phospholipid bilayer that can encapsulate the DNA to be delivered.

The bacteria with antibiotic resistance are then selected and grown in culture. To study the gene of interest, scientists isolate and analyse the plasmids. This technique of bacterial transformation is also used to insert genes that code for useful proteins into bacteria so that the bacteria will then make the protein for human use.

Transformation is also used to produce human growth hormone, which is used to treat people with a certain form of dwarfism. Before this technique, the hormone was extracted from the pituitary glands of human corpses.

SOUTHERN Biological Developed by Southern Biological

INVESTIGATION 3.2

Bacterial transformation

DNA can mutate spntaneously or afer an error occurs in DNA pliaion.Botechnolgists have developed methods of contlled DA mutaton suh as intentionally mutaing Ato lter w the cell behaves. It is also possible to transfer DNA from one organism ino anothe. This method, called gene tic tranformation uses an ngineered molecule of DNA to transfer a gene or genes fom one organism to anothe r so that the organsm is capable of producing the protein encoded by the transormed gene.

Am

To perform abcterialtransformation using the green fluorescent proten plasmid pGreen

Time reuirement

50minutes

Materas

- *E. coli* MM294
- pGree pasmid (10µL)
- 2 agr plas cotaining Luria broth
- 2 agr plas cotainin Luri h with ampicillin
- StileLuria broth (10mL)
- Stile 50 M $CaCl_2$ (10mL)
- Stile water (20mL)
- 2 serile trasformation tubes
- 3 serile 1mL tansfer pipettes
- 10µL mcroipette
- 3 serle disosale ioculation loops
- 4 serle disosale inoculation spreaders
- Stie tips for 10 µL mcrpipette
- Water bath
- Ice bath
- ine point marker pen
- Stopwatch
- Test-tube rack
- Adhsive tape (to seal plates)
- Thermometer
- Incubator
- Lab coat
- Safety glasses
- G loves
- sinfectant
- Ulight

What are the rsks n dong th is investigation?	How can you manage these rsks to stay safe?
Some bacteria may cause disease so assume them to be pathogenic. (Note: *E coli* MM294 is a commonly used laboratory strain of *E coli* and s safe to use n schoos)	Wear appropriate personal protective equipment at all times ncudng eye protecion and love. Wash your hands thoroughly at the end of the investigation. Decontaminate benches before and after the investigation Food any sps wth beach
Micro-organisms will grow on the agar patesDo not op	en agar plates once they are securely tapd. Dispose of agar plates appropriately after autoclaving.
Disinfectnts r bleah may leave a corosve rsidue.	Afte wiing the bench clean with bleach, wipe off the residue. Ensure your lab coat sleeves are rolled down and wear gloves.

Method

Noe: To useaseptic ehnique, wipe your bench downwith ethanol (or bleach), and keep your work near the Bunsen burnert waf potential contaminants away from your maeals.

Preparng the transformaton souton

1 Lael the transforation tubes ‘ +’ (+ plasmid and ‘ – (– plasmid) (Figur 3.31). Keep the tubs cod y placng hem prigh in the ice bath. Keep the tubes cappedt all timesexcet when in use.

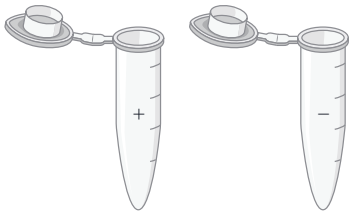


Figure 3.31 Label the transformation tubes ‘+’ and ‘–’.

2 Add 250 µL (.25 m) of ice old $CaCl_2$ sltion to each tranformton tube using a serie transfer pipette. Maintain the temperatureb placing th tubesback ino the ice bath.

Suspendng the bactera

1 Use a strle iouation loop to trnsfer a single colony of *E coli* from the starter plate totheice cold $CaCl_2$ sltion n th ' + plasmid' transfomation tube. To dislodge the *E. coli* cels from te oo, spn thelo rapilyinthe solution. Check whether your *Ecoli* has transferred scessfully – t shoud bevsbl in your ube.
2 Suspend the *E. coli* n the $CaCl_2$ sltion bydrawin thesolution in and out of apipette by squeezng and releasing the ulb seeral tims. Do nt incoprate air bubbles in th e qid or llow ayliqui to splashup the sides of the tub. The oution hould start to ecome milky whe asthe cellmass is suspended. Tocheck that there are no lumps or partices in the tb, hold it tothe light; then retur the tube to the ice.
3 Repeat steps 1 and 2 to tr nsfr a single colony of *E coli* from the starter plate to te ice cold $CaCl_2$ sltion in the –Plasmid' transformation tube.

Addng the pasmd

1 Your teacheror lab echnian willbrng the plasid t you workstation. Using a micropipette, transfer 10 µL (001 mL) o plamid soluio tothe '+ plasmid' transforaton tube Ad he plasmid directly to the liquid in the tube wthoualoing it to touch the sides.
2 mmediately return the tube to the ic bathand ix the plasmid ino thebateriasuspension by placing a sterile nocuationloop in the liqudan rpl spinning it. Incubae the two tubes on ice for 15 minutes.
3 Lael the tw agar ates containing Luria broth 'LB + plasmid and 'LB – plasid'. Label the two agar plates contning uria broth wimpicillin 'LB/amp + plasmid and 'LB/amp – plasmid' (Figur 3.32).

Heat shock

1 After 1 minute of ncubation, remove the wo tubes from the ice bath and transfer both of them to a warm watr bat (42°C). Hold them in the bath for 90 seconds and make sure the tube capso not becme fully submerged in the watr. ently agitate te tubes whiletheyare waring up in the water.
2 mmediately transfer the tues to the ice ath wenhe time is up. Allow the tubes to rest in te ice bath for at east 1minute beforecotnuing.

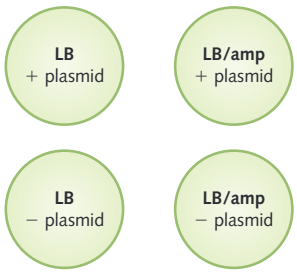


Figure 3.32

Recovery

1 Usng a strie plastic pipette, transfer 250 µL (.25 mL) of Luria broth toeach tube. Mix te iquids at the base of eah tube by gently grasping the top and tapping the base with your finger.
2 Alow the tubes to recoverfor 1 mintes in a microtube rack at room temperature.

Pate nocuaton

1 Use a strie plastic pipette to traser two drps of liquid from the ' + plasmid' tubeto the 'LB + plasmd'plate. Use a stile spreader t qickly sprd he lquid eveny over the plate.
2 Use a strie plastic pipette to tranfr two dros of liquid from the ' + plasmid' tubeto the 'LB/amp + plasmd'plate. Use a strile spreader o uickly sprd he lquid evenl oer the plate.
3 Use a strie plastic pipette to tranfr two dros of liquid from the ' – plasmid' tubeto the 'LB – plasmd'plate. Use a stile spreader t qickly spre the liquid evenl oer the plate.
4 Use a strie plastic pipette to tranfr two dros of liquid from the ' – plasmid' tubeto the 'LB/amp – plasmd'plate. Use a strile spreader o uickly sprd he lquid evenl ove theplate (Figure 3.33).

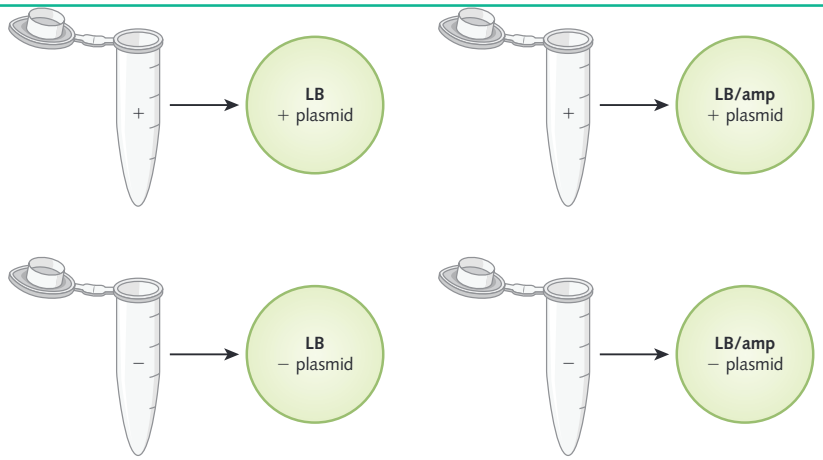


Figure 3.33

5 Secure te lid of eachPeti dishto it base with tape. Leave the plates o rest on the bench for 5 minutes and then pace them pside down (agar o top) ina 33°C incubator for 24–36 hours. Inspectthe growth after this time. You shoud see ether a bacter allawn, inglecoonies, or no growth on the ndvdulplaes. Take th plates into a dark room to observe evidence of fluorescence n the transfre colonies. Usof a UV light may enhance the fluorescence.

6 To count the numbe o ndiida clonie, mark the id of the Petridish above the coly with a marker as you count it. f ll growth is too densetocunt individ ual cloie, mark tat plateas a lawn. Record you rsults inthe sults table.

Resuts

1 Copy th resut tabl into our logbook. Record thereslts ofyour experiment in the table.

Bactera coony resuts

Plate	Result
LB + plasmid	
LB – plasmid	
LB/amp + plasmid	
LB/amp – plasmid	

2 What growth and phenotypes can you observe?

3 Describe what you see on your lates wen you looktyour plates under UV light.

Dscusson

1 Wh is the plamid olution pl aced o ice for 5minutes?

2 Wic plate is th ontrol inthis experiment? xplain your answer.

3 Explain th functionof t e Luria broth.

4 What is the purposeof incbating the cells at room temperature?

5 Explain what plasmid is.

6 Explain how th DAplamid is pu n th bacteria. What isthe advntag of being able to do this? (Consider what theplasmi DNA allow the acteria to do.)

7 Explain how we ar able to identf tht the plasmd DNA is in the bacteria.

Concuson

Wite a conclusion ofyou invstigation, ncluing a short discussion of our results.

Takin it further

nvestigate ow geeti engineerig and bacterial transformationenbles the advancement of medical treatments for exampein sulin retion.

WB 3.7.2 SYNTHESISING THE HUMAN INSULIN GENE PAGE 74

Synthesising the human insulin gene

Before inserting a DNA fragment containing the insulin coding sequence into a plasmid, the human insulin gene must be copied and produced in adequate quantities to be manipulated. This can be done by PCR, but first some alterations need to be made to the PCR template sequence so the gene can ultimately be expressed in bacteria. Bacterial DNA does not contain introns, and bacteria do not have the machinery to splice them out of mRNA. For a eukaryotic gene such as the insulin gene to be expressed in *E coli*, the inserted DNA must contain the exons (coding regions) only. In this situation the nucleic acid template for PCR amplification is mRNA molecules (exons only). These are first converted back into DNA strands, now called copy DNA (cDNA). Primers for PCR must be designed so that they bind on either side of the cDNA region to be amplified. One primer binds to the template strand and the other to the complementary strand of the cDNA. The PCR reaction then proceeds and produces billions of copies of the insulin gene.

WB 3.7.3 PLASMID X PAGE 75

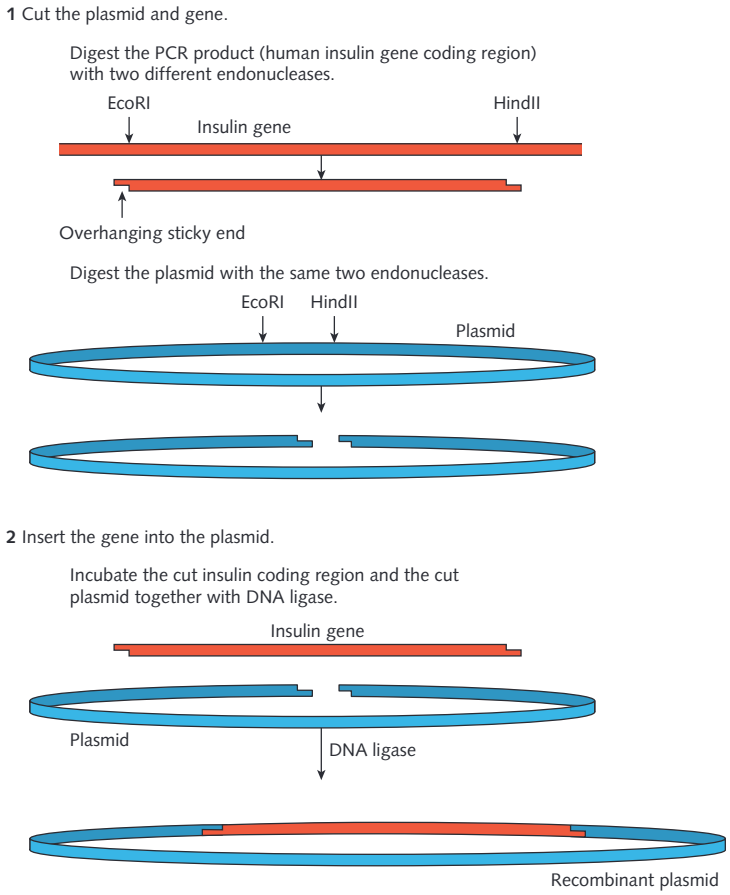


Figure 3.34 Inserting the human insulin gene into a plasmid

Creating a recombinant plasmid with the insulin gene

Once the human insulin gene has been amplified by PCR, it can be inserted into a plasmid vector. The PCR product (the insulin gene) is cut with two different endonucleases that give it different sticky ends at either side. The plasmid can be cut with the same endonucleases, and so the sticky ends of the insulin gene hydrogen bond with the complementary ends of the plasmid and the gene slots into the plasmid in the correct orientation. Then, DNA ligase seals the nicks in the DNA backbone and the recombinant plasmid is complete. The steps involved in creating a recombinant plasmid with the insulin gene are summarised in Figure 3.34.

Quality control of the recombinant plasmid

DNA ligation in the laboratory is not usually 100% efficient. Following the restriction digest reaction and the ligation reaction, four possible DNA fragments may be found in the tube. These are the:

- plasmid with the insulin gene incorporated into it (the recombinant plasmid that is the desired product)
- cut PCR product (insulin gene), which has not been inserted into the plasmid
- cut plasmid, in a linear conformation
- plasmid, with cut ends re-ligated to themselves.

The recombinant plasmid containing the insulin gene will be used to insert the gene into bacteria. This is separated from the other DNA fragments by DNA gel electrophoresis. The digested PCR product, digested plasmid and uncut plasmid are run in parallel wells as controls to compare sizes. The circular plasmid runs slightly further than the linear plasmid because it is more compact. The recombinant plasmid travels the shortest distance because it is the longest fragment. It can be cut out of the gel and purified out of the agarose. The DNA sequence of the region of the plasmid containing the inserted insulin gene is checked to ensure the insulin gene is inserted in the correct orientation and no mutations have arisen during the PCR reaction used to amplify the gene. The sequence is compared to public genomic databases to check that it aligns and that there are no mismatches.

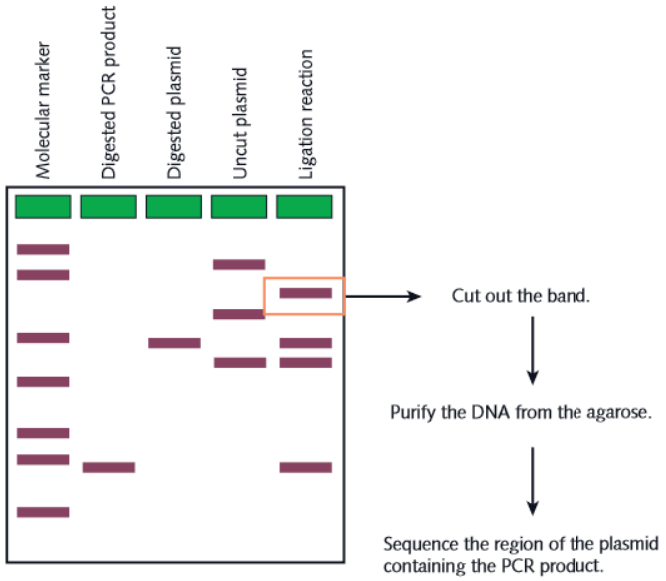


Figure 3.35 Isolating the recombinant plasmid by gel electrophoresis

Sequencing the regions where the DNA fragment joins the plasmid can show whether the DNA fragment has been inserted in the correct orientation. This is especially important when the ligation reaction has joined blunt ends. Together these steps ensure that the DNA being cloned is correct. A single base pair mismatch can completely change the function of a gene product.

Scaling up production

Once the recombinant plasmid containing the human insulin gene is purified, it is ready to be inserted into *E. coli* cells. Bacteria are kept ice-cold to halt their metabolism, and their cell walls are compromised by chemicals or electrical pulses so that DNA can be taken up more readily. The cells are then 'shocked' by a rapid increase in temperature to close the cell walls and to kick-start the cell cycle. The cells are put in a selection medium containing an antibiotic. Because the plasmid encoding the insulin gene also codes for a gene for antibiotic resistance, only cells that have taken up the plasmid can survive in the presence of the antibiotic, and these selected cells rapidly reproduce in large fermentation tanks. While growing, the transformed bacteria containing the recombinant plasmid express the insulin protein. Once the cells reach an optimal density in the tanks, they can be filtered out and lysed to rupture their walls and release the insulin protein. These steps are summarised in Figure 3.36.

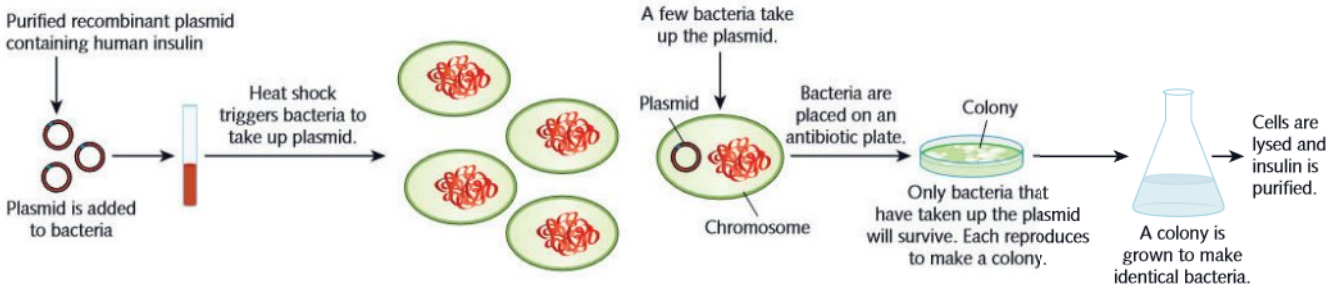


Figure 3.36 The steps involved in transformation of *E. coli* bacteria with the plasmid containing the human insulin gene

Figure 3.37 A person with diabetes using an nsun pen to dever a dose of insulin

The protein is harvested and purified, and then packaged into insulin pens for medicinal use (Figure 3.37).

Islet transplantation: a cell-based therapy for type 1 diabetes

The production of recombinant human insulin has meant that type 1 diabetes is no longer a fatal disease. However, ongoing research aims to lower or completely remove the need for insulin injections. In Australia, islet transplantation is a cell-based treatment for type 1 diabetes. Pancreatic islets are groups of cells in the pancreas. They include beta cells, which produce insulin. In islet transplantation, islets are purified from the pancreas of a deceased donor, then infused into the recipient (the patient) through the portal vein into the liver by using a catheter (a thin, flexible tube). X-rays and ultrasound help to guide the catheter from an incision in the upper abdomen to the portal vein into the liver. The islets establish in the liver and, in the following weeks, new blood vessels form and connect the transplanted islets to the recipient's blood vessels. The beta cells produce insulin and release it into the bloodstream.

People with type 1 diabetes who want an islet transplant need to satisfy certain criteria. For example, they must have had diabetes for 5 years or more and be over the age of 18. The major criterion is that the person has severe hypoglycaemic unawareness. This means that they are unable to detect when they have a large drop in blood sugar, because they do not secrete the hormone epinephrine (which generates the characteristic symptoms of low blood sugar).

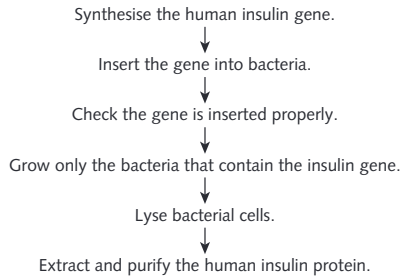
Although islet transplantation is very successful at managing type 1 diabetes, it also has several challenges. For example, recipients must take immunosuppressants (drugs that suppress the immune system) to prevent their immune cells from attacking and destroying the transplanted islets. Immunosuppressants have side effects that affect quality of life. Current research aims to find ways to suppress the immune response without the need for immunosuppressive drugs.

KEY CONCEPTS

- Gen cloing i an lternative to CR for generating many opiesof DNA. It ue bacterial plasmids to produce many copiesof a gene.
- A DNA fragmet can e inserted into a plasmid and repicated as te baceria carryngthe plasmid divide. Theplamid ishen caled areobinant plasmid.
- Recombian plasmids can beusd to produce insulin for use by people with type 1 diabetes.
- Recombian plamids usualyhave an antibiotic resistance gene addedthat allows hemto be selected. Oly the plasmid-carying bcteria can grow in the presence of t antibiotic.

Concept questions 3.7

1 Lst the seps involvein synthsising a recombinant paid.
2 Recominant plasmis are caled 'vectors'. What does tis meanin tis pplication?
3 Define transfomation' in the conext of genetic enineing.
4 Gene lning is use asan alernative to PCR. How are theydifferent?
5 The gene for huannsulin cn be nserted into a plasmid ad replicated as the bacteria carrying the now recominant plasm d divi.The following flow chart sumarises the stes making nsulin for use in human. Copy the flow chart and add note escribing each step.



HOT Chaenge

6 Transformation is not10% effective. What can biotechnlogists do to en sure that theplasmids of transformd bacter they are daling with are only the ones that have taen up the foreign DNA (e.g. the nslin gene)?

3.8 Genetic engineering in agriculture

WB
3.8.1 LITERATURE REVIEW P. 77

One of the most common applications of genetic engineering is the use of GMOs in agriculture, such as growing crops, managing the pasture of livestock, and aquaculture (farming fish). Genetic engineering provides ways to meet the great, and growing, global demand for food and other agricultural products by:

» increasing yield
» decreasing pesticide use
» improving food quality
» providing disease resistance
» increasing shelf life
» increasing nutritional value
» increasing tolerance to environmental stress (such as drought).

The process used for most of these applications is transformation – the process of transferring a gene from one species and into another to obtain a desired characteristic, producing knock-in or transgenic GMOs. The use of GMOs is controversial and, before their widespread use, there are extensive investigations into their potential impacts on the environment, and the possibility they may be toxic or cause allergies. Ethical considerations are important, particularly animal welfare. Here we will discuss some examples of GMOs currently used or being developed for use in agriculture to increase yields and provide resistance against disease.

Inserting genes to protect crops against pests and disease: Bt crops

The United Nations estimates that 20–40% of global crops are lost to insects pests each year. Insecticides are widely used to kill insects and improve crop yields (Figure 3.38). However, insecticides are a potential risk to humans and the environment.

In the mid-1990s, new GMO crops gave plants intrinsic resistance to insects and avoided the need for insecticides. These crops, including cotton, corn, sweet corn and potatoes, were engineered to express genes taken from the bacterium *Bacillus thuringiensis* (Bt), which encode toxins harmful to a range of common crop pests. The Bt genes were engineered to be controlled by promoters from the plants that resulted in the Bt genes being expressed in the appropriate plant tissues, at the appropriate stage of plant development. The US Environmental Protection Agency found that the Bt protein behaves like a normal dietary protein and is not toxic when ingested, even in high doses. Over time, with increased use of Bt corn, insecticide use in the US decreased (Figure 3.38a).

After 20 years, patents expire and generic brands using the same technology begin to appear. Because the Bt technology is so commercially important, the company that sells it (Bayer) has developed new improvements to the genetic modifications, which produce new intellectual property and new patents, preventing generic copies from taking a significant share of the market. Bt cotton, in its third iteration, now contains three transgenes from *Bacillus thuringiensis*: *cry1Ac*, *cry2Ab* and *vip3A*. The proteins encoded by these genes are lethal to common Lepidopteran pests (moths and butterflies) when ingested. Resistance of the pests to the Bt crops is minimised by having the three proteins, as the chances of the pests developing resistance to all three is much lower.

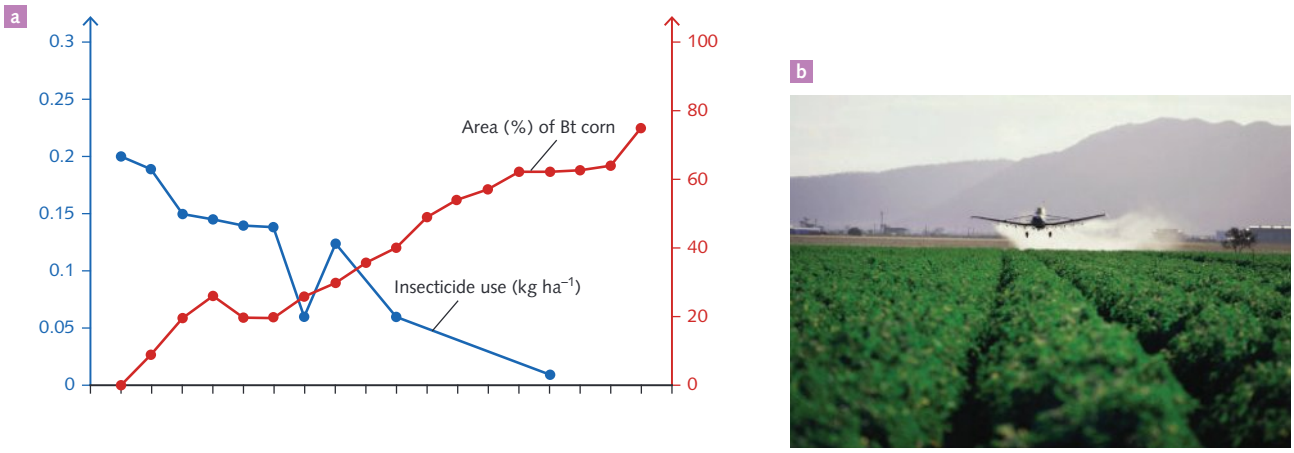


Figure 3.38 Pesticide application in agriculture. **a** In the US, insecticide use (blue) is inversely correlated with the use of Bt corn (red) i.e. insecticide use decreased over time as Bt corn use increased. **b** Insecticide being applied to crops.

Inserting genes to improve growth and yield: AquAdvantage salmon

In 1989, a US company created the genetically modified AquAdvantage salmon, which grows twice as fast as wild salmon (Figure 3.39). This was achieved by introducing a growth hormone gene from a Pacific Chinook salmon into the salmon genome, under the control of a promoter sequence from another fish, the ocean pout. The promoter sequence switches on the growth hormone permanently, rather than only in the spring and summer, the normal growth periods for wild salmon. It would normally take 3 years for salmon to grow big enough for consumption, but the genetically modified salmon reaches the required size in 1.5 years.

Figure 3.39 The genetically modified AquAdvantage salmon is twice the size of non-genetically modified salmon at the same age.

This has commercial advantage for the company, but caused concerns that if AquAdvantage salmon escaped into the wild they may out-compete their smaller wild counterparts. AquAdvantage salmon are housed in land-based farms far from streams or the ocean and enclosed by a series of dam barriers.

AquAdvantage eggs are also treated with high pressure to produce triploid eggs (containing three sets of chromosomes rather than the usual two). This makes the salmon sterile (unable to reproduce because their cells cannot achieve homologous pairing of chromosomes in meiosis) and redirects metabolic energy into growth instead of reproduction.

ACTIVITY 3.2

GMOs in science and society

Before gentilly mdi fied samon was permtted to be grown and slin the US and Canada the US Food and Drug Admnstraton nvestgated wh ether ths new GMO met strct safety reuiremets.This comprehesive aalsis was based on scent fic evdence and determned that the genetcay mod fied samon was safe to eat and that the genetc modificaton was a safe n terventon to the fish tsef

n Austalia, he CSIRO hs a etensive selectie breein program t iprove Atlantic salmon. This involves anaysing the performance o samon to select which fish to breed for the next gneation. The progrm aims to select key performance traitssuc as groth, resistanctodisease and carcass quality.

You areolicymakers, and ave been appointed to a food and safetyregulatory bard to advise ether genetically modified almonshoul be importdand sl in ustrali, or ifthe selecive reeding program should be given more fudig. You taski tcritically ompare and evaluate the AquAdvantae salmon strategy and the CSIRO breeding program and construct an argument to spprt your opinion. (R emember that when yo comare, you look at how they are iilar and different.)

Am

To detemine whethe r the genetcay mod fied samon shoud be mpo rted and sod n Austraa or f the seectve breeing program shuld be prortsed by crtca y anaysng reasons for and aganst both strateges

Youll need

iteboard

» W
» Whiteboard markers

What to do

1 In your grop,llocate who w argue for AquAdvantage slmon and wh o w argue for the seectve breeing program.
2 Summaise the AquAdvantage or slecive bree dng program ith a paragraph and dagram
3 Compare and debate AquAdvantage samon versus the seectv e breeing progrm. As pects to consder ncude
   » benefits of getically modi fied slmon
   » potetia envirnental impact
   »impact if getically modi fied almon esaped to the wild
   » conainmenaciit required.
4 After a iscusio, evluate the arguments on bothsides to make your own choce
5 As a personl re flecton actvty, go through the same process to evauate Bt crop, Glden ice and Roundup Ready crop. You may need to do some research on te Interet.

KEY CONCEPTS

» GMOs are used requntly i agriculture. They can be enineered to ave incrased yield, reduced need for peicide and incrased utritional value.
» Ehial issues surround the use of GMOs and must be specificlly ealuated in each case.

Concept questions 3.8

1 What typeof GMO is the fast-growing salmon?
2 GMOs have been conoversil since heir introduction. Wha might be some of the bene fits and risksof using GMOs?
3 *Bacillus thuringiensis* (Bt) has genes tha are toxic to a number of crop psts. Ho has this information been harnessed by the griultral community to improve crops?

HOT Chaenge

4 Large chmica companiespant te intellectual property assoiated with number of GMOs. Eventully these patet expire.
   a Why do companies patent GMOs?
   b What happens when the patents expire?
   c How can cemcal ompnies limitthe resistance of the pests to the GMO crops?

BRANCHING OUT

CRISPR-Cas9 reveals we don't know cancer drugs like we thought we did

Cancr is a major cause of deathnAstrala Intense medical re searh aims to discover the genes that cancers depend on to aggresively grow ad spred. Tese discveries can reveal potetally drggable targets to treat acer (Figure 3.40). Most cancer drugs target proteins that are essential for cancer lldvsion and urival.

US sietists wre trying to find gene involved n reguaing cance growh. They inclded a positive control n their exermet. This was gene called *MELK* whch i important for cncer ell growth. The researchers sed CRISR-Cas9 to disable the genes they were suding.Sikingl, they didn't see any effect ofknocking out *MELK* on cancer ll groth.

To doble check this ffect, the researchers tested a drug dvelopeto inhibitthe protein encoded by *MELK* Even ithout *MEL,* the drug ws still very effetive killing ancer cells.

Tis perlexin result suggested that the drug nteracte with ohe moleculesthan that which it was dsigne for to kill ancercells. Puzzled, the researchers proceeded to test oher cancer drugs, most of whc like the *MELK* nhbitr, showedeffectiveness n prelinca studies and hd ented ntoclinical trials. Each drug gave thesame result when the speci fic target prtein was knocked ot usingCRISPR-Cas9, the drugt killed cner cells. These re suts demonstrated that the mechanism by whch researchers thought most cancer drugs workd wa incorct.How is it possible that tis new research s strongly conradicted previous findngs? Before CRISPR-as9, mot scienti fic eidence for the drug targs was collecte d from expriments that usd a chnique called RN interference (A), ich silences gene expresion byihbitiR A molc les. However, this technique can have off-targt ffcts, alteing the expression of other genes thatcould pntially ex plain te diffeence i rsults.

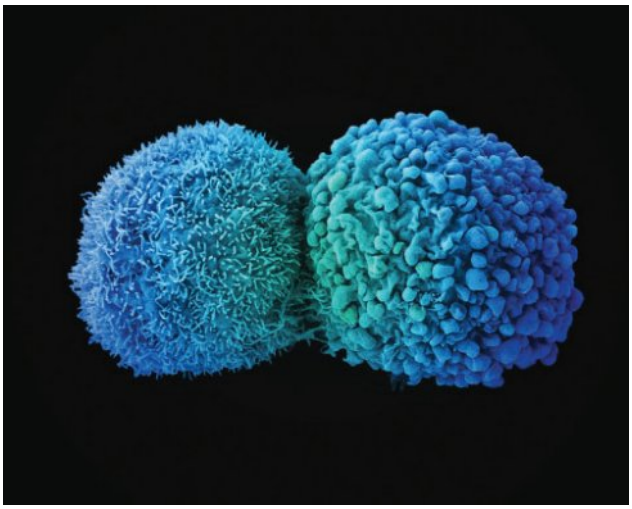


Figure 3.40 Lung cancer ces Cancer drugs aim to inhibit cancer cells from growing and spreading.

Tis research has n implications for ance drug developent. The process o etting a cancer drug to the clinic is on. There ar strict and robust tests that a drug needs to pass to show tht it is safe and ftiv Clinical trials are th e ast, major part of this long proess tests, involving te sing of the copound in people who have onsented. Consider theiical rils in which rugs from tis study were used. Th ese drus alone were usd i 29clincal trials, with each ta iming to have u to 1000 voluneers. Unfortunately, ma ny drugs do notmake it trulinical trial for reasons that are oftenunclea, despite larg mounts of promising re search beforete trial Perhaps this esearch might provide someexpanation as to why mst drugs don' aei hrough clinical trials.

Questions

1 Decribe ow appying the CRISPR-Ca system or genome editig has cotributed to understanding the actions of new drugs to treat cancer.

2 The cancer dugs in tis experiment ee tested on olated, cultured cells *in vitro* (n th laboratory) that were geneticllymodi fied sing te CRISPRCas9 system.
   - **a** Wh might the findngs difer in a human subject?
   - **b** Ouline a process to de fine the drug targe that s, th protein to hich th drug binds, beginning in the aboratory and con firing the trget inhuma tissues.

3 Imagine you ar decision makerin Theraptic Goods Australa (TGA), the body that regulates the pre-market testng, supply and manufacture ofdugs in Australi. Youhave ben tasked with updating the guidelines for the assessment of th reclinical esearch studies of new drugs that aresubmitted to the TGA for approval for use in cancer.
   - **a** ist someneguideliesyu mght include inthe new era of CRISPR.
   - **b** Cosider a drug that shos effectveness in clinical tria s for cancer treatment,but wich CISPR studies later show not to work though tspreviously de fined target potein. As aTGA decision maer,what new information would you needto decide whether or not to approve the new drug?

wutaue . emVnui e, dC
Chapter 3: Summary of key concepts

3 Summary of key concepts

3.1 Genetically modified organisms

KEY CONCEPTS

p81

- Geneticengineering refers toe use of living things to make new products or systms y switching eneson or off, removing genes or ntroucing genes fromonespecie into another.
- Oranisms tht are altered or prducd y genetic engineering tecniques are known aseneically modi fied oranisms (GMOs).
- Gentaly modi fied oranimsinclude knock-ot organisms, knoc-in organisms and transeni organisms.
- Knock-ou organisms have ad a gene deleed or its function interruptd.
- Knok-in rganisms have had a gene or fragment of DN inserted int apaticular locus in the genome.
- Transenic organisms have had a gene or fragent of DNA inserted nto a non-speci fic ocs.

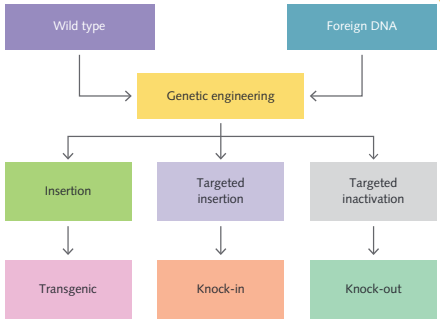


Figure 3.3 Genetically modified organisms include knock-out, knock-in and transgenic organisms

3.2 Enzymes for modifying DNA

KEY CONCEPTS

p84

- DNA polymera es catalyse the foration of new DA molecules from free nulotides ad a templat DNA strand.
- The technology that recombines DNA fromdifferent sources o modify the DNA sequenc is alled ecombinant DNA technolgy.
- The cutin tools used are enzymes known as retrictio endnucleases, or restrction ezymes.
- Endoncleases can gnerate blunt or sickyends.
- DN liase is an enzym used to join dfferent ieces of DNA together.
- Coplementry sticky ends help DNA strand bind to each oher via hydrogen bondig.

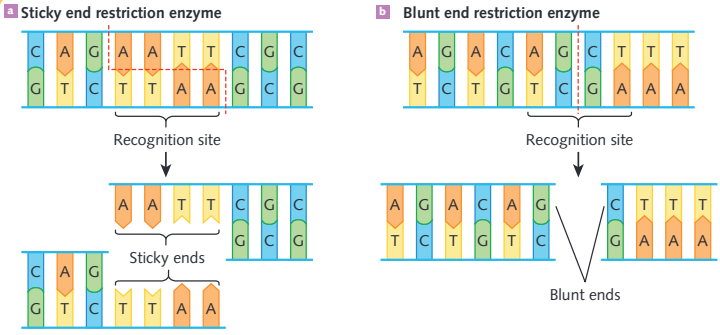


Figure 3.5 a Sticky ends produced by cutting DNA with the endonuclease EcoRI. **b** Blunt ends produced by cutting DNA with the endonuclease AluI

9780170452533

3.3 CRISPR-Cas9

KEY CONCEPTS

p87

- The RISPR system i acterial immune defence mecanis agist vira infection.
- CRISPR-Ca9 is applied to edit genomes to preiely create knock-outor knok-in genetic modifications.
- Aliationsof CRISPR-Cas9 technology nclude editin falyallelesin diease, improving agrculture nd making newmouse strains for sieti fic research.
- The use o CRISPR-Cs9 tchnoogy raises serious biotica questions.

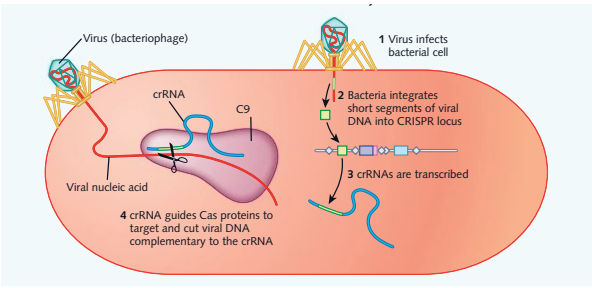


Figure 3.8 The CRSPR-Cas9 system n bactera and ts appcaton to genetcay mo dfy DNAA short mlecle of RNA (gude RNA) bnds to the target gene and gudes the Cas9 enzyme to the gene the Cas9 enzyme breaks the DNA and the bacter a ce tres to repar the break Ths can resut n a fram eshft mutaton – deeton or nserton of a gene – f a donor DNA fragmentis ncorporated

3.4 Amplifying DNA

KEY CONCEPTS

p92

- PC is a process tht ampli fies a specific DNA sequence foralysis.
- The sequence ofthe primers determines the DNA sequence to bempli fied.
- The stpsinvolved in PCR aredenaturation, annling andextension. These steps are repeated may tis t yield a large number of dentical DA oecules.

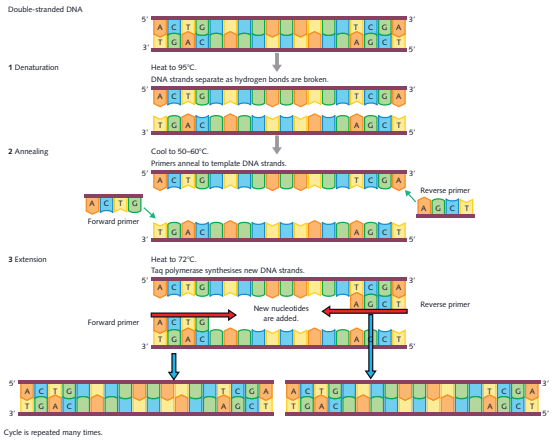


Figure 3.12 Amplifying DNA using PCR

3.5 Gel electrophoresis

KEY CONCEPTS

p95

- Ge electrophoresis separates DNA moecues by sze.
- Negtively charge DNA travels through gel atrix towds a positive electrde.
- DNA s vualied with aDNA binding dye.
- Thesizes of DNA fragments can be etimated y cmparing their movement through he gel with that of fragments now sizes, referred to as mlecuar siz markers.

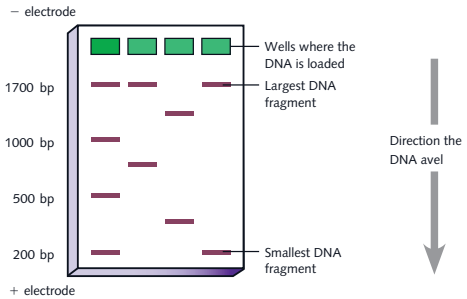


Figure 3.15 Standards are molecular markers of known size that are run alongside samples and allow estimation of the size of the DNA fragments migrating through the gel

3.6 DNA profiling

KEY CONCEPTS

p. 98

- DNA profiling is used to solve crimes, determine family relationships and identify human samples.
- By examining STRs in non-coding regions of DNA, a profile can be obtained that can be compared to a database of samples given by family members.

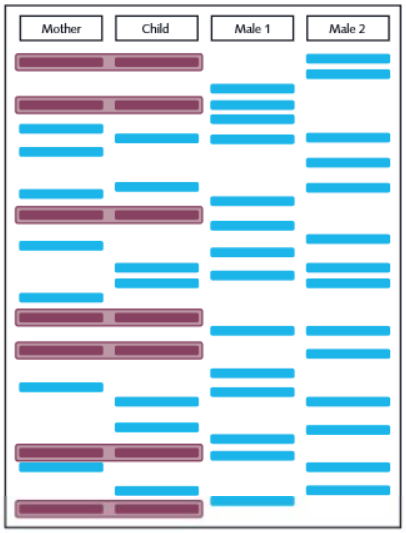


Figure 3.23 This gel electrophoresis run reveals the DNA profiles of the four individuals in case study 2. Primers for seven STR regions were used to generate these DNA profiles

3.7 Recombinant plasmids and human insulin

KEY CONCEPTS

p. 105

- Gene cloning is an alternative to PCR for generating many copies of DNA. It uses bacterial plasmids to produce many copies of a gene.
- A DNA fragment can be inserted into a plasmid and replicated as the bacteria carrying the plasmid divide. The plasmid is then called a recombinant plasmid.
- Recombinant plasmids can be used to produce insulin for use by people with type 1 diabetes.
- Recombinant plasmids usually have an antibiotic resistance gene added that allows them to be selected. Only the plasmid-carrying bacteria can grow in the presence of the antibiotic.

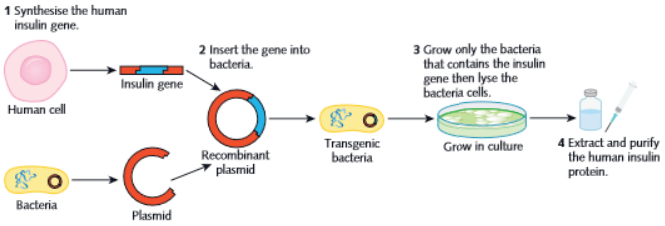


Figure 3.25 The steps involved in producing insulin in bacterial cells

3.8 Genetic engineering in agriculture

KEY CONCEPTS

p. 115

- GMOs are used frequently in agriculture. They can be engineered to have increased yield, reduced need for pesticides and increased nutritional value.
- Ethical issues surround the use of GMOs and must be specifically evaluated in each case.

Figure 3.39 The genetically modified AquAdvantage salmon is twice the size of non-genetically modified salmon at the same age

WB
3.9.1 KEY TERMS PAGE 79

3 Chapter glossary

agarose gel a gel matrix used for electrophoresis

annealing a process used in the polymerase chain reaction to join separate strands of DNA together as a result of hydrogen bonds pairing; occurs when the temperature is lowered

antibiotic selection growing bacteria in the presence of an antibiotic so only cells containing a gene for antibiotic resistance (encoded on a recombinant plasmid) can grow

bacteriophage a virus that can infect bacteria and replicate

bioethics the study of ethical issues emerging from advancements in biology

biotechnology the use of living organisms and biological systems and processes for human benefit

blunt end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at the same position on both strands

Cas9 protein an endonuclease that cuts double-stranded DNA at a target location in the genome

CRISPR-Cas9 a bacterial immune defence mechanism in which short RNAs target complementary sequences in viral genomes to guide Cas9 proteins to destroy an invading virus

crRNA (CRISPR RNA) RNA transcribed from the CRISPR locus; guides Cas9 proteins to their complementary sequence in the invading viral DNA genome, targeting it for destruction by Cas9

DNA ligase an enzyme that catalyses the formation of a phosphodiester bond between two pieces of DNA

DNA profiling comparison of individuals based on patterns of non-coding base sequences in the genome

DNA sequencing the process of establishing the nucleotide sequence of a piece of DNA

frameshift mutation a mutation in DNA caused by the addition or deletion of a nucleotide or nucleotides resulting in a change in the amino acid sequence and protein being made

gel electrophoresis a technique that separates DNA fragments according to their size and charge

gene cloning the process of using plasmids and bacteria to make numerous identical copies of a gene

genetic engineering manipulation of genetic material, including altering DNA in an organism to suppress or enhance a gene's activity, or combining genetic material from different species

genetically modified organism (GMO) an organism whose genome has been genetically engineered

guide RNA RNA that guides the Cas9 protein to the target sequence in a genome for gene editing

knock-in organism an organism in which DNA has been inserted into a specific locus

knock-out organism an organism whose DNA has been modified to disable the expression or function of a gene product

molecular size marker a set of pieces of DNA of known length that is used to estimate the size of other DNA fragments in a gel

polymerase chain reaction (PCR) a cyclical reaction in which DNA polymerase is used to copy a DNA template, making millions of copies of the same piece of DNA

polymorphism a variation in DNA sequences among individuals

primer a single-stranded DNA molecule that acts as the start of the amplification process

recombinant DNA technology the process of transferring a gene from a cell of one species to the cell of a different species

recombinant plasmid a plasmid with foreign DNA inserted into it

reporter gene a gene that enables visualisation or quantification of gene expression

restriction digest reaction a reaction in which restriction enzymes are incubated with DNA to cut the DNA into fragments at specific restriction sites

restriction endonuclease (restriction enzyme) an enzyme that cuts DNA at a specific restriction site

restriction fragment a short fragment of DNA generated after the cutting of a longer DNA fragment by a restriction enzyme

restriction site a specific nucleotide sequence (usually 4–8 bp) that is recognised as a cleaving site for a restriction enzyme

short tandem repeat (STR) a short non-coding region of DNA of up to five bases that is repeated many times in the genome of an organism; the number of times an STR is repeated is variable and can be used in DNA profiling

sticky end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at different positions on each strand

transformation the process by which the genetic material of an organism is changed by the addition of new genetic material

transgenic organism an organism that has been modified by incorporating a piece of foreign DNA into its genome

vector a vehicle used to transfer DNA sequences from one organism to another

wild type the genotype or phenotype that is most common, or standard, in natural conditions, in contrast to an atypical or mutant form

WB
3.9.2 EXAM PRACTICE PAGE 82

3 Chapter review

Remembering

1 Match each term in the first column with a description in the second column. Use each item only once.

DNA ligase	Small circular self-replicating DNA molecule
Vector	Sorts DNA molecules by size and charge
Primer	Joins two single-stranded sections of DNA together
Blunt end	Specific site at which restriction enzymes cut DNA
Plasmid	Vehicle to introduce DNA into a host cell
Restriction site	Enzyme that catalyses the synthesis of DNA
Gel electrophoresis	Results from cleavage by a restriction enzyme in the middle of the recognition sequence (restriction site)
DNA polymerase	Synthetic short, single-stranded DNA molecule

2 Recall the features of a plasmid vector.
3 What makes the features of a plasmid vector useful for biotechnology?
4 How could DNA ligate to itself?
5 State why the temperature is lowered to 50–60°C during the annealing phase of PCR.
6 How do biotechnologists use the concept of wild type?
7 In forensic science, what method is used to distinguish one sequence of DNA from another?
8 What is gene cloning used for?

Understanding

9 Consider Figure 3.41. Predict whether the cuts made by restriction enzymes produce sticky or blunt ends. (The lines show the cuts lines in the double-stranded DNA.)

Figure 3.41

10 What would happen if the electrodes on a gel electrophoresis tank were accidentally swapped?
11 For how long would you apply a current to the gel during electrophoresis?
12 Agarose gels can be made with different concentrations of agarose. If increasing the concentration of agarose results in a denser gel matrix, what would be the effect on DNA migration speed?

Applying

13 Predict the minimum band-sharing percentage in the DNA profiles of a mother and her baby.

14 Consider Figure 3.42. Four samples (a, b, c and d) containing DNA fragments of the sizes given below, were accidentally swapped around while being loaded into the gel. Identify which lane corresponds to each sample.

- **a** 200, 250 and 900 bp
- **b** 150, 400 and 600 bp
- **c** 50, 450 and 650 bp
- **d** 100, 100 and 450 bp

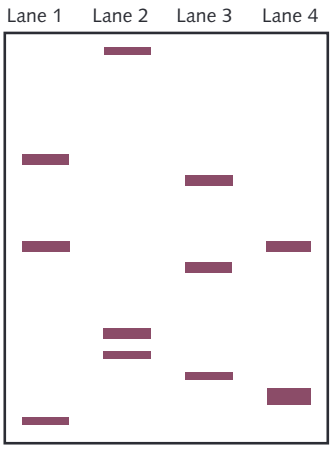


Figure 3.42 Gel electrophoresis

15 Predict whether digestion of the human genome by AluI or by EcoRI would result in the larger number of fragments and explain why.

16 The following section of DNA shows a sequence of 120 bases in one strand of DNA. Refer to Table 3.1 on page 84 for restriction sites.

ATATGTGTGGATCCGTCTTAGGTTATCGAATTCTAGAGCT
ATGGCCTATTAGCTTCCTGGATCCA ACCTGTATAGAGCTA
CTCGTCAGCTATTGCTACGGGATCCTAGCTGATTGGATTC

- **a** How many BamHI and AluI restriction sites are there in the sequence?
- **b** If the sequence was cut by BamHI, how many fragments of DNA would be produced?
- **c** If the sequence was cut by AluI, how many fragments of DNA would be produced?
- **d** If the sequence was cut by both BamHI and AluI, how many fragments would be produced?
- **e** If this piece of DNA was circular and not linear, how many cuts would have been made by BamHI to get the number of fragments stated in part **b**?

17 What is ethidium bromide used for in gel electrophoresis?

18 It has been said that restriction enzymes form part of the 'immune system' of bacteria. Use the information in this chapter to explain your understanding with reference to viruses.

Analysing

19 When conducting PCR, some unwanted DNA molecules are sometimes present.

- **a** Identify the possible consequences of having an unwanted DNA molecule in the PCR.
- **b** Identify two possible sources of this contamination.
- **c** Suggest what could be done to prevent this contamination.

Evaluating

20 A gene with unknown function, named *Taurin*, has been cloned and used to generate a *Taurin* knock-out mouse strain. In mice, the *Taurin* gene contains three exons and two introns. The knock-out mutation was achieved by inserting a small region of irrelevant DNA into exon 1 of the *Taurin* gene, causing a frameshift mutation that resulted in a premature stop codon and prevented translation of *Taurin* into a protein. The knock-out mouse strain was analysed for any abnormalities by comparing the knock-out mice to wild-type littermate (sibling) control mice. It was noted that the knock-out mice developed tumours characterised by B cells proliferating in an uncontrolled manner (Figure 3.43).

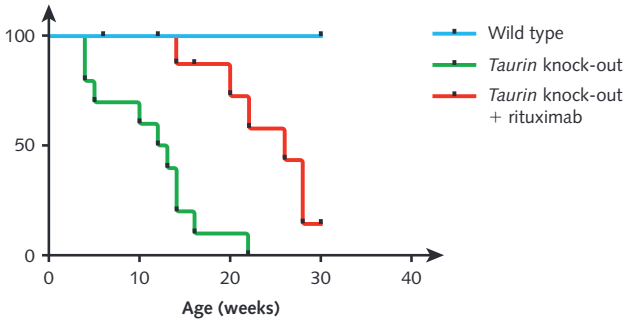


Figure 3.43 Tumour-free survival in Taurin knock-out and wild-type control mice

- **a** What is the function of the *Taurin* protein that can be inferred from this observation?
- **b** Why are the knock-out mice compared with wild-type littermate controls?

9780170452533

c Draw diagrams of the *Taurin* gene locus in wild-type mice and the same locus in the knock-out mice.

d How could you use PCR to distinguish between wild-type mice and mice carrying the knock-out mutation? Draw PCR primer binding sites in the diagrams you drew for part **c**.

e The knock-out mice were treated with rituximab, a monoclonal antibody used to deplete B cells. What can the survival curve of the treated mice tell you about your answer to part **a**?

f What important control is missing from the experiment represented in Figure 3.43?

21 Consider Figure 3.44. It shows a developed agarose gel from electrophoresis of a sample taken from a crime site. Decide whether the defendant is guilty or not guilty and justify your reasons.

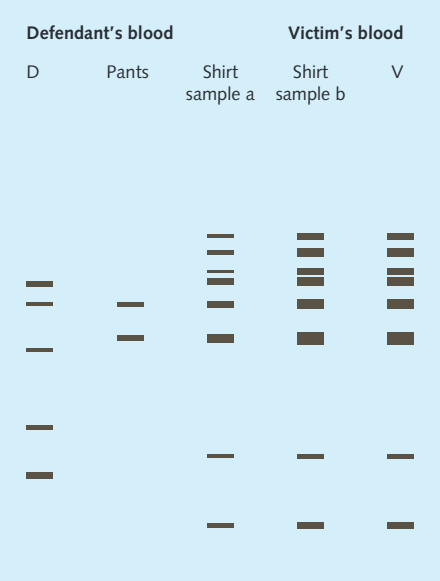


Figure 3.44 An electrophoresis of a sample taken from a crime site

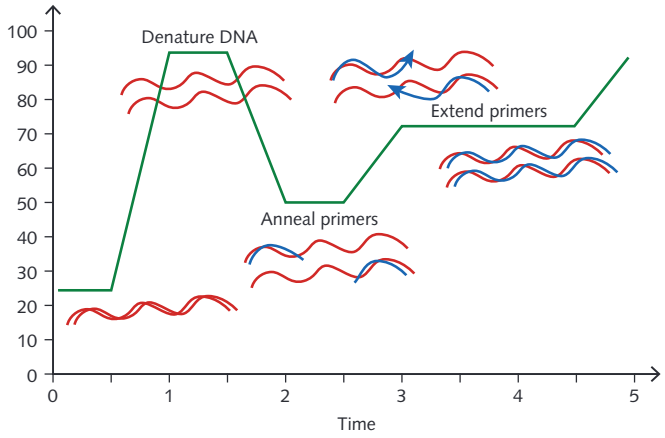


Figure 3.45 The polymerase chain reaction

22 Interpret the graph in Figure 3.45 by summarising the activity being undertaken. Make sure you refer to the data in the graph in your analysis.

Creating

23 A new restriction enzyme has been discovered, called StarI. Design a strategy for large-scale production of StarI for use in research laboratories. Draw a flow chart outlining the strategy.

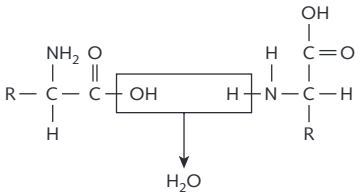
Reflecting

24 Many companies have started labelling their food products 'GM free'. Considering your knowledge of biotechnology techniques and applications, what do you think of the use of this type of labelling for consumers with a non-scientific background?

Unit 3, Area of Study 1 review

Multiple choice

Question 1 ©VCAA 2018 Q3 ADAPTED EASY



The diagram above represents adjacent amino acids being joined together.

The joining of adjacent amino acids

A results in the formation of a dipeptide.
B is a reaction that releases energy.
C is catalysed by the enzyme DNA polymerase.
D is a combustion reaction.

Question 2 ©VCAA 2018 Q2 ADAPTED EASY

The proteome is

A the total set of proteins present within a single organism.
B a complete set of chromosomes found inside a sex cell of an organism.
C the entire set of DNA expressed by an organism at a given time.
D the entire set of tRNA in a cell's cytosome.

Question 3 ©VCAA 2008 Q4 ADAPTED MEDUM

The genetic code is described as a universal code.

This means that

A each amino acid is only coded by one specific codon.
B some amino acids may be encoded by more than one codon.
C a single nucleotide cannot be part of two adjacent codons.
D in almost all organisms the same DNA triplet is translated to the same amino acid.

Question 4 ©VCAA 2009 EXAM 2 Q17 ADAPTED HARD

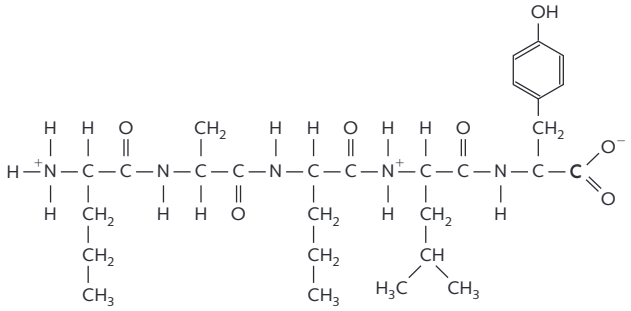
The process of gene expression involves

A transcription, which follows translation.
B four nucleotides on each tRNA molecule, that carry one. specific amino acid.
C every cell of the organism that contains a particular gene, all undergoing identical action simultaneously.
D the production of a polypeptide.

Question 5 ©VCAA 2008 EXAM 1 Q3 ADAPTED MEDUM

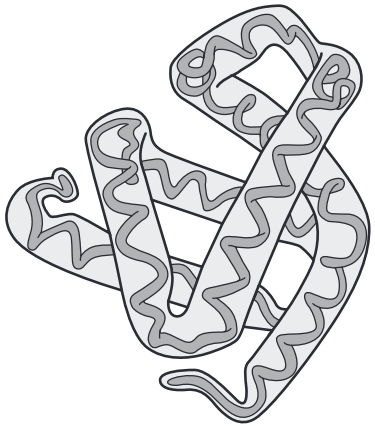
The tertiary structure of a protein is represented by

A

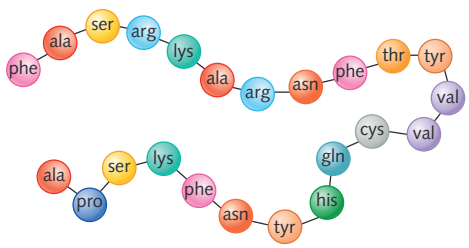


B

C



D



Question 6 ©VCAA 2019 Q38 ADAPTED EASY

DNA ligase

A forms phosphodiester bonds between the two fragments of DNA to join them together.
B cuts DNA molecules at specific nucleotide sequences.
C is an enzyme involved in protein synthesis.
D separates two DNA strands during translation so that a copy can be made.

Question 7 ©VCAA 2017 Q2 ADAPTED MEDUM

To begin transcription of the five structural genes in the *trp* operon, RNA polymerase needs to bind to the

A promoter gene.
B structural genes.
C regulatory gene.
D operator gene.

Question 8 ©VCAA 2014 Q25 ADAPTED MEDUM

Genes are often transferred from one species to another using bacterial plasmids. The process can be represented as follows.

bacterial plasmid cut

foreign gene and plasmid mixed

plasmid with inserted foreign gene

These steps are facilitated by enzymes.

Identify the two enzymes required for the first and last steps of the process, from the following table.

	Cuts plasmid	Inserts genes
A	Restriction enzyme	DNA polymerase
B	Restriction endonuclease	DNA ligase
C	DNA ligase	DNA polymerase
D	DNA polymerase	Restriction enzyme

Question 9 ©VCAA 2017 Q34 ADAPTED MEDUM

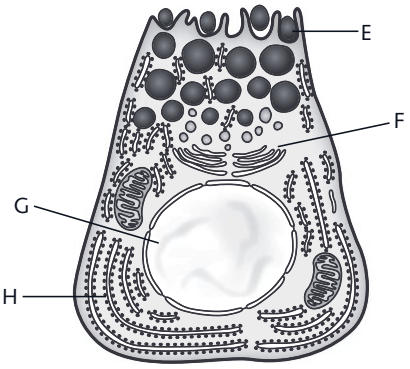
The polymerase chain reaction (PCR) is a process that involves repeated cycles made up of several steps.

During PCR, the

A first step in each cycle is to denature the DNA at a high temperature.
B final step of each cycle involves the use of DNA ligase.
C second step in each cycle is to heat the DNA to a high temperature.
D temperature must be lowered to 37°C before the beginning of each cycle.

Question 10 ©VCAA 2012 EXAM 1 Q7 ADAPTED EASY

Examine the following diagram of a cell in the digestive system, which secretes the hormone leptin, which regulates appetite.

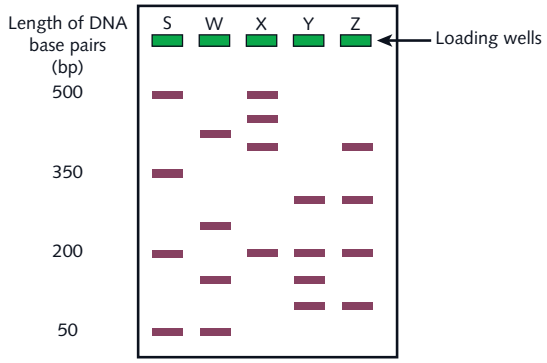


The secretion of proteins from the cell requires organelles operating in which order?

A E, F, G, H
B H, G, E, F
C F, E, H, G
D G, H, F, E

Use the following information to answer Questions 11 and 12.

Four samples of DNA were loaded into four different wells in lanes W, X, Y and Z. A standard ladder (DNA of known lengths) was loaded into the well in lane S. The results of gel electrophoresis are shown below.



Question 11 ©VCAA 2018 Q30 ADAPTED EASY

Which lane represents a sample that was loaded with DNA fragments of four different lengths: 50 bp, 150 bp, 250 bp and 450 bp?

A W
B X
C Y
D Z

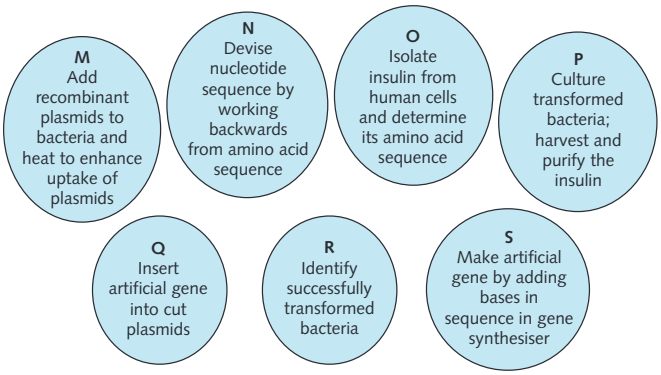
Question 12 ©VCAA 2018 Q31 ADAPTED MEDUM

Which sample lane contains the band that is closest to the positive electrode?

A W
B X
C Y
D Z

Question 13 ©VCAA 2013 Q34 ADAPTED MEDUM

Insulin is made in commercial quantities by using cultured bacteria that have been transformed with an artificial insulin gene. The steps taken to produce insulin by this genetic engineering are summarised below. The order of the steps has been mixed up.



The correct sequence of steps when producing the insulin is:

A P, R, Q, M, O, S, N.
B M, S, N, R, P, Q, O.
C P, R, O, M, Q, N, S.
D O, N, S, Q, M, R, P.

Question 14 ©VCAA 2017 Q40 ADAPTED MEDUM

Many plant crops are affected by viruses. Some viruses can affect many different crops. Scientists have trialed a spray treatment for one such virus that infects tomato crops. This treatment does not alter the DNA of the tomato plants.

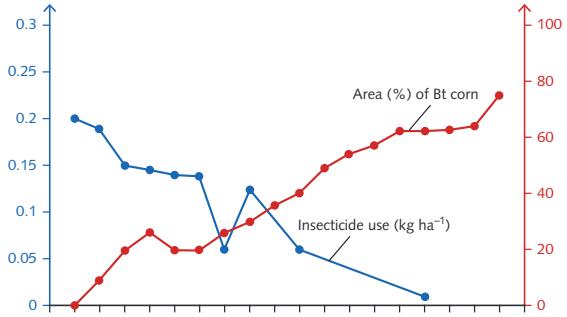
Clay nanoparticles containing double-stranded RNA (dsRNA) are applied to the surface of the growing tomato leaves. The dsRNA released from each of the clay nanoparticles enters the plant cells where it silences a gene from the virus by causing the breakdown of viral RNA.

This technique

A demonstrates dsRNA has a nucleotide sequence complementary to a section of DNA nucleotides in the tomato plants.
B causes dsRNA to stop expression of the viral gene by preventing translation.
C would not be effective on other crop species.
D would result in the tomato being defined as a GMO.

Question 15 ©VCAA 2019 Q37 ADAPTED MEDUM

Bt corn expresses a protein that acts as an insecticide to protect from insect attack.



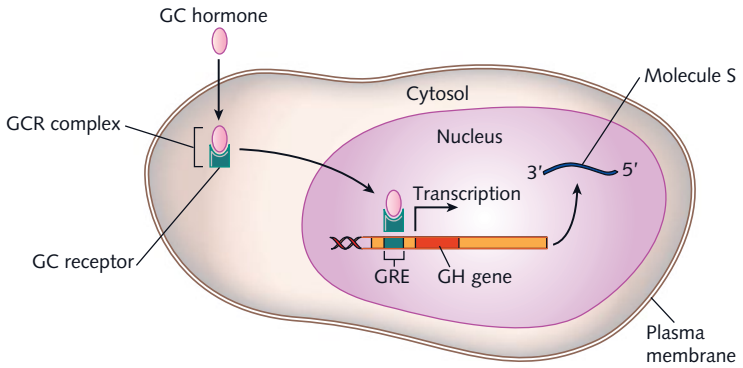
Based on the data in the graph above and your knowledge, what is a benefit of using Bt corn?

A Less insecticide is used with Bt corn crops.
B Bt corn is more expensive to produce than non-Bt corn.
C Negative impacts on ecosystems would be increased.
D More farmers are predicted to plant Bt corn in the future.

Short answer

Question 1 ©VCAA 2015 SECTON B QUESTON 7 ADAPTED EASY

The diagram below shows signal transduction of glucocorticoid (GC) – a hormone in humans. GC binds to a receptor in the cytosol. The glucocorticoid–receptor complex (GCR–complex) then moves into the nucleus and attaches to the DNA, causing transcription to begin.



The glucocorticoid response element (GRE) is the location where the GCR–complex attaches to the DNA. The GRE is located approximately 250 base pairs upstream of the growth hormone (GH) gene. After the GCR–complex has attached to the GRE, an enzyme catalyses the transcription of the gene.

a Name the enzyme that catalyses transcription.

1 mark

b State the role of the transcription product, molecule S.

1 mark

c Describe the processing that molecule S undergoes before it exits the nucleus.

2 marks

d In the human pituitary gland, GC stimulates the production of the human growth hormone protein. However, in the human liver, GC stimulates the production of the enzyme CYP3A4. Explain how the production of distinct proteins in different cell types could occur, given that the genetic sequence is identical in all somatic human cells.

2 marks

e If a rat gene is inserted into the DNA of human pituitary gland cells, these genetically engineered cells can be used to produce rat growth hormone. What characteristic of the genetic code enables a rat protein, such as rat growth hormone, to be made by human cells?

1 mark

Question 2 ©VCAA 2018 SECTON B Q 1 A B ADAPTED

Trypsin is an enzyme that is released from human pancreatic cells.

Nucleic acids encode instructions for the synthesis of trypsin in a pancreatic cell.

a Outline the steps of translation of trypsin synthesis.

1 mark

b After being synthesised, trypsin is released from pancreas cells via exocytosis.
Copy and complete the table below by naming three different organelles directly associated with the transport of the synthesised trypsin within or from pancreatic cells. State the role of each organelle in this process.

2 marks

Organelle	Role

Question 3 ©VCAA 2009 EXAM 1 SECTION B Q2 ADAPTED

a A section of the template strand of a DNA molecule has the sequence of bases shown.

1 mark

DNA: G T G A C A T T A C T C

Copy the table below and enter the sequence of bases in the corresponding mRNA that is complementary to this DNA.

mRNA												

b The percentage of base G in a molecule of DNA is 20%. What is the percentage of T bases in the same DNA molecule?

1 mark

c Another type of nucleic acid is rRNA.

i Where is rRNA found in a cell?

1 mark

ii Describe the role of rRNA.

1 mark

d The table shows the names of six amino acids together with some of their DNA codes.

Amino acids	DNA triplet(s)
Cysteine	ACA, ACG
Histidine	GTA, GTG
Aspartic acid	CTA, CTG
Asparagine	TTA, TTG
Leucine	GAA, GAG, GAT, GAC
Methionine	TAC

Use the information in the table and write the order of amino acids coded for by the DNA sequence given in part **a**.

1 mark

e Nucleic acids are made up of nucleotides. Each nucleotide consists of three components – nitrogen base (B), phosphate (P) and sugar (S) – linked together in a particular way.
Draw a diagram to show the way the three components are joined to make a nucleotide.
Use the following symbols in your diagram. 1 mark

Enzymes and the regulation of biochemical pathways

4

By the end of this chapter you will have covered the following material.

Key knowledge

Regulation of biochemical pathways in photosynthesis and cellular respiration

- the general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product pp. 137–141
- the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration pp. 142–144
- the general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors pp. 145–150

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify independent, dependent and controlled variables in controlled experiments pp. 147–148
- predict possible outcomes pp. 147–148

Plan and conduct investigations

- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 147–148

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 147–148
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 147–148

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 147–148
- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 147–148
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 147–148

Analyse and evaluate data and investigation methods

- identify and analyse experimental data qualitatively, handling where appropriate concepts of: accuracy, precision, repeatability, reproducibility and validity of measurements; errors (random and systematic); and certainty in data, including effects of sample size in obtaining reliable data pp. 147–148

Construct evidence-based arguments and draw conclusions

- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 147–148

Online Chapter Map
Chapter 4 map

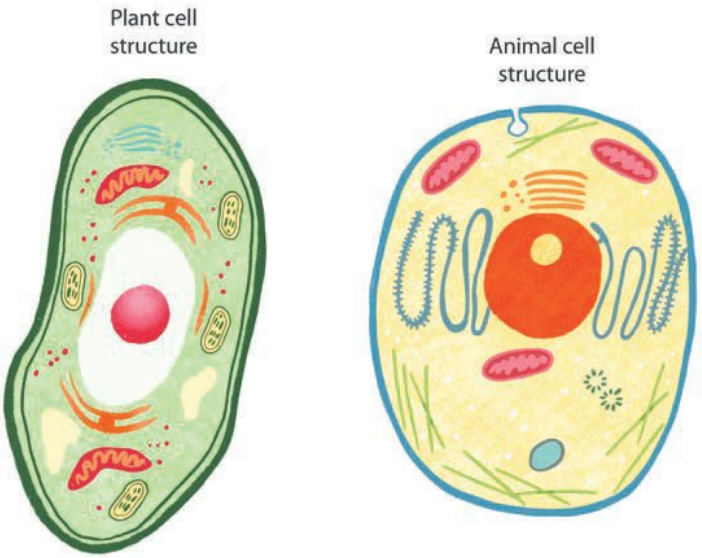
4 Enzymes and the regulation of biochemical pathways

Your body can only carry out all its necessary functions because you have the correct enzymes. Enzymes are proteins, which are encoded in DNA and made by ribosomes.

41
Bochemca pathways for ce metabosm

A the chemca reations that occur n a ce are known as ceuar metabosm Some of these reations use energy(.g. photosyntesis) and some rlease energy(.g.elular repirtio). Thesebiochmial pathways are regulatd in a step-ke fashon wth each step contrlled by enzymes

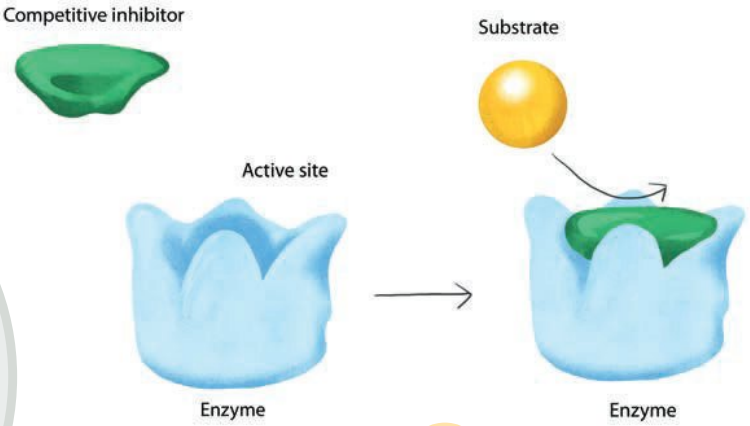
p 137



p 145

43
Photosynthess and ceuar respraton

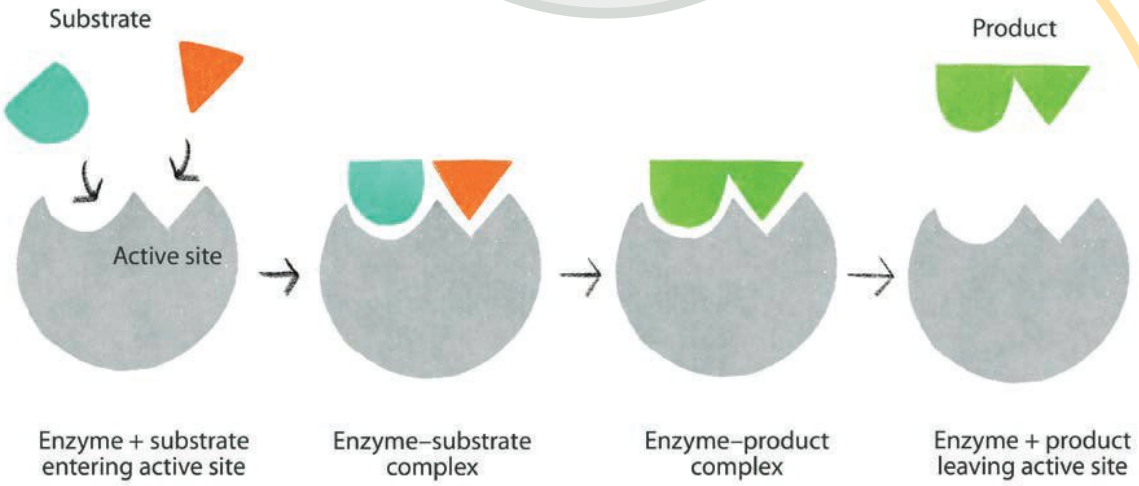
Enzymes are proeis, so enzyme functon s affected by envronmentl factors such as temperatur. Heat can denature enzymes and ower temperatures can sow reacions dow. Other mlecles can bock enzyme actvty, whch stops or lows down metaboc reacion.



p 142

42
Enzymes
the key to controng
bochemca pathways

Without enzyme, our boies wold
functon very sowy, f at a Enzymes
speed up chemca re actons by arrangng
the correct substrate moecues so that they
fit together ke a ock and key.Different
substrates reuir di fferent enzyms.
Once the chemca reation has
occurre, the enzy mes ativesite
s freed so the enzyme can
work agan



You have a lot to thank enzymes for. Without them, chemical reactions would occur very slowly, or not at all. Life would not be possible without enzymes.

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Online Chapter Mapv
- Chapter 4 map (p. 134)

Onne.e:Ker, m v
- Chapter 4 flashcards (p. 136)

Weonkm v
- Enzymes, active sites and cofactors (p. 143)
- Enzyme-mediated reaction under different conditions (p. 145)

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- Enzymes in biochemical pathways (p. 143)
- Enzyme catalysis (p. 145)

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- Chapter 4: Summary of key concepts (p. 152)

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Chapter 4 flashcards

Know your key terms

activation energy
active site
adenosine diphosphate (ADP)
adenosine triphosphate (ATP)
aerobic cellular respiration
allosteric site
anabolic reaction
anaerobic cellular respiration
ATP synthase
biochemical pathway
catabolic reaction
catalyst
cellular metabolism
cellular respiration
chlorophyll
chloroplast
coenzyme
cofactor
competitive inhibitor
electron transport chain
endergonic reaction
endothermic reaction
exergonic reaction
exothermic reaction
$FADH_2$
feedback inhibition
glycogen
glycolysis
induced-fit model
lactic acid
loaded
lock-and-key model
NAD^+
NADH
$NADP^+$
NADPH
non-competitive inhibitor
optimum pH
optimum temperature
photosynthesis
reactant
unloaded

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 Enzymes are biological catalysts that speed up chemical reactions.
2 Photosynthesis and cellular respiration are biochemical pathways that rely on enzymes to drive each step in their reactions.

WB
REMEMBER
PAGE 85

Your cells use enzymes to power many fundamental reactions in your body. Advances in technology are bringing us closer to using enzymes instead of metal catalysts to provide energy for powering portable devices. Researchers at the University of Oxford in the UK have used two enzymes, one from a bacterium and the other from a fungus, to catalyse reactions in hydrogen fuel cells. The scientists were able to capture the energy from this reaction and use it to power a digital watch. Using enzymes found in nature provides many advantages: the enzymes are biodegradable and cheap and can be a reliable source of power.

In your body, thousands of reactions build up and break down chemicals. The reactions are catalysed by enzymes, and together they are known as cellular metabolism. The rate of cellular metabolism varies among organisms. The rate of cellular metabolism also affects the life span of an organism. For example, the antechinus, a small Australian mammal, has a very short life span partly because it has a very high rate of metabolism (Figure 4.1a). Trees have a lower rate of metabolism and can live for hundreds of years (Figure 4.1b).

All living things on Earth require a source of energy. Energy released in breaking-down metabolic reactions provides cells with the energy needed for efficient functioning and survival of the cells, and ultimately the organism. Enzymes are the key to speeding up these reactions so that they take place at a rate that will sustain life.

a

b

Figure 4.1a An antechinus marsupial and **b** a eucalyptus tree have different rates of cellular metabolism.

4.1 Biochemical pathways for cell metabolism

WB 4.1.1 ORGANISING KEY TERMS PAGE 86

Metabolism includes a range of biochemical processes that occur in living organisms. **Cellular metabolism** is the sum of all the chemical reactions taking place in all living cells. It consists of two major types of reactions: catabolic and anabolic reactions.

In **anabolic reactions**, atoms and simple molecules are joined to make more complex molecules. Anabolic reactions require an initial input of energy so they can start to form new chemical bonds. Reactions that require an initial input of energy to get them started are called **endergonic reactions**. Photosynthesis is an anabolic reaction in which the simple inorganic molecules water and carbon dioxide react to produce the complex organic molecule glucose. It is an **endothermic reaction** because light energy is absorbed from the surroundings and used to form the bonds in the glucose molecules.

CONNECT

For more information on photosynthesis and cellular respiration, go to Chapter 5.

In **catabolic reactions**, complex molecules are broken down into simpler molecules. Catabolic reactions are spontaneous reactions that do not require an initial input of energy to get them started, so they are called **exergonic reactions**. Cellular respiration is a catabolic exergonic reaction in which glucose, the main initial reactant, is broken down to release the energy stored in the glucose and produce simpler products. Reactions that release energy are called **exothermic**. If oxygen is available and used in the reaction, it is called **aerobic cellular respiration** and the products are water and carbon dioxide. If no oxygen is available or it is not used, it is called **anaerobic cellular respiration**, and the products are **lactic acid** in animal cells, and ethanol and carbon dioxide in plant cells.

Note:

Make sure you understand the difference between exergonic and exothermic, and endergonic and endothermic.

WB
4.1.2 BIOCHEMICAL PATHWAYS AND COENZYMES PAGE 87

Biochemical reactions: pathways with regulated steps

The metabolic reactions that occur in cells do not take place randomly. They are all controlled and regulated to maintain cell functions and to meet the needs of the cell. To achieve this, chemical reactions in cells occur in a series of regulated steps, collectively called **biochemical pathways**, as illustrated in Figure 4.2. These reactions must occur at a rate that allows the cell to function efficiently.

The initial **reactants** are the substrates, the molecules that enter the reaction and are acted upon by enzymes to speed up their conversion into products. At each step, a substrate–enzyme complex is formed, which then separates and releases the new product for that step. The products or outputs of the first step become the reactants or inputs in the next step until the final products are reached.

CONNECT
Activation energy, coenzymes and cofactors are discussed more on page 143.

Each step in the pathway is controlled by a specific enzyme (a protein that speeds up the rate of a chemical reaction without being used up or destroyed in the reaction). Enzymes catalyse reactions by lowering the **activation energy**, which is the energy required for a reaction to start. Without enzymes, the biochemical pathways of the cell, including both cellular respiration and photosynthesis, would occur too slowly to maintain life. Enzymes often require the assistance of other molecules to ensure that reactions can take place and be maintained. Molecules that assist enzymes are called **cofactors** or **coenzymes**.

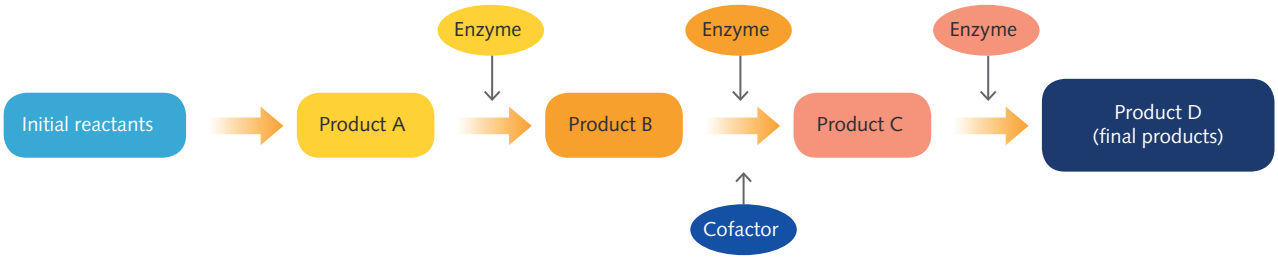

Figure 4.2 A biochemical pathway. The products or outputs of the first step become the reactants or inputs in the next step until the final products are reached. Each step is regulated by a specific enzyme. Cofactors may be involved.

Biochemical pathways can be compared to other systems. A system has inputs, which are processed through a series of steps, and outputs. Cellular metabolism is the sum total of all the biochemical pathways or systems that occur in all living cells. Different cells have different requirements and rates at which they carry out processes. For example, heart muscle cells have a high rate of metabolism to keep the heart beating. The oxygen that is transported in blood is used for aerobic cellular respiration in the cardiac muscle cells to provide enough energy to keep the heart pumping.

Chemical reactions are reversible under certain conditions, and it is important that products are removed from a cell so that they do not accumulate and slow down vital metabolic reactions. To achieve this, cell biochemical reactions go through a series of steps in which the product of one step becomes the reactant for the next step (Figure 4.3, p. 139). In this way, a product from one reaction is continually removed by being the reactant for the next reaction.

EXAM TIP
Make sure that by the end of this chapter you can explain the ways in which metabolic reactions are regulated.

Cells have ways of removing the final product so that a biochemical pathway keeps operating in the right direction. In a plant cell, the final product of photosynthesis is glucose. Glucose, a soluble substance, is converted into the insoluble polysaccharide starch and other substances, which are stored by the plant. Thus, the plant can continue to produce and store more glucose. In cellular respiration, the products of the breakdown, carbon dioxide and water, diffuse from cells and are expelled into the atmosphere by different means.

In some cell biochemical pathways, when the product reaches an adequate or excess amount, the reaction slows down or stops temporarily. This occurs because the product is acting as a reversible inhibitor on one of the enzymes in the reaction (p. 149). If the enzyme is prevented from acting as

a **catalyst**, the reaction will no longer occur, so no more product will be formed. Inhibition of the reaction is essential to conserve cell resources and energy by not making excess product. When the amount of product in the cell decreases as it is used up or removed from the cell, the inhibitory effect no longer exists and the biochemical reaction can begin again (Figure 4.3).

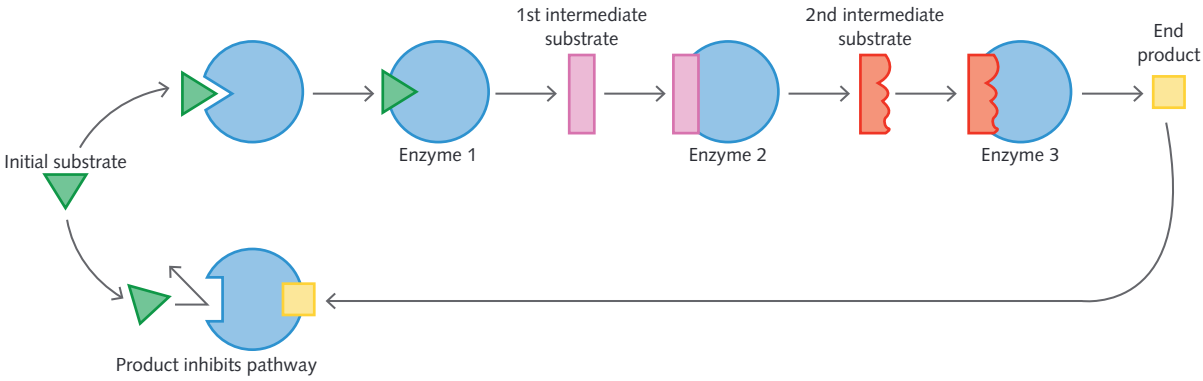

Figure 4.3 **A biochemical pathway showing initial reactants, substrate–enzyme interactions, specific enzymes and final product. Excess end product can act to inhibit the reaction temporarily by binding to the enzyme.**

KEY CONCEPTS

- » Clular mtabolism fers to ll th chemical reactions that ocrin liig cells.
- » Anaolic eactions are ndoermic, e.g. photosynesis Cat bolic reactos are exothermic, e.g.cllula respiration.
- » Exegonic eactionsoccur wi thout an ntial input of enery; endrgonicreactions reqire aiial input of energy to sart.
- » iochmical react ons occur inpaways that series of regulated teps contrlled by enzymes.

Concept questions 4.1a

1 Ditnguish betwenthefollowing pairs of energy terms and povide an example of each term.
   - **a** Analic and ctabolic
   - **b** Endothrmic and xothermic
   - **c** Exergonic and edergonic
2 Define metblism'.
3 Explain the stemen: 'Endergonic reactions and exergnic rection are interdeendent'.
4 Why do cemical reatis in cell proceed in a series of stepcalld abichemical pathway?
5 What isabological catalyst?

HOT chaenge

6 Enzymes often equire cofactors or coenzmes to work. What efft will the absene of a speci fic cofactor or coenzyme have on catalyse bichemical reaction?

Photosynthesis and cellular respiration

Photosynthesis and cellular respiration are both complex biochemical pathways, consisting of a series of steps from the initial reactants to the final products. Each step is catalysed or sped up by specific enzymes. Coenzymes facilitate these steps. Conditions in the internal environment of cells can affect enzyme function. Factors that affect enzyme function include temperature, pH, substrate concentration and inhibitors.

Photosynthesis

Photosynthesis is the process by which photoautotrophs capture light energy and use it to convert water and carbon dioxide to glucose, water and oxygen. The light energy that is captured is stored as chemical energy in the bonds of the glucose molecule. The glucose can then be used as a structural or storage molecule for the plant, or as a fuel in cellular respiration to provide energy for the plant. Photosynthesis is summarised by the following equations:

$$12H_2O + 6CO_2 \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} 6O_2 + C_6H_{12}O_6 + 6H_2O$$

OR

$$6H_2O + 6CO_2 \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} 6O_2 + C_6H_{12}O_6$$

$$\text{Reactants/inputs} \longrightarrow \text{Products/outputs}$$

CONNECT

Photosynthesis will be discussed in detail in Chapter 5.

The process of photosynthesis occurs in **chlorophyll** molecules of plants and some single-celled protists. Photosynthesis is often shown as a simple reaction but it is actually a complex series of reactions, with each step within each reaction being catalysed by a specific enzyme or enzymes.

Cellular respiration

WB 4.1.3 SCIENTIFIC LITERACY PAGE 88

The energy trapped from the Sun and stored in the chemical bonds in glucose during photosynthesis can be released by the process of cellular respiration. Glucose is the main energy source molecule for multicellular organisms. Plants produce their own glucose to power cellular respiration, whereas animals ingest glucose and other complex substances that can be digested into glucose, in their food. As already discussed, the bonds within glucose molecules require energy to form; this energy is released when those bonds are broken. Glucose can be packaged into more dense forms for longer-term energy storage, such as **glycogen** in animals and starch in plants.

Cellular respiration is the process of breaking down glucose, either completely in aerobic cellular respiration using oxygen, or incompletely in anaerobic cellular respiration with no oxygen used. The purpose of this process is to release the energy stored in the bonds of glucose to form the energy-storage molecule **adenosine triphosphate (ATP)** – the 'energy bank' of cells, which can make energy available to cells when it is needed. ATP is the universal energy storage molecule for all living organisms, from bacteria to humans. ATP contains an adenosine molecule, attached to a sugar group (ribose), which is bound to a chain of three phosphate groups. ATP is formed by the addition of the third phosphate group to an **adenosine diphosphate (ADP)** molecule in a reversible reaction catalysed by the enzyme **ATP synthase** according to the following equation:

$$ADP + P_i \underset{}{\overset{\text{ATP synthase}}{\rightleftharpoons}} ATP$$

where P_i represents an inorganic phosphate group.

The process of aerobic cellular respiration is summarised by the following equation:

$$C_6H_{12}O_6 + 6O_2 + 36ADP + 36P_i \longrightarrow 6CO_2 + 6H_2O + 36ATP$$

Cellular respiration occurs in the cytosol and mitochondria of all living eukaryotic cells. It occurs in a series of steps, each catalysed by specific enzymes. Cells that carry out anaerobic respiration also do so in a series of steps; however, the process is simpler and results in the incomplete breakdown of glucose. These types of anaerobic respiration will be discussed in more detail in Chapter 5. The following word equations summarise anaerobic respiration in plant and animal cells:

- Plant cells: glucose $\longrightarrow$ ethanol + carbon dioxide + 2ATP
- Animal cells: glucose $\longrightarrow$ lactic acid + 2ATP

The energy given off when glucose is broken down is captured and stored in ATP molecules, ready for powering other biochemical pathways. The energy from ATP is used to build complex molecules, in cell division and growth, to maintain cell organisation, to move substances and in many other cell functions and activities (Figure 4.4).

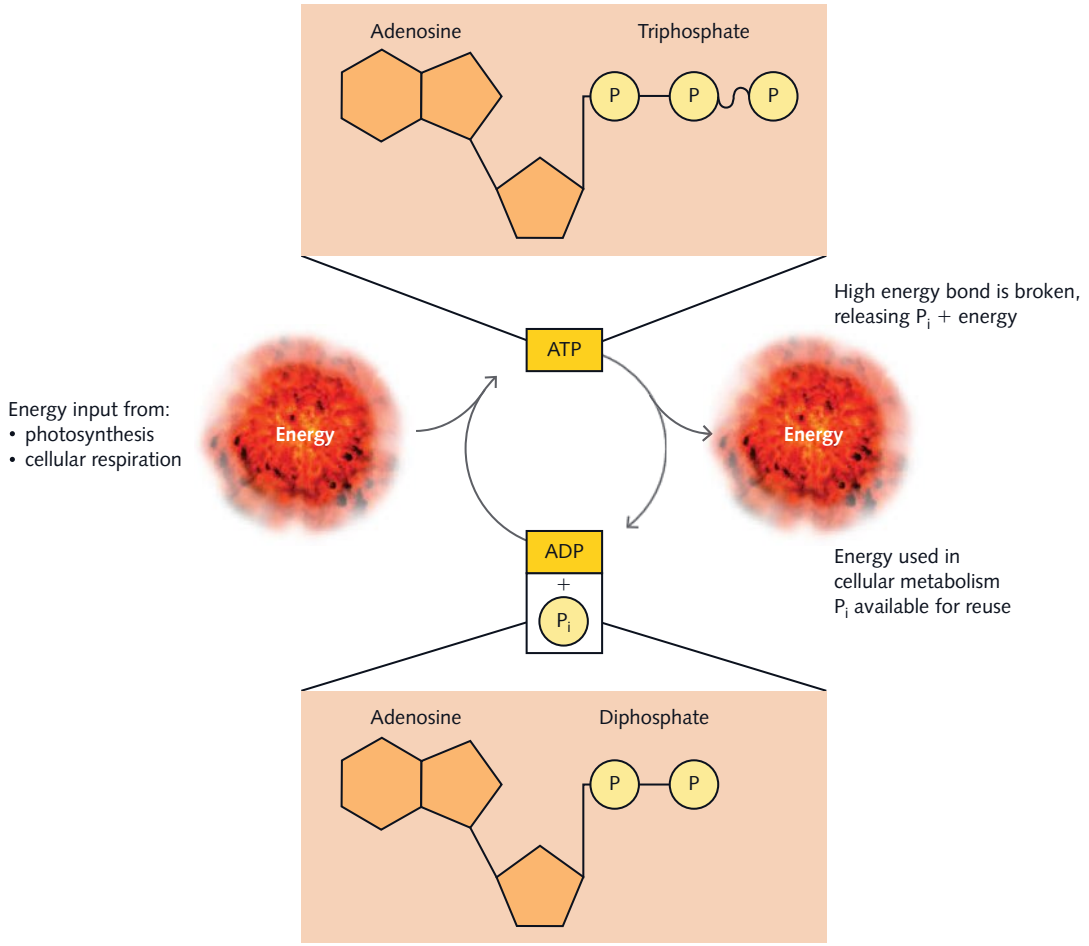

Figure 4.4 The ATP–ADP cycle. The ATP–ADP system links energy-yielding processes to energy-requiring processes within the cell by conserving, transferring and releasing energy. Both photosynthesis and cellular respiration rely on the ATP–ADP conversions.

Cells capture the chemical energy released from certain reactions to fuel other reactions that happen simultaneously. Because these reactions do not always occur in the same place within the cell, energy must be transferred between reactions. This transfer is achieved by molecules of ATP, but these molecules never leave the cell to transfer energy to other cells. All cells must independently carry out cellular respiration to produce ATP for their needs. In the process of cellular respiration, much of the energy, up to 60%, is lost as heat energy, which either helps maintain a relatively constant body temperature, or is lost to the environment.

KEY CONCEPTS

- Thebiocemicalpahays inve inputs of initial reactats, ormation of enzymesubstrate complexes at each step, and final outputs or proucts.
- Photosynhesisd celular respiration are both essnil bochemcal rctios fr life and involve biocheical pathways that are regulated by enzymes n a series o steps.

Concept questions 4.1b

1 Whatwould happe to abochmical reaction if the final product were not removed?
2 Lst five features of the ATPADP system.
3 Would an endrgonic reaction produce ATP or ADP mlecules? Explainyour answer.
4 Wher is the P_i that is used to ynthesise ATP sourced fro n:
   a aials
   b plants?
5 ATP synthase a be classi fied as what type of mlecule? How do you know?

HOT chaenge

6 Cellar rspirationisstrictly classi fied as ocurring n the presence of oxyge, cmonly clled aerobic respiaon. It produces mo ecles of TP. naerobic resirtin also prouces ATP.Anaerobic respiration does not occur in the presenc of oxygen. When anaerbic esirationoccursin the cytosol of an aial o plat cell, howmay molecules of ATP are produced pe mlecule of glucose bing catabolised? What happens to the rest of theenergy that is not released?

4.2 Enzymes: the key to controlling biochemical pathways

It is very busy inside living cells: billions of molecules are randomly moving around, colliding with each other. Most molecules bounce off and continue with their motion but when a specific substrate collides with the correct enzyme at the right orientation, an enzyme–substrate complex is formed, and then dissociates to release the product.

Enzymes are not consumed in the reaction, but are recycled. Enzymes are the 'workhorses' of the cell. Without enzymes, the reactions that occur in living organisms would be so slow as to hardly proceed at all, which could result in cell death.

EXAM TIP
If you see a word ending in '-ase' it is probably an enzyme, but be aware that not all enzymes end in '-ase'.

Normally, an enzyme is named by attaching the suffix '-ase' to the name of the substrate on which it acts: for example, proteases act on proteins, and ATPase acts on ATP. However, not all enzymes end in '-ase-; for example, Rubisco, an enzyme involved in photosynthesis.

Each step in a biochemical pathway is controlled and regulated by a specific enzyme. More than 1000 different reactions can take place in an individual cell at any given time. Enzyme specificity is why enzymes can control each step in a biochemical pathway. There are a multitude of enzymes in living organisms, and each enzyme acts on a specific substrate or small number of substrates.

Each enzyme can also be regulated by:
» the availability of its substrate
» cofactors or coenzymes that it needs to be able to function
» other enzymes that physically modify its structure to control its activity
» inhibitors
» environmental factors, such as temperature and pH.

This means that a highly complex series of reactions can be carefully regulated, switched on and off according to the needs of the cell.

Enzyme specificity

WB
4.2.1 SPECIFICITY OF ENZYMES PAGE 90

Most enzymes are large globular proteins. In Chapter 3, you examined the tertiary structure of proteins and learnt how their shape determines their function. The folding of the polypeptide chain in an enzyme into its tertiary structure forms a groove or pocket called its **active site**. The shape of each active site is highly specific for a particular substrate, which must have a compatible shape for binding to occur. This model of enzyme action is known as the **lock-and-key model** (Figure 4.5a).

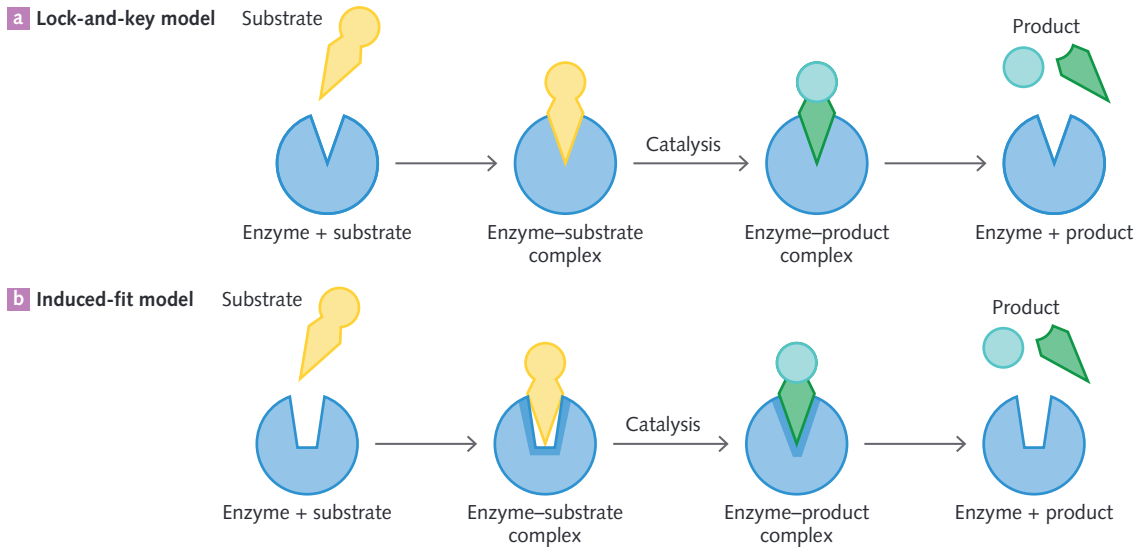

Figure 4.5 Enzyme action. **a** In the lock-and-key model, the binding of the substrate into the active site of an enzyme mirrors a door's lock-and-key mechanism. The substrate's shape is complementary to the shape of the active site within the enzyme. **b** In the induced-fit model, the substrate molecule enters the enzyme's active site, causing the enzyme molecule to change shape so that the two molecules fit together more closely.

EXAM TIP
When you discuss the active site, ensure that you describe the active site within an enzyme. There is no active site on a substrate.

The bonds that form between an enzyme and its substrate can also modify the shape of the active site so that the substrate can fit 'snugly' into the active site. This interaction is called the **induced-fit model** of enzyme action (Figure 4.5b). In this situation, the bonds within the substrate molecule are stretched and bent and, as a result, the activation energy required to initiate the reaction is dramatically lowered and new product molecules are formed at a faster rate. As the product molecules are not specific to the active site, they no longer bind to the enzyme and are released.

Weolink
Enzymes, active sites and cofactors

Onne Wyrkm heet
Enzymes in biochemical pathways

WB
4.2.2
ENZYMES NEED HELP: COENZYMES AND COFACTORS
PAGE 91

Cofactors and coenzymes

Some enzymes are inactive until they bind with other molecules or ions that change their conformation. This alters the shape and the charge of the enzyme's active site so that it can capture substrate molecules and catalyse reactions more efficiently. Two classes of substance bind to enzymes or to substrates to activate enzymes: cofactors and coenzymes.

Cofactors are inorganic molecules that include metal ions such as magnesium (Mg^{2+}), zinc (Zn^{2+}) and iron (Fe^{2+}).

Coenzymes are non-protein organic substances. They are relatively small molecules compared with enzymes. During these biochemical pathways, coenzymes are reversibly **loaded** and **unloaded** with the groups of atoms they carry. Examples of coenzymes are **NADH** (nicotinamide adenine dinucleotide), **NADPH** (nicotinamide adenine dinucleotide phosphate), **$FADH_2$** (flavin adenine dinucleotide) and ATP. They are essential for many of the steps in photosynthesis and cellular respiration. These molecules can accept electrons and protons and chemical groups such as phosphates during biochemical reactions and transfer them to another reaction in a different step of the process. These coenzymes recycle as they transfer a chemical group from one molecule to the next molecule. In this way, energy is transferred within different stages of the biochemical reactions of photosynthesis and cellular respiration.

CONNECT
The coenzymes involved in photosynthesis are discussed in more detail in Chapter 5.

The unloaded form of a coenzyme accepts an electron, a proton or a chemical group. It is now loaded because it is storing chemical energy in the bonds between the coenzyme and the chemical group. The energy to drive the reactions in the intermediate steps of the pathways comes from the bonding and releasing of the protons that occur along a concentration gradient. ATP acts as a

EXAM TIP
Make sure you know the loaded and unloaded forms of each coenzyme in Table 4.1.

Table 4.1 Loaded and unloaded forms of some coenzymes

Loaded	Unloaded
ATP	ADP
NADH	NAD^+
$FADH_2$	FAD
NADPH	$NADP^+$

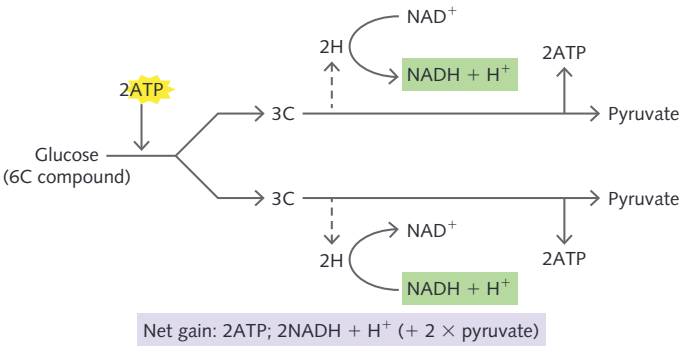

Figure 4.6 The first stage of cellular respiration is glycolysis.

coenzyme and relies upon ATP synthase to cycle between ATP and ADP and inorganic phosphate. ATP and other coenzymes are essential to drive reactions of photosynthesis and cellular respiration.

Many intermediate steps in the photosynthesis and cellular respiration biochemical pathways rely on coenzymes. Coenzymes lower the activation energy of each step in the pathway to ensure each reaction is initiated and occurs. For example, in the first stage of cellular respiration, which is called **glycolysis**, glucose is broken down into two pyruvic acid molecules, releasing energy and two hydrogen (H) atoms. The unloaded forms of the coenzymes, **NAD^+** and ADP, pick up the H atoms and energy to produce the loaded coenzyme molecules, NADH and ATP (using another inorganic phosphate ion) (Figure 4.6). When oxygen is present, the two pyruvic acid molecules move into the mitochondria and a series of reactions in the Krebs cycle produces more loaded NADH and another coenzyme, $FADH_2$. All these loaded coenzyme molecules from the two stages drive the reactions that occur in the **electron transport chain**. In this way, coenzymes are loaded and unloaded to ultimately generate the necessary ATP required by the cell. (See Table 4.1.)

EXAM TIP
Remember that an enzyme's active site is changed during denaturation; it is not the substrate's shape that changes.

Photosynthesis involves a series of steps in two major stages. In the first stage, water molecules are split by light energy into oxygen, protons and electrons, and energy is released. They are used to convert the unloaded coenzymes ADP and **$NADP^+$** into the loaded coenzymes, ATP and NADPH. These coenzymes are then used in the reactions of the second stage of carbon fixation to ultimately produce glucose. In this stage, ATP releases energy and NADPH releases H^+ ions to be used in carbon fixation, thus producing ADP and $NADP^+$ coenzymes that can be recycled and used again in the first stage.

KEY CONCEPTS

- Enzymes e iological atalyts that interact with substrat molecues to increase the rt of a reaction.
- Enzyms lower theactivation energy required for a biocheical reactionto proceed.
- Substrae olecules fit nto the speci fic atie site of an enzye, acordig to lock-and-key or induced- fit moels.
- Coenzymes may be rversibl loadd and unloaded. They are rquired for enzye ctivtion in cellular respiration and photosynthesis.

Concept questions 4.2

1 Define catayst'.
2 What is te role ofenzyms in a cell?
3 What happens to an enzye afterit has catalysed a reaction?
4 How do enzymes affect he activation energy of a reaction?
5 Explain hat is meat by the induced- fit modl of enzyme ation.Ho isthis different rom the lock-and-key mdel?

HOT chaenge

6 Table 1 list exaples o loaded and unloaded coenzymemoeules.
   a For eacheample, name the particle that has been unloaded to form the unloaded version of the coenzyme.
   b NADH is a coenzym inolved in otosynthesis. What do yu think the source ofthe H may be in th loaded molecule?

4.3 Photosynthesis and cellular respiration

Enzymes for photosynthesis and cellular respiration function in the intracellular environment of the cell, in both the cytosol and the organelles – the **chloroplasts** for photosynthesis and the mitochondria for cellular respiration. The environment in the cells must provide the conditions necessary for optimal functioning of the enzymes. This will ensure efficient cell functioning and therefore cell survival. Enzymes are sensitive to changes in substrate concentration, temperature and pH and the presence of substances such as inhibitors.

Weolink
Enzyme-mediated reaction under different conditions

Onne Wyrkm heet
Enzyme catalysis

WB
4.3.1 EFFECT OF TEMPERATURE
PAGE 93

Effect of temperature

The activity of enzymes increases with increasing temperature. This is because molecules gain more energy, move around more quickly, and collide more often. As the number of collisions increases, so does the frequency of substrate molecules entering the active sites of enzyme molecules. Therefore, the rate of the reaction increases as the temperature rises. As temperature continues to increase, enzyme activity reaches a peak at the **optimum temperature** (Figure 4.7). This is the temperature at which the enzyme works at its fastest. However, if the temperature gets too high, the bonds that determine the three-dimensional shape of the enzyme proteins will break. As a result, the protein loses its functional shape. It becomes permanently denatured and the substrate can no longer fit into the active site. The enzyme's activity stops.

At low temperatures, molecular movement slows and so fewer collisions occur between the substrate and enzyme molecules. The rate of the reaction decreases. If the temperature increases again, the molecules begin to move faster as they gain energy. Since the binding in the enzyme protein and its three-dimensional shape have not been altered, the rate of the biochemical reaction will begin to increase.

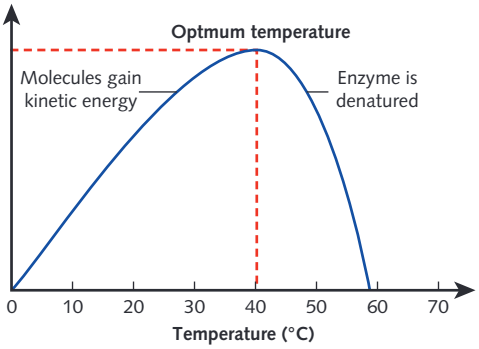

Figure 4.7 The effect of temperature on enzyme activity.

Different enzymes have different optimum temperatures. This reflects the conditions in which the organism is normally found and if it can regulate its body temperature. Enzymes in the human body work best at temperatures of about 37°C, which is the relatively constant core temperature of the body. This means cellular respiration in human cells is optimum at this temperature. The enzymes of other mammals and birds have optimum temperatures that reflect their body temperatures.

WB
4.3.2 EFFECT OF CHANGING pH
PAGE 96

Effect of changing pH

The pH of the solution surrounding enzymes also affects their structure and the activity of their active site, as well as their interactions with substrate molecules. Each enzyme has an **optimum pH** at which it works fastest (Figure 4.8). Some enzymes can work in a wide range of pH environments, while others are very sensitive and will only work in a narrow pH range. Most enzymes work most effectively around a neutral pH of 7. Like optimum temperature, the optimum pH relates to the environment in which the enzyme works.

A neutral pH of 7 is optimum for both photosynthesis and cellular respiration. Cellular solutions need to be buffered because proteins influence the pH of a solution by donating hydrogen ions or hydroxyl ions. A buffer solution usually contains a weak acid and one of its salts, and its pH remains relatively stable while hydrogen ions are released or absorbed during chemical reactions. Changes in pH affect the amino acids making up a protein and therefore the enzymes. If the charges on the amino acids in

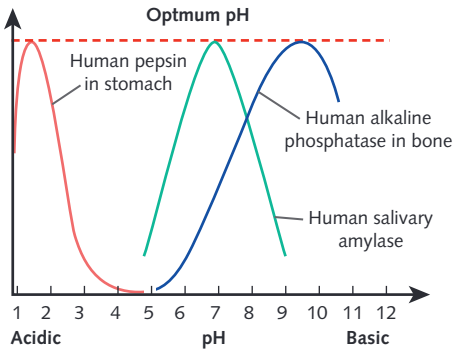

Figure 4.8 The pH range for three different enzymes: pepsin digests proteins in the acidic juices of the stomach; salivary amylase digests carbohydrates in the mouth at a neutral pH; and alkaline phosphatase catalyses reactions in the relatively alkaline environment of the bone.

a protein change, then the bonds that maintain the three-dimensional structure of a protein may be broken. In most cases, if the pH varies, the protein shape is altered so much that the enzyme becomes denatured and can no longer catalyse a reaction. A buffered solution prevents fluctuations in pH while chemical reactions are releasing and taking up hydrogen ions, and this protects the structure of enzymes. Because the chemical reactions in photosynthesis and cellular respiration involve uptake and release of hydrogen ions from the coenzymes, it is important that cells contain buffered solutions so that the enzymes are not affected by changes in pH.

Effect of substrate and enzyme concentration

WB
4.3.3 EFFECT OF SUBSTRATE AND ENZYME CONCENTRATION PAGE 99

The amount of enzyme present in cells can limit the reaction rate and amount of product formed. With a certain fixed amount of enzyme in the cell, increasing amounts of substrate result in more product being made, until the substrate concentration reaches a point where all the enzyme molecules are working at their maximum capacity (Figure 4.9). This is the saturation point.

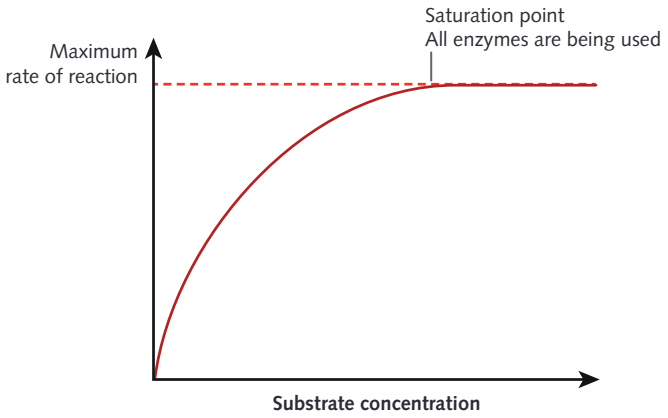

Figure 4.9 The effect of increases in substrate concentration on the rate of an enzyme-catalysed reaction. At the saturation point, further increases in substrate concentration do not increase the rate of the reaction.

At higher concentrations of carbon dioxide, photosynthetic cells increase their rate of reaction if adequate light and water are available. However, at a certain point, the enzymes in the photosynthetic cells may reach saturation point. If this happens, the rate of photosynthesis will become constant. For example, during the second stage of photosynthesis, carbon dioxide is the substrate for the enzyme Rubisco, which converts carbon dioxide and ribulose bisphosphate (a five-carbon molecule) to a six-carbon molecule that ultimately splits into two three-carbon molecules that form the building blocks for glucose. As the concentration of carbon dioxide increases, the rate of photosynthesis also increases, until a plateau is reached. The rate of photosynthesis most likely becomes constant because Rubisco is saturated and cannot work any faster.

This also applies to all living cells that undergo cellular respiration. If glucose is available in unlimited supply, enzymes may limit the rate of the reaction if the amount of enzyme available in the respiring cells becomes saturated, as seen in the graph in Figure 4.9.

Enzyme concentrations are regulated in response to the needs of a cell. This regulation is achieved by:

- controlling the expression of the enzyme
- controlling the rate of degradation of the enzyme
- activating the enzyme in response to a stimulus.

SOUTHERN Biological Stress-tested by Southern Biological

INVESTIGATION 4.1

Effect of temperature on enzyme activity

Enzymes are roteins and are therefore affected by the same factors thataffec all prein;or example, heat. All enzymes havean optimum temperture at which they wo rk bst. The enzym stdied i tis nvestigation is an ntralular enymethat is, it speed up anconrls etabolism within cells.

Am ylase breaks d ow n s tarch mo lecu les into separate glucose mcll. oine ia staintht urns blue-black in the presence of stach, but says yellow-brown in theabsence of starch. Therefor e, th colourof oine indicates wheher starch is present .

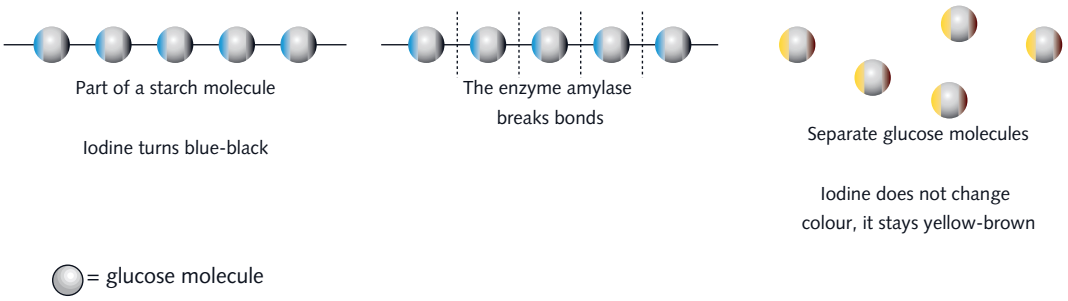

Figure 4.10 Amyase breaks down starch to gucose

Am

To test the effect of temperatur on enzyme function

Materas

- 2% amylaesolution
- 5% starc solution
- Idin solution
- 10 test tubes and test-tube rack
- Large beakes to hold test tubes
- lockor timer
- Thermometer
- hpicks
- 5 m pipette
- 10 mL pipette
- Too
- Spttng tile
- Ice water
- 4 water baths

What are the rsks n dong th s nvestgaton?	How ca n you manage these rsks to stay safe?
odne can stan skn an d cothngTake care toavoid sp	ng odne on skn and cothng
Hot water baths can burnDo not touch thesi	des of the water bath or the water n t

Method

1 Set up four water baths a20C, 40°C, 60°C and 80°C. Put some ice wter in a eker; this is your °C water bath.
2 Add 10 mL of sarch solution to five test ubes.
3 Add 5 mL of amyae solution to another five test ubes.
4 Pace one starch andone amylas test tube into each ater bath for 5 minutes so that the enzyme and substrate both reach thedesired teperaure. You will need o use beakes to hold the test tubes.
5 Pace 1 drop of idie into eac well on te sotting tile.
6 Add the amylase to thestarch in th 0°C water bath.

7 Start thetimer.
8 Everyminue, removea sample of te stach–amylase solton and addit dop of iodine on the white tile. Mix wth aclean tothpick.
9 Repeat step 8nti the iodine no longerchange colour. This is when hre is no starch left.
10 Record your rslts.
11 Repeat steps 6–10 for each of the temperatures.

Resuts

1 Copy th resut tabl into our logboo. Rcord you results in the table.

Resuts of amyase actvty at dfferent temperatures

Temperature (°C)	Time to digest starch (min)
0	
20	
40	
60	
80	

2 Draw sutable graph to how the time taken fo r starch t be digeted at differnt temperatures.

Dscusson

1 Lst the dependnt and indepenentvaiabls n his investigation.
2 Lst three extraeous variables and descrbe how they were controlled.
3 Explain how the colour prdue wih iodine indates the actiiy of te enzyme. Explain why the colour changes throughout th tetng time in ome test tubes.
4 Preict the optimum temperatre for amylase ac tiity. Jstify your answer fro the results.
5 Are there any temperatureswhere amylase does no appr to function? xplain why this might occur.
6 Describe one improveent you ould make to thismthodto show that itis the action of amylase that is causing the breakdown of starch to lucose.

Concuson

Draw a onlusion on the effect of temp eratue in detrmining amyas activity.

Takin it further

1 Modify the above method todesign an exeriment that investigatethe ffct of pH on enzyme activity. If possible, carry out theexpriment.
2 Fever may cause aperson's body emperature to rise above the noa lvel. Discuss ho w tis culd affet cellular acvityand in turn the ntire body.

Enzyme inhibitors

Enzyme inhibitors change the rate at which a chemical reaction occurs, either slowing it down or stopping it completely.

WB
4.3.4
ENZYME INHIBITORS
PAGE 100

Non-competitive inhibitors

Non-competitive inhibitors are molecules that bind to a part of the enzyme that is not at the active site, called the **allosteric site** (Figure 4.11b). This alters the structure of the enzyme in such a way that the active site of the enzyme changes shape, and no longer has the complementary shape for the substrate to bind. Some enzymes have two or more active binding sites. These enzymes can move between their active and inactive state when inhibitor or activator molecules bind with them.

EXAM TIP
Ensure you can label a diagram to show how the attachment of an inhibitor prevents the substrate from binding to the active site of the enzyme.

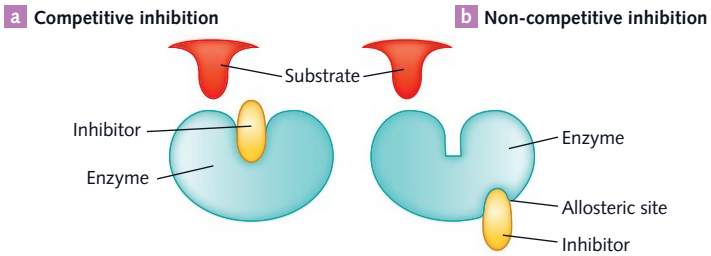

Figure 4.11 a A compettve nhbtor molecule blocks the enzyme's active site. **b** A non-competitive inhibitor binds to the allosteric site, which is not the active site.

The activity of many enzymes is regulated by **feedback inhibition**, in which the output of a process is used to limit the production of more of the product (Figure 4.3). If a large amount of product is present in the cell it will act as an inhibitor by binding to a site on the enzyme other than the active site, thus slowing the rate of reaction. If the product is removed, then inhibition will be reduced, the substrate can enter the active site, and the product will again be formed. This helps cells keep the concentration of products within a certain range.

The importance of non-competitive inhibition can be shown when a cell no longer requires ATP – cellular respiration is switched off to prevent the cell wasting resources and energy. This is also an example of feedback inhibition. ATP acts as a non-competitive inhibitor of another key enzyme, pyruvate kinase, which catalyses the final step in glycolysis. The inhibition of pyruvate kinase stops the cells from breaking down more glucose when a certain threshold of ATP is present in the cell. This prevents the overproduction of ATP and stops unnecessary cell resources and energy from being used.

Competitive inhibitors

Competitive inhibitors compete directly with the substrate for space in the active site and prevent the substrate from binding (Figure 4.11a), either lowering the rate (Figure 4.12) or stopping the reaction from taking place. The shape of the inhibitor molecule is complementary to the shape of the active site of the enzyme, so it can bind in place of the substrate.

Cyanide is a competitive inhibitor of a key enzyme, cytochrome c oxidase, which is involved in the electron transport

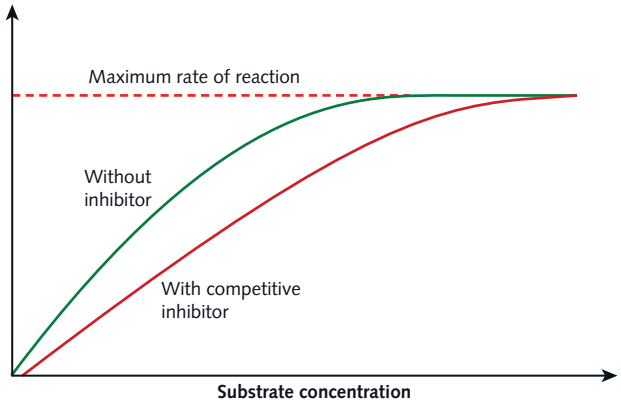

Figure 4.12 The effect on rate of reaction with and without a competitive inhibitor. Addition of a competitive inhibitor results in fewer enzyme–substrate complexes forming and lowers the overall rate of a biochemical reaction. If there is plenty of substrate (towards the right of the horizontal axis), the inhibitor is effectively outnumbered, the substrate gains entry to all the active sites of the enzyme molecules and the maximum rate of reaction is reached.

chain – the last stage of aerobic cellular respiration. When cyanide binds to the key substrate, cytochrome c, cells can no longer produce the full complement of ATP. Therefore, cells need to switch to anaerobic respiration. This results in a build-up of lactic acid in the blood, which can be fatal.

Herbicides used on plants block the action of key enzymes involved in the biochemical pathways of the two stages of photosynthesis. Most of these inhibiting molecules block the transfer of electrons from one molecule to the next; without electron transfer, plants cannot produce glucose.

CONNECT

The Krebs cycle is discussed in detail in Chapter 5.

The second stage of cellular respiration, called the Krebs cycle, can be controlled by the enzyme fumerase, which acts as a competitive inhibitor on intermediate molecules. If the concentration of products starts to build up, this pathway can be slowed down; if the concentration of the products is low, the process can be sped up (Figure 4.13).

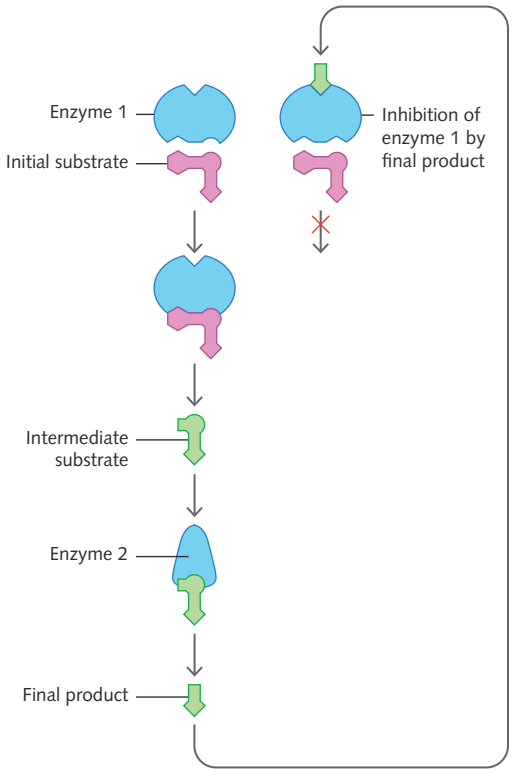

Figure 4.13 An example of end product inhibition

KEY CONCEPTS

- Enzymes a sensitive t temperature. Lower temperatures rede civity; higher temperatures can denature enzyes making them erm nently inactive.
- Enzymes ar ensitveto p, which will affect rates of bochmial eations.
- The eative concentrations of enzyme and substrate can affect the rate ofa iocheical reaction.
- Temperature, pH and oncenration of inputs can affect the ate f iochemcal reactions in photosnthessand clllar respiration.
- A non-comtii inhiitor alter an nzyme's activity by changing its cnfor aionwithout binding to its acivesite.
- A comtiive inhbitor bloc s the active site of an enzye.
- The ation of inhibitors ca e reversible or rreversible and can block key components of both cellar respiration and phosynthesis.

Concept questions 4.3

1 How can the amount of product produced in a reacion affect a enzye's activity?
2 Ditnguish between ann-copetitive inhibitor and a competieihibitor.
3 A human protease works be t at 37°C.
   a Whatwould happen to te enme's activity at very low temperatures?
   b How doe this differ frmthe activity of the enzyme at vry high temperatures?
   c What has happened t the ative site in both cases?
4 Explain what happens tothe protein structure of an enzyme that becomesdenatured.

HOT chaenge

5 Explain, uing diagram, how he aturation point of an enzyme can bereached. Make sure you discuss any contiuting factors and what patthe substrate plays.

BRANCHING OUT

Drug design

Sietists ae ntiully sriving to discover nw drugs o trea dieases. B looking closely at the molecular structure of a target prtein (or anenzym), scietist can designa molecule tha can bndto thetarget and interfere with its normal functon. Mch like fiting jigaw piece ito the comlementary g in a groingpuzzle, a carefully designed drug can fit the exact shape of a taget prtei andbok its action. This s clledraionaldrug desi gn Rational drug design was used when an Atralianteam of scientists at CSO and Monas Unversity, led by Dr Peter Colman, developed a drug clled Relenza ® to prevent the influenza vrus fro infecting uman ng cells.

A dru is a bilogical sbstance that is take for non-dietary need. This substance can occur naturally or be syntetic (man-made). Whe drugs re introduced into an organ sm they produce a charcteristic action or effect that alters soe boiy functon Thicn include relieing ymptom ofa iseas, even cuing it in some circumstances.

Some dugs, suc as vaccines, may revnt a particular dis ease from ocurring. Many studnts confuse drugs with antibdies. A drug y have a smilar moe oaction t an antidy, in how they inhibit the activity of a pathogen, but drugs an atibodies are not the same. A vaccine can be con sdered a drug hat indces the immune system to produce antibodies anmemory clls against speci fic pathogns.

Drugs wok by intracting with eceptor molecules on tar get celsin our bde.This iteraction leads t a change in the targ ellactiviiesthat is usually bene ficial to ourhelh. Thismayinvov ethersmlatig or inhibiting certain acities othe cell.

Tisis much the same f naturally occrring chemical mess engerswihin thebody, such as hormone. In many ways, a dru is a cheical messng but, unlie hormones, drugs ar e synthesise outside he body. Fr xample, insulin is a hormone that isormaly snthesised in th e bod in response to igh lood glucose le vs. Peole with ype 1 diabetes do not produc inlin – but they can administe r synteticnulin, a fomf insulin thatcould be considered a drug.

The mehanismsa principes of how cells detect and re spond to igals pply in much the same way, except the siulus and messngr molecule rethe drug itself.

Drugs can ave side effects. Many of the e occ r when the drugs interact wihreceptors that are not on target cells, or nhbit the nrm activitof th cell, pro ucing ufavourabe reat ons. One of the challenges in discovering new drugs is to find those that ae speci fic to the targtcells, anddo not disruptthe ormal funcioning of the host.

Questions

1 How is thespeci ficity of a dru determined?
2 How is the study of enmes hepng the evolution of rugs to treat human diseases?
3 Elain the advanagesof using ational drgdesign overcoventinal methods of designing drugs.

Online ke: TynTeptm
Chapter 4: Summary of key concepts

4 Summary of key concepts

4.1 Biochemical pathways for cell metabolism

KEY CONCEPTS

p 137

- » Clular mtabolism fers to ll the chemical reacions that cr in ing cells.
- » Anaolicreactions ar ndothermic, eg. photosnhess. Ctabolic reactions are exothmic,.. celular respiration.
- » Exegonic eactionsoccur wi thout an ntial input of enery; endrgonicreactio ns reqire aiial input of energy to sart.
- » iocemcalreact ons occur inpa ways that involve a series of egulated sts controled by enzymes.
- » Thebiocemicalpahays involve inputs of ntial reacants formation of enzyme–substrate complexes at eachstep, and final outputs or products.
- » Photosynhesisd celular respiration are both essnil bochemcal rctios fr life and involve biocheical pathways that are regulated by enzymes in aseries of steps.

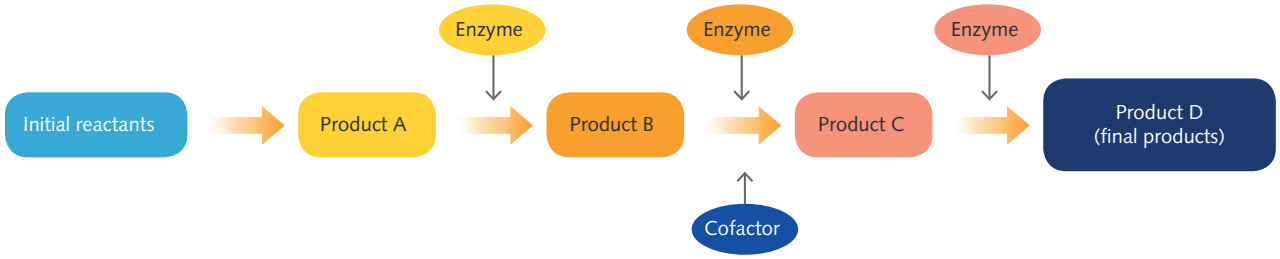

Figure 4.2 A biochemical pathway. The products or outputs of the first step become the reactants or inputs in the next step until the final products are reached. Each step is regulated by a specific enzyme. Cofactors may be involved.

4.2 Enzymes: the key to controlling biochemical pathways

KEY CONCEPTS

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- » Enzymes e iological atalyts that interact with substrat molecues to increase the rt of a reaction.
- » Enzyms lower theactivation energy required for a biocheical reactionto proceed.
- » Substrate olecules fit nto the speci fic acive site of an enzme, ccordig to lock-nd-key or induced- fit moels.
- » Coenzymes may be rversibl loadd and unloaded. They are rquired for enzye ctivtion in cellular respiration and photosynthesis.

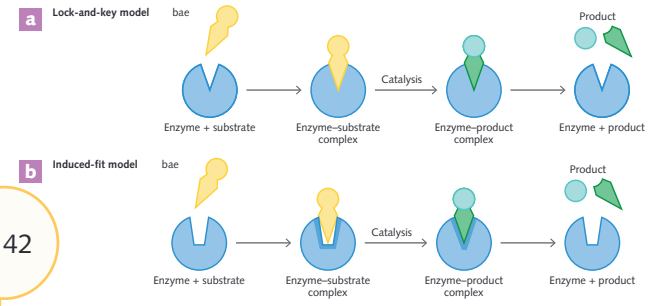


Figure 4.5 Enzyme action. **a** In the lock-and-key model, the binding of the substrate into the active site of an enzyme mirrors a door's lock-and-key mechanism. The substrate's shape is complementary to the shape of the active site within the enzyme. **b** In the induced-fit model, the substrate molecule enters the enzyme's active site, causing the enzyme molecule to change shape so that the two molecules fit together more closely.

4.3 Photosynthesis and cellular respiration

KEY CONCEPTS

p 145

- Enzymes ar ensitive to tmperature. Lower temperatures reduce actiity; higher temperatures can denature enzyms,making them prmaetly inactive.
- Enzymes ar ensitveto p, which will afect rtes fbiochemical reactions.
- The eative concentrations of enzyme and substrate can affect the rate o biocemical reaction.
- Temperature, pH and oncenration of inputs canaffct the rte of bichemical reactions in photosynthesis and cellar respration.
- A non-cptitive inhibitor lters an enzyme's actiity by chnging is cnfrmation without binding to its active site.
- A comtii inhiitor block the actie site of an enzyme.
- The ation of inhibitors ca e rversile or irrversible and can blk key components of both cellular respiration and photosynthesis.

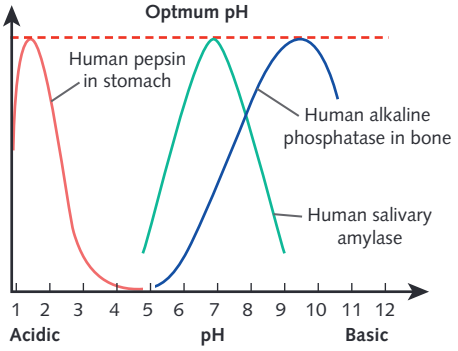


Figure 4.8 The pH range for three different enzymes: pepsin digests proteins in the acidic juices of the stomach; salivary amylase digests carbohydrates in the mouth at a neutral pH; and alkaline phosphatase catalyses reactions in the relatively alkaline environment of the bone.

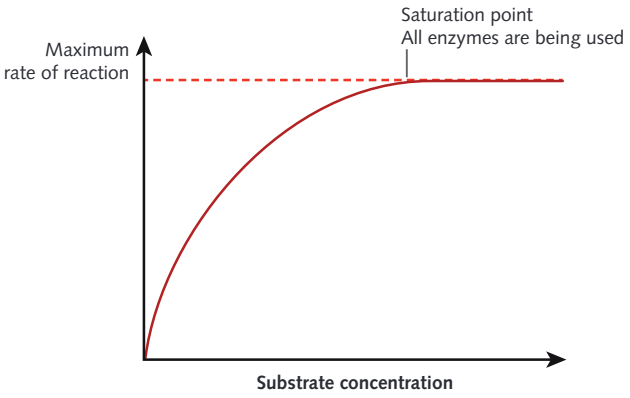


Figure 4.9 The effect of increases in substrate concentration on the rate of an enzyme-catalysed reaction. At the saturation point, further increases in substrate concentration do not increase the rate of the reaction.

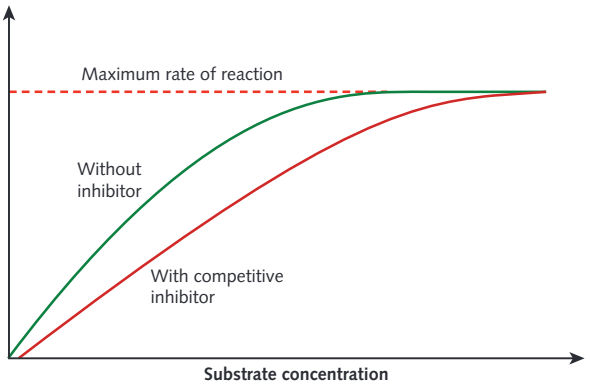


Figure 4.12 The effect on rate of reaction with and without a competitive inhibitor. Addition of a competitive inhibitor results in fewer enzyme–substrate complexes forming and lowers the overall rate of a biochemical reaction. If there is plenty of substrate (towards the right of the horizontal axis), the inhibitor is effectively outnumbered, the substrate gains entry to all the active sites of the enzyme molecules and the maximum rate of reaction is reached.

WB
4.4.1
KEY TERMS
PAGE 102

4 Chapter glossary

activation energy the energy required to initiate a reaction
active site the place on the surface of an enzyme molecule where substrate molecules attach
adenosine diphosphate (ADP) a low-energy compound made of adenine and ribose with two phosphate groups attached; it is converted to ATP for energy storage when it gains a phosphate group
adenosine triphosphate (ATP) a high-energy compound made of adenine and ribose with a chain of three phosphate groups attached; it releases energy for cellular reactions when its last phosphate group is removed and it is converted to ADP
aerobic cellular respiration a metabolic reaction that requires oxygen to produce energy for the cell
allosteric site a binding site on a protein (usually an enzyme), that is not part of the active site; binding of a specific molecule at this site results in a change in activity of the protein
anabolic reaction a reaction in which atoms and small molecules are joined together to make larger molecules
anaerobic cellular respiration cellular respiration in the absence of oxygen
ATP synthase an enzyme that provides energy for the cell through synthesis of ATP
biochemical pathway a series of chemical reactions, each controlled by an enzyme, that brings about the step-by-step conversion of an initial substrate molecule to a final product
catabolic reaction a reaction in which larger molecules are broken down into smaller molecules
catalyst a substance that increases the rate of a reaction without itself undergoing any permanent chemical change
cellular metabolism the sum of metabolic reactions in a cell
cellular respiration a process occurring in all living cells where large molecules are broken down to release energy
chlorophyll the green pigment in plant chloroplasts; it absorbs light energy, making it available for photosynthesis
chloroplast a membrane-bound organelle containing chlorophyll and found in the cytoplasm of plants and algae; its main function is photosynthesis and storage of carbohydrates
coenzyme a small molecule that assists enzyme activity by carrying groups of atoms to or from the reaction
cofactor a molecule that assists enzyme activity by helping the enzyme to fold properly or to facilitate the reaction
competitive inhibitor a substance that competes with a substrate for an enzyme's active site and thereby reduces the enzyme's activity
electron transport chain the process involving the stepwise transport of electrons to a final electron acceptor, such as oxygen (in aerobic cellular respiration); ultimately, it creates an electrochemical gradient across membranes to drive the addition of phosphate to ADP to yield ATP
endergonic reaction a chemical reaction that requires the input of energy for it to proceed
endothermic reaction a reaction that absorbs energy from its surroundings
exergonic reaction a spontaneous reaction that releases energy
exothermic reaction a chemical reaction that releases energy, usually in the form of heat or light
$FADH_2$ the loaded form of flavin adenine dinucleotide, a coenzyme that acts in both cellular respiration and photosynthesis
feedback inhibition a control mechanism used by cells in which an enzyme's activity is stopped or reduced by the product
glycogen an energy-storage polysaccharide in animals that is made of many connected glucose molecules
glycolysis an energy-yielding process occurring in the cytosol of cells in which glucose is partially broken down to pyruvate in enzyme reactions that do not require oxygen; this first stage of cellular respiration produces two ATP molecules
induced-fit model a model of enzyme action that explains that the shape of an enzyme's active site undergoes specific changes, induced by the substrate, to achieve a high degree of specificity with the substrate
lactic acid a product of anaerobic cellular respiration in animals
loaded carry protons, electrons or chemical groups that are needed for anabolic reactions to occur
lock-and-key model a model of enzyme action that suggests that the shape of a substrate molecule is an exact fit to the shape of an enzyme's active site
NAD^+ the unloaded form of the nicotinamide adenine dinucleotide, a coenzyme that has a role in cellular respiration
NADH the loaded form of nicotinamide adenine dinucleotide, a coenzyme that has a role in cellular respiration
$NADP^+$ the unloaded form of nicotinamide adenine dinucleotide phosphate, a coenzyme that has a role in photosynthesis
NADPH the loaded form of nicotinamide adenine dinucleotide phosphate, a coenzyme that has a role in photosynthesis
non-competitive inhibitor a molecule that binds to an enzyme at a site other than the active site; this changes the shape of the enzyme so that the substrate can no longer bind to the active site
optimum pH the pH at which an enzyme works fastest
optimum temperature the temperature at which an enzyme works fastest
photosynthesis the anabolic reaction in which light energy is captured by chlorophyll molecules and used to split water molecules, releasing oxygen and hydrogen atoms, which are joined to carbon dioxide to form glucose
reactant the inputs of a chemical reaction that are required to form products or outputs
substrate a substance on which an enzyme acts; a reactant for an enzyme-controlled reaction
unloaded can accept protons, electrons or chemical groups that are released from catabolic reactions

WB
4.4.2
EXAM PRACTICE
PAGE 103

4 Chapter review

Remembering

1 Identify each of the following as an anabolic or a catabolic process. Justify your choice.
   a Protein synthesis
   b Digestion
   c DNA synthesis
   d Photosynthesis
   e Cellular respiration

2 What is added to ADP to produce ATP? What is the significance of this in maintaining a cell's energy supply?

3 Enzymes are responsible for the production of sperm and male sex hormones in the testicles of human males. Some of these enzymes have an optimal temperature of about 33°C, which is about 4°C lower than body temperature. If this temperature is increased or lowered, sperm and testosterone production is adversely affected.
   a Why would an increase in temperature affect sperm production?
   b Draw a graph to show reaction rate of the enzyme responsible for sperm production against temperature.

Understanding

4 Amylase, pepsin, trypsin and lipase are human enzymes. For each type of enzyme, find out:
   a the general substrate
   b where it is most active in the human body
   d the optimum temperature
   e the optimum pH.

5 DNA ligases catalyse the formation of a phosphodiester bond between single strands of DNA. DNA ligases are present in all living organisms. In eukaryotic cells, the cofactor is ATP. ATP is broken to down to AMP (adenosine monophosphate). From your knowledge of the formation of ATP, how might the formation of AMP occur in this process?

6 Why are DNA ligases mostly found in the nucleus of a cell?

7 What is the difference between a loaded coenzyme and an unloaded coenzyme?

8 The activation energy of a reaction is important. If the activation energy is not reached, then the reaction does not proceed. How do enzymes aid in that process?

9 The following equation is sometimes used to summarise enzyme action. Use your own knowledge to interpret what this equation means.

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

Applying

10 The naturally occurring enzyme polyphenol oxidase causes many cut fruits to brown quickly when exposed to air. Rubbing freshly cut fruit with lemon juice can prevent the brown discoloration. Explain why this happens.

11 Cyanide binds to the enzyme cytochrome oxidase, preventing it from transferring electrons to the final acceptor molecule, oxygen, in aerobic cellular respiration.
   a Where in the cell would cyanide target this enzyme?
   b Explain why cyanide is such a fast-acting poison that results in the death of the organism.

12 The pH of human blood and body fluids (excluding gastric juices) is 6.8–7.0. Explain why maintaining this pH is important.

13 During a heart attack, blood flowing to the heart muscle is interrupted by a blockage of a coronary artery. How would you expect the metabolism in the heart muscle to change?

14 After a heart attack, people often have small amounts of lactate in the blood, which comes from the damaged heart muscle. Suggest an explanation for this observation.

15 The rate of photosynthesis is directly related to temperature, which affects the enzymes involved. Discuss the effect of temperature on the rate of photosynthesis in a plant exposed on a hilltop from sunrise to sunset during the course of a hot summer's day.

Analysing

16 The graph in Figure 4.14 demonstrates the progress of a reaction with and without a catalyst. The black arrows depict the amount of activation energy required to start the reaction.

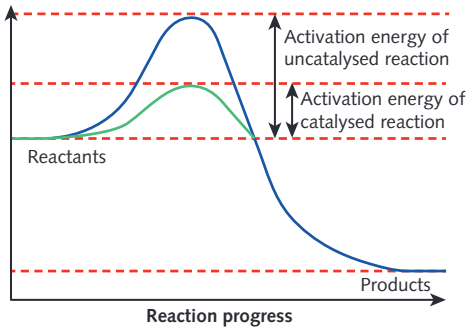


Figure 4.14 The progress of a reaction with and without a catalyst

a Which reaction has the lower activation energy?

b Is this reaction endothermic or exothermic? How can you tell?

17 Figure 4.15 shows a computer model of the enzyme catalase. Catalase degrades hydrogen peroxide in the cell before hydrogen peroxide can do any damage. When degraded by catalase, hydrogen peroxide produces free oxygen and water. Catalase is found in high quantities in the liver.

Draw a flow chart for this reaction that demonstrates the substrate, the enzyme–substrate complex and the products.

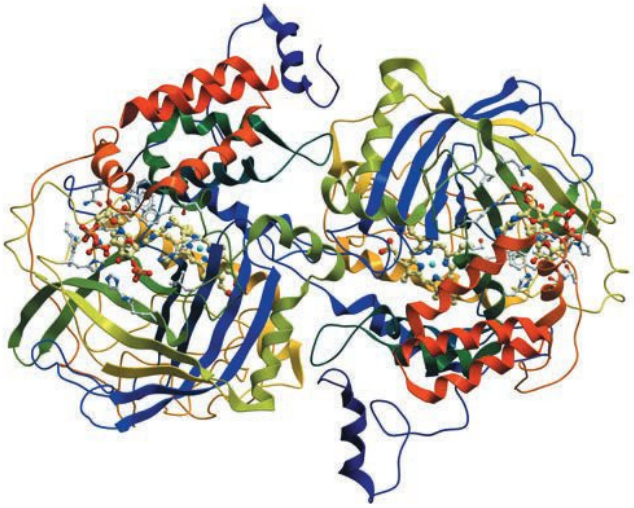


Figure 4.15 A computer model of the enzyme catalase

18 You are given two test tubes (A and B) containing two types of yeast cells that are the same in every way except that one can only carry out aerobic respiration and the other one can only carry out anaerobic respiration. The yeast in tube A grows rapidly, whereas the yeast in tube B grows slowly. Which tube contains the cells that can carry out only aerobic respiration? Justify your choice. Devise an experiment to support your choice.

Evaluating

19 Organisms such as the bacterium *Thermophilus* can thrive in hot springs at about 80°C. Use resource materials to find out why some enzymes are more heat stable than others.

20 Figure 4.5 (p. 143) demonstrates two models for enzyme catalysis. Copy and complete the following table by stating 'yes' or 'no'.

	Lock and key	Induced fit
One substrate only		
Requires enzyme and substrate		
Exact fit only active site		
Active site changes to help substrate fit		

Creating

21 Investigate the use of a commercial enzyme and record your findings under the following headings.

- Source of enzyme
- Properties or action of enzyme
- Industrial or commercial applications

Some tradenames of commercial enzymes are Neutrase, Lipolase, Lactozyme and Termamyl.

22 Design a simple investigation to find out whether catalase is present in a particular bacterium. You have the following reagents and equipment:

- dilute hydrogen peroxide
- bacterial isolate
- light microscope
- microscope slide plus coverslip
- dropper.

If catalase is present, the hydrogen peroxide will degrade to free oxygen and water.

a Write a word equation to describe the possible reaction you are investigating.
b How will you measure any reaction activity? (What will you look for and how will you measure it?)
c What do you expect to happen if catalase is not present?
d What is the independent variable in this investigation?
e What is the dependent variable?
f Write a hypothesis for the investigation.

5 Biochemical pathways: photosynthesis and cellular respiration

By the end of this chapter you will have covered the following material.

Key knowledge

Photosynthesis as an example of biochemical pathways

- inputs, outputs and locations of the light dependent and light independent stages of photosynthesis in C_3 plants (details of biochemical pathway mechanisms are not required) pp. 163–168
- the role of Rubisco in photosynthesis, including adaptations of C_3, C_4 and CAM plants to maximise the efficiency of photosynthesis pp. 166–167
- the factors that affect the rate of photosynthesis: light availability, water availability, temperature and carbon dioxide concentration pp. 168–169

Cellular respiration as an example of biochemical pathways

- the main inputs, outputs and locations of glycolysis, Krebs Cycle and electron transport chain including ATP yield (details of biochemical pathway mechanisms are not required) pp. 172–181
- the location, inputs and the difference in outputs of anaerobic fermentation in animals and yeasts pp. 181–183
- the factors that affect the rate of cellular respiration: temperature, glucose availability and oxygen concentration pp. 184–185

Biotechnological applications of biochemical pathways

- potential uses and applications of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields pp. 186–187
- uses and applications of anaerobic fermentation of biomass for biofuel production pp. 187–188

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify, research and construct aims and questions for investigation pp. 170–171
- formulate hypotheses to focus investigation pp. 170–171
- predict possible outcomes pp. 177–181

Plan and conduct investigations

- determine appropriate investigation methodology: case study; classification and identification; controlled experiment; correlational study; fieldwork; literature review; modelling; product, process or system development; simulation pp. 170–171, 177–181
- design and conduct investigations; select and use methods appropriate to the investigation, including consideration of sampling technique and size, equipment and procedures, taking into account potential sources of error and uncertainty; determine the type and amount of qualitative and/or quantitative data to be generated or collated pp. 177–181
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 170–171, 177–181

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 170–171, 177–181
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 170–171, 177–181
- demonstrate ethical conduct when undertaking and reporting investigations pp. 170–171, 177–181

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 170–171, 177–181
- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 170–171, 177–181
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 170–171, 177–181
- plot graphs involving two variables that show linear and non-linear relationships pp. 170–171, 177–181

Analyse and evaluate data and investigation methods

- process quantitative data using appropriate mathematical relationships and units, including calculations of ratios, percentages, percentage change and mean pp. 177–181
- identify and analyse experimental data qualitatively, handling where appropriate concepts of: accuracy, precision, repeatability, reproducibility and validity of measurements; errors (random and systematic); and certainty in data, including effects of sample size in obtaining reliable data pp. 177–181
- identify outliers, and contradictory or provisional data pp. 177–181
- repeat experiments to ensure findings are robust pp. 177–181
- evaluate investigation methods and possible sources of personal errors/mistakes or bias, and suggest improvements to increase accuracy and precision, and to reduce the likelihood of errors pp. 170–171, 177–181

Construct evidence-based arguments and draw conclusions

- distinguish between opinion, anecdote and evidence, and scientific and non-scientific ideas pp. 177–181
- evaluate data to determine the degree to which the evidence supports the aim of the investigation, and make recommendations, as appropriate, for modifying or extending the investigation pp. 177–181
- evaluate data to determine the degree to which the evidence supports or refutes the initial prediction or hypothesis pp. 177–181
- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 170–171, 177–181
- identify, describe and explain the limitations of conclusions, including identification of further evidence required pp. 177–181
- discuss the implications of research findings and proposals pp. 177–181

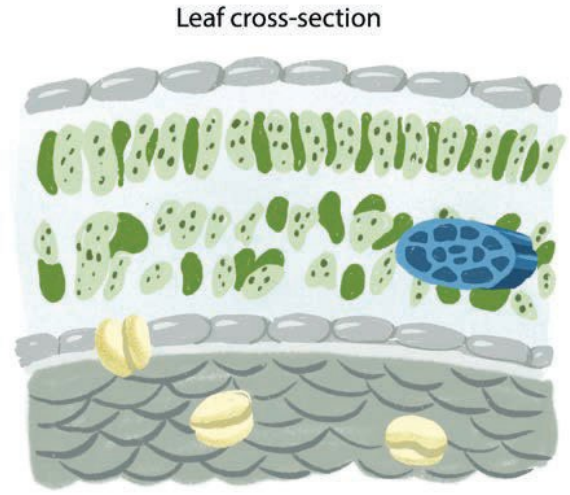
Analyse, evaluate and communicate scientific ideas

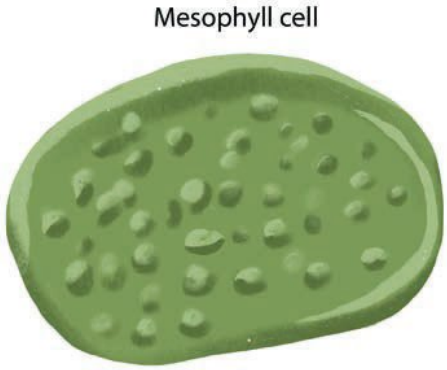
- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement pp. 170–171, 177–181
- discuss relevant biological information, ideas, concept, theories and models and the connections between them pp. 170–171, 177–181

Online Chapter Map
Chapter 5 map

5 Biochemical pathways: photosynthesis and cellular respiration

Two of the major biological biochemical pathways are photosynthesis and cellular respiration. Photosynthesis uses CO_2 and produces O_2. Cellular respiration uses O_2 and produces CO_2. Thus, they form nature's perfect recycling system.





p 163

51 Photosynthess as a bochemca pathway

Photosynthess o ccurs wthn the choroplasts ofplant ce s Each step of ths bochemca pathwa y s contrlled by enzymes Some steps reuir light and occur n the granum Other steps do not reuir light and occr in the stroma. For these steps to occur at an optma rate ntensty of ght temperature and concentraton of CO_2 are limportan.

p 172

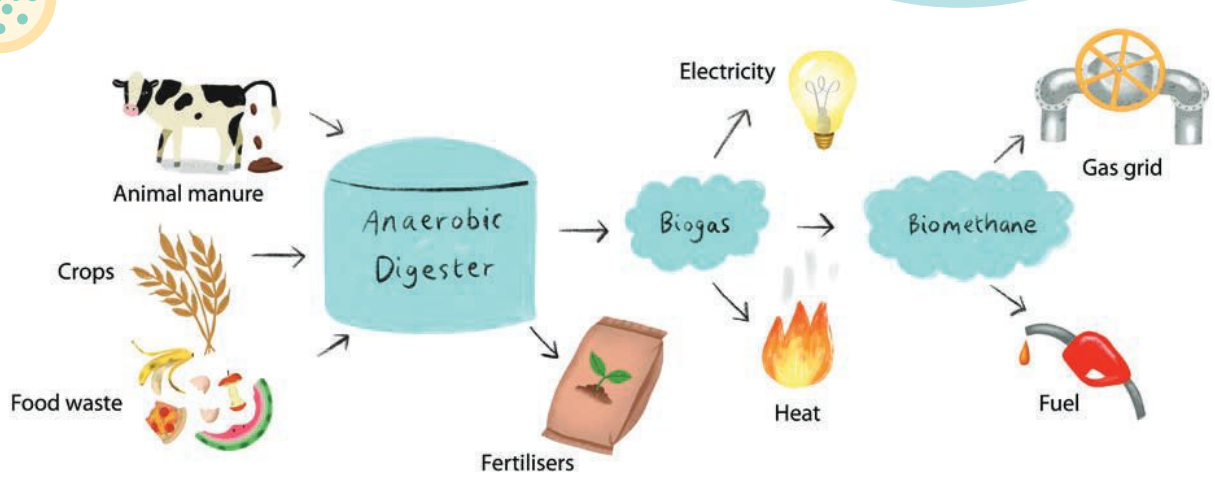
52 Ceuar respraton as a bochemca pathway

Witin the itochon dra of a vng ces ceuar repirtion breaks down gucose to rlease the energy storedwihin the bonds When oxygen s presen,large amounts of ATP are produced When oxygen s absent sma amounts of ATP are prodced. Each step n ths bochemca pathway s contrled by enzyme, wich work best at optma temperatures and lucose and O_2 concentratons

53 Botechnoogca appcatons of bochemca pathways

p 185

Scentsts use bo technooges to manpuate photosyn thess and ceuar repirtion to makeplants more ef ficent and mprove crop produc ton CRSPR s used to remoe, add or change sequences of DNA wthn a pant genome to ncrease prodcivty. Anaerobic lular r espraton of bomass produces iogas and can be used to replace foil fels for transport and to produce eectrcty.



Does biotechnology hold the key to our future? Governments and scientists are pinning their hopes on being able to feed and clothe billions of people through DNA manipulation. Are humans interfering with nature and producing future problems? What are your thoughts?

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 5 map (p. 160)

Online Key Terms:
- Chapter 5 flashcards (p. 162)

Weblinks:
- Two stages of photosynthesis (p. 169)
- Cellular respiration (p. 175)
- Ecological Justice Hub (p. 189)

Online Worksheets:
- Two stages of photosynthesis (p. 169)
- Cellular respiration (p. 175)

Video:
- Photosynthesis and cellular respiration? (p.184)

Online Key Concepts:
- Chapter 5: Summary of key concepts (p. 190)

Online Key Terms
Chapter 5 flashcards

Know your key terms

acetyl CoA
aerobe
alcoholic fermentation
anaerobe
autotroph
biofuel
biomass
C_3 plant
Calvin–Benson cycle
CAM (crassulacean acid metabolism) plant
carbon fixation
chemoautotroph
cristae
cytochrome
grana
heterotroph
Krebs cycle
lactic acid fermentation
light-dependent stage
light-independent stage
limiting factor
matrix
mitochondrion
photoautotroph
photorespiration
pigment
pyruvate
stroma
thylakoid membrane

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 Photosynthetic autotrophs use the Sun's energy to synthesise organic compounds.
2 ATP is the main energy carrier in a cell.
3 Chloroplasts are organelles that are the site of photosynthesis.
4 The outputs of photosynthesis are the inputs for aerobic cellular respiration.
5 Cellular respiration produces ATP.

WB
REMEMBER
PAGE 105

The 'biosphere' is the collective term for all life forms on Earth. Nearly all life on Earth obtains energy directly or indirectly from the Sun. Solar radiation is transformed into other types of energy that flow through the biosphere as organisms live, grow and reproduce. Much of the energy is lost as heat energy because no chemical process is 100% efficient at converting one form of energy to another.

Living things can be grouped according to how they obtain their organic molecules. **Autotrophs** can manufacture their own complex organic molecules from simple inorganic molecules taken in from their surroundings, using an external energy source. **Heterotrophs** obtain their organic molecules by feeding on other organisms and their products, which they then digest into simpler substances.

Autotrophs that capture solar energy are called **photoautotrophs**. They contain **pigments** that capture light energy and use carbon dioxide as the sole source of carbon to produce organic molecules, such as glucose, in photosynthesis. Autotrophs include all plants, photosynthetic protists, and photosynthetic bacteria (called cyanobacteria). However, not all autotrophs rely on solar energy as their primary energy source. Many bacteria, called **chemoautotrophs**, still convert one or more carbon-containing molecules (usually carbon dioxide or methane) and nutrients into organic matter. They obtain their energy from inorganic chemical reactions (oxidation), using various inorganic materials, including hydrogen sulfide, hydrogen gas and iron compounds, as their primary energy source.

Heterotrophic organisms ingest complex organic molecules, such as carbohydrates, lipids and proteins (Figure 5.1). All animals, fungi, some protists and most bacteria are heterotrophs. Both autotrophs and heterotrophs use organic molecules to fuel metabolic reactions. The organic molecules are broken down to release the stored energy in their chemical bonds in the process of cellular respiration. Thus, the energy stored is released for building structures and efficient functioning of the cell.

Figure 5.1 Cycling energy in the biosphere: autotrophs capture solar energy to build cell structure (photosynthesis), which is eaten by heterotrophs to provide them with energy, which is released during cellular respiration.

5.1 Photosynthesis as a biochemical pathway

Plants use either the C_3 or C_4 carbon fixation pathway in photosynthesis. **C_3 plants** are more common than C_4 plants. As you learned in Chapter 4, photosynthesis produces complex organic molecules from inorganic molecules, using light as an energy source. Light is captured by chlorophyll and converted into chemical energy in the bonds of glucose (a six-carbon, or C_6, molecule). Photosynthesis occurs as a series of steps in a biochemical pathway, each catalysed by specific enzymes, which take place in specialised membrane-bound organelles called chloroplasts.

WB
5.1.1 STRUCTURE AND FUNCTION OF CHLOROPLASTS PAGE 106

Chloroplast structure

Chloroplasts (Figure 5.2) are organelles in eukaryotic cells of green plants and some protists and are the sites of photosynthesis. Chloroplasts have an outer and an inner membrane. Enclosed by the inner membrane is the **stroma**, a gel-like matrix that is rich in enzymes. Suspended in the stroma is a third membrane system called the **thylakoid membranes**. Thylakoids are flat, sac-like structures grouped together into stacks called **grana** (singular: granum).

EXAM TIP
Not all plant cells contain chloroplasts. Root cells and phloem are some examples of plant cells that do not contain chloroplasts.

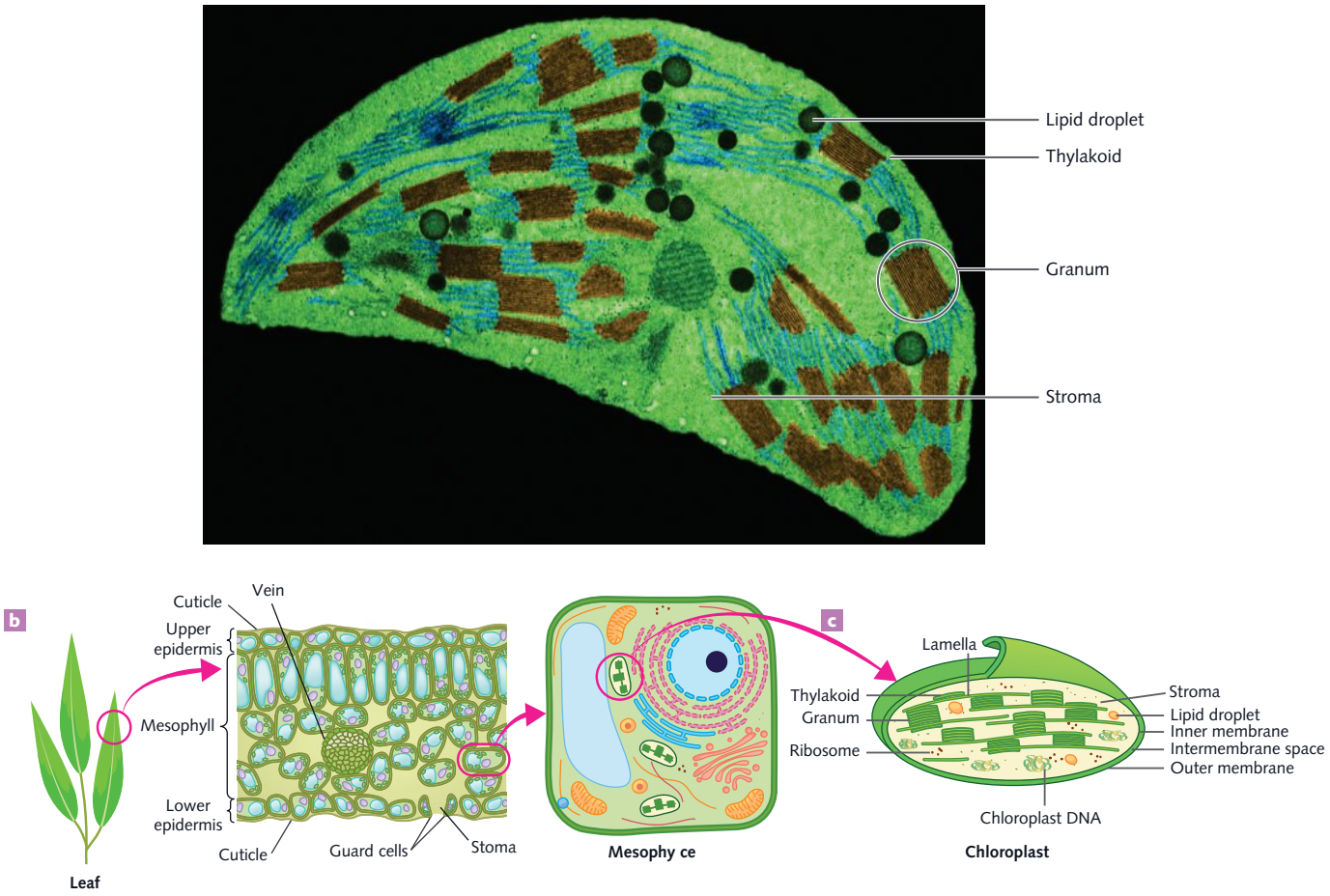


Figure 5.2 a A cross-section of a leaf ch oropast taken wth a transmss on eectron mcr oscope (TEM) **b** A leaf cross-section showing the location of mesophy ces that contan choropasts **c** A generased dagram of a chloroplast showing the main components. Note the presence of both DNA and rbosomes

EXAM TIP
VCAA also accepts the alternative equation for photosynthesis shown on page 140.

Stages of photosynthesis

Photosynthesis is summarised by the following equation:

$$\text{Water} + \text{Carbon dioxide} \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} \text{Oxygen} + \text{Glucose} + \text{Water}$$

Reactants/inputs Products/outputs

Photosynthesis consists of many reactions divided into two distinct stages: the **light-dependent stage** and the **light-independent stage**. Each stage takes place in specific sites within the chloroplast. The first stage (the light-dependent stage) requires light energy to be absorbed.

Light-dependent stage

The light-dependent reactions occur on the thylakoid membranes of the chloroplast grana, where the chlorophyll is located. When a chlorophyll molecule absorbs light energy, it uses it to produce ATP. Water molecules are split in photolysis, producing protons (H^+), electrons and oxygen gas. Water is an input and oxygen is a by-product of this stage of photosynthesis.

The energised electrons are transferred and relayed through many molecules in an electron transport chain and are eventually conveyed to an acceptor molecule. This acceptor molecule is the coenzyme $NADP^+$ (nicotinamide adenine dinucleotide phosphate), which accepts a proton (H^+) and a pair of electrons and becomes NADPH. Another coenzyme is formed when ADP and an inorganic phosphate ion (P_i) form ATP in a reaction cataysed by ATPase. Both $NADP^+$ and ADP + P_i are inputs and NADPH and ATP are outputs of the light-dependent stage (Figure 5.3). The two coenzymes, NADPH and ATP, are used in the next stage of photosynthesis, the light-independent stage.

A simplified summary equation of the light-dependent stage is:

$$\text{Water} + NADP^+ + ADP + P_i \xrightarrow[\text{Chlorophyll}]{\text{Light}} \text{oxygen} + NADPH + ATP$$

Inputs Outputs

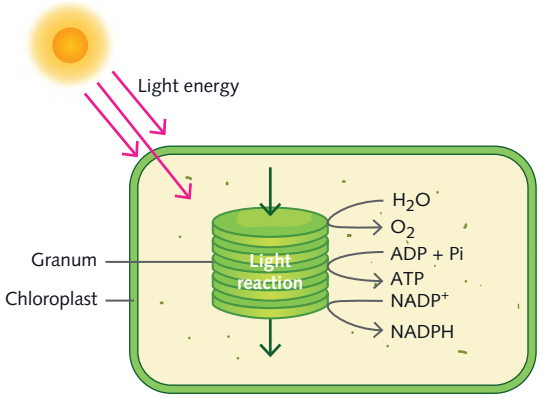


Figure 5.3 Light-dependent reactions occur in the thylakoids of the grana. Light energy is absorbed by chlorophyll pigments and splits water molecules. Electrons move along the electron transport chain. ATP is generated from ADP and P_i, in a reaction catalysed by the enzyme ATPase. $NADP^+$ picks up an H^+ ion to become NADPH. The products of the light-dependent reactions (ATP and NADPH) are used for carbon fixation to produce glucose in the light-independent stage.

Note:

Chloroplasts contain their own genetic material (DNA and RNA) and ribosomes that are independent of those of the nucleus and cytoplasm. Chloroplasts have several characteristics in common with prokaryotic cells, which has led biologists to propose the endosymbiotic theory. This suggests that chloroplasts evolved from ancient prokaryotic cells that were taken up by endocytosis by a much larger primitive nucleated host cell and subsequently established a symbiotic relationship within the eukaryotic cell.

KEY CONCEPTS

- Autotrophs areorganisms that can anufacture their own complex orgni molecules rm simple inorganic mlecle, using an energy sorce. They include photoautotrophs and chemoauttrophs.
- Heterotrophsobtain their comlex organic substances by conuming othr organisms andtheir products.
- Photosythesis is theprocess by which photoautotrophs produceglucose from crbn dioxide water and light energy.
- Photosynhesis ccur in chloroplasts and has a ght-dependentnda light-independent stage.
- Thlight-dependent stage of hotosnthesis occurs in the thyakoids that make up te gana in a chloroplast.
- In te light-dependent stage o hotosynthesis, chorophl absors light enery to slit water, releasing hydrogen ion (protons) into the electron transport cain, electrons and the by-product oxygen.
- Wær ADP, P_i and $NADP^+$ areinputs for the ght-dependen stage.
- AP, NADPH and oxygen gas are outputs of the ght-dependen stage.
- ATP and NADP become inputs for the next stage of photosnheis, he light-inependent stage.

Concept questions 5.1a

1 Ditnguish between a photoautotroph and a chemoauotroph.
2 Recll the gnerl ovrall equation for photosynthesis.
3 Describe thestructural fatres of chloroplasts.
4 Describe the ole ochlorophyl n photosynthesis.
5 What is the water ued r in the light-dependent stage of photosynthesis?

HOT chaenge

6 Produce a tble tha summaises the light-dependent stage of photosynhesis n terms of:
» oc ation
»inputs (equirements)
» outputs (proucts).
In your bl, highlight the products or outputs of the ght-dependen reaction thatare sed as inputs in the ght-independent eaction of phtsynthesis.)

Light-independent stage

The light-independent reactions occur in the stroma of chloroplasts. In these reactions, glucose molecules are produced from carbon dioxide in a biochemical pathway called the **Calvin–Benson cycle** (also known as the Calvin cycle) (Figure 5.4). This reaction requires a supply of carbon dioxide gas, hydrogen ions and chemical energy in the form of ATP. The loaded acceptor molecule NADPH is the source of hydrogen ions, and ATP molecules provide the chemical energy for the conversion of carbon dioxide to glucose molecules. Both the coenzymes NADPH and ATP, which were formed in the light-dependent stage, are used in this light-independent stage and are recycled to the grana as $NADP^+$, ADP and P_i to be used again in the light-dependent stage.

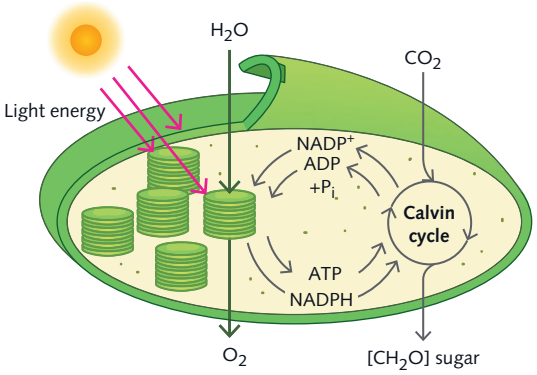


Figure 5.4 The close relationship between the light-dependent stage and the light independent Calvin–Benson cycle.

Water is an output of the light-independent stage. The water is produced by rearrangement of the oxygen atoms in carbon dioxide. Half of the oxygen atoms from the carbon dioxide are incorporated into the carbohydrate, the other half into water. Therefore, this water is different from the water consumed as an input in the light-dependent stage. For this reason, water is represented on both sides of the photosynthesis equation (p. 164).

The following equation is a simplified summary of the light-independent stage.

$$\underset{\text{Inputs}}{\text{Carbon dioxide} + \text{NADPH} + \text{ATP}} \rightarrow \underset{\text{Outputs}}{\text{glucose} + \text{water} + NADP^+ + \text{ADP} + P_i}$$

EXAM TIP
Make sure you remember the inputs, outputs and locations of the light-dependent and light-independent stages of photosynthesis in C_3 plants. You do not need to know the details of the chemical pathways.

The first step of the Calvin–Benson cycle involves attaching carbon dioxide to a five-carbon molecule called ribulose bisphosphate (RuBP). This step is called **carbon fixation** and is catalysed by the enzyme ribulose bisphosphate carboxylase/oxygenase (commonly known as Rubisco) – the most abundant enzyme. After several more steps that involve coenzymes ATP and NADPH, the six-carbon molecule glucose is produced. Glucose is a vitally important output of photosynthesis because it is the key building block of the plant's carbohydrates: sucrose, starch and cellulose.

All plants use the enzyme Rubisco to catalyse reactions that create organic carbon out of inorganic carbon dioxide. Rubisco might be the most plentiful enzyme on Earth but it is highly inefficient. In C_3 plants, Rubisco fixes carbon dioxide by joining it to a five-carbon sugar. Then it cuts the new six-carbon sugar chain into two identical three-carbon molecules, and hence the name C_3 plants. On hot, dry days stomata close so that C_3 plants can conserve water, but then carbon dioxide cannot diffuse into the leaves. The carbon dioxide level drops and carbon fixation slows down. Rubisco

begins to react with oxygen that is building up in the leaves rather than with the small supply of carbon dioxide. This is called **photorespiration**, and results in the production of carbon dioxide by Rubisco. This means that C_3 plants are able to live across a greater temperature range being found in both temperate and tropical climes. They represent about 83% of the world's total flora.

WB
5.1.2
PHOTOSYNTHESIS IN C_3, C_4 AND CAM PLANTS AND THE ROLE OF RUBISCO
PAGE 107

In C_4 plants, carbon dioxide is 'harvested' in the mesophyll cells. It is joined to a three-carbon molecule to form a four-carbon molecule, and hence the name C_4 plants. This fixed carbon moves out of the mesophyll cells into specially adapted bundle sheath cells. Here the carbon dioxide is liberated and then fixed by Rubisco in the Calvin cycle. In this way the carbon dioxide gradient stays low in mesophyll cells so that it will continue to diffuse in from the outside, even when the stomata are almost closed. This partitioning also means that C_4 plants move the Calvin cycle into an area with a high carbon dioxide concentration. Why is this important? The enzyme Rubisco will spend more of its time fixing carbon dioxide in photosynthesis than fixing oxygen in photorespiration. In C_3 plants, photorespiration increases as the temperature rises, so carbon fixation by Rubisco slows. This does not happen in C_4 plants, so they are more competitive in high temperatures. C_4 plants are found mainly in grassland areas and make up only 3% of the world's total flora. C_4 plants include maize and sugar cane, which is renowned for its high glucose production.

In **CAM (crassulacean acid metabolism) plants**, carbon dioxide is 'harvested' at night and fixed to form malate. This again is a four-carbon molecule, but CAM plants differ from C_4 plants. CAM plants do not transport malate away from the mesophyll cells but store it during the night and then liberate carbon dioxide from these molecules and use it in the Calvin-Benson cycle during the day. This use of malate requires four more ATP molecules than the C_3 pathway, so these plants tend to grow more slowly than other plants. They lose up to 95% less water than C_3 plants as they only open their stomata at night. CAM plants are found in deserts (and occasionally in tropical areas), so this adaptation is very useful. Interestingly, CAM plants can swap to the C_3 pathway if there is a period of rainfall, giving them a sudden growth spurt. They can also keep their stomata closed all night and day during drought conditions and exist by fixing carbon dioxide that is released from respiration reactions within the plant. CAM plants make up about 10% of the world's total flora. The pineapple is an example of a CAM plant.

KEY CONCEPTS

- In th liht-independent stage of poosynthesis, coenzymes ATP and NAD from the light-dependent stage povide the hemical energy and protons to fix carbon in t Calvin–nson cycle.
- Inputs ofhelight-independent stage are carbon doxde, NADPHand ATP.
- Outputs o the light-independet stage are glucose, water, $NADP^+$ ADP and P_i.
- C_4 and CAM plants have adaptd photosynthetic pathways tht enable the to survive in hot and dry enironmens.

Concept questions 5.1b

1 Where does the Calvin–Bensoncycle take place?
2 What is the purpose the Cavin–Benson cycle?
3 Wh is th alvin–Bnson cyclenown as the light-ndependent stage of photosynthesis?
4 Why does the Calvin–Benson cycle need ATP and NADPH and where do they come from?
5 Can te light-independent stage of hotosynthesis occur urin daylight evn hough it is referred to as th 'darkrection'?
6 Compare the photoynthetic pathways of C_3 C_4 and CAMplnts. How are their respective pathways an adapttion totheir environment?

HOT chaenge

7 Glucose is termed a C_6 molele,yet in C_3 plats, photosnthesis makesthe energy-rich C_6 organic mlecue glucose. What do C_6 and C_3 refer to and what does C_3 plants mean?

WB 5.1.3 INPUTS AND OUTPUTS OF PHOTOSYNTHESIS PART A PAGE 109

Inputs and outputs of photosynthesis

The two stages of photosynthesis require certain inputs (water and carbon dioxide) and produce outputs (oxygen, glucose and water). Coenzymes cycle between the two stages in loaded and unloaded forms. For the number of coenzymes cycling between the two stages, 12 cycles of light-dependent reactions must occur for every six cycles of light-independent reactions. These inputs and outputs are presented in Tables 5.1 and 5.2.

Table 5.1 Summary of inputs and outputs for the light-dependent stage of photosynthesis, which occurs in the chloroplast thylakoid (for 12 cycles of light-dependent reactions)

Inputs		Outputs	
Molecule	Total number	Molecule	Total number
H_2O	12	O_2	6
$NADP^+$	12	NADPH	12
ADP P_i	12 12	ATP	12

Table 5.2 Summary of inputs and outputs for the light-independent stage of photosynthesis, which occurs in the chloroplast stroma (for six cycles of light-independent reactions)

Inputs		Outputs	
Molecule	Total number	Molecule	Total number
CO_2	6	H_2O	6
NADPH	12	$NADP^+$	12
ATP	12	ADP P_i	12 12
		Glucose	1

Comparing the inputs and outputs for both stages of photosynthesis reinforces the fact that some components are recycled between the two stages. NADPH and ATP formed during the light-dependent stage are used during the light-independent stage. The $NADP^+$, ADP and inorganic phosphate (P_i) produced during the light-independent stage are returned as inputs to the light-dependent stage. Because these components are recycled, they are not shown in the photosynthesis equation on page 164.

WB 5.1.4 FACTORS THAT AFFECT THE RATE OF PHOTOSYNTHESIS PAGE 112

Factors affecting rate of photosynthesis

The main factors affecting photosynthesis are environmental – light, carbon dioxide and temperature – although other factors, such as the presence of inhibitors and changes in pH, can also change the rate of photosynthesis. This means that the factors become limiting.

Light

In the light-dependent phase, photons (the basic units of light) are needed to excite the chlorophyll molecules. These then split water molecules, freeing electrons to enter the electron transport system. Therefore, the amount of light available is an important factor that limits the rate of the first reaction in photosynthesis. The rate of photosynthesis increases as light intensity increases, but eventually a maximum point is reached and adding more light does not increase the rate of photosynthesis. This point is called the light saturation point (Figure 5.5) and happens when more ATP and NADPH are produced in the light-dependent stage than can be used in the light-independent stage.

White light is a mixture of all the different wavelengths of the visible spectrum. The wavelengths of white light available to a plant affect its rate of photosynthesis. Light energy of various wavelengths is absorbed by different pigments within the thylakoid membranes of chloroplasts. These pigments include chlorophylls (green), carotenoids (orange) and xanthophylls (yellow). Chlorophylls absorb blue and red wavelengths and reflect green wavelengths, which is why plants appear green. All green protists and

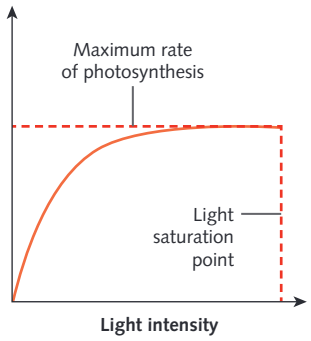


Figure 5.5 The effect of light intensity on the rate of photosynthesis: as light intensity increases, photosynthetic rate increases up to a maximum rate – this level of light is known as the light saturation point.

plants have chlorophylls as their major photosynthetic pigments. Figure 5.6 shows that there are three main pigments involved in photosynthesis – chlorophyll a, chlorophyll b and β-carotene. Each absorbs light in a specific range of wavelengths. Chlorophyll a and chlorophyll b are the main photosynthetic pigments that absorb light in the violet, blue and red wavelengths of white light.

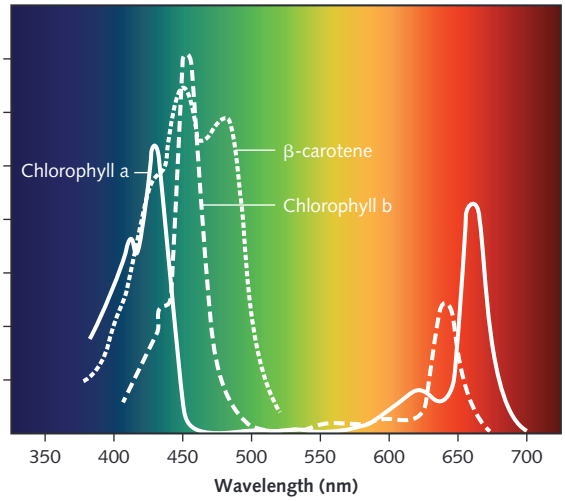


Figure 5.6 Absorption of light at different wavelengths for the various pigments found within chloroplasts of plants used in photosynthesis. Optimal absorption of light occurs at different wavelengths for different pigments.

Weblink
Two stages of photosynthesis

Online Worksheet
Two stages of photosynthesis

WB
5.1.5
INPUTS AND OUTPUTS OF PHOTOSYNTHESIS
PART B
PAGE 115

Carbon dioxide

The main **limiting factor** in photosynthesis is usually the availability of carbon dioxide. Ambient carbon dioxide concentrations are relatively low, at 0.04% of Earth's atmosphere. During the light-independent stage, carbon dioxide is the substrate for the enzyme Rubisco, which converts it to an unstable six-carbon compound that splits into two three-carbon compounds, which then form glucose. As the concentration of carbon dioxide increases, the rate of photosynthesis increases, provided there are enough active sites on the enzyme available to catalyse the reactions (p. 142), and there is enough ATP and NADPH from the light-dependent stage to fuel the light-independent reactions. Increasing the carbon dioxide concentration raises the light saturation point because then the light-independent reactions can use more ATP and NADPH and the rate of photosynthesis can increase overall (Figure 5.7).

Temperature

The third main factor that influences the rate of photosynthesis is temperature. At low temperatures, the enzymes for photosynthesis and the substrate molecules move slowly, few collisions occur, and the rate of photosynthesis is low. As the temperature increases, the molecules have more kinetic energy so they move faster and collide and interact more frequently, increasing the rate of photosynthesis. However, as with all proteins, above a certain optimum temperature the enzymes become denatured and the photosynthetic reactions stop (Figure 5.8).

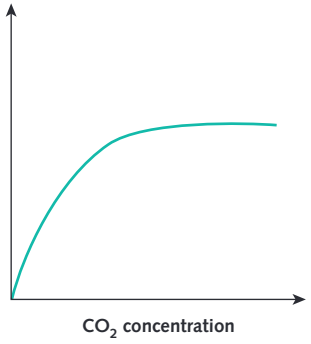


Figure 5.7 The effect of carbon dioxide concentration on the rate of photosynthesis: as CO_2 concentration increases, photosynthetic rate increases up to a maximum rate.

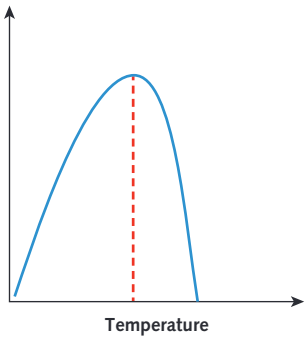


Figure 5.8 The effect of temperature on the rate of photosynthesis: as temperature increases, photosynthetic rate increases up to the optimum temperature. Above this, photosynthetic rate decreases because of denaturation of enzymes.

Developed by Southern Biological

INVESTIGATION 5.1

Effect of light wavelength on photosynthesis

In this investigation, you will use algal balls to test whether the wavelength of light affects their photosynthetic rate. Algal balls consist of many algal cells trapped in a jelly-like substance called sodium alginate, which immobilises the algae but allows them to keep photosynthesising.

Aim

To test the effect of light wavelength on photosynthesis

Materials

- 60 algal balls
- 4 × 7 mL empty dram vials
- Light source
- 40 mL (approx.) hydrogen carbonate indicator
- Plastic pipette
- Set of pH standards or colour chart
- Red, purple and green cellophane
- Strainer
- Spoon
- Disposable gloves (optional)

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Some algae pose an environmental hazard.	Know and follow all regulatory guidelines for the disposal of laboratory wastes.
Disposable gloves can cause allergic reactions in sensitive people.	Use a type of glove that has no allergy risk and is suitable to use with the chemicals in this investigation.

Method

1. Separate the algal balls from the surrounding liquid using the strainer. To do this, pour the algal balls into a strainer over a small beaker.
2. Use the spoon to place an equal number of balls into each dram vial.
3. Using a plastic pipette, fill all the vials with the hydrogen carbonate indicator. Make sure the caps are secured.
4. Keep one vial to act as your control.
5. Copy the results table into your logbook and complete column A by comparing the colour of the hydrogen carbonate indicator to the set of standard references shown in Figure 5.10. (You can use a colour chart if standards are not available.)
6. Place each vial approximately 10 cm away from your light source (Figure 5.9). Make sure the vials do not get hot.
7. Wrap a different piece of coloured cellophane around the other three vials.
8. After 40 minutes complete column B of the results table by comparing the colour of the hydrogen carbonate indicator to the set of standard references shown in Figure 5.10. (You can use a colour chart if standards are not available.)

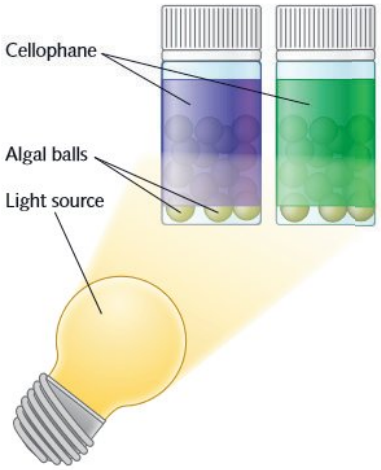


Figure 5.9 The experimental set-up

Southern Biological

Figure 5.10 Standard reference colours for hydrogen carbonate indicator

9 Coplete the final clumn of the esults table by subtracthe amount in columnA frm the amount in column B.

Resuts

Resuts tble

Ceophane coour	Colour of solution before exposure (A)	Clour o olution after exposure (B)	Clour change (B – A)
None (control)			
Red			
Purple			
Green			

Dscusson

1 Carbn ioxidedissolved in waterforscarbonic acid. Hy drogen carboae indcator is used tomeasure the acidity of a sysem. The pH of th system is low (yllow) when hee is a ot f dissolved CO_2 As CO_2 s removed, the pH rises and he colour becomespuple Use this information to construct a bar graph of CO_2 changes as afunction of wavele gth.
2 Why do weinclude a cnrol in te experiment? What does tis contol represnt in ter of lght wavelength?
3 Describe the process ht is appening in the vi als ith regards to photoynthesis nd respiration.

Takin it further

Lght aaability is another factor that affects photosynth es. esign a experiment o test hodistance from light effects the rate of phoosynthesis.

KEY CONCEPTS

- Almitingfactor is factor that limits the rate of a reacin.
- Thlmiting actors inphotynthesisare light intensity, carbn ioxide concntration andtemperature.

Concept questions 5.1c

1 Lst th imiting factors on the re of photosynthesis.
2 n the context of enzme ctvty, explain why the rate of photosynthesislevels off at hig carbon dioxide concentrations.
3 At lolight itnsities, th rate o increase in photosnthsisis linear. What happens to the rate at ver hgh lght nensities?
4 What occursat the igt saturaton point of photosynthesis? denif tis point onthe grh in Figure 5.5.
5 Explain why the rate of potosynthesis varies as the temperature aries.

HOT chaenge

6 Would the concenration o oxygn in the ambient enironment of a C_3 plant ever b consided a limiting factor? Why or why not?

WB
5.2.1 CELLULAR RESPIRATION PAGE 117

5.2 Cellular respiration as a biochemical pathway

As discussed in Chapter 4, the complex series of reactions involved in the process of cellular respiration provides the ATP required by all living organisms (Figure 5.11). Most organisms use glucose as the primary source of energy to drive anabolic cellular reactions and for cellular activities and functions. During cellular respiration, the chemical bonds in glucose are broken, resulting in the release of energy. The energy is used to convert ADP and P_i into ATP, where the energy is temporarily stored, even for a fraction of a second, before it is released for cell use.

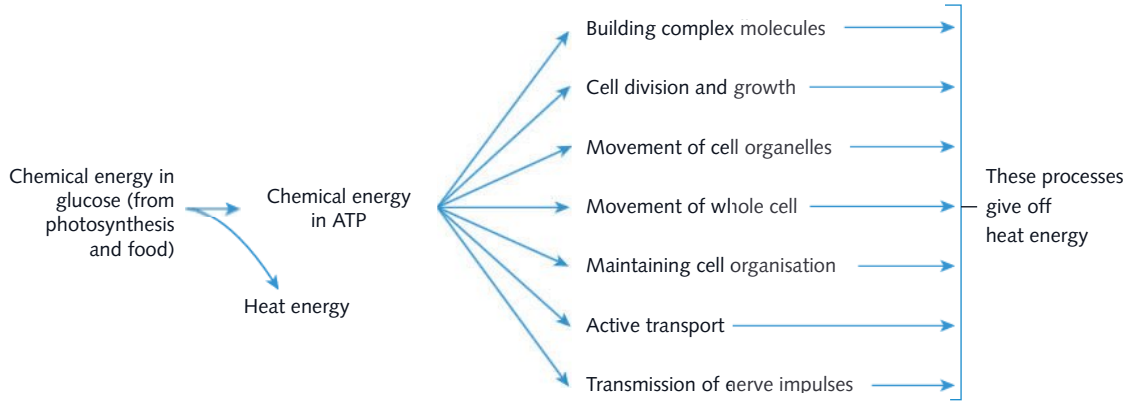


Figure 5.11 Uses of energy in the cell

Most animals, plants, protists and fungi are called **aerobes** because they require oxygen for aerobic cellular respiration. Bacteria and some other micro-organisms are **anaerobes** because they do not use oxygen and instead carry out anaerobic respiration.

For all organisms, the oxidation of glucose to supply the cell with energy, regardless of whether oxygen is used or not, starts with the biochemical pathway glycolysis.

Glycolysis occurs in the cytosol

The biochemical pathway of glycolysis occurs in the cytosol of the cytoplasm. Glycolysis consists of 10 reactions, each step controlled by a specific enzyme. The initial reactant is the six-carbon compound glucose, which is broken down into two molecules of a three-carbon compound, **pyruvate** (or pyruvic acid). During this process, two unloaded acceptor molecules, NAD^+, are loaded with hydrogen to form two loaded NADH molecules, and a net of two ATP molecules are formed from ADP and P_i, using the energy released in the exergonic reactions splitting the glucose into two molecules (Figure 5.12). This process occurs at a fast rate and the ATP molecules are available for immediate use by the cell if required. This ATP production may be adequate for the needs of certain micro-organisms, or plant and animal cells under certain conditions, but it is not adequate for most multicellular organisms. The fact that nearly all organisms carry out glycolysis, either as their sole source of energy or as the first step in more elaborate pathways to gain sufficient ATP for their needs, points to glycolysis being one of the earliest reactions to produce energy for the cell.

The by-product of glycolysis, pyruvate, is used in the further reactions of aerobic or anaerobic cellular respiration. This reduces the concentration of pyruvate, which is toxic to cells if it accumulates. What occurs to break down pyruvate after glycolysis differs between prokaryotic and eukaryotic cells and depends on whether oxygen is present or absent.

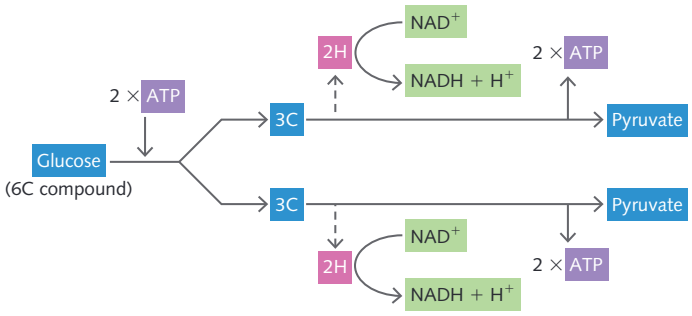


Figure 5.12 Glycolysis is the first stage of cellular respiration. Glucose is broken down into pyruvate, and coenzymes ATP and NADH are produced.

A simplified summary equation of glycolysis is:

$$\underset{\text{Inputs}}{\text{Glucose} + \text{NAD}^+ + \text{ADP} + \text{P}_i} \rightarrow \underset{\text{Outputs}}{\text{pyruvate} + \text{NADH} + \text{ATP}}$$

Table 5.3 summarises the inputs and outputs of glycolysis.

Table 5.3 Summary of inputs and outputs of glycolysis

Inputs		Outputs	
Molecule	**Total number**	**Molecule**	**Total number**
Glucose	1	Pyruvate	2
ATP	2	ADP	2
		P_i	2
ADP	4	ATP	4
P_i	4		
NAD^+	2	NADH	2

Note that 2 ATP molecules are used to initiate glycolysis and 4 ATP molecules per glucose molecule are produced. Overall, there is a net output of 2 ATP molecules for each glucose molecule.

KEY CONCEPTS

- The purposeof celular rspiration is to release the energy stored in th bonds of glucose to produce ATP, whch canprovide energy uel elular reactions.
- lycolysis occurs ith ctosol. It involves the breakdown of glucose to two pyruvate moecle, two ATP moecles and two loaded acepo molecules, NADH.
- ATP isvailabl for immediatee by the cell. Pyruvate s procesed in subsequnt reactons that differ betweenorganisms and depen the availability of oxyen.

Concept questions 5.2a

1 Wite the heical equatin o glycolysis.
2 What is nitial ubstat i the glycolysis pathway? What is the final product?
3 Where does lycolsistaelace in all cells?
4 Gyoysis is n anaerobic process. What does this mean?
5 Glycolysis produces four moecules of ATP. Why is the calculated net number of molecules produced by glycolysis stated as 2?

HOT chaenge

6 Glycysis, the procs of synhesising new ATP, reqires hich of tefollowng s an input: a six-carbn sugar, two tree-carbon sugars, NADH or oxygen?

WB 5.2.2 CELLULAR RESPIRATION USING OXYGEN PAGE 118

Cellular respiration using oxygen

In eukaryotic cells that have oxygen available, the two molecules of pyruvate per glucose molecule formed in glycolysis enter organelles called mitochondria (singular: **mitochondrion**). The pyruvate molecules are the initial substrate in a series of reactions that use oxygen to produce a much larger amount of ATP than in glycolysis. Mitochondria are often described as the 'energy powerhouses' of the cell because inside them many ATP molecules are produced in two further biochemical pathways called the **Krebs cycle** (or citric acid cycle) and the electron transport chain.

EXAM TIP
Remember that the total number of ATP molecules produced per molecule of glucose is through the additive combination of the processes of glycolysis, Krebs cycle and electron transport chain.

Structure of mitochondria

Mitochondria are small organelles that are found throughout the cytosol of eukaryotic cells. They are attached to the cytoskeleton. Each mitochondrion consists of an outer smooth membrane and a highly folded inner membrane. The folds in the inner membrane, called **cristae**, protrude into the inner space of the mitochondrion, which is filled with a protein-rich fluid, the **matrix**. The space between the outer and inner membranes is also filled with fluid and is called the intermembrane space (Figure 5.13).

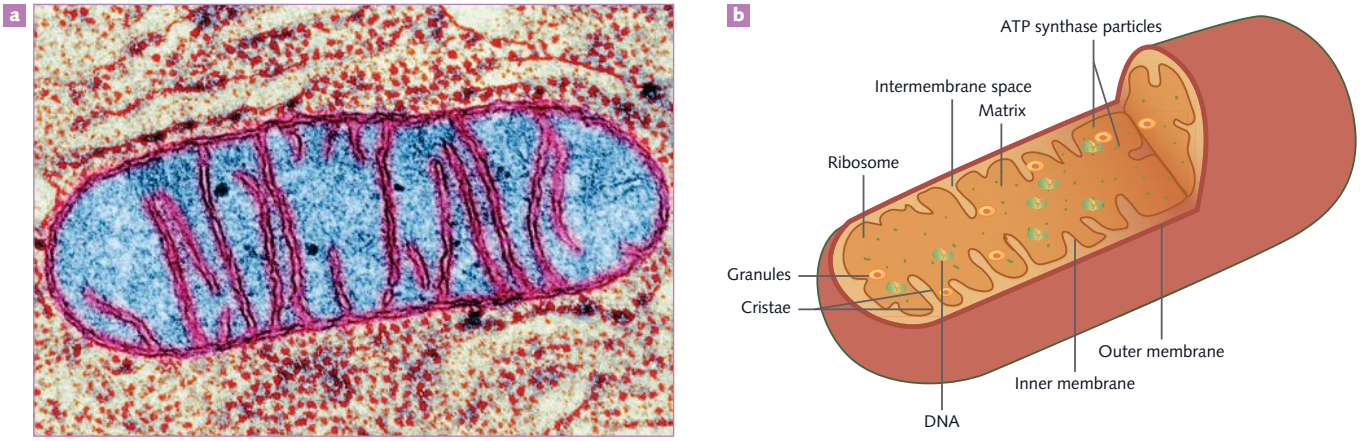


Figure 5.13 a An electron micrograph of a mitochondrion and b a diagram of part of a mitochondrion. Note the presence of mitochondrial DNA and ribosomes.

Mitochondria, like chloroplasts, have their own genetic material – mitochondrial DNA (mtDNA) and RNA – and ribosomes. Like chloroplasts, they are also capable of division independently of the cell's nucleus, so they make copies of themselves and have many characteristics of prokaryotic cells. This evidence points to their origins as independent organisms.

Krebs cycle occurs in mitochondrial matrix

The two three-carbon pyruvate molecules formed in glycolysis are transported into the mitochondrial matrix. Pyruvate undergoes an intermediate reaction that results in the formation of two molecules of the loaded coenzyme **acetyl CoA** (a two-carbon molecule), which are then broken down. The final products from the pyruvate that entered the Krebs cycle are carbon dioxide, ATP formed from the energy released in the reactions, and two types of loaded acceptor molecules, NADH and $FADH_2$.

The following is a simplified summary equation of the Krebs cycle:

$$\underset{\text{Inputs}}{\text{Pyruvate} + \text{ADP} + P_i + \text{NAD}^+ + \text{FAD}} \longrightarrow \underset{\text{Outputs}}{\text{carbon dioxide} + \text{ATP} + \text{NADH} + \text{FADH}_2}$$

Table 5.4 summarises the inputs and outputs of the Krebs cycle.

Table 5.4 Summary of inputs and outputs for the Krebs cycle, which occurs in the mitochondrial matrix. Values are per glucose molecule

Inputs		Outputs	
Molecule	**Total number**	**Molecule**	**Total number**
Pyruvate	2	CO_2	6
NAD^+	6	NADH	6
FAD	2	$FADH_2$	2
ADP P_i	2 2	ATP	2

Electron transport chain occurs in mitochondrial cristae

The loaded acceptor molecules produced in the Krebs cycle (NADH and $FADH_2$) enter the electron transport chain, which takes place in the inner mitochondrial membrane on the cristae. NADH and $FADH_2$ are coenzymes that contribute to the production of ATP and water in the electron transport chain. With the addition of the loaded coenzymes, electrons are transferred through a series of enzymes and compounds called **cytochromes** that are embedded within the inner mitochondrial membrane. NADH and $FADH_2$ donate the H^+ to become NAD^+ and FAD to be recycled and used again. H^+ is taken up by oxygen, which is an electron acceptor, to form the by-product, water. The energy released during this electron transport chain is used to drive the production of ADP and P_i into ATP, catalysed by the enzyme ATP synthase. There is a net 32 ATP molecules per glucose molecule produced in one cycle (Figure 5.14), but the amount can vary depending on the cell type.

The following is a simplified summary equation of the electron transport chain:

$$\underset{\text{Inputs}}{\text{Oxygen} + \text{ADP} + P_i + \text{NADH} + \text{FADH}_2} \rightarrow \underset{\text{Outputs}}{\text{water} + \text{ATP} + \text{NAD}^+ + \text{FAD}}$$

Table 5.5 summarises the inputs and outputs of the electron transport chain. Table 5.6 summarises the inputs and outputs of aerobic respiration.

EXAM TIP
The specific details of the glycolysis, the Krebs cycle and the electron transport chain aren't required by VCAA. But you must know the inputs, outputs and locations of each process.

Table 5.5 Summary of inputs and outputs of the electron transport chain (values are per glucose molecule)

Inputs		Outputs	
Molecule	**Total number**	**Molecule**	**Total number**
O_2	6	H_2O	6
ADP P_i	32 32	ATP	32
NADH	10	NAD^+	10
$FADH_2$	2	FAD	2

Weblink
Cellular respiration

Online Worksheet
Cellular respiration

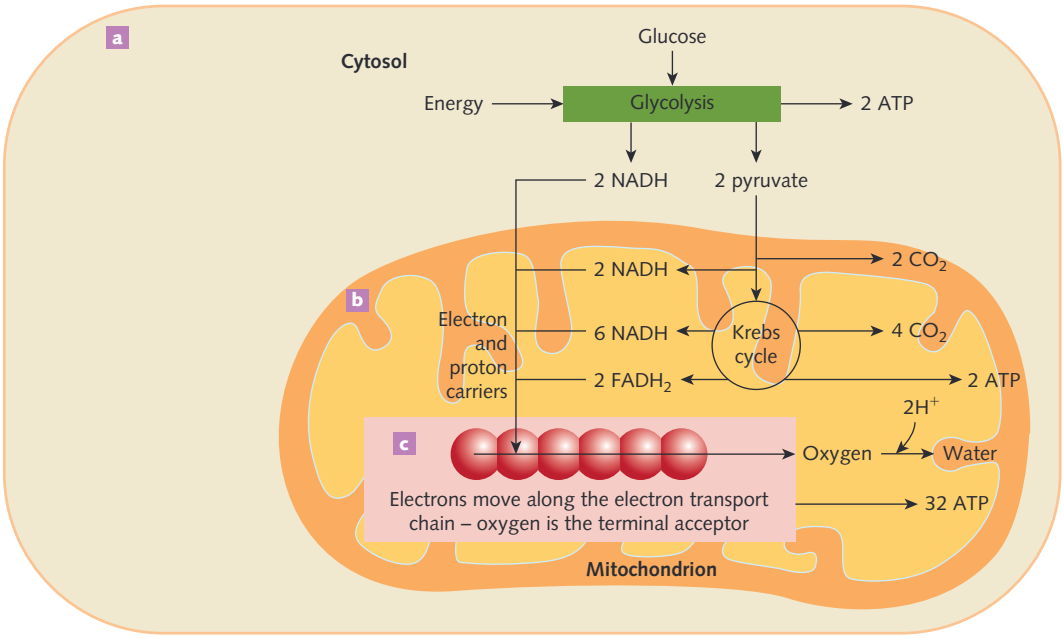


Figure 5.14 a Glycolysis, the first stage of cellular respiration, occurs in the cytosol. In glycolysis, glucose breaks down to pyruvate. **b** Pyruvate enters the mitochondria. In the presence of oxygen, the Krebs cycle and **c** electron transport chain occur.

Table 5.6 Total inputs and outputs for cellular respiration in the presence of oxygen (aerobic respiration)

Process	Location	Inputs	Outputs
Glycolysis	Cytoplasm	1 glucose 2 ADP + 2 P_i 2 NAD^+	2 pyruvate molecules 2 ATP 2 NADH
Krebs cycle	Mitochondrial matrix	2 pyruvate molecules 2 ADP + 2 P_i 6 NAD^+ 2 FAD	4 CO_2 2 ATP 6 NADH 2 $FADH_2$
Electron transport chain	Mitochondrial inner membrane, cristae	Oxygen 8 NADH 2 $FADH_2$ 32/34 ADP and 32/34 P_i	Water 8 NAD^+ 2 FAD 32/34 ATP
Total			36/38 ATP per glucose

9780170452533

INVESTIGATION 5.2

Investigating aerobic and anaerobic respiration

Am

To investigte aerobi andanaerobic respiration

Time reuirement

45minutes

Materas

- lucose solution 5 mL
- Janus Green B oxgen indcator solution (5 mL)
- 80–100 Yast balls
- iilled water 5 mL
- 2Glucose test strips
- 2Plastic pipettes
- 2Glass beakers (for 37°C water baths)
- latic spoon
- imer or stopwatch
- Marker
- Paper owel
- St rring rods
- 2 Syringes (10–20 mL)
- 2 Syringe stands
- 2 Mtal washers
- Straine (ptional)

What are the rsks n ths n vestgaton?	How can you manage these rsks to stay safe?
Janus Green B may cause eyeiritaio. W	ear lab coats safety glass es and gloves wash hands thorougly at end the end of the investigation
Yeast can cause an allergic reaction in sensitive people	Wash hands after ue. Do not ea inclas, due to the possbt y of contamination Be aware of any allergis.
Dsposabe loves may posallergy risk Use	type of glove that r emoves allergy risk and is suitable to chemicals being use.
Disposable gloves can cause allergic reation in sestive pepe.	Use a type of gove that has no aergy isk andis sitale to use ith the cheiclsin this investigation.

Method

Determnng the nta gucos e concentraton of the souton

1. Use a lsic pipette to add two drops of Janus Green B ox ygeninicator to he glucs solutin. Swil gently to mix.
2. Cllect a glucoseteststriand dip the yellw quare ab at the tip into th glucose solution for 2–3 seconds.
3. To remove eces liquid, pat te test strip dry with a paper toel. Leavethe strip to ret or three minute.
4. To detemine nitial oncentration glucose in milligrams per deilires, compar the colour ofthe test strip with the olour chart on thepakaging.
5. Copy th resutstables int your logbook.Record the initial glucose concentrton in esult s table 1 for th respiromee containing yeast bal + glucose soution.
6. Repeat steps 1–4 wth dsted water (n pace of gucose souton) to determne the gucose concentraton of the contr. Record the resuts in thetble.

Assembng the resprometers

1. Remove the whte plunger frm the clear chamber of a syringe.
2. Pace a meal washer over the bas of the syinge stand to enable the respirometer toremain submerged in a wter bath during data coletin (Fiure5.15).

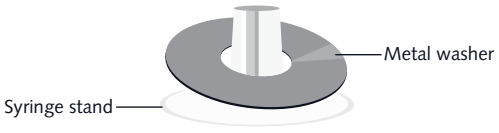


Figure 5.15 Place a washer over the base of the syringe.

Figure 5.16 Add yeast balls to the 1 mL mark of the syringe

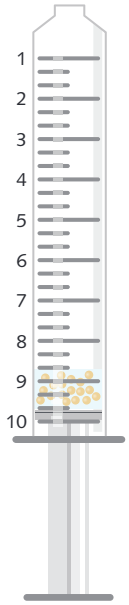


Figure 5.17 The inverted respirometer

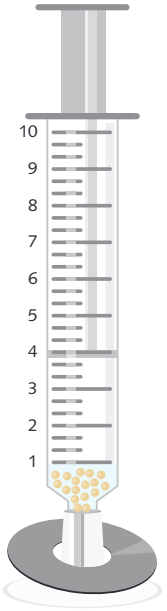


Figure 5.18

3 Careuly placethe clear syringe camberon te syringe stand. Then, use a plastic spoon to fi the syringe chamber wth yeat balls t the 1.0 mL mr (Figure 5.16). Do nt transfer excess water into the chamber as you add the yeast bal; use a triner if necessary.
4 Careuly insertth whiteplunger nto the yringe but only en ough to connect the two pices (aproximately 2 mm). Remove the repiromete (cler syring chamber + white plunger) from the bae o te syringe stand and invert it (Figure 5.17).
5 Dislodge the east bals from th tip o the sring by lighly tapping the clear chamber.
6 Gradualy compress the plungeron the inerted respiromter to the 1 mL mark. Make sure no yest ballsre expelled through the syringe.
7 Pace thetip of te syrine n the olution and lowly draw up(aspirate) 3 mL of glucose + oxygn ndicao solution untl th solution reachs the 4.0 mL mr on th sringe. The ttal volume f the yeast balls and oxygen indicator sltin is now mL.
8 Pace the respirometer back onto the syringe stan with the washer. D notdepress the white plunger.
9 Repeat steps 6–13 to prepare the contro respirometr, dawn liquid from th e glclose and Janus Gre Bsolution. La e thisrespirometer E fo experiment.

Data coecton from th e resprometer system

1 Colect two glass beakers containing 200 mL of warm (approximately 37 °C).
2 Lael one beaker ‘east balls + glucoe’ and the othe‘Yeast balls + watr’ (cntrol).
3 Careully submerge ech resirometer intothe ppriately labelled beaker.
4 Record the saring volume fr each respiometer as 4 mL.
5 Using the graduted olume markings o the syringe chamber, recod he volue o each syringe, and the indicator solution colou r everyminute fo 30 inutes ecord hs informaion in eultstables 1 and , alongwith any other observations you make.
6 To alculate the chage in gas olue a eah minuteinterval, calcule the difference between the initial reading (4 mL) and the final reaing on the syringe.
7 When the plunger reaches the10 mLark, stop collecting data and emove the respirometers from their water baths.
8 Todisassemble he repirometers cover themwith a paer towel and crefully remove the white plunger from the clear chambe ontaininyeast balls.
9 To detemine the final concentrtionof guose withn each respiromet er, ip the end o a glucose tst strip into the sltin in eachsyringe and copare the colour of the test st ripswith te colour chart on th packaging. Record the final lucose concetrtonlevels inyourresults tables.

10 Share youridividualexperment results wth the class to generte a class data set and use the class daa to calculate the mean volume fo eah ti interval in the glucose re spirometr. Record te esult n the 'Class mean CO_2 vlue' columnin the rsultstbles.

Resuts

Copy the resuts tabes nto yo ur ogbook Compete the resuts tabes and graph your resuts

Resuts tabe 1 Gucose repirometer results

Water bath temperature (°C) _____

ntial colour f solution _____

Fnal clour of olution _____

ntia glucose cocentration (mg/dL) _____

Fnal lucose concntration (mg/dL) _____

Tme minReading on pipette (mL)	–	tialreading	=	CO_2 volume (your group)	Clour of nicator soution	Observations	Class mean CO_2 volume
0		–		=				
1		–		=				
2				=				
3		–		=				
4		–		=				
5		–		=				
6		–		=				
7		–		=				
8		–		=				
9		–		=				
10		–		=				
11		–		=				
12		–		=				
13		–		=				
14		–		=				
15		–		=				
16		–		=				
17		–		=				
18		–		=				
19		–		=				
20		–		=				
21		–		=				
22		–		=				
23		–		=				
24		–		=				
25		–		=				
26		–		=				
27		–		=				
28		–		=				
29		–		=				
30								

Resuts tabe 2 Non-gucose res prometer reults

Water bath temperature (°C) _____

ntial colour f solution _____

Fnal clour of olution _____

ntia glucose cocentration (mg/dL) _____

Fnal lucose concntration (mg/dL) _____

Tme minReading on pipette (mL)	–	tialreading	=	CO_2 volume (your group)	Clour of nicatorsolution	Observations
0	–		=			
1	–		=			
2	–		=			
3	–		=			
4	–		=			
5	–		=			
6	–		=			
7	–		=			
8	–		=			
9	–		=			
10	–		=			
11	–		=			
12	–		=			
13	–		=			
14	–		=			
15	–		=			
16	–		=			
17	–		=			
18	–		=			
19	–		=			
20	–		=			
21	–		=			
22	–		=			
23	–		=			
24	–		=			
25	–		=			
26	–		=			
27	–		=			
28	–		=			
29	–		=			
30						

Dscusson

1 Were the esults of te nvestigationonsistent among all groups Ifnot, what are me possible reasons for the varaion i results? Were thee any experimenta errr or uncontrolled variables?
2 Explain why the aerage value of reuts from several investig aion is morreliable thn a single se o test results.
3 How doe his nvestgation design adress the suggestion th at caro dioide releaseddid not require the presence of carbohydrate?
4 Explain wh the indictor soluton in the resirtor changed colour.
5 Proide tw pieces of evidenc fro te investigation that lustrate hat glucose was fermntedby yeast balls during cellar respration.
6 Yeascells serve s a odel oanism for cellular rocesses occrring in other eukayotic cells, such as those of humans. Explain whyhe cells of oubody normally do not carry out fe rmentaion, yt we give of carbon doxide when we exhale.

Takin it further

Fllwing the same mtho atis eeriment, s t possibl that carbn dioxide can be released from sugar without the presence of yeast? Din an ditional controlled experiment thatwould allow yo totest this idea.

KEY CONCEPTS

» Pyruvate rm lycolyss enter the mitochondria where t enters the Kreb ycle.
»In the Krebscycle,pyrvte is competely broken down, releasing ca bon dioxide as a byproduct and producing ATP and he loaded coenzymes NADH and $FADH_2$.
» On th cristae of themtochndria, a complex series of steps iolving cytochrome enzymes transfers the electrons to theoxygen, whih reacts with H^+ from the coenzymes to producewater, in the electron transport chn.
» The net output of ATP fom aoic celluar respiration s 36/38 ATP per gluosmolecule.

Concept questions 5.2b

1 What is te role of xygn ineobiccelular respiration?
2 What is the source of the by-prdct carbon dioxide in aerobic lularresiration?
3 How many nt molecules of AT are produced in one cycle ofaeroi celluarresiraton? In practice, the numberis someha less. Suggest a eson for this.
4 Where does cetyl CoA come fromad what is its function?
5 What molecule is th terminal acceptor at the end of theelectron tranport chain?

HOT chaenge

6 Wate is produced at the en of the electron transport chain.It is an outu of celulrrespiration. What are the source and form of the oxygen and of the hydrogen that are combined to make water?

Cellular respiration without oxygen

WB 5.2.3 CELLULAR RESPIRATION WITHOUT OXYGEN PAGE 119

The first energy-releasing pathways evolved around 3.8 billion years ago when there was not much free oxygen in the atmosphere. The process was essentially anaerobic because it could be completed in the absence of oxygen. Many bacteria and protists still live in places where oxygen is absent or not always available, and they produce ATP by anaerobic pathways. Such organisms have evolved biochemical pathways that allow glycolysis to continue in the cytosol by using molecules other than oxygen as the final electron acceptor. Prokaryotes have evolved many different anaerobic pathways.

As you have seen, oxygen is required for aerobic respiration in eukaryotic cells. If oxygen is absent, the Krebs cycle and the electron transport chain in the mitochondria shut down. The cell then relies entirely on glycolysis to maintain a supply of ATP for its energy needs. However, without the Krebs cycle and electron transport chain to remove them, the products of glycolysis build up in the cytosol. These products

are pyruvate and the loaded coenzyme NADH. The problem is that NADH must be continually unloaded, forming NAD^+, to sustain glycolysis. Eukaryotic cells have solved the problem by reacting NADH with pyruvate in the processes of **alcoholic fermentation** or **lactic acid fermentation**.

Alcoholic fermentation

Many micro-organisms, including yeast and some bacteria, carry out alcoholic fermentation (Figure 5.19). The step producing ATP is still glycolysis, but a second step converts the potentially toxic pyruvate together with NADH molecules into carbon dioxide and ethanol. In the second stage, no additional ATP is made, so the net output remains at two ATP molecules per glucose molecule produced in glycolysis. The equation for alcoholic fermentation is:

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow \text{ethanol} + \text{carbon dioxide} + 2\text{ATP}$$

Humans make use of these metabolic waste products in the production of wine, beer and bread, using anaerobic cellular respiration by yeast (Figure 5.20). However, plants cannot use ethanol. It cannot be reconverted into carbohydrates, nor can it be broken down in the presence of oxygen. Ethanol is an alcohol, which is toxic to cells and cannot be allowed to accumulate. Many plants (or parts of plants) can respire anaerobically for a short time, such as germinating seeds and roots living in water-logged soil, where there is little oxygen. However, before the concentration of ethanol reaches a certain level, the plants must revert to aerobic respiration; otherwise, the plants will be poisoned by the ethanol.

Yeast uses anaerobic respiration, but respiration is better under aerobic conditions. If too little oxygen is present, the ethanol concentration rises so much that the yeast cells are killed. When making beer and wine, it is important to not let conditions become too anaerobic. Industrial fermentation microbiologists work to develop new strains of yeast that tolerate high concentrations of ethanol.

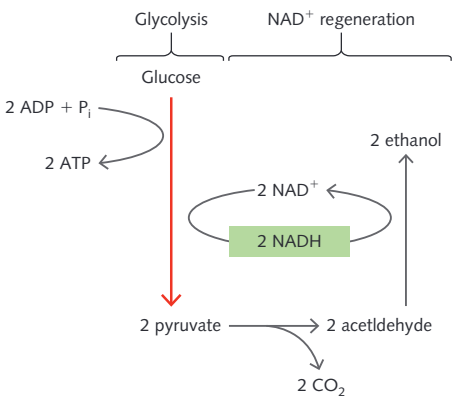


Figure 5.19 Alcoholic fermentation involves the breakdown of pyruvate generated from glycolysis into carbon dioxide and acetaldehyde, which accepts protons from NADH to form ethanol (alcohol).

Figure 5.20 Plants and yeast produce ethanol and carbon dioxide in the anaerobic respiration process of alcoholic fermentation. This reaction has been used by industry to produce bread and wine. In bread, the carbon dioxide makes the bread rise while the ethanol evaporates during baking.

Lactic acid fermentation

Lactic acid, or lactate, is the end product of anaerobic cellular respiration in animal cells. In this process, glycolysis produces two ATP molecules per glucose molecule, followed by the breakdown of the pyruvate to produce lactic acid (Figure 5.21). Animal muscle cells respire anaerobically when they need a short, rapid burst of energy or if not enough oxygen is available. Lactic acid fermentation cannot be sustained for very long because the build-up of toxic lactic acid will result in cell death.

After strenuous exercise, muscle soreness and cramps may develop. In 1929, Archibald Hill proposed that these symptoms were the result of lactic acid building up in the muscle tissue. However, the latest theory gaining widespread acceptance in the scientific world suggests that these symptoms are a result of an increase in extracellular potassium ion concentration, which leads to the observed muscle fatigue cramps.

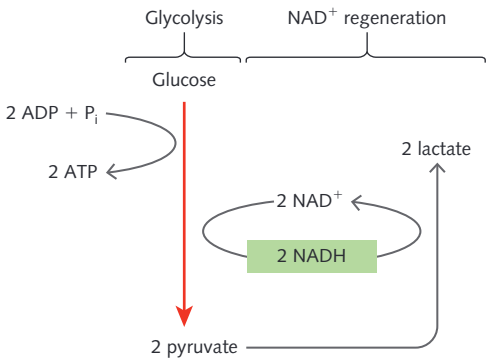


Figure 5.21 Lactic acid fermentation occurs in muscle cells. In the process, NADH is converted to NAD^+, allowing glycolysis to continue.

Comparing aerobic and anaerobic cellular respiration

Aerobic cellular respiration involves the complete breakdown of glucose to release enough energy to produce 36/38 ATP molecules per glucose molecule. In anaerobic cellular respiration, glucose is only partly broken down and enough energy is released to form only two ATP molecules per glucose molecule. The remainder of the energy is still trapped in the bonds of the complex molecules of ethanol or lactic acid. In animals, the lactic acid molecules can be converted back into pyruvate, which can then be broken down into carbon dioxide and water in the Krebs cycle and electron transport chain (Figure 5.22). Table 5.7 summarises anaerobic and aerobic cellular respiration.

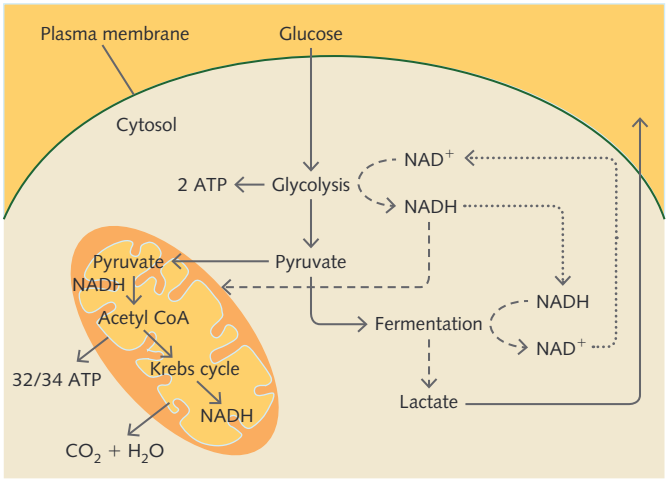


Figure 5.22 A summary of aerobic and anaerobic respiration in an animal cell

Table 5.7 A summary of anaerobic and aerobic cellular respiration

	Anaerobic	Aerobic
Reactants	Glucose	Glucose/oxygen
Amount of ATP produced per glucose molecule	2 ATP	(Varies according to cell type) 36/38 ATP
Location	Cytosol	Cytosol (glycolysis), mitochondrial matrix (Krebs cycle) and cristae of mitochondria (electron transport chain)
Stages	Glycolysis and conversion	Glycolysis, Krebs cycle, electron transport chain
Products	Animals: lactic acid Yeast: ethanol and CO_2	CO_2 and H_2O
Speed of reaction	Faster for short burst of energy	Slower for longer, sustained energy supply

Putting photosynthesis and aerobic cellular respiration together

Photosynthesis and aerobic cellular respiration are closely related and interdependent – that is, the outputs of one are the inputs of the other (Figure 5.23). In plants and other autotrophs, the two processes occur in the same cells when both chloroplasts and mitochondria are present. But many cells in green plants, such as root cells, do not have chloroplasts. These cells and those of heterotrophs depend on the products of photosynthesis to carry out cellular respiration. Thus, there is a dependency between autotrophs and heterotrophs.

EXAM TIP
Cellular respiration occurs in all living cells all the time. Photosynthesis occurs in living cells of plants and autotrophs in the presence of light energy.

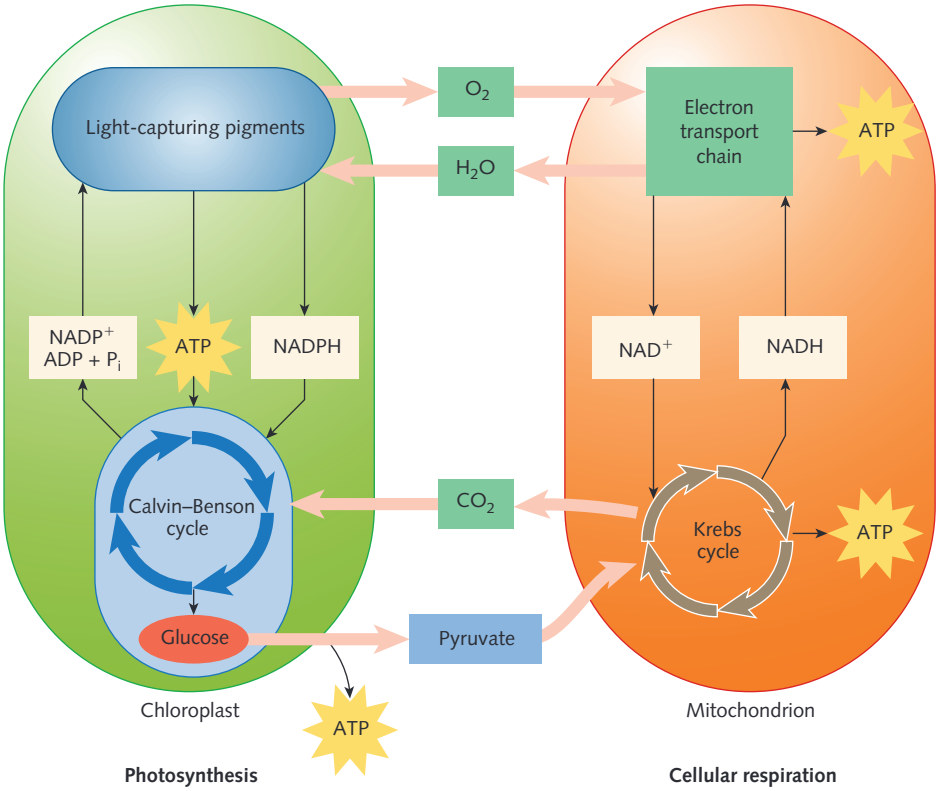


Figure 5.23 **Photosynthesis uses the products of cellular respiration and cellular respiration uses the products of photosynthesis.**

Factors affecting rate of cellular respiration

The rate of cellular respiration is influenced by both environmental and physiological factors: temperature and the concentrations of glucose and oxygen. As with all biochemical reactions, as the concentrations of substrates increase, the rate of cellular respiration increases up to a saturation point, at which time other factors limit the reaction rate. These other factors may include the amount of enzyme available, the pH of the intracellular environment, and the presence of cofactors, coenzymes, and competitive and non-competitive inhibitors of the enzymes.

Yeast is an accessible organism for observing the effect of various factors on the rate of cellular respiration. When mixed with a glucose and water solution, yeast undergoes anaerobic cellular respiration, producing carbon dioxide that forms foam. Several independent variables can be altered to observe the effect they have on anaerobic cellular respiration, including temperature, concentration of glucose solution, and the availability of oxygen. The amount of foam produced can be measured as the dependent variable.

WB
5.2.4 PUTTING PHOTOSYNTHESIS AND AEROBIC CELLULAR RESPIRATION TOGETHER PAGE 120

n.
video
Photosynthesis and cellular respiration?

Temperature

The rate of cellular respiration increases as temperature increases because of increased molecular movement and frequency of collisions between the substrate and enzyme molecules. At a certain high temperature, the enzymes involved begin to denature and the rate of cellular respiration slows down and almost stops. Organisms reach the upper limit of their tolerance range and cells and tissues start to shut down.

At lower temperatures, the kinetic energy of all the molecules involved is lower and this affects the speed of their reaction. When it is cold, plant growth rates decrease and some animals go into hibernation to minimise their energy requirements.

Glucose

Cellular respiration depends on an ongoing supply of glucose so cells must constantly replenish their glucose stores. Photosynthetic cells can produce their own glucose, while other non-photosynthetic plant cells rely on transport of glucose to them. In multicellular organisms, glucose can be stored in specialised cells and released and transported, as required, to the rest of the tissues. Plants store glucose as starch grains in the cells of roots, stems and even leaves. Animals accumulate and store glucose as glycogen in liver and muscle cells. The need for glucose must be communicated between the cells that demand it and the cells that store it. If glucose stores become depleted, the body draws on alternative sources to meet the energy needs of its cells such as lipids or proteins.

Oxygen

The availability of oxygen affects the type of cellular respiration carried out by the cells, whether aerobic or anaerobic. During anaerobic respiration, the ATP is supplied only from glycolysis, which generates a much smaller amount due to incomplete glucose breakdown than from aerobic respiration (two ATP compared to 36/38 ATP per glucose molecule).

However, ATP production by anaerobic respiration is faster than that of the more complex set of reactions involved in aerobic respiration. Consequently, if the demand for ATP outweighs the supply from aerobic respiration, eukaryotic cells shift to anaerobic respiration. This occurs in the muscle cells of athletes, such as sprinters, when they perform brief bursts of strenuous exercise. The switch can only be temporary because the accumulation of lactic acid in cells can denature enzymes and inhibit metabolism. It must be removed to restore normal cell functioning, which takes time to achieve.

KEY CONCEPTS

- Eukaryotes have two anaerobic lular esiration pathways for poducing TP from glucose in the absence of xygen.
- Yeats, plants an some bactera carry out alcoholic fermentaion; omeanimal cll carry out lactic acid fermenation.
- The products f aneroic respiration are toxic at hgh concentations and so anaerbicrepiation is ony a short-term olutionfr obtainng chemical energy.
- The rate o ellular rspiration is affected by temperatre, glucose and xen avalability as well as other factors.
- Photosynhesisd cellular re spiration ae linked – the nputs of one are the outputs of the other.

Concept questions 5.2c

1 Lst two differences between arobic respiration and fermenation.
2 Compare the products of nerobic respiration with those of erobic rspiation in:
   a aialcells
   b planclls.
3 Describehw cellla respration i altered when:
   a temperature is very hig or very low
   b oxygn lvels arevery high or very low
   c glucose lvels arevery highor very low.
4 Drawa imple sheatic diagram to show how photosnthessand clllar repirtion are linked.
5 Comlete arobic rspiration of n glucose molecule releases enough energy to ake36/38 ATP molecules. n anaerobic esiation, one gluose molecule releases enough energy to create 2 ATP. What has happened to the remining energ in the glcose molecule in anaerbicrepiration?

HOT Chaenge

6 n eukarot ells, ifoxygen is ot present, aerobic respiratioill not proceed. The producs of glycolysis are converted to other outputs through a further anaerbic process and the otheoututs collect in the ll. What are the her possible outputs and what effects can they hav n te cell? Is this further anaerbic proces eversible?

5.3 Biotechnological applications of biochemical pathways

The world's population is estimated to reach about 9.7 billion by 2050, which will affect the world's ability to produce enough food. In addition, the challenges of climate change, and increases in pests and diseases to crops already under threat, may contribute to lower crop production rates in Australia and around the world. Lower crop production rates are expected to have negative impacts on the amount of food available for people across the world. It is hoped that development of new technologies, many of which use biotechnology and genetics, may help to meet the increasing demand to feed the world's growing population.

WB
5.3.1 APPLICATIONS OF CRISPR-CAS9 TECHNOLOGIES PAGE 121

Using CRISPR-Cas9 to improve photosynthesis

The CRISPR-Cas9 gene-editing tool is a technology that has enormous potential to enhance crop yields. CRISPR (clustered regularly interspaced short palindromic repeats) is a process mediated by the protein Cas9. Many scientists are researching and experimenting to determine how the CRISPR-Cas9 technology can be used to enhance photosynthesis in plants to increase crop yields. The CRISPR-Cas9 protein acts as an accurate 'cut and paste' tool for geneticists so they can introduce specific DNA sequences into plant genomes that code for more efficient photosynthetic traits. CRISPR is efficient and easy to use. Possible applications of CRISPR to increase yield include enhancing light availability (by altering plant architecture), increasing light capture and energy conservation. CRISPR can be used to directly modify a trait, by targeting the exact genes or regulatory genes to be modified. These techniques are cheaper and faster than both traditional cross-breeding methods and other transgenic methods.

CONNECT
CRISPR is discussed in detail in Chapter 3.

Tobacco (*Nicotiana* species) is used widely for this research because tobacco plants reproduce quickly, are easy to grow and can be engineered easily by modern transgenic methods. Once the transfer of certain genes can be accomplished in tobacco, scientists are hoping to transfer these genes to crop plants such as soybeans, potatoes, maize, rice and wheat in order to increase their biomass, which in turn could increase the amount of food available for the world's growing population.

Applications so far include engineered plant pigments that use a broader spectrum of light wavelengths from the Sun's radiation, allowing plants to absorb sunlight more effectively. Too much light can damage a plant so, under extreme light conditions, plants have adapted a mechanism to 'switch off' the process of photosynthesis. However, the mechanism to then 'switch on' photosynthesis is slow. Scientists have used CRISPR to engineer a process to speed up this transition and to allow plants to maintain a more consistent rate of photosynthesis. This has achieved enhanced production in tobacco plants and scientists are hoping this can be transferred directly to crop-yielding plants.

Scientists are also looking to enhance the efficiency of Rubisco, one of the key enzymes involved in driving photosynthesis. Rubisco is a protein that binds to carbon dioxide during photosynthesis. However, oxygen and carbon dioxide molecules compete for the same binding site on Rubisco and approximately 20% of the protein is susceptible to oxygen binding in place of carbon dioxide (this process is known as photo*respiration*). In addition, when Rubisco binds to oxygen, a toxic compound is produced that must be recycled, and this costs the plant further energy and resources that could otherwise be used for growth. Scientists aim to make Rubisco more efficient at binding to carbon dioxide to increase productivity. Scientists who have engineered an alternative pathway for photorespiration to decrease this wasted energy and enhance photosynthesis estimate that the savings in resources for plant growth could be as high as 40%.

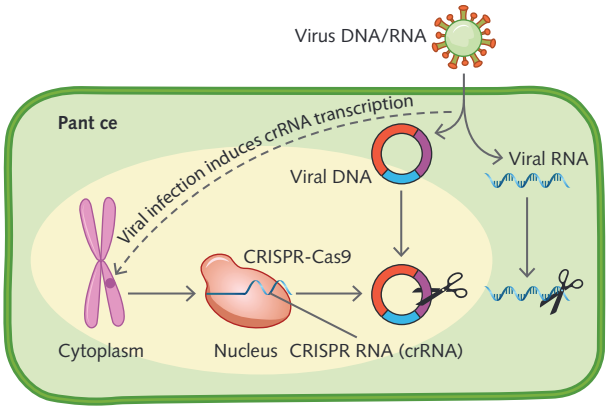


Figure 5.24 Using the CRISPR-Cas9 system to defend against viral pathogens. Coding sequences for Cas9 and the CRISPR RNA (crRNA) are integrated into the plant genome, and their transcription is induced by infection. The crRNA targets the CRISPR-Cas9 complex to cut the DNA or RNA of the virus, conferring resistance to the invading virus.

Other areas of potential applications for the CRISPR-Cas9 system include increasing the yield of crops under particular biotic and abiotic stresses by providing disease-resistant genes (Figure 5.24); and to improve tolerance in crops growing in drought and salinity-affected areas. Rice, barley, wheat and maize are staple food crops for more than half of the world's population, and scientists hope that CRISPR-enhanced crops can significantly produce greater yields of biomass in these crops by conferring resistance against external stresses.

CRISPR is also being researched to improve fibre quality in cotton production. Other potential uses for CRISPR technology include protein seed storage in soybean, virus resistance in tomatoes, starch yield in potato crops, disease resistance in citrus fruit, and pest resistance in grapes.

Other potential applications include introducing novel genes into plants to produce new 'behaviours' to enhance their efficiency. Artificial DNA sequences, including regulatory and

transcriptional elements, can be inserted into plant genomes so that the plant can undertake processes such as nitrogen fixation, a protein production method that is exclusive to legumes. If a plant can fix nitrogen, then it can maintain growth and biomass in nitrogen-deficient soils, and globally could reduce the need for inorganic fertilisers, in turn reducing pollution from fertiliser run-off. Other novel areas of interest include using CRISPR to reverse the effects from hundreds of years of inbreeding in species such as maize and rice, to counter the reduced genetic variation in these species. Such historical reduction in genetic variation has had a negative impact on the plant's ability to survive under specific environmental stresses. CRISPR could be used in crops to manipulate specific traits that are under the control of only one gene in the wild species, so they would be controlled by a system of polygenes to increase genetic variation, so that in times of stress these plants have alternative pathways to keep thriving and producing high yields.

Genome editing using CRISPR will no doubt be a powerful tool for specific crop improvement within agriculture. Not only is the technique simple and efficient, it also provides the precise ability to edit regulatory and structural genes to enhance yield, resistance to pests, increase quality of biomass and increase the ability of crops to improve photosynthetic yield under stressful conditions.

Biomass for biofuel production

WB
5.3.2
ANAEROBIC FERMENTATION OF BIOMASS FOR BIOFUEL PRODUCTION
PAGE 122

In the summer of 2019–2020, Australia experienced a series of bushfires that killed 75 people and an estimated 3 billion animals, burned more than 11 million hectares of land, and destroyed almost 2000 homes. There has been much discussion about climate change and its contribution to this and other natural disasters. British naturalist Sir David Attenborough says it is time for governments across the globe to address this issue. He has called on countries such as China to take action, in the hope that other countries will follow and make carbon emissions reduction a priority. Protests against inaction on climate change have increased in recent years and people are thinking about how to reduce their carbon footprint.

One way to reduce the use of fossil fuels is to harness the energy in plant and animal **biomass** to produce **biofuels**. Anaerobic fermentation using biomass is often referred to as 'anaerobic digestion' because it uses micro-organisms to decompose plant and animal waste products in the absence of oxygen (like detrivores in a food web), and as a measure of organic waste management. Anaerobic digestion occurs naturally in particular ecosystems, such as deep lakes and ocean basin residues, due to their lack of oxygen.

The waste product of such decomposition is methane gas, a flammable organic gas produced by bacteria, which can be collected and used as a fuel source. This fuel resource is known as biogas and has been produced since 1859 in India and the 1870s in England. In modern times, communities in developing nations have used biofuel from fermentation 'generators' on a household scale. Vegetable waste and animal manure is used in these fermenters to produce methane gas for heat and light production. Fermenters are often made from relatively inexpensive plastics and pipe and can be kept safely in trenches beside rural homes. The ability of various livestock animals to produce such biogas varies. For example, in warm climates, 1 kg of cattle dung can produce 40 L of biogas in a day, whereas 1 kg of chicken droppings can produce up to 70 L.

Biogas consists of only 50–75% methane compared to natural gas, which consists of 80–90% methane. However, in biogas, all the carbon in the methane being burned to produce carbon dioxide has already been drawn down from the atmosphere as the original plant photosynthesised. On the other hand, burning of natural gas extracts carbon that has been stored for millions of years and adds this to the carbon already existing in the modern atmosphere. Biogas production, therefore, is a carbon neutral process, where the entire path from plant to livestock to manure to decomposition is considered a closed system. Biogas-generated methane burns readily in oxygen to produce carbon dioxide and water vapour but leaves no carbon footprint.

Applications of biogas on a larger scale are being developed, particularly in Germany (Figure 5.25). Rural farmers may sustain heat for cooking and gas lamps for light from their biogas generator while engineers in Germany are using biogas in two ways to generate electricity:

» methane fuel cells, which are costly to build and require clarified gas
» conventional combustion engines to drive electrical generators.

Conversion of biogas to electricity is becoming a standard technology and an ethical, sustainable and cost-effective method to produce energy from waste products.

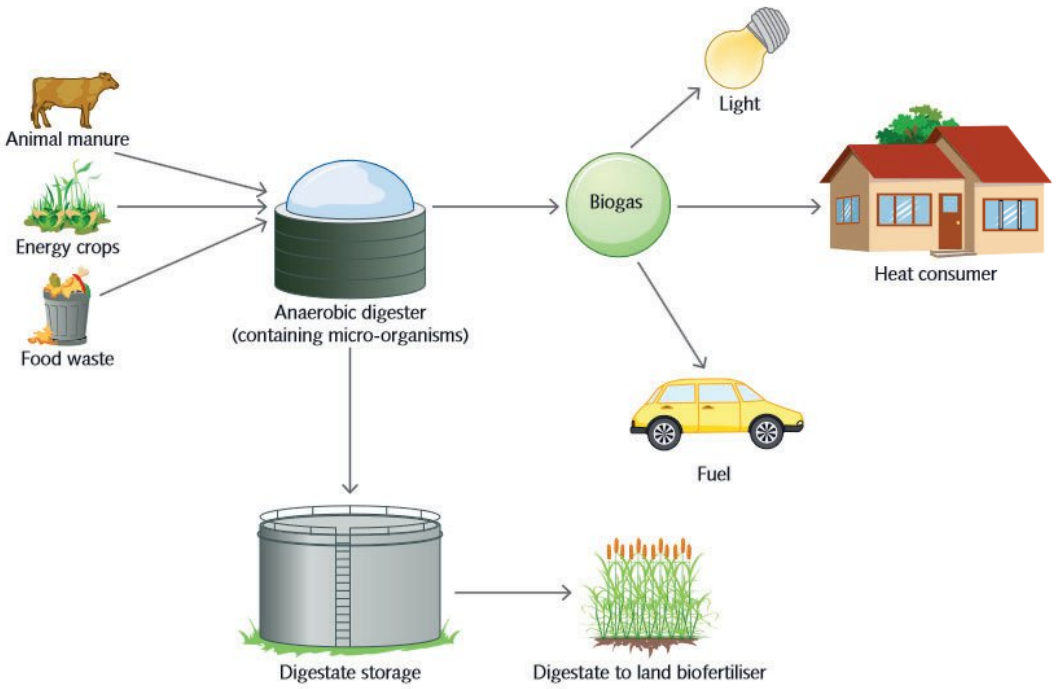


Figure 5.25 Biogas can be converted to electricity. Organic material is broken down (digested) to produce biogas, which is used to produce heat, electricity and fuel for cars. Residue is used as fertiliser.

KEY CONCEPTS

- The CRISPR-Cas9 protein acts as a 'cut and paste' tool for geneticists so they can introduce specific DNA sequences into plant genomes; for example, to code for more efficient photosynthetic traits.
- Possibilities of CRISPR-Cas9 include increasing crop yield by enhancing light availability (by altering plant architecture), increasing light capture and energy conservation.
- Anaerobic fermentation of biomass by micro-organisms produces biogas, which is used as a fuel for transport and electricity.
- Biogas production is a carbon neutral process, where the entire path from plant to livestock to manure to decomposition is considered a closed system.

Concept questions 5.3

1 List some potential uses for CRISPR biotechnology tools in growing more efficient food crops. Distinguish what aspects of the plants would be targeted.

2 CRISPR-Cas9 protein is a natural 'cut and paste' tool. Where does it come from?

3 The increased growth of biomass crops to produce biofuels in the Amazon rainforest in Brazil has been shown to be both a great leap forward and a disaster. Research the pros and cons of this newer cropping program.

4 Australia has some of the most nutrient-deficient soils in the world, yet produces more than $75 billion worth of food through cropping, grazing and fishing. Australia only requires currently $25 billion worth of food to feed its own citizens. The surplus food production is mostly exported to other countries. In Australia, why might a decreased need for fertilisers to supplement the deficient soils engineered through the use of CRISPR be a positive step for crop production?

5 Rubisco is the most abundant enzyme on Earth, but can easily be inhibited.
   a What are the main issues with the Rubisco process in plant photosynthetic pathways?
   b How might biotechnical engineering help to increase plant photosynthesis rates by 40%?

HOT challenge

6 In Australia, cotton cropping is controversial because it requires huge amounts of water and pesticides. How has the process of CRISPR reduced some of these concerns, and what might be the future for cotton in Australia?

BRANCHING OUT

A closer look at global issues with local solutions

Figure 5.26 **Stuart Muir Wilson is project coordinator at the Ecological Justice Hub in Brunswick, Victoria.**

Stuar Mir ilson is theproject coodiaor atheEcoical Justice Hub in Brunswick, Vicoria (Figure 5.26). Suat is an architect bioenergy expert and humanitarian who has worked n ecological catastrophs for moretn 11 years in many countries, including Austalia, Gerny India, Nepal andMexico and ountries in Eastern Africa.

Hs projects ave icuded building iny omes' and runing a community garden tha provides fresh vegetabls to hmeless eope. The aim of Ecological Justie isto bring boutsocia and environmenal justice through projects, eduction a training t serve arinased and vulnerable people.

Projects at the ub include courses for the comunity such as mushroom grown, woodwork andimber recyclingzero waste harvesting, cooking and composting demontrations. Many of the project aim to reuceglobl warming through community awareness and to proid ides, eucation and support to help regenerateenvironentalrecovery.

The Hubalso hs a biogas reactor signed an buit by Stuart (Figure 5.27). t uses bioass in the form of compost to produe methane that is then convertd ino ectricity and heatfor neghouring dwellings. The bioreactor works by anaroicallyferenting biomass waste and captures methane and carbon doxde. The cabn dioxide is fitered out and the metane gas is re fined s it can be used as asore f electricity. The bioreactor provies aclose loop olution that links the carbon footrint from waste oelcricity. This means that one of ts min bene fits to commntieswoud beess reliance on foss fuels fo electricty needs.

The wder mpcations for aaerobic ermentaton producing bi ogascould mean farmers and industry are offered a cost-effe tive wst disosal optionthat doesn't require the use of expensive and unnecessary land fi Governments could offer farmersand indusy subsidies to take up an oportunity touse aboreactor.Cosieig their low cost and relative simplicit y and that humans produce a lt of organic waste, biogas genera tors culd ell be te e lcalsolutonto global warming.

Figure 5.27 **The portabe boreactor at the Ecoogca Justce Hub n Brunswck produces e ectrcty from bomass waste products suppyng eectrcty w thout the use of foss fues**

Weblink
Ecological Justice Hub

Question

1 iscuss the approach that tuart is akingto the comple mental ustice in terms of consequences-basedbiothics.

2 Identify any socia and econmic factors relevan o he ork of the Ecological Justice Hub.

Online Key Concepts
Chapter 5: Summary
of key concept

5 Summary of key concepts

5.1 Photosynthesis as a biochemical pathway

KEY CONCEPTS

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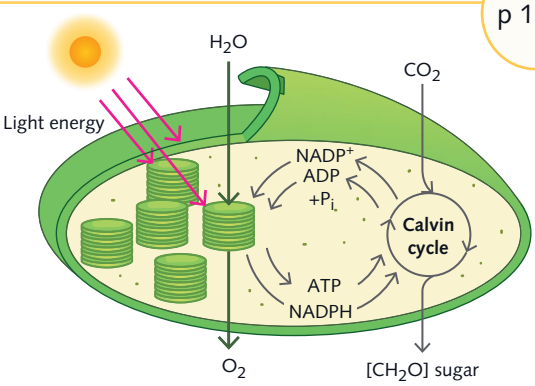

Figure 5.4 The close relationship between the light-dependent stage and the light independent Calvin–Benson cycle.

- » Autotrophs areorganisms that can manufacture their own coplex orgnic molecles fom simple inorganic mlcules, using anenerg source. They include photoautotrophs and cheoautotrophs.
- » Heterotrophs btin teir cmplex organic substances by consuing otherorganisms and heir products.
- » Photosynhesis is the pocess by which photoautotrophs produc glucose from aron dioxe, water and light energy.
- » Photosynhesi occus in chlo plasts and has a light-dependent ad a ight-indeendent stage.
- » Th light-dependent stageof photsynthesis occurs in the thylakoids that make p th gran in a chloroplast.
- »In th light-dependent stage fphotosthesis, chlorophyll absobs light engy t splitwater, releasing hydrogen ions (protons) into te electron ansport chain, electrons and the by-product oxygen.
- » Waer ADP, P_i and $NADP^+$ areinputs fothe light-dpendent stage.
- » AP, NADPH and oxygen gas areutputs of th light-dependent stage.
- »In th light-independent stag f photosynthesis, coenzymes ATP and NADPH from the ight-dependent sage provde the chemical energy and protons to fix carbon in e Calvin–Benson cycle.
- »Inputs of te lght-independent stg are carbon dioxide, NADPH and ATP.
- » Outputs the light-indepe dentstae are glucose, water, $NADP^+$, ADP, and P_i.
- » C_4 and CAM plants have adapted photosynthetic pathas that enable them to survive in hot and dry enironmnts.
- » Almitingfactor is factor that lmits the rate of a reaction.
- » Thlimiting factors in potosynthsis ae ight intenity, carbon dioxide concentration and temperature.

9780170452533

5.2 Cellular respiration as a biochemical pathway

KEY CONCEPTS

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- The purpose ocellua rspiraion is to release the energy stoed in the bons of glucose to poduce ATP, whch can provide energy to fue ceuar rations.
- lycolysis ocurs inecytosol. It involves the breakdown of glucose to two pyuate moleues, two ATP molecules and to loaded accetrmolecles, NADH.
- ATP isvailabl for immediatse by the cell. Pyruvte is processed in subsequent reactions tha differ between organisms and depend on the avibility o oxygen.
- Pyruvate enter the mitochondr a where t s broken don.
- In the Krebscycle,pyrvte is completely broken down, releasing cabon dioxide as a by-prouct and producing ATP and he loaded coenzymes NADH and $FADH_2$.
- On th cristaeof themitocondra, a complex series of step involving cytochrome enzymes transfers th e electrons to th oygen, wich reacts with H^+ from the coenzymes to produ watr, in the electron transport chain.
- The net output of AT fr aerobic cellular respirai is 36/38 ATP per glucose molecule.
- Eukaryotes have woaerobiccellular respiration pathwas for producig ATP from glucose in absence of oxygen.
- Yeats, plants an some bactia carry ut alcohoic fermetatin; some animal cells carry out lactic acid fermentaion.
- The products of aaerbic repiraion are toxic at highconcntatios and so anaerobic respiration is only a short-term olution or obaining chemical energy.
- The rate ocellular esiration is affecte by temperatu,lucoe and oxygn availability as well as other factors.
- Photosynhesi nd clula respiraion are linked – the inputs of one are the outputs of the other.

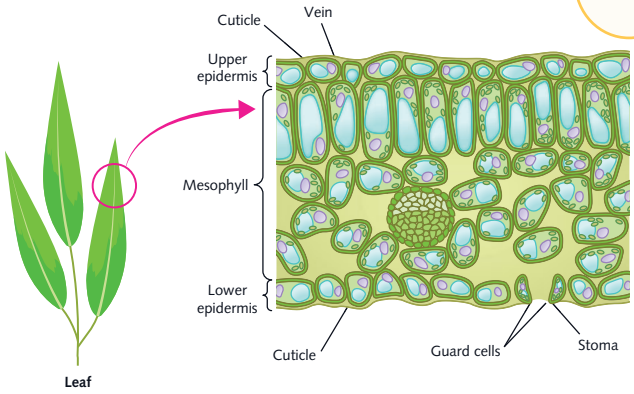

Figure 5.2 b A leaf cross-section showing the location of mesophyll cells that contain chloroplasts.

5.3 Biotechnological applications of biochemical pathways

KEY CONCEPTS

p 185

- The CRISPR-Cs9 protein acts as a 'cut and paste tool or geneticists so they can introduce specific DNA sequecesinto plant genomes that code for more efficient photosnthsic traits.
- Posibilties icludeicreasing crop yield by enhanin lig aailabilit (by altering plant arhitecture) ireasing light capture and energy conservaion.
- Anaerbicrepiration of biomass produces bioa, hich is use as a fuel for transport and eectricty.
- iogas prodction is a carbonneutral process, where the etire pathfrom lant to livestock to manure to decompstin is cnsidered a closed system.

Figure 5.27 The portable bioreactor at the Ecological Justice Hub in Brunswick produces electricity from biomass waste products, supplying electricity without the use of fossil fuels.

WB
5.4.1
KEY TERMS
PAGE 124

5 Chapter glossary

acetyl CoA a molecule used to convey carbon atoms to the Krebs cycle

aerobe an organism that requires oxygen to survive and reproduce

alcoholic fermentation a form of anaerobic respiration (no oxygen present); glucose is converted to ethanol, a type of alcohol

anaerobe an organism that does not require oxygen to survive and reproduce

autotroph an organism that makes its own food from inorganic substances, using light (through photosynthesis) or chemical energy (through chemosynthesis); includes green plants, algae and certain bacteria

biofuel a fuel that has used biomass as its original source

biomass the total dry weight of organic material

C_3 plant a plant that directly uses CO_2 as an input for photosynthesis

Calvin–Benson cycle a biochemical pathway in which sugar molecules are produced using carbon dioxide

CAM (crassulacean acid metabolism) plant a plant that shuts its stomata during the day and fixes carbon during the night when its stomata are open; an adaptation to hot dry environments

carbon fixation the conversion of atmospheric carbon dioxide into carbohydrates in the stroma of chloroplasts in eukaryotic cells

chemoautotroph an organism that makes its own food from inorganic substances, using chemicals as the primary energy source

cristae the folding of the inner mitochondrial membrane into the matrix, thus increasing the total surface area of the inner membrane

cytochrome a family of membrane-bound proteins that carry out electron transport; located in the mitochondrial inner membrane and in chloroplast thylakoid membrane

grana the stack of thylakoid membranes in a chloroplast that contain chlorophyll

heterotroph an organism that cannot make its own organic compounds from simple inorganic material; it depends on other organisms for nutrients and energy requirements

Krebs cycle a biochemical pathway that requires oxygen and takes place in the mitochondria as part of cellular respiration; acetyl CoA, the product of glycolysis, is broken down to produce carbon dioxide, water and energy in the form of ATP

lactic acid fermentation a form of anaerobic respiration (no oxygen present) that occurs in animal cells and some anaerobic bacteria; glucose is converted to lactic acid

light-dependent stage the first stage of photosynthesis; it requires light energy that is absorbed by chlorophyll to split water molecules to produce oxygen, hydrogen ions and ATP

light-independent stage the second stage of photosynthesis; through a series of reactions, carbon dioxide, hydrogen ions and ATP produce carbohydrate

limiting factor the factor that limits the rate of a reaction

matrix a gel-like fluid in mitochondria, where the Krebs cycle (citric acid cycle) of cellular respiration takes place

mitochondrion an organelle within the cytoplasm that is the site of aerobic cellular respiration, which releases energy for the cell

photoautotroph an organism that makes its own food from inorganic substances, using light as its primary energy source

photorespiration the process in which plants take up oxygen and release carbon dioxide

pigment a molecule that absorbs certain wavelengths of light and reflects all others

pyruvate a three-carbon molecule that is the end product of glycolysis

stroma the jelly-like, semifluid interior of a chloroplast

thylakoid membrane the interconnected, folded membrane within a chloroplast

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WB
5.4.2
EXAM PRACTICE
PAGE 126

5 Chapter review

Remembering

1 Why is chlorophyll seen as green?
2 When do plants respire?
3 What are the reactants in aerobic cellular respiration?

Understanding

4 Figure 5.28 shows a generalised cross-section of a leaf.

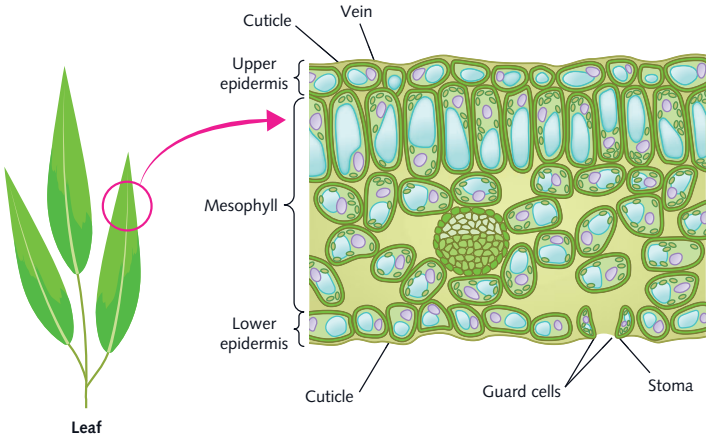


Figure 5.28 A cross-section of a leaf

a State the photosynthetic equation.
b What structures bring water to the leaf?
c What structures engage in gaseous exchange? What gases are exchanged with the atmosphere?
d Roots usually do not have chloroplasts in their cells. Why are they vital structures for photosynthesis?

5 What are the reactants in photosynthesis? What are the products in photosynthesis?
6 Which molecule absorbs the energy required to power photosynthesis?

Applying

7 Why are plants the ultimate recyclers when it comes to photosynthesis and respiration?
8 What effect does increasing the temperature of the intracellular environment of plant cells have on photosynthesis?

Analysing

9 In an investigation into photosynthesis, a student added sodium hydrogen carbonate to the water. Explain why.
10 An investigation was made into photosynthesis of the pondweed *Elodea*. How would you account for the following results?
» Experiment A produced 81 bubbles of oxygen.
» Experiment B produced 9 bubbles of oxygen.

11 Mitochondria are known as the powerhouses of the cell. What structures in mitochondria lend themselves to this claim and how?

12 Find out which cells in the human body might have a net production of 36 ATP and which might have a net production of 38 ATP. Discuss why this might occur.

13 How is energy stored in ATP?

14 What is considered to be a waste product of cellular respiration? Is it harmful? How does the body remove it?

15 Which pair of molecules are products of aerobic and anaerobic cell respiration in some organisms?

	Aerobic cellular respiration	Anaerobic cellular respiration
a	ATP	CO_2
b	CO_2	$C_6H_{12}O_6$
c	O_2	Pyruvate
d	Lactate	ATP

16 During exercise under low oxygen conditions, muscles can switch to only anaerobic forms of respiration. What aspects of this process are useful and what aspects are dangerous?

Unit 3, Area of Study 2 review

Multiple choice

Question 1 ©VCAA 2019 Q12 ADAPTED MEDUM

During which process would the production of ethanol be observed?

A Aerobic cellular respiration
B Photosynthesis
C Fermentation in yeasts
D Fermentation in animals

Question 2 ©VCAA 2014 Q7 ADAPTED MEDUM

During photosynthesis in chloroplasts, energy is used to convert carbon dioxide into carbohydrates. This occurs

A during the light-dependent reaction in the grana.
B during the light-independent reaction in the stroma.
C on the membrane of the thylakoids during the light-independent reaction.
D on the surface of the outer chloroplast membrane during the light-dependent reaction.

Question 3 ©VCAA 2014 Q8 ADAPTED MEDUM

When the atmospheric CO_2 level increases, the resulting increase in the rate of photosynthesis is due to the rate of the

A light-independent reactions on the thylakoid membranes of the chloroplasts increasing.
B light-dependent reactions on the thylakoid membranes of the chloroplasts increasing.
C light-independent reactions in the stroma of the chloroplasts increasing.
D light-dependent reactions in the stroma of the chloroplasts increasing.

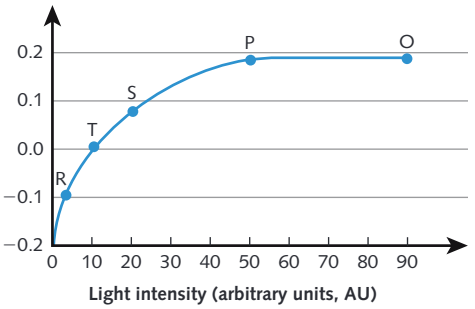
Question 4 ©VCAA 2018 Q9 ADAPTED MEDUM

The respective inputs and outputs of the Krebs cycle in an animal cell are

	Inputs	Outputs
A	NAD^+, pyruvate, ADP, P_i, FAD^+	CO_2, NADH, ATP, $FADH_2$
B	NADH, ADP, water, P_i, $FADH_2$	ATP, NAD^+, oxygen, FAD^+
C	NADH, ADP, oxygen, P_i, $FADH_2$	ATP, NAD^+, water FAD^+
D	NADPH, ADP, pyruvate, P_i, $FADH_2$	$NADP^+$, ATP, oxygen, FAD^+

Use the following information to answer Questions 5 and 6.

The graph below shows the net amount of oxygen that geranium leaves release as light intensity is increased in an experiment. The temperature is always kept constant.



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Question 5 ©VCAA 2017 Q13 ADAPTED MEDUM

Which one of the following conclusions can be made based on the graph?

A At point O, all photosynthesis has ceased.
B The least amount of photosynthesis occurs at point T.
C At point P, the amount of oxygen output is twice that at point R.
D Above 10 AU of light, the plant is absorbing more CO_2 from the atmosphere than it is producing.

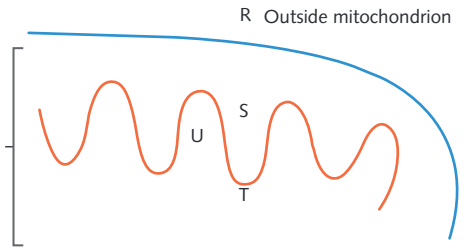
Question 6 ©VCAA 2017 Q14 ADAPTED MEDUM

The rate of oxygen output remains constant between points P and O because

A lactic acid build-up has denatured the enzymes involved in the photosyntheis reactions.
B the rate of photosynthesis is limited by the concentration of available carbon dioxide.
C the light has stimulated more respiration in the mitochonria.
D oxygen has stopped being produced around the geranium leaves because of an accumulation of oxygen there.

Question 7 ©VCAA 2018 Q8 ADAPTED MEDUM

The diagram below shows a section through a part of a mitochondrion



The sites of the pathways in aerobic respiration are

A R – glycolysis, S – Krebs cycle, T – electron transport chain.
B R – glycolysis, U – Krebs cycle, T – electron transport chain.
C U – glycolysis, T – Krebs cycle, R – electron transport chain.
D T – glycolysis, R – Krebs cycle, S – electron transport chain.

Question 8 ©VCAA 2019 Q13 ADAPTED EASY

In human cells, the rate of aerobic cellular respiration may increase if the

A temperature of the cell is increased from 37°C to 40°C.
B rate of facilitated diffusion of glucose into the cytosol of the cell decreases.
C carbon dioxide concentration in the cytosol of the cell increases.
D amount of glucose available to the mitochondria increases.

Question 9 ©VCAA 2017 Q11 ADAPTED HARD

Radioactively labelled glucose was added to a culture of animal cells. The cells were then monitored for three minutes. After this time, radioactively labelled atoms would be present in which cellular chemical?

A Inorganic phosphate
B Adenosine triphosphate
C Oxygen
D Carbon dioxide

Question 10 ©VCAA 2010 EXAM1 Q15 ADAPTED HARD

The starting compounds and end products of a chemical reaction that results in a net reduction of ATP are

	Starting compounds	End products
A	DNA	Nucleotides
B	Glucose	Starch
C	Peptides	Amino acids
D	Triglycerides	Fatty acids and glycerol

Question 11 ©VCAA 2011 EXAM 1 Q20 ADAPTED HARD

The reaction ADP + P_i → ATP

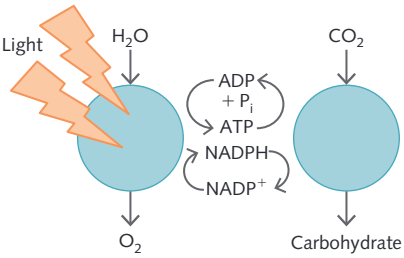
A is not reversible.
B only occurs in eukaryotic cells.
C occurs in the absence of enzymes.
D occurs in yeast cells during anaerobic respiration.

Question 12

In the three stages of aerobic cellular respiration, the yield of ATP per glucose molecule is:

	Glycolysis	Krebs cycle	Electron transport chain
A	32	2	2
B	2	2	34
C	4	32	2
D	36	1	1

Question 13 ©VCAA 2018 Q15 ADAPTED HARD



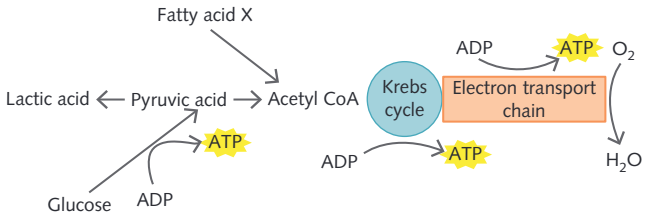
From the diagram above you can tell that

A the light-dependent reaction builds ATP and the light-independent reaction releases ADP and P_i.
B $NADP^+$ is the loaded form of NADPH.
C the oxygen released by photosynthesis comes from the carbon dioxide.
D the light-independent reaction has no output other than carbohydrate.

Question 14 ©VCAA 2014 Q12 ADAPTED HARD

If a cell has insufficient glucose for cellular respiration, fatty acids can be changed to acetyl CoA. Each fatty acid X molecule produces eight molecules of acetyl CoA.
The pathways for the breakdown of fatty acid X and glucose are summarised in the diagram below.

The number of molecules produced in each step is *not* shown.



Referring to the information above and your knowledge of cellular respiration, which one of the following conclusions can be made?

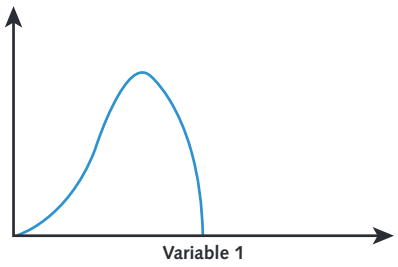
A More ATP is made in the Krebs cycle than in glycolysis.
B One glucose molecule produces less ATP in aerobic conditions than one fatty acid molecule.
C No ATP is formed from the breakdown of glucose under anaerobic conditions.
D Pyruvic acid is converted to lactic acid under aerobic conditions.

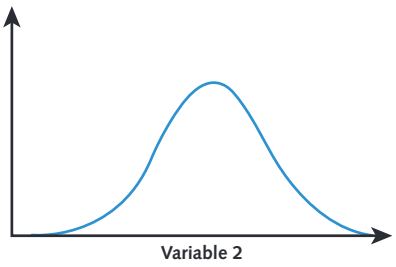
Question 15 ©VCAA 2018 Q7 ADAPTED EASY

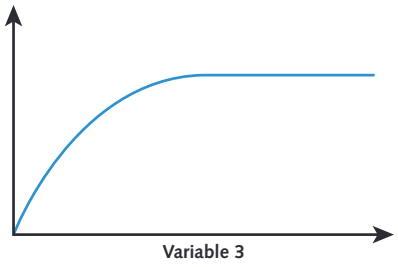
A series of experiments were performed to investigate the effect of four different variables on the rate of an enzyme-catalysed reaction that normally occurs in the human body. The students each changed one of the following variables: substrate concentration, pH, temperature and enzyme concentration. After recording their data, the results were displayed in a series of graphs, as shown below. Each graph is a line of best fit for their data.

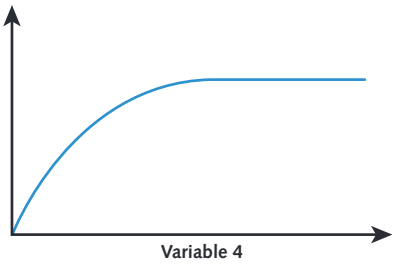
The students did not label the horizontal axis on any of their graphs. From your knowledge of enzyme functioning you can conclude that if

A more enzyme were added to the enzyme–substrate mixture, the graph of Variable 1 would show an increase in rate of reaction.
B more substrate were added to the enzyme–substrate mixture, the graph of Variable 2 would show an increase in rate of reaction.
C the enzyme–substrate mixture were cooled to 25°C, the graph of Variable 3 would drop to zero.
D the enzyme–substrate mixture were heated to 45°C, the graph of Variable 4 would drop to zero.









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Short answers

Question 1 ©VCAA 2013 SEC B Q1 ADAPTED

Yeast is a single-celled, microscopic fungus that uses sucrose as a food source. The cellular respiration by a particular species of yeast was investigated in an experiment. Yeast cells were placed in a container and a sucrose solution was added. The experiment was carried out at room temperature. An airtight lid was placed on the container. The percentages of ethanol and oxygen in the container were recorded over a one-hour period. The results are shown in the following table.

	Oxygen (%)	Ethanol (%)
Start of experiment	21	0
End of experiment	18	4

a Explain why a change in the oxygen level was observed during the experiment.

1 mark

b Name the process that would account for the change in the ethanol level observed during the experiment.

1 mark

c The experiment was allowed to continue for the next 24 hours. Predict what you think would happen to the levels of oxygen and ethanol over this extended period and provide an explanation of your prediction.

2 marks

Question 2 ©VCAA 2013 SEC B Q1 ADAPTED

Increasing the efficiency of photosynthesis in food crops has always been of interest to scientists, particularly the effect of the concentration of carbon dioxide on photosynthesis.

a During which stage of photosynthesis is carbon dioxide used?

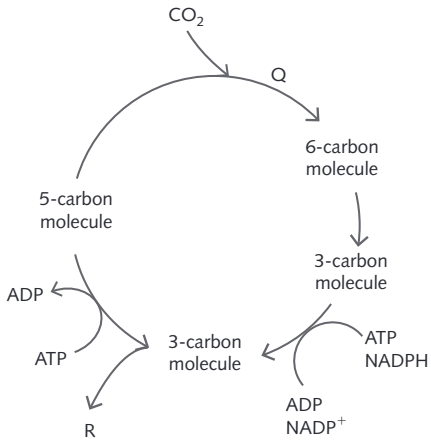
1 mark

b Name the stage of photosynthesis that does not require carbon dioxide as an input. List two inputs of this stage and describe the role played by each in this stage of photosynthesis.

2 marks

Question 3 ©VCAA 2007 E1 SEC B Q4 ADAPTED

The light-independent reactions (Calvin–Benson cycle) of photosynthesis are summarised in the diagram below.



a State the name of Q.

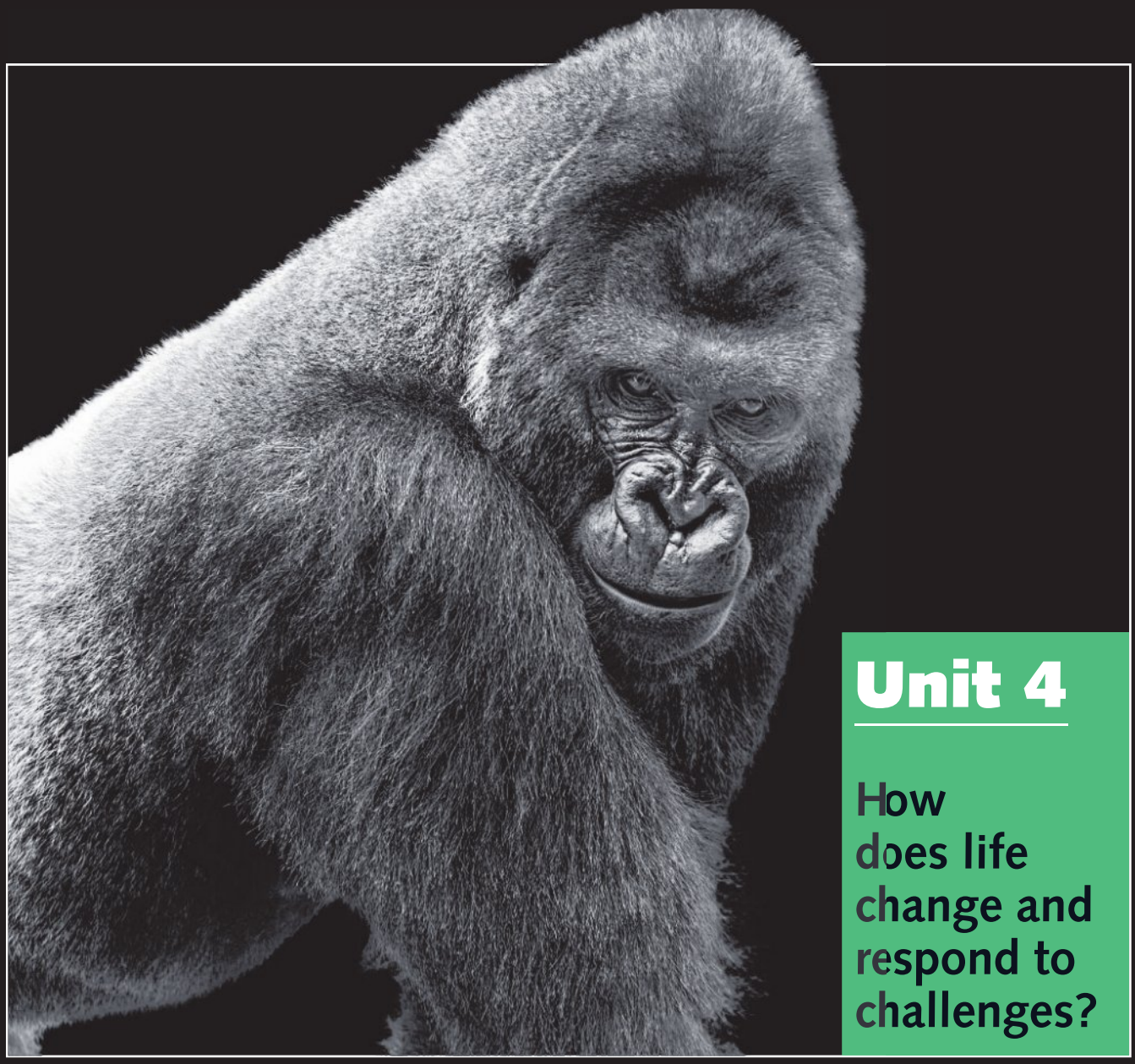
1 mark

b NADPH and ATP are used during the light-independent reactions. What are the roles of NADPH and ATP?

1 mark

c What is compound R?

1 mark



Unit 4

How does life change and respond to challenges?

Area of Study 1: How do organisms respond to pathogens?

Area of Study 2: How are species related over time?

Area of Study 3: How is scientific inquiry used to investigate cellular processes and/or biological change?

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Responding to antigens

6

By the end of this chapter you will have covered the following material.

Key knowledge

Responding to antigens

- physical, chemical and microbiota barriers as preventative mechanisms of pathogenic infection in animals and plants pp. 204–214
- the innate immune response including the steps in an inflammatory response and the characteristics and roles of macrophages, neutrophils, dendritic cells, eosinophils, natural killer cells, mast cells, complement proteins and interferons pp. 214–223
- initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens pp. 222–233

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- formulate hypotheses to focus investigation pp. 208–210

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 208–210

Generate, collate and record data

- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 208–210

Analyse and evaluate data and investigation methods

- process quantitative data using appropriate mathematical relationships and units, including calculations of ratios, percentages, percentage change and mean pp. 208–210
- repeat experiments to ensure findings are robust pp. 208–210
- evaluate investigation methods and possible sources of personal errors/mistakes or bias, and suggest improvements to increase accuracy and precision, and to reduce the likelihood of errors pp. 208–210

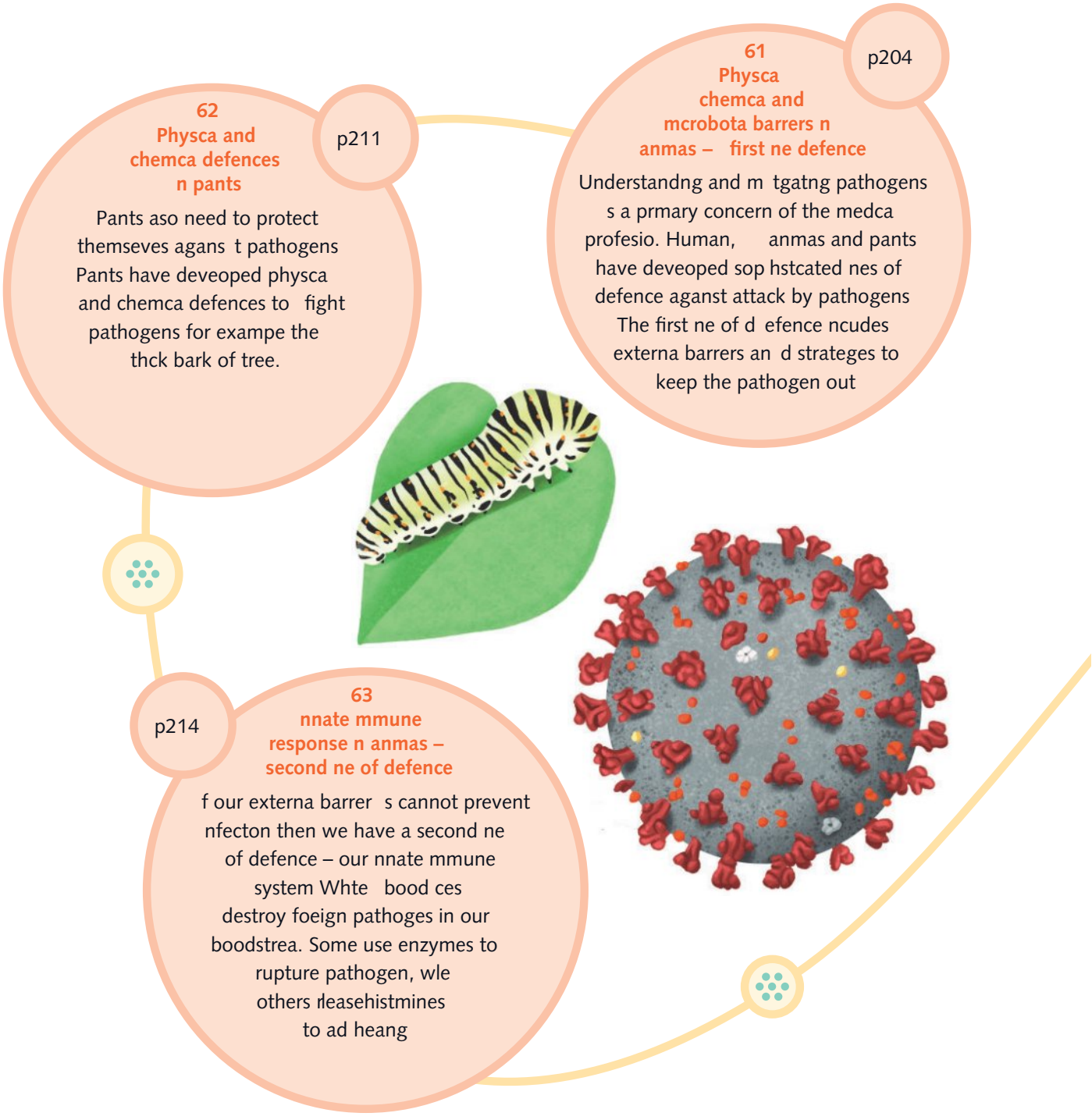
Construct evidence-based arguments and draw conclusions

- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 208–210

Online Chapter Map
Chapter 6 map

6 Responding to antigens

How does the flu virus enter your body? What does your body do about it?



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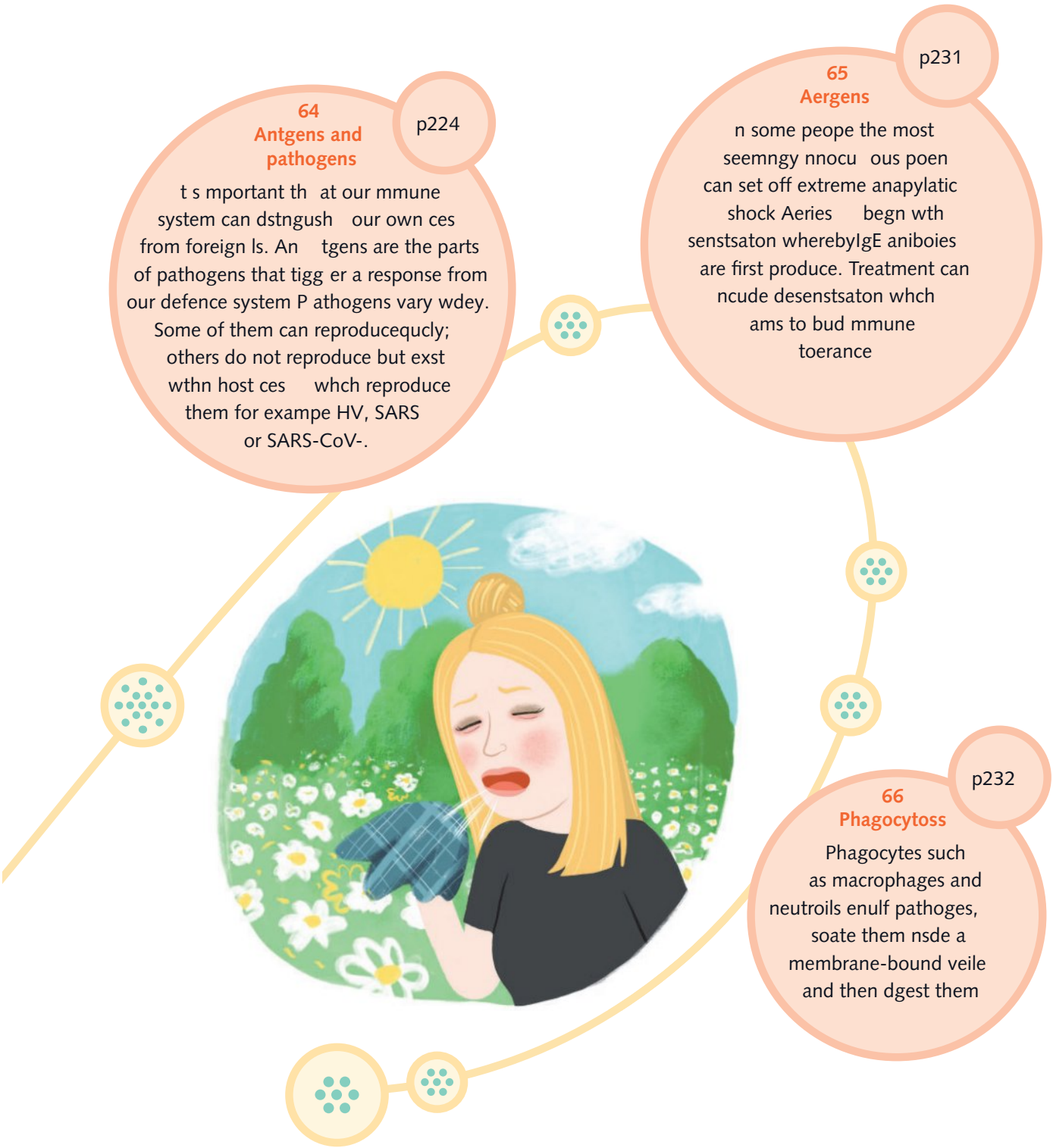

Your body has three lines of defence to protect you from infection and disease. The first line of defence consists of chemical and physical barriers to prevent invaders entering your body. If this fails, the second line of defence destroys invaders in a generalised way. If that does not work, your body activates the third line of defence, which targets specific invaders.

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Online Chapter Mapv
- Chapter 6 map (p. 200)

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- Chapter 6 flashcards (p. 202)

Weonkm v
- The body's first line of defence (p. 208)
- Immune response in plants (p. 211)

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- First line of defence (p. 208)
- Plant immunity (p. 211)

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- Chapter 6: Summary of key concepts (p. 236)

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Chapter 6 Flashcards

Know your key terms

adaptive immune response
allergen
allergy
anaphylactic shock
antibiotic
antigen
apoptosis
bacteria
bacterial capsule
cellular pathogen
chemokine
chemotaxis
cilia
companion plant
complement
cytokine
defensins
degranulation
dendritic cell
desensitisation
disease
eosinophil
first line of defence
flagellum
fungus
granulocyte
histamine
host
immune system
immune tolerance
infectious disease
inflammation
innate immune response
interferon
keratin
leukocyte
lymphocyte
lysis
lysozyme
macrophage
mast cell
microbiome
microbiota
microflora
monocyte
mucous membrane
natural killer cell
necrosis
neutrophil
non-cellular pathogen
non-self antigen
non-specific response
obligate parasite
opsonisation
pathogen
phagocyte
phagocytosis
phagolysosome
phagosome
platelet
prion
protist
second line of defence
secondary metabolite
self-antigen
sensitisation
sterile inflammation
transmitted
vasodilation
virus

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 A disease is any condition that affects the structure or normal functioning of an organism.
2 A bacterium is a prokaryotic organism that can cause diseases such as gastroenteritis and cholera.
3 Plants have structures, such as thick waxy cuticles and hairs, that assist in preventing disease-causing pathogens from entering.
4 Plants have stomata on their leaf surface, which open and close to allow gas exchange. Disease-causing pathogens can enter the plant through the stomata.

WB
REMEMBER
PAGE 129

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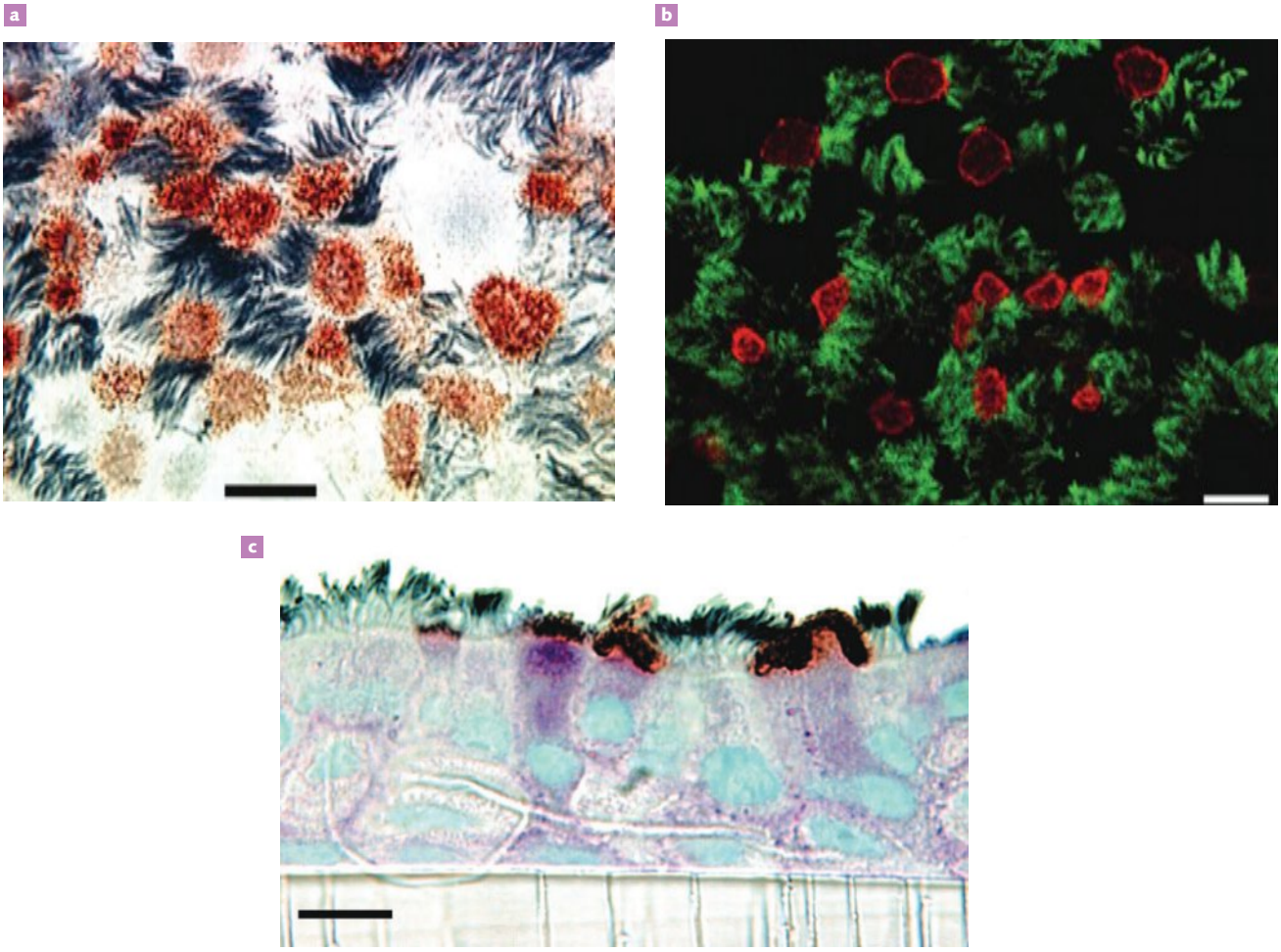


Figure 6.1 Influenza virus particles binding to cilia cells in the respiratory tract as seen by **a** light microscopy (virus is red and cilia are grey), and **b** fluorescent confocal microscopy (virus is red and cilia are green). **c** Influenza virus shown targeting secretory cells (virus is red and cilia are grey). Bars = 10 µm.

A **disease** is any condition that interferes with how an organism, or any part of it, functions. Diseases can be grouped according to their cause. An agent that can be passed from one organism to another causes **infectious diseases**. The infected organism is the **host**. An infectious agent that causes disease is called a **pathogen**.

Disease is often described in terms of battles and wars – attacks on the body and invasions by pathogens. The immune system is commonly referred to as the defence system, fighting pathogens using lines of defence, like a walled city under siege. If the first line of defence is breached and pathogens enter the body, they are attacked by second and third lines of defence. Hence, despite significant exposure to invading micro-organisms and parasites, in most cases we are able to resist infection. All organisms have evolved various types of defence mechanisms to inhibit the entry of pathogens and to deal with them should they gain a foothold. This is often referred to as an 'evolutionary arms race' between pathogens and their hosts. Even simple, single-celled organisms such as bacteria can defend themselves against viruses by using the CRISPR-Cas9 system.

Influenza is an acute infectious condition. It makes an individual ill quite quickly, but hopefully clearing within a relatively short time period. The clearance of influenza from the body is not a passive process. It involves a highly coordinated series of responses from molecules, cells and the body as a whole.

CONNECT
You will learn about the third line of defence in Chapter 7.

CONNECT
See page 87 for more about CRISPR-Cas9 in bacteria.

Coughing and sneezing are common ways in which your body tries to rid itself of the influenza virus before it reaches your cells. This is called the **first line of defence** of your body's immune system. As part of the first line of defence, cells in mucous membranes produce mucus, which traps viral particles, and ciliated epithelial cells in the trachea beat their cilia, moving the contaminated mucus away from the lungs and towards the throat for swallowing. Some influenza virus particles may escape these mechanisms and infect the epithelial cells in the respiratory tract to initiate an infection. The symptoms that usually follow include muscle aches, fatigue and fever. These are also signs that the body's **second line of defence** has been initiated. In the second line of defence, chemicals are released that ultimately hinder the virus's ability to reproduce inside your cells. The second line of defence is a **non-specific response** from the immune system in that it detects and responds to the pathogen the same way each time it encounters the pathogen. A suite of signalling molecules are released that protect surrounding cells from further invasion and activates other cells to destroy the cells that are infected, safely containing the infection.

6.1.1 PHYSICAL AND CHEMICAL BARRIERS PAGE 130

6.1 Physical, chemical and microbiota barriers in animals – first line of defence

The most effective way of preventing the colonisation of an organism by pathogens is to keep them out of the body in the first place. This first line of defence comprises various physical, chemical and microbiota barriers that stop the entry of pathogens and other foreign substances. The scales of reptiles (Figure 6.2), the exoskeleton of arthropods such as insects and crustaceans, the shells of eggs and human skin are examples of physical barriers that protect the animal from invasion.

Figure 6.2 Reptiles such as this iguana have tough, scaly skin that helps defend against the entry of some pathogens.

Skin: a tough physical barrier

The skin is the largest organ in the human body, and acts as a tough physical barrier between the body and the outside world. Like all the inner and outer linings of the body, the skin is made from epithelial cells. The epithelial cells become keratinised, a process in which the structural protein **keratin** is deposited, and form a hard outer layer that is impervious to water and micro-organisms (Figure 6.3a). The importance of the skin as a barrier can be seen in burns victims who lose a large proportion of their skin. If they survive the effects of heat and dehydration, they may still die as a result of multiple infections caused by invading micro-organisms that overwhelm the immune system.

Damaged skin is an ideal site for infection. In addition to burns, other injuries such as cuts and abrasions provide a potential site for the entry of pathogens. When the skin is cut and blood vessels are damaged, cell fragments in the blood, called **platelets**, are quickly attracted to the site of the wound. As they stick to the damaged tissue, they send out chemical messages. These messages trigger the formation of a web-like mesh of fibrin protein that stabilises the aggregation of platelets and traps red blood cells to form a clot. This plugs the break in the vessel wall, forming a scab that seals the wound and keeps out micro-organisms while the skin is healing. As long as it remains unbroken, our tough waterproof skin is an effective barrier against invaders.

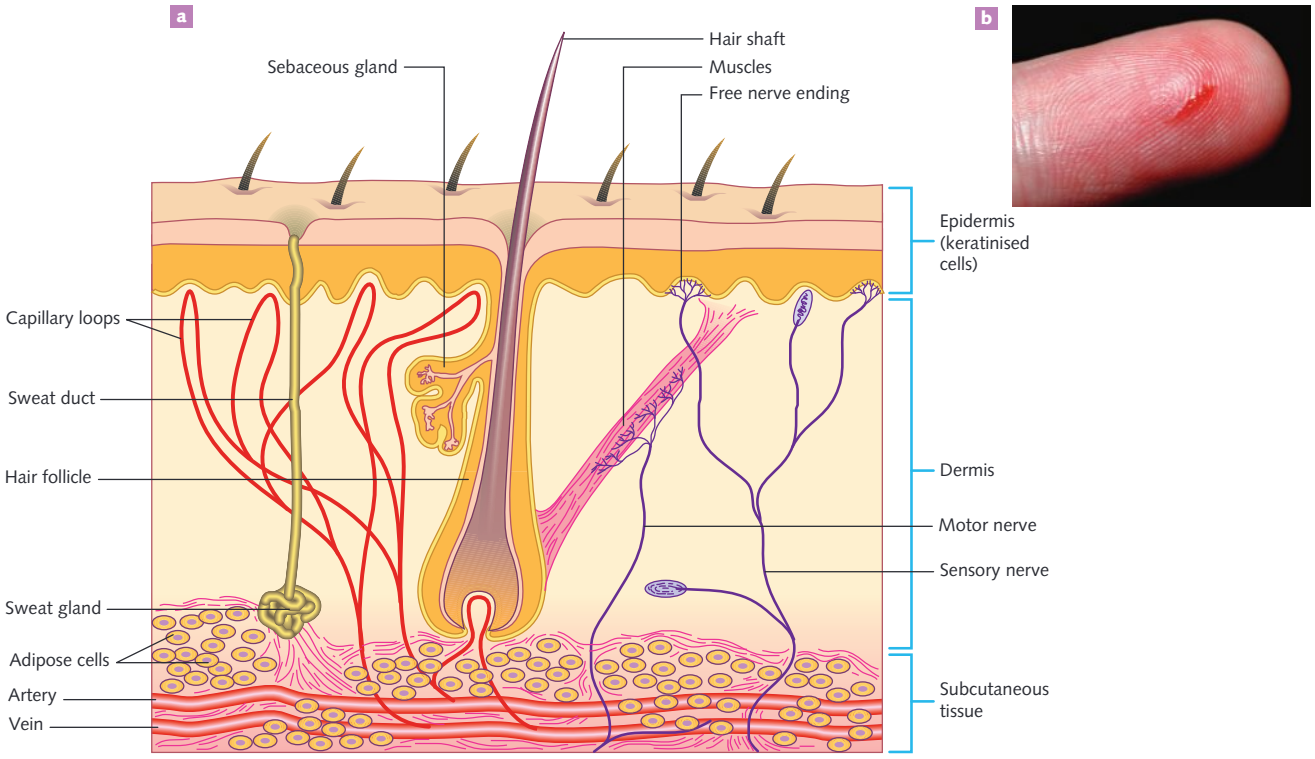


Figure 6.3 a A cross-section of human skin. **b** A cut or scratch breaks the barrier of the skin and can be a site of pathogen entry into the body.

Tissue secretions

The external openings of the respiratory, digestive, excretory and reproductive systems are ideal entry points for micro-organisms. Tissues secrete substances that physically trap and expel invading micro-organisms and other foreign particles. These tissue secretions include mucus, sebum (an oily secretion) and tears.

The human respiratory, gastrointestinal and reproductive tracts are lined with epithelial cells that secrete mucus. For this reason, they are called **mucous membranes**. Slender hair-like structures called **cilia** line the respiratory tract (Figure 6.4). Their beating pushes mucus up to the throat,

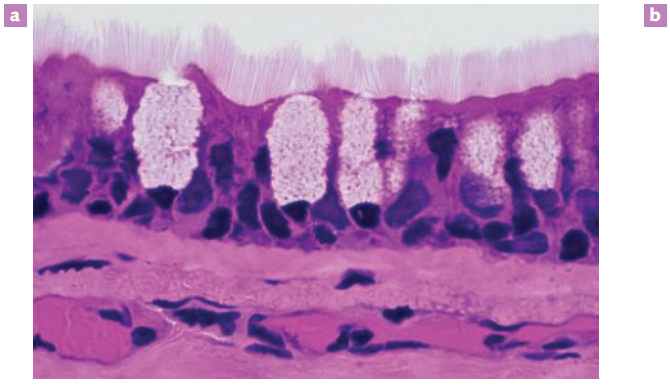


Figure 6.4 a A light micrograph of a mammalian trachea, showing, in vertical section, the cilia lining the wall. Cilia help to trap pathogens and move them up and out of the body **b** A scanning electron micrograph showing the cilia of cells lining the respiratory system; the structures between the cilia are mucus-secreting cells.

where it can be coughed or sneezed out or swallowed. People with defective mucus secretion or inhibited ciliary movement frequently develop lung infections caused by **bacteria** colonising the epithelial surfaces.

The eyes have ducts that secrete tears, which help to flush pathogens from the eye surface. **Lysozyme**, which is an enzyme in tears, saliva and mucus, acts as an antimicrobial agent, breaking down the cell wall of certain types of bacteria and causing them to undergo **lysis**, or bursting, as shown in Figure 6.5.

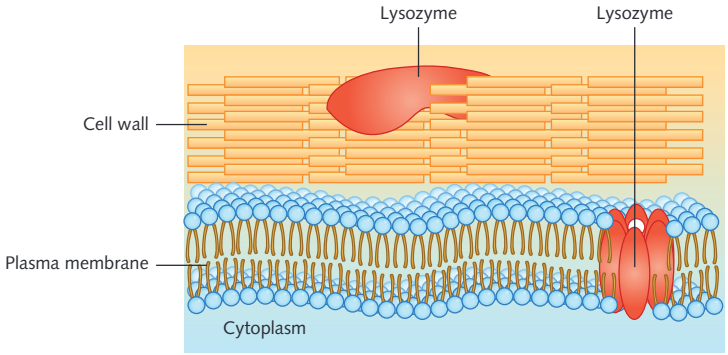


Figure 6.5 **The action of lysozyme on the bacterial cell wall as it binds to the cell wall, breaks apart bonds and exposes the plasma membrane to further action of the enzyme.**

The skin secretes sebum, which has antimicrobial properties to protect from surface invaders.

Urine passing through the urethra has a flushing effect on micro-organisms that are trying to enter the body via the urethra.

Skin secretions such as sweat and oil mean that skin has a pH of 3–5, which is acidic enough to prevent colonisation by many pathogenic species. The low pH of the vagina also prevents the overgrowth of infectious agents. The highly acidic environment of the stomach kills many micro-organisms in food and drinks, as do the digestive enzymes secreted by the stomach and small intestine.

WB
6.1.2 MICROBIOTA AS A BARRIER PAGE 130

Microbiota as a barrier

During birth, a baby acquires micro-organisms from its mother that become permanently associated with it. The symbiotic micro-organisms that live in close association with each other on and in our bodies are our normal **microbiota** (also called **microflora**) and include bacteria, fungi, protists and viruses. By taking up space and using nutrients, our normal microbiota prevent colonisation by other micro-organisms that may be pathogenic. Through the substances they produce, normal microbiota also set up a chemical micro-environment that supports the growth of other beneficial, non-pathogenic, micro-organisms. These bacteria are known as commensals because they help the human body in a variety of ways, including digestion and assisting the immune system; for example, by consuming invading pathogens. This results in a microbial community of species that contributes to our health, and effectively forms a barrier against pathogenic bacteria. When non-pathogenic bacteria are killed by **antibiotic** treatments, any pathogenic micro-organisms with antibiotic resistance may replace them and cause disease.

Other first lines of defence

In the gut, peristalsis is an important mechanism for keeping both food and infectious agents moving through. Failure of peristalsis is typically accompanied by overgrowth of bacteria within the intestinal lumen.

Figure 6.6 and Table 6.1 summarise the first lines of defence of humans.

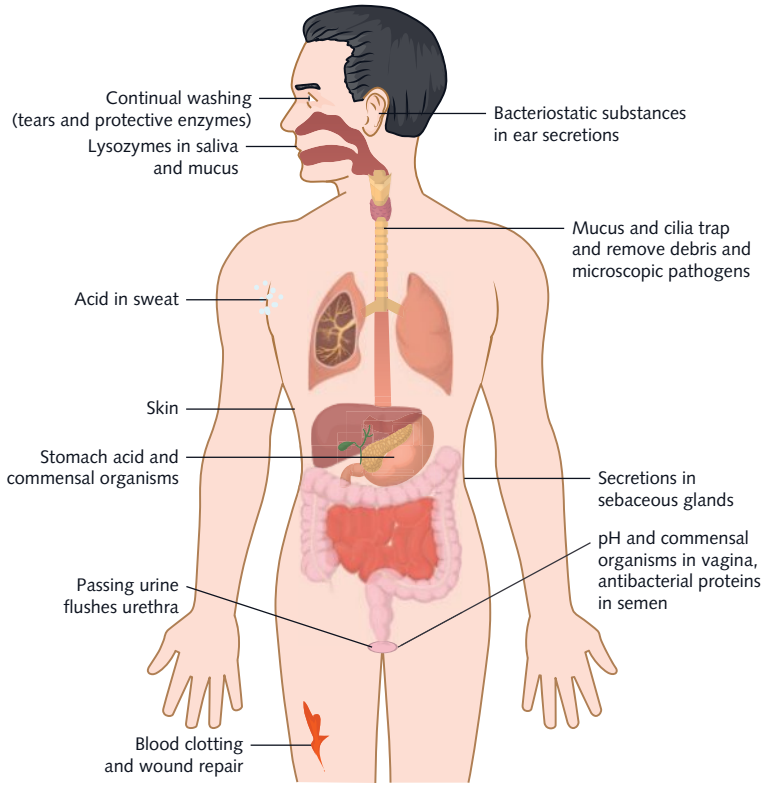


Figure 6.6 A summary of the physical and chemical barriers to pathogenic infections in a human. Peristalsis also helps by moving food and infectious agents through the digestive system.

Weolink
The body's first line of defence

Onne c yrkm heet
First line of defence

EXAM TIP
Make sure you know the first lines of defence in humans and can provide an example of each.

Table 6.1 A summary of first lines of defence barriers in humans

Type of barrier	Location	Barriers or mechanisms to prevent entry of pathogen
Physical	Skin	Keratinised skin cells, rapid blood clotting, rapid wound healing, antiseptic action of acidic secretions
Chemical	Skin	Secretion of sebum, which contains antimicrobial substances such as lysozymes
Chemical	Digestive system	Lysozymes in saliva and mucus, enzymes and strong acids in stomach
Physical and chemical	Respiratory system	Mucus traps dirt and small pathogens; cilia lining trachea move this upwards
Physical and chemical	Reproductive tract	Mucus with acidic pH moving fluids flush out pathogens
Chemical	Urinary tract	Urine flushes out pathogens and its acidity inhibits bacterial growth
Physical and chemical	Sense organs	Ear wax and hairs, eyelashes and nostril hairs trap pathogens; tears wash away pathogens and contain lysozyme
Microbiota	Skin and digestive tract	Their presence prevents the growth of pathogenic micro-organisms

INVESTIGATION 6.1

Second-hand data analysis: is lysozyme an effective barrier against bacteria?

Eyes are war n moist, which akes them an idealentry point fo bacteriainto the human body. Tears contain the powerfl anbacteial ezye lysozyme, which estroys pahoes rpidlyby lysing their cells. This investigation uses aga pates spreadwith a culre of bacteria,t cmpare the bateriidaleffctiveness of lysozyme, an antiseptic and a dsinfectant. Bacteria grow on agar plat es produce a bacteia'lan', a cloudy fim oflions of bcteria on the surface of the agrplate. If paper dics cnaining antibacterial sustances are plaed on the agar, they produce clear areas, known as zone of nhbition, where bactria cannot grw (Fiur 6.7).

Youll nalyse he results obtanedfromthe investigation. Th materials and method are provided so that you can reflect on them as part o ur analysis.

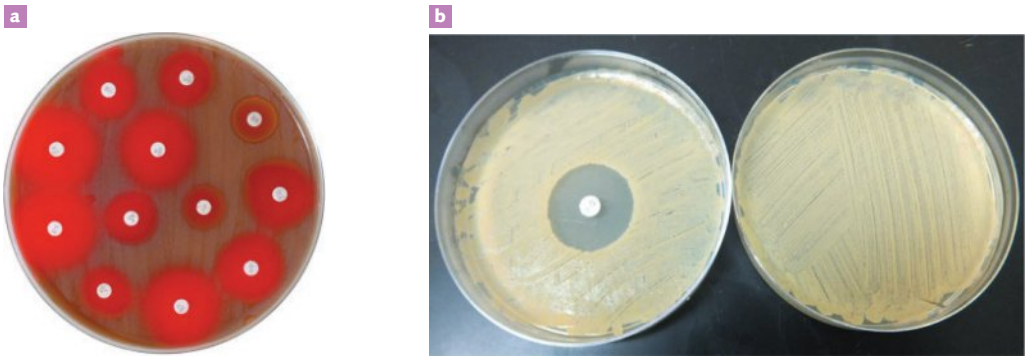


Figure 6.7 Examples of bacterial plates with zones of inhibition shown as cleared areas around antibiotic-soaked paper discs. The size of the zones of inhibition indicates the sensitivity of the bacteria to the antibacterial substance on the discs. a Red areas are blood agar where no bacteria is growing. Blood agar contains 5% sheep blood and provides a range of nutrients for bacteria that wouldn't otherwse grow *n vtro* . b In this example, the bacterial lawn is off-white and the agar is clear.

Am

To compare the aibacerial efectiveness f lysozyme from tear ith that of an antiseptic and a disinfectant

Materas

Per lss:

- Brot culture of *Escherichia coli*
- Incubator set to 25°C
- Lab coats
- Safety glasses
- G loves

Per grou:

- 3 nurient aar plates
- ilter paper
- O
- 10 mL each disinfctan, anteptic and distilled water
- ilutedsinfectnt olution; fr example, bleach
- icky tape
- Stile 5 L pipette
- Forceps
- lass spreader
- Bunsen burner
- S
- Ruler

What are the rsks n dong th s nvestgaton?	How ca n you manage these rsks to stay safe?
Although lab strains are usually harmles, bacteria may cause disease so assume them to be pathogenic.	Wear lab coas, safety glasses and glovs; wash hands thoroughly at the end of the investigation. Decontaminate benches before and after the investigation. Flood spills with bleach.
Micro-organisms will grow on the agar patesDo not op en plates once they are securely tapd. Dispose of plates appropriately after autoclaving.	
Onions contain substances that irritate the eyes and nose	Ensure the nio is eld cose to eyes but does not actuay come n contact with face or eyes.
Ethanol may be used to sterilise the bench top and s hghy flammable.	Be careful to avoid igniton of ethano qud or fumes when usng the Bunsen burner.
Disinfectants or bleach may leave a corrosive residue.	After wiping the bench clean with bleach ensure the residu iswiped of; ensure lab coat sleeves are rolled down and gloves are won.

Method

Record your hypothesisbfore eginnngtis investigation.

Noe: To inimis cnamination, wipe the be nch ith bleach or thanol beforeyou start.

1. Fld apiece of fiter paper into quarters anduse a hole punch to make four fiter-pape dics.
2. Lael the base of neagar plate with the date and the name of yourgroup, d then diide the agar nto four quarters. Near the edge f the plate, label eacho thfur quaters: atr', 'Lyozyme', 'Antiseptic' and 'Disinfectant'.
3. Remove 1 mL of *E. coli* cuture ith te pettelift the ld of the labelled plate and transfer the bacteria to the surface of the gar.
4. Ether rplace he idquickly and spread th e qud evnly by wrling, or spadthe iqud evenly h the sterilised glass spreader, then eplacee lid. Lave the plate on the bnch for 2 minutes to allow thebacteria to penetrate the agar.
5. Make your eyes wer by hoding a cutonionear them, an blink to release tears.
6. Stilise the foceps in the Bunsen burner flame allow them cool, then pick up a fiter-pape disc and arflly dip it nto one of the tars Quickly touch the edge of the disc to the remains of the folded fiter paper tobot, thn gently place the disc on the quarter of the gr late belled 'Lysozyme'.
7. Prepar sma quantitie (10 mL) of disinfectant and antiseptic slutions ydiluting accodin to directionson the bottles.
8. Restilise the forcep and mistena is by dipping it in to the antseptc an blottn, thengently place the disc on the corret labelled quarter f the agar plate.
9. Repeat step 8 for t disinfectan ad the istilled water.
10. Repeat steps 1–9 twice to make a total f tree replicates.
11. Seal the pats with sicky tape and incubate at 25°C for 24 hours.
12. Ensure the bechis wipeddownwith bleach and wash you hands thoroughly.
13. The nextday, observe the plates for the presence or asnce of growth near the discs.
14. Measure te diameter of th zon of nhiitio, which is the clear area around each disc. This shows the degree of sentivity of the bacteria to eah substance.

Resuts

Tis tble shows the data that one group of students obt ained whenolowing this ethod. opy the table into your ogbook Calculate te mean vales and draw a suitable gaph to represent the data.

Data for a group of students

	Dameter of zone of nhbton (mm) for each substance			
Trial	Lysozyme	Antiseptic	Disinfectant	Water
1	11	13	15	1
2	16	17	13	2
3	12	12	16	1
Mean				

Anayss of method

1 What seps in the method were taken to ensure therewas no cross-contamination?
2 Explain the role f he isc diped in water.
3 Explain the purpose of th three agar plates.
4 dentify one othr risk and how ou woudmanage it.

Anayss of resuts

Describe he resuts by stating the order of effe civeness of each of he solution sbactericides.

Dscusson

Composea discussion of the findngs(minimum of 300 words) as perthe scienti fic met od.

Concuson

Draw a onlusion fr his nvetigation.

KEY CONCEPTS

» Strucural,chemil and biological features can act as barriers to pathogens as a firsline of deence.
» The ki is a tough hsicl barrier made from kerinisd thlial cells that can prevent the entry of pathoens.
» When the arrier ofte skin s roken, pltelets quickly form apug, o scab,that upolds he barrier until the skn s repaird.
» Mucus traps pathogens that invade mucous membranes, d cilia beat themucus to a place where t can be plld. Urine and tears flush out mcro-organisms an pistalsis keeps them moving through the gut.
» Chmcal barriers that preven pathogen colonisation ncludelow (idic) H onskin and inthe stomach, dgestive enzymes poduced n the gut, and lysozyme n tears, aliva and mucus.
» Syboticorganisms frmourmicrobiota, which take up space and use utriens. This reens colonisation by pathgens.

Concept questions 6.1

1 Name the three types of barriers that form the firsline of defence aaint diease.
2 Lst threeopeings n the skinhat can allow the entry of pathogns.
3 Ouline te role of mucous embranes.
4 Describe threeways in which the body can flush out micro-orgaisms.
5 Recount therole fpltlets i blood clotting.

HOT Chaenge

6 There are svrl main places in the human body where ow pHlls pathogens as part of the firsline of defenc. Draw u a tale that lists these places and the normal pH o these sites. Rsearch what chemicals deterine the pH at ech site.

6.2 Physical and chemical defences in plants

Plants are prone to attack by parasites, pests and disease just as animals are. They are subject to attack by a huge array of mites, insects, nematodes (roundworms), fungi, bacteria and viruses (Figure 6.8), yet plants often survive these attacks. Plants also have mechanisms of defence. An understanding of plant defences may help scientists to reduce crop losses caused by plant disease. This research is critical to the wellbeing of humans, because plants are a vital component of our ecosystems. We depend on plants for food, as well as for valuable materials such as wood, plastics, textiles, medicines, dyes, inks and industrial chemicals.

a

b

Figure 6.8 a Leaves of a healthy tobacco plant, *Ncotana syvestrs* , and **b** a plant infected with tobacco mosaic virus

First line of defence

Plants have physical and chemical barriers to infection that form their first line of defence against invaders. As these are present before contact with the pathogen, they are termed 'passive defences'.

Physical barriers

Physical barriers in plants include the thick bark of stems and a thick and waxy cuticle (leaf surface) (Figure 6.9). Waxy cuticles and vertically hanging leaves may also prevent the formation of moisture films on leaves. This inhibits bacteria and roundworms that require water for motility, and fungal spores that germinate only in water. Hairs and thorns may also deter vectors of particular pathogens. Stomatal openings are weak spots, because they offer an entry point. Some plants have hairs that guard these openings and others may have sunken stomata that make access difficult.

WB
6.2.1 PLANTS – FIRST LINE OF DEFENCE PAGE 133

n.
Weolink
Immune responses in plants

Onne Wyrkm heet
Plant immunity

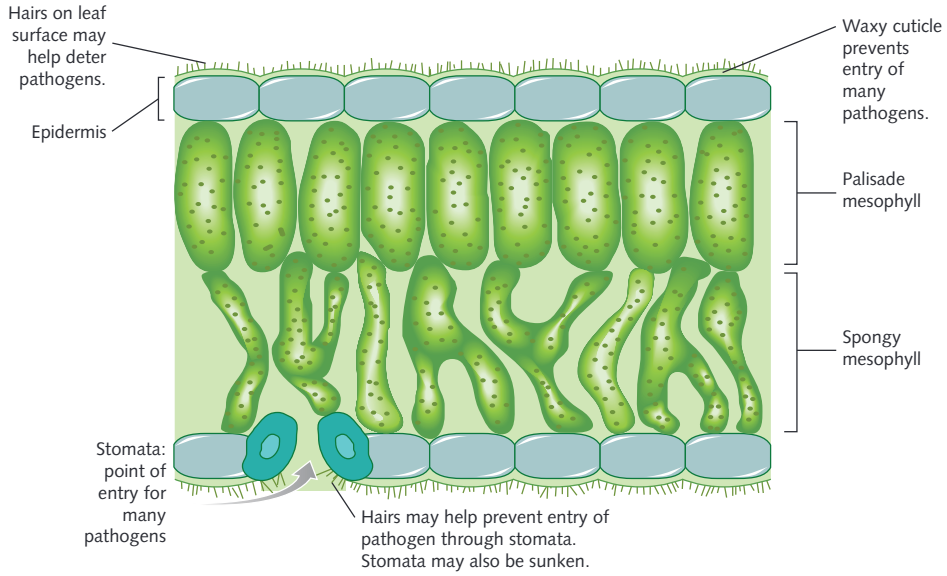


Figure 6.9 A cross-section of a typical dicotyledon leaf showing some barriers to pathogens found in plants

Chemical barriers

The first line of defence in plants also includes chemicals that inhibit the growth and development of pathogens. These chemicals include enzymes that help to destroy pathogens, peptides that inhibit their growth, and organic compounds called **secondary metabolites** that are not involved in normal growth, development and reproduction, but which are used as defence mechanisms by the plant.

Some secondary metabolites are released into the environment. For example, asparagus plants produce asparagusic acid, which inhibits the eggs of nematode parasites from hatching. The chemicals that plants such as asparagus secrete into the soil are toxic to nematodes, making the plants good **companion plants** for tomatoes, which are commonly attacked by these parasitic roundworms (Figure 6.10). Other substances remain in the plant, ready to stop invaders. These substances include wetting agents that destroy fungal plasma membranes, and phenols and other compounds on leaf surfaces that discourage herbivore feeding and inhibit many potential pathogens.

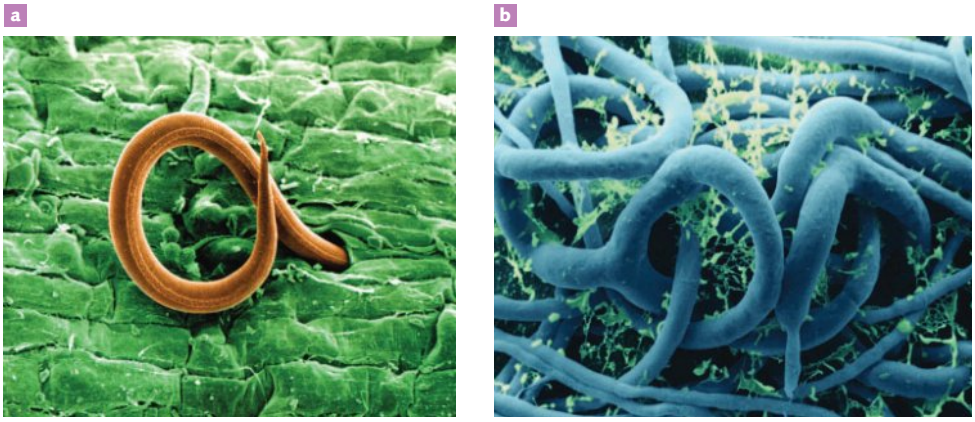


Figure 6.10 a The root knot nematode is a common plant pathogen, infecting more than 2000 plant species and causing about 5% of global crop losses. **b** Nematophagous fungi trap and eat nematodes such as the root knot nematode and are considered to be biological control agents of nematodes.

Defensins

Defensins are small, stable peptides that can inhibit the development of fungi, as well as bacteria, viruses and insects. More than 300 defensin-like genes have been identified in plants. Defensins may constitute up to 10% of the total proteins in some types of seeds, and they are also present in the cells of flowers, leaves, fruit, bark and tubers. Their antimicrobial action comes from their ability to reduce membrane permeability and inhibit the action of enzymes and ribosomes. Because of their anti-feeding activity against insects, defensins can also provide a defence against insect-transmitted viruses.

As well as protecting plants from pathogens, defensins appear to be involved in cellular signalling, growth regulation and heavy metal tolerance. Many defensins accumulate during normal plant development; others are produced in response to attack by pathogens or environmental stress such as drought, salt and cold. Defensins have been shown to inhibit the human cancer cell cycle, so they may potentially be used to treat human diseases.

WB
6.2.2
PLANTS – SECOND LINE OF DEFENCE
PAGE 211

Second line of defence

Despite the many barriers, pathogens still enter plants. When they do, plants mount a strong defence. These innate immune responses may be very rapid, with host gene expression beginning minutes or even seconds after exposure to pathogens. Unlike animals, plants do not have a circulatory system that can efficiently transport their defence mechanism. Instead, their responses tend to be more localised, with most cell types retaining the capacity to express a broad range of antimicrobial defences.

Detection of plant pathogens

Plants recognise invaders in much the same way as the cells of animals do. The broad molecular patterns commonly shared by pathogens (such as flagellin, glycoproteins, lipopolysaccharides and chitin) are recognised as being foreign to the plant. These pathogen-associated molecular patterns (PAMPs) are recognised by pattern recognition receptors (PRRs) that are found on the surface or in the cytoplasm of a variety of cells. PRRs also detect components of plant cells and tissues that should not normally be released, but which can be present after damage to the plant. These damage- or danger-associated molecular patterns (DAMPs), such as breakdown products of plant cell walls, can also stimulate the defence responses of plants, preparing the plant for attack by invading pathogens and initiating the clearance of compromised cells and repair of damaged tissues.

Note:

Information about PAMPs and DAMPs has been included to complete the story about the defence against pathogens but you do not need to know it for the VCE Biology exam. You do need to know about receptors and antigens.

Responses to plant pathogens

Once invaders have been detected, plants can synthesise a toxic cocktail of antimicrobial compounds that includes defensins and phytoalexins. Phytoalexins are low molecular weight antimicrobial compounds that can puncture cell walls, delay maturation, disrupt metabolism, or prevent reproduction of the pathogen. The effectiveness of these defences is shown by plants in which phytoalexin biosynthesis is inhibited. Such plants are more susceptible to infection and are extensively colonised by pathogens. More than 350 phytoalexins have been found in more than 100 plant species from 30 families of plants.

Note:

Chemicals in the first line of defence in plants are secreted almost continuously. Chemicals in the second line of defence are secreted in response to detection of a pathogen.

Another chemical response to invasion is the production of a burst of highly reactive oxygen molecules. These substances have a direct antimicrobial action, and are also highly toxic to plant cells. They cause rapid and localised programmed cell death (**apoptosis**) at the site of pathogen invasion. This has the effect of producing a physical barrier around the area of infection, which acts to isolate the pathogen from the rest of the plant.

Several other mechanisms help stop the spread of infection through the plant. Some plants shed infected parts such as leaves and branches (Figure 6.11), an option not available to animals. Wounds caused by a pathogen can be quickly plugged by resin, and cells can thicken and fortify their walls, thereby preventing the spread of pathogens into nearby cells.

Figure 6.11 Plants shed infected leaves to rid themselves of infection.

After the initial reaction to invasion, plant tissues may become resistant to a broad range of pathogens for an extended period of time. This is called systemic acquired resistance. It occurs because a signal travels through the vascular system to activate synthesis of antimicrobial proteins in distant tissues when a pathogen attacks a plant. This brings about a heightened state of readiness in which the whole plant, not just the part initially attacked, is prepared for further invasion. Systemic acquired resistance is effective against a broad range of plant pathogens, making it fundamentally different from the adaptive immune response of mammals.

Despite these many plant defences, pathogens frequently reduce plant growth, reducing productivity and yields in crops. Crop breeding programs often select plants with strong innate defences that will provide the individual plant with resistance to one or more important diseases.

KEY CONCEPTS

- lants have pysica nd hemical barriers to prevent nvsion by pathogens. hese inclde thick bark, waxy cutils, hairs and prouction of molecules such as defensns that are toxic to pahogens.
- lants use PRRs (pattern recontion receptors) to detect PAMPs (pathogen-asociated molecular patterns on nvading pthogens. They mount a raid innate immune resposeto invasion by pathoens.
- After ivasion by apathogen, plants attain a state of sysemicacquired resistance that makes them more prepared to figh inasion by a broad range of pathogns.

Concept questions 6.2

1 Proide three reasonsthatexlin why it is important for us to understad disease ad efene in plants.
2 Describe five physical daptations that prevent the entry of pathogen int plants.
3 Summaise thechmical defeces of plants in a sutable le incuding their naes and the ways in whch the act.
4 Describe th interactios o a cmpanion plant with a crop lnt. Use n example to suppot your response.

HOT Chaenge

5 Describe te mechanismbyich defensins kill nvdin microorganisms and why the response may be loaised.

6.3 Innate immune response in animals – second line of defence

If a pathogen breaches an animal's first line of defence, it will be detected and dealt with by the host's immune system. The **immune system** is often described as having two components. The initial response to a pathogen is rapid and general and occurs in the same way every time any pathogen invades the body. This response is called the **innate immune response** and is sometimes described as being the second line of defence. The third line of defence is the **adaptive immune response**, which develops into a potent, coordinated battle against a pathogen and involves the activation of immune cells called **lymphocytes**. These cells have the capacity to 'remember' the pathogen and make a faster, stronger response to it the next time it appears.

WB
6.3.1 SOME SPECIFIC CELLS OF THE INNATE IMMUNE RESPONSE PAGE 135

Some specific cells of the innate immune response

Several different cell types are involved in mediating innate immune responses, and collectively these cells are called **leukocytes**, or white blood cells. Figure 6.12 and Table 6.2 summarise these different cell types. Leukocytes that engulf and digest foreign pathogens are called **phagocytes** (meaning 'eating cells'). **Granulocytes** are leukocytes that have granules in their cytoplasm. The granules contain enzymes, which help digest pathogens.

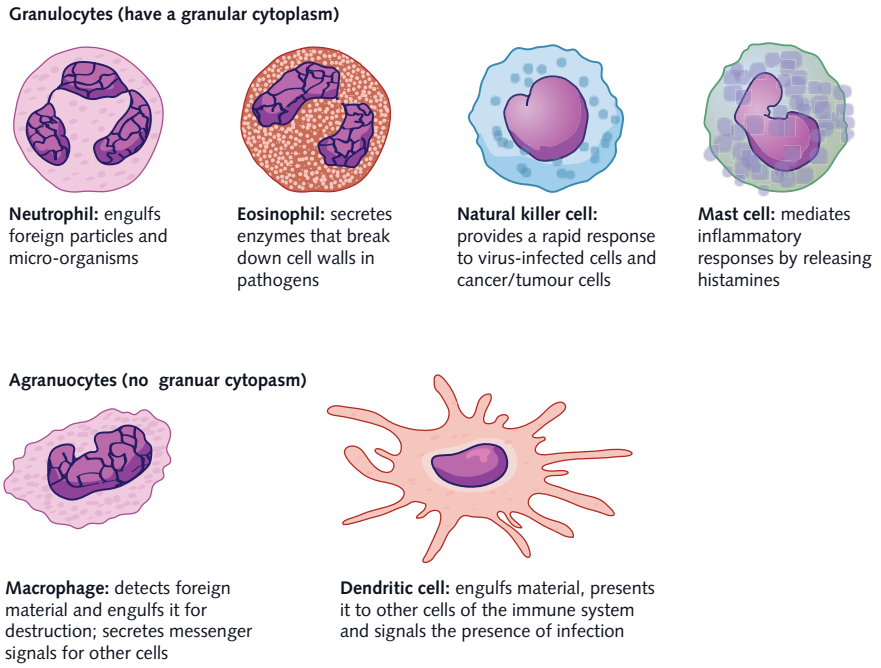
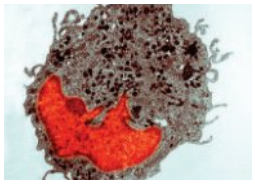
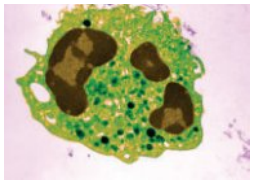
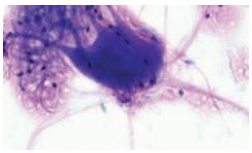
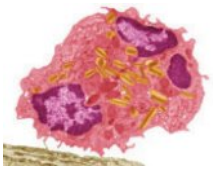
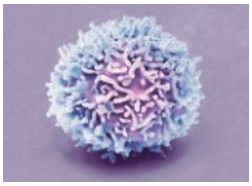
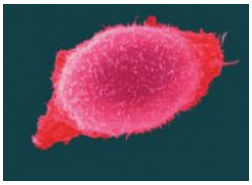


Figure 6.12 Different types of white blood cells (leukocytes)

Table 6.2 The cellular components of the immune system

Cell	Function
Leukocyte	A general term describing white blood cells. Includes all of the cell types below.
Phagocyte	A general term describing a white blood cell that engulfs and digests foreign pathogens in a process called phagocytosis. Macrophages, neutrophils and dendritic cells are phagocytes.
Granulocyte	A general term describing white blood cells that are granulated (neutrophils, basophils and eosinophils).They have granules in their cytoplasm, which contain enzymes that digest pathogens.
Macrophage 	A large phagocyte found in body tissues that becomes a powerful stimulator of an immune response when it engulfs a pathogen.
Neutrophil 	Classed as a granulocyte and a phagocyte and found in the blood and tissues. Rapidly enters sites of inflammation, engulfing the pathogen and then dying in large numbers. Pus contains the debris of dead neutrophils.

EXAM TIP
Even though spelling is not assessed in the VCE Biology exam, make sure you know how to spell key terms because incorrectly spelt terms lack clarity and may lead to a failure to gain marks.

Dendritic cell 	A phagocyte with membranous extensions that engulf pathogens, process them and present them to other cells of the immune system.
Eosinophil 	A granulocyte with secretory vesicles that contain powerful enzymes that rupture (lyse) cell walls of pathogens. Important in combating parasites such as worms and flukes. Their chemicals are toxic to the tissues of both parasites and host.
Natural killer cell 	A granulated lymphocyte that secretes chemicals that lyse cancer cells and cells that are infected with viruses. They attach to the glycoproteins on the surface of infected cells, and kill them.
Mast cell 	A granulocyte that releases histamines; also involved in healing wounds. Concentrated within the respiratory and gastrointestinal tracts, and within the deep layers of the skin.

Macrophages

Macrophages are a type of white blood cell. They tend to survive for a long time, sometimes months. They develop from **monocytes** that have left the blood vessels and entered the tissues in response to signs of infection.

As potent activators of other immune cells, macrophages are specialised to switch on inflammation. Macrophages have receptors on their surface that recognise **antigens** (substances that trigger an immune response) on invaders, such as bacteria, that are not normally found in the body. Macrophages activated in this way produce a number of cell signalling molecules called **cytokines**, which include **interferons** and various interleukins. Cytokines are important because they act as signalling molecules, stimulating cells to move towards the sites of inflammation. Macrophages also destroy pathogens and clear apoptotic cells and damaged tissue by **phagocytosis**.

Neutrophils

A **neutrophil** is a type of granulocyte that is abundant in blood. Neutrophils have irregular, multilobed nuclei and granular cytoplasms. Neutrophils rarely survive longer than a few days, so reinforcements from the blood are constantly required. At least 80 million neutrophils are produced by the bone marrow every minute. Like macrophages, neutrophils carry out phagocytosis (so these cells are sometimes collectively called 'phagocytes'). They produce a wide range of cytokines that can induce **chemotaxis**, which is the movement of cells towards the source of a chemical gradient. Cytokines can also trigger mast cells to release **histamine**, a chemical that stimulates inflammation. Neutrophils also produce defensins, which are peptides that act as powerful natural antibiotics with wide antimicrobial activity.

An important feature of neutrophils is that, as well as having a very short lifespan, they die rapidly after they have phagocytosed a pathogen. This adaptation ensures that pathogens cannot propagate in

neutrophils and spread through the body. Pus contains the cellular debris of large numbers of neutrophils that have died in this way. Some pathogens are able to evade death during phagocytosis. In as little as 30 seconds after ingestion, *Rickettsia*, an intracellular parasite of phagocytes, uses an enzyme to free itself into the cytoplasm. *Legionella* bacteria survive by preventing lysosomes from fusing with the phagosome, and pathogenic *Streptococci* cause lysosomal granules to explode and release their lethal contents into the cell, thus killing the phagocyte and releasing the pathogen.

Dendritic cells

Dendritic cells are derived from monocytes. They are large cells with a non-granular cytoplasm and are named for their dendrites, or finger-like structures, which reach in between cells in tissues and sample whatever is in the microenvironment. If this contains antigens, the dendritic cell becomes activated and can engulf and destroy invaders, and send out signals to other cells of the immune system to bring about the beginning of a coordinated immune response. Dendrites on the surface of the cell help them to take up, process and present antigens to lymphocytes efficiently.

Dendritic cells have similar functions to those of macrophages, including phagocytosis of antigens and secretion of cytokines that bring about an immune response. However, dendritic cells differ in that they are highly specialised to take up the pathogen at the site of the infection and transfer it to organs of the lymphatic system. This is where the dendritic cells present the antigen to lymphocytes, cells of the adaptive immune system. Dendritic cells are therefore important in linking innate immune responses with the adaptive immune system.

Eosinophils

Eosinophils are leukocytes with a role in the innate immune response. Eosinophils secrete powerful enzymes that can form destructive holes in the cells of multicellular pathogens such as blood flukes and parasitic worms.

Natural killer cells

Natural killer cells circulate around the body, acting as security guards. They check the cells they encounter for suitable surface markers that identify the cell as self. Any suspicious cells whose markers have changed, such as those infected with virus or transformed by cancer, are destroyed by an attack on their plasma membranes. This leads to apoptosis, ensuring the destruction of both the cell and the virus inside. The importance of natural killer cells in the initial response to infection by a virus is shown by patients deficient in natural killer cells being highly susceptible to the early phases of *Herpes* infection.

Mast cells

Physical damage such as a cut in the finger can rupture body cells and release danger signals that stimulate **mast cells**. Mast cells are located in the tissues. When activated by the danger signals, or the detection of an antigen through binding to surface receptors called IgE (p. 231), they release their granules, which are loaded with histamine, a major stimulus for the initiation of inflammation. Mast cells also secrete heparin, which prevents blood clotting in the injury site, although a clot forms around the outside of the injury site to prevent the spread of the pathogen.

Histamine, together with cytokines (both are signalling molecules) released by macrophages, promotes **vasodilation** (widening of blood vessels, especially arterioles) in the damaged region. With increased blood flow comes a battalion of cells and chemicals to fight off an infection. This increased blood flow is the cause of the redness and swelling seen at the site of an injury. As blood also transfers heat, swollen areas often become very warm. Histamine changes the permeability of capillaries in the inflamed area, making it easier for leukocytes, blood plasma and blood proteins to squeeze out through the walls and into affected tissue. This will be discussed further on page 220 in the section on inflammation.

Complement proteins

The **complement** system gets its name from its role in helping, or complementing, the immune system. The complement system consists of many small proteins that circulate in the blood and tissue fluids.

These proteins are secreted mainly by the liver, but also by macrophages, monocytes and other body cells. Complement proteins are normally inactive and are designated by a C and a number (e.g. C3). The inactive precursor proteins become activated immediately when they encounter antigens from a foreign body, such as an invading bacterium. Activation of a complement protein (e.g. inactive C3 becomes activated C3a and C3b) has a cascade effect, stimulating the activation of other complement proteins (e.g. inactive C5 becomes activated C5a and C5b), which then activate other proteins. This is known as a complement cascade.

These proteins have three main ways of defending the body.

» An important product of the complement cascade is the membrane attack complex (MAC). MAC forms pores in the membranes of target cells, disrupting the phospholipid bilayer. With membrane integrity destroyed, osmotic cytolysis occurs because of a sudden influx of fluid, which causes the cell contents to spill out into the extracellular fluid. Cell death follows (Figure 6.13).

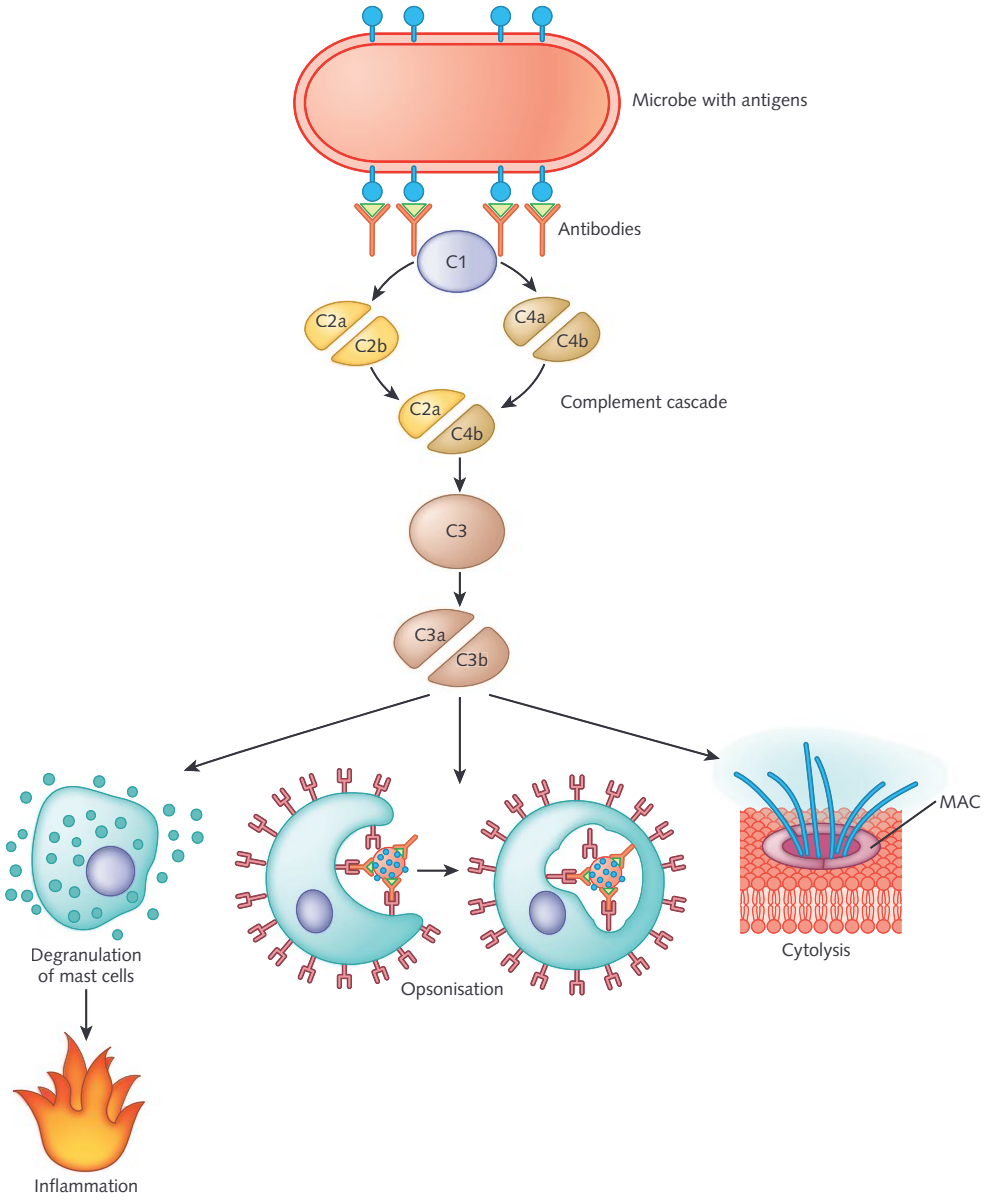


Figure 6.13 **Activation of the complement system leads to a complement cascade, which causes opsonisation, cytolysis and inflammation.**

» C3b molecules coat the surfaces of microbial cells such as yeasts and bacteria and function as opsonins, acting as a tag to help their detection and uptake by phagocytes, which have complement receptors on their surface. C3b molecules induce chemotaxis by creating concentration gradients that attract phagocytes and other white blood cells to the damaged or infected site. Complement activates these phagocytes by increasing their ability to ingest and destroy pathogens. This process is called **opsonisation** (Figure 6.13).

» Degranulation of mast cells (release of their granules) leads to inflammation (p. 220).

With its powerful and potentially dangerous effects, the complement system is tightly controlled. One important safeguard is that activated complement proteins are rapidly inactivated unless they bind to the surface of a pathogen. In addition, MAC-inhibitory proteins are expressed on all body cells. This protects the body's own cells by inhibiting MAC formation in their membranes. People lacking the gene for the MAC-inhibitory protein suffer episodes of intravascular red blood cell lysis caused by activated complement.

Interferons

Complement proteins and lysozymes are extremely effective against bacteria but cannot destroy viruses. Instead, some virus-infected cells secrete cytokines called interferons, which induce resistance to viral infection in the surrounding cells. Interferons act as warning signals from the doomed cell and cause changes in the surfaces of the surrounding cells, making it more difficult for a virus to infect them. By targeting multiple points in the viral life cycle, interferons also prevent replication of virus particles inside the host cell.

Interferons also enhance many of the functions of other cells of the immune system. For example, natural killer cells are activated by the release of interferons and cytokines targeting macrophage responses. Natural killer cells contain cytotoxins that are released to eliminate both virus-infected cells and cancer/tumour cells. Within their cytoplasm are granules containing toxins such as perforin and other proteases that, on release, induce apoptosis. Apoptosis produces small blebs (bulges in the plasma membrane) of cell contents, which are efficiently engulfed and processed by phagocytes. Apoptosis is the preferred method of cell eradication because it ensures that both the infected cell and its contents are eliminated without further contaminating surrounding tissue. In necrosis, the plasma membrane has no control over the process, so when the membrane is breached both the cell contents and virions (virus particles outside a cell) are released and can then move into surrounding cells (Figure 6.14). This would do little to stem the further progression of the virus into healthy cells and tissue.

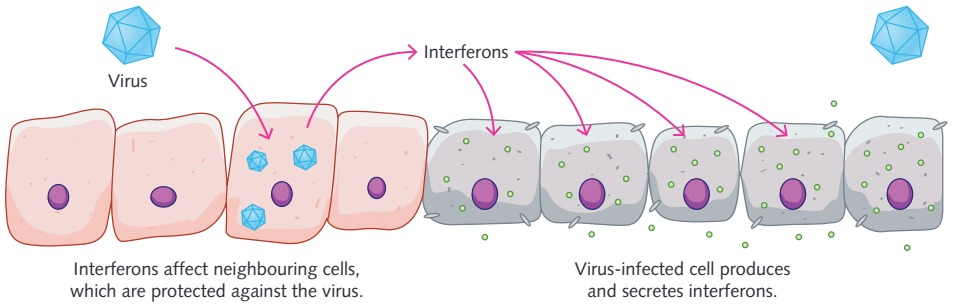


Figure 6.14 **Interferons are secreted by virally infected cells and act on neighbouring cells to prevent viral entry and viral replication. Cells shown in grey are in a protected state.**

Note:

Cytokines such as interferons also play an important role in the adaptive immune response.

Because of their particular effects on cells, individual interferons have proved useful in the treatment of a number of diseases such as multiple sclerosis, rheumatoid arthritis, hepatitis C and some cancers. Like complement proteins, interferons are non-specific in their effects, being secreted in response to the detection of any viral invader.

KEY CONCEPTS

- »Innae immune responses are on-speci fic and are nborn features of the body.
- » Leukocyts (whitelood cels) ediate the innate immune response by engulfing and digestng forein pathogens.
- »Immune lls such as macrophage begin the immune response after their receptor sites bind with foreign antigens.
- » Neutrphils are a type of phagocyte that are abundant n blood. Th redily migratrom caillaries to sites of nfecton, where they phagocytose pathogens and then elf-destruct to stoppathogen spread.
- » The complement sysem consists ofa number of small mlecules inthe bloo that ave important roles in inflammtion, ncluding osonisatin and deposition of the membrane attck comlex (MAC).
- »Interferons are producd y viru-infected cells and alertneighbouing cells t th danger, causing changes n gene expresion that make the more resistant to vralinfetion an relication.
- » llplants and anmals ount innate immune responses to pathogns.

Concept questions 6.3a

1 Define innate responss toinection' and state the type oforganism that can generatethese responses.
2 Describe the role f phagocytic leukocytes in the mmune sysem.
3 Lst onesilarity nd one differnce between:
  a macrophages andeosophils
  b neutropils and bsophils.
4 State three outcomes whe the complement system s acivted.

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5 Suggest why phgocytes, such asacrophages, typically contin large numbes of ribsomes and lysosomes.

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6.3.2 INNATE IMMUNE RESPONSE – INFLAMMATION PAGE 139

Innate immune response – inflammation

Inflammation is a major feature of the innate immune response. It consists of a complex series of reactions and processes involving the activities of many different cell types and signalling molecules, which act together to initiate and maintain inflammation until the danger is cleared.

Inflammation has two roles:

- » to destroy the cause of the infection and remove it and any products it has produced from the body. If this fails, then inflammation works to limit the spread of the infectious agent by confining the infection to a small area
- » to replace or repair tissue damaged by the infection by improving blood flow.

The first signs of infection in a cut finger are usually pain, heat, redness and swelling. These are the four physical signs of inflammation. Inflammation occurs in tissues where cells are killed or damaged by physical injury or invading pathogens, and is the key weapon of the innate immune response. The pain, heat, redness and swelling occur because blood vessels in the inflamed area become permeable (leaky), allowing cells of the immune system to move out of the blood vessels into the area to mount a strong response against invading pathogens. Once the pathogen is cleared, the cells die or migrate elsewhere, the four signs of inflammation disappear and the site returns to normal.

Initiation of inflammation

Inflammation is triggered by the recognition of a non-self organism or part of a non-self organism. If pathogens enter the body, they are likely to encounter white blood cells (such as macrophages and dendritic cells). It is important to note that even though these cells are generally classed as white blood cells, many occur in all body tissues, especially in the skin, liver, lungs, kidneys, spleen and lymph nodes, where they act as resident sentinels.

Infection by a pathogen is not essential for inflammation. Intracellular molecules, which are usually hidden from the immune system, can be released through injury or tissue damage. These can be detected as DAMPs by PRRs and may trigger **sterile inflammation** (inflammation arising in the absence of infection). This type of inflammation may be more in anticipation of infection, and with a greater emphasis on promoting effective wound healing.

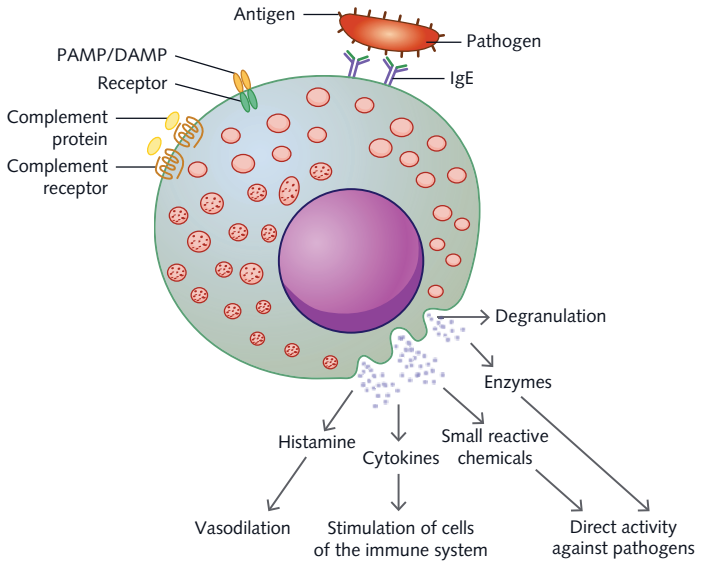

Figure 6.15 Mast cells degranulate in response to inflammatory signals, releasing histamine and other molecules. IgE stands for immunoglobulin E, an antibody.

Inflammation takes place in the following steps.

1. Blood flow increases to the site of damage. This causes redness and swelling and brings more immune cells to the site.
2. Mast cells release histamine. Histamine makes blood vessels permeable and causes them to dilate (vasodilation). This allows immune cells such as phagocytes to move out of the blood vessel and enter the tissue.
3. Blood clotting occurs. Platelets and fibrin (protein) form a clot, which prevents other pathogens entering the damaged site.
4. Phagocytes engulf pathogens or foreign material.
5. Other immune cells detect antigens and produce immune signalling molecules, including interferon and complement. This brings more immune cells to the site to help with the immune response.

Inflammation involves cellular migration

During the resting state, and especially once an immune response is initiated, cells of the immune system undergo chemotaxis. This is an important way in which cells involved in the inflammatory response are recruited from the blood to sites of infection or tissue damage. During chemotaxis, white blood cells move towards increasing concentrations of cytokines called **chemokines**, which are any molecules that induce chemotaxis. Chemokines include molecules released by micro-organisms, activated macrophages and other cells. There are many types of chemokines, each with specific receptors expressed by particular target cells. Only cells expressing a particular chemokine receptor will undergo chemotaxis in response to that chemokine.

In response to chemokines, two types of leukocytes, monocytes and neutrophils, squeeze out through the capillary walls into the tissues. After monocytes enter the tissues, they mature into macrophages. The

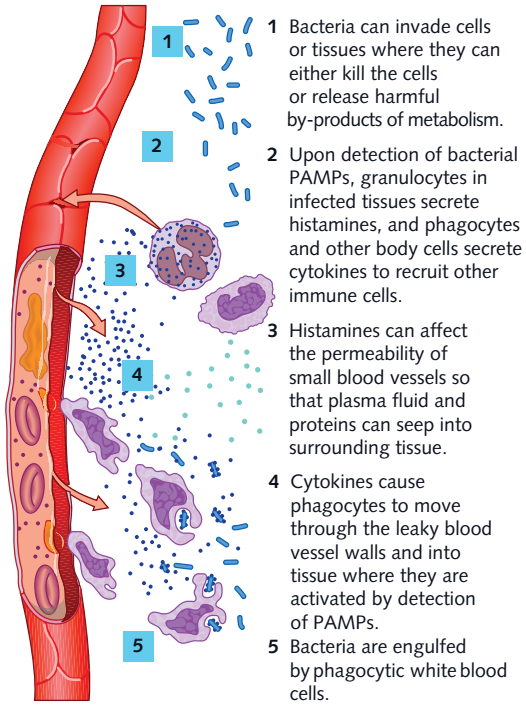

Figure 6.16 The steps that occur in acute inflammation after invasion by a bacterial pathogen

swelling caused by the action of histamine, which increases leakage of blood plasma and leukocytes into the inflamed area, also causes some localised pain. Feeling pain is an important process, as it reduces voluntary movement in that area, thus limiting further tissue damage and speeding up the epair process.

Resolving inflammation

After an inection, in flammation does not resolve passively but in a highly coordinated, active process that is controlled by several factos, including cytokines and othersignalling molecules. These switch off movement of leukocytes to the site of inflammation, reverse vasodilation, and reduce the permeability of fine blood vessels to the level before the inflammation. They also stimulate macrophages to safely dispose of material that has accumulate at the site of infection. This includes dead netrophils, fibrin and exudate, the fluid that leaks out of blood vessels at the site of inflammation. During phagocytosis, macrophages detect and recognise a molecule on the surface of neutrophils that have died by apoptosis. This triggers the release of cytokines, including those that promote the resolution of inflammation. Sometimes the signal for apoptosis fails and the neutrophils die by **necrosis**, unprogrammed cell death that occurs as a result of injury or infection (Figure 6.17). Whereas apoptosis is an immunologically 'silent' form of cell death that does not normally activate the immune system, necrosis stimulates inflammation, and macrophages release cytokines that further enhance inflammation rather than suppressing it.

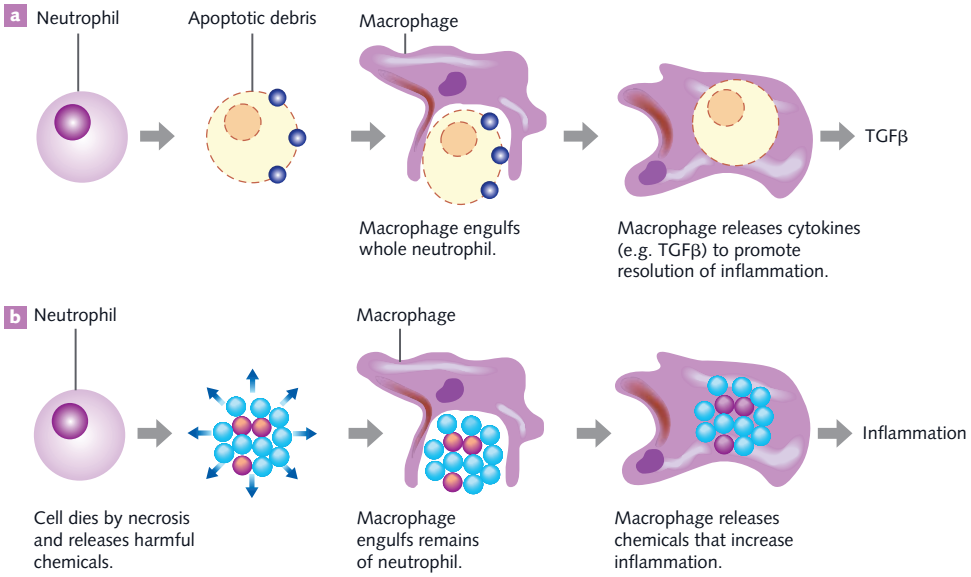

Figure 6.17 Different signals, from a apoptosis and b necrosis, can resolve or prolong inflammation.

Successful resolution of inflammation limits excessive tissue injury and reduces the opportunity for chronic, or long-term, inflammation. Defects in these clearance mechanisms appear to be associated with persistent tissue inflammation and autoimmune responses directed against cellular contents. Repeated bouts of inflammation, as happens with autoimmune conditions such as Crohn's disease and rheumatoid arthritis, result in ongoing tissue damage.

Distinguishing self from non-self

In order for an organism to detect when a pathogen has gained access, the organism must be able to distinguish self from non-self. Both plants and animals are alerted to invasion of pathogens by physical and chemical changes that occur in their cells or tissues, which enable them to distinguish self from non-self. The presence of foreign molecules, either on the outer surface of the invaders or in the toxins and enzymes they secrete, stimulates host immune responses that usually lead to the destruction and removal of the pathogen.

Cells of the immune system recognise non-self molecular patterns (PAMPs) that are characteristic of microbes but are not found on host cells. Immune cells have evolved receptors (PRRs) to recognise these molecules because they are unique to pathogens, their structure is probably essential for the pathogen to function properly.

KEY CONCEPTS

- Inflammton is chracterisedby pain, heat, redness and elling. In flammton is the key weapon of th innae immune resonse,detrying invading pathogens before they cn establis an infection.
- Chemotxis is the procs of cllular migration towards reions o higher concntrationsof chemokines. Each cytkine has a peci fic receptor tht is expressed by partcuar leukocte types.
- Inflammto sinitiated when acrophges, dendritic cels or other lls detec antigenr signalling mlecules ad begin to produce cytokines that bring in and ativateother iue cells.
- Pain reduces voluntary movement toassist the repair proces.
- Immune ll recruitmnt to sites of in flammton is assisted by hstamine, which is sereted by mast cells an increases theermealt of capillaries.
- The reslutin of in flammton isa ighly active process that is necesary to lmit and repair tissue damage caused by the inflammatory response.
- Th innate and dative immune rsponses rely on bingable tell self fro non-self.

Concept questions 6.3b

1 Lst the four hscal igns of in flammation.

2 Some whie blod ces are calle granulocytes. Descrie:
   - **a** one feature tht ditingushes granulocytes from other wite loo cells
   - **b** their fnction.

3 Explain the steps that ause yr ankle to swell if you sprai it.

4 Cytkines re a large grou o proeins, peptides or glycopoteins that are sereted by speci fic cels of th immune sytem.Cytokines are a category of sgnling moleculesthat mediate and regulate mmunt in flammtion and other pocesses. L-10 (iterleukin10) is a cytkine with potent ani-in flammatory poperties, repressing the expresion of oher in flammatorycytokines by ac ivated macophages.So chemokines initiate inflammtion; othes sht it own.Why is this important?

5 Discuss wa i inolvedinthe resolution of inflammtion andwhy his is important for mitig tissue daage a sitsof infection.

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6
   - **a** Describe te mechanism bywhich macrophages, haing detected a angesgnal (i.e. a PAMP or a DAMP by PR), aler other ells of the immune system of th danger.
   - **b** Describe the importace of an organism being ale toditnguish beween sel and non-self.

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6.4 ANTIGENS AND PATHOGENS PAGE 140

6.4 Antigens and pathogens

The trigger that initiates an immune response is the presence of antigens derived from a cellular or non-cellular pathogen. Some responses are specific to a particular antigen and others are more generalised, and responses can be localised or systemic (throughout the systems in the body, not just at the site of infection). Some antigens are **allergens**, substances that trigger an allergic response. An **allergy** is a specific type of immune response to normally harmless stimuli such as food, pollen or house dust mites. More harmful unwanted immune responses occur when the antigen is a **self-antigen**; that is, a substance that is normally present in the body. This can cause an autoimmune disease, in which the body's own cells and tissues are mistakenly targeted for destruction by the immune system. Appropriate and effective immune responses depend on the ability of the immune system to distinguish self-components from **non-self antigens**, derived from a foreign agent such as a pathogen.

Antigens can be made of many different substances, including proteins, peptides, lipids or polysaccharides, and are often embedded in the membranes of cells or the outer coat of viruses. Antigens can also be intracellular components of pathogens, including nucleic acids, proteins, carbohydrates and lipids. The genetic information of the organism to which they belong determines their exact shape and structure. Just as DNA is unique to an individual, it follows that the antigens produced by an organism are unique to that organism.

To a normally functioning immune system, self-antigens are invisible, and the immune system does not mount a response when it encounters them.

Pathogens are sources of non-self antigens. An immune response will be launched against the non-self antigens with the aim of destroying or neutralising the pathogen. Pathogens can be cellular or non-cellular. **Cellular pathogens** are able to reproduce and function by themselves. Cellular pathogens include bacteria, fungi, protists and parasites. Fungi cause diseases such as thrush and tinea; bacteria cause sore throats and gastroenteritis. Protists are the disease-causing agents in malaria, amoebic dysentery and giardiasis. A parasite is an organism that lives on or in its host for all or part of its life, causing harm to and gaining nutrition from the host. Examples of parasites are arthropods (mites and ticks) and helminths (worms and flukes).

Non-cellular pathogens are not made of cells and cannot reproduce and function without being inside a host, in most cases inside a host cell. Non-cellular pathogens include prions and viruses. Viruses are responsible for diseases such as smallpox, anthrax, HIV/AIDS, and coronavirus diseases such as SARS-CoV, which causes severe acute respiratory syndrome, and SARS-CoV-2, which causes COVID-19. Prions cause kuru and mad cow disease.

Bacteria

Bacteria may have been the first cellular life form on Earth, and today they are still the most abundant and most diverse group of organisms. Only a relatively small number of bacteria cause disease. Billions of bacteria live on your skin and in your body, many of which are commensal. All of the genomes of the microbes in this microbiota are collectively referred to as the **microbiome**, and this can be used to identify the organisms of the microbiota.

Structure of bacteria

Typically bacteria are 1–10 µm (micrometres) in length and 0.20–2 µm in diameter. Like all cells, bacteria have a plasma membrane that encloses the cytoplasm. The plasma membrane is surrounded by a cell wall (Figure 6.18). As they are prokaryotes they have no membrane-bound organelles or nucleus; however, bacteria do have ribosomes and a single circular chromosome. The cell wall is made of peptidoglycan (a protein–carbohydrate compound). These are known as gram-positive bacteria. Some bacteria also have an additional outer membrane made of a lipopolysaccharide component. These are known as gram negative bacteria.

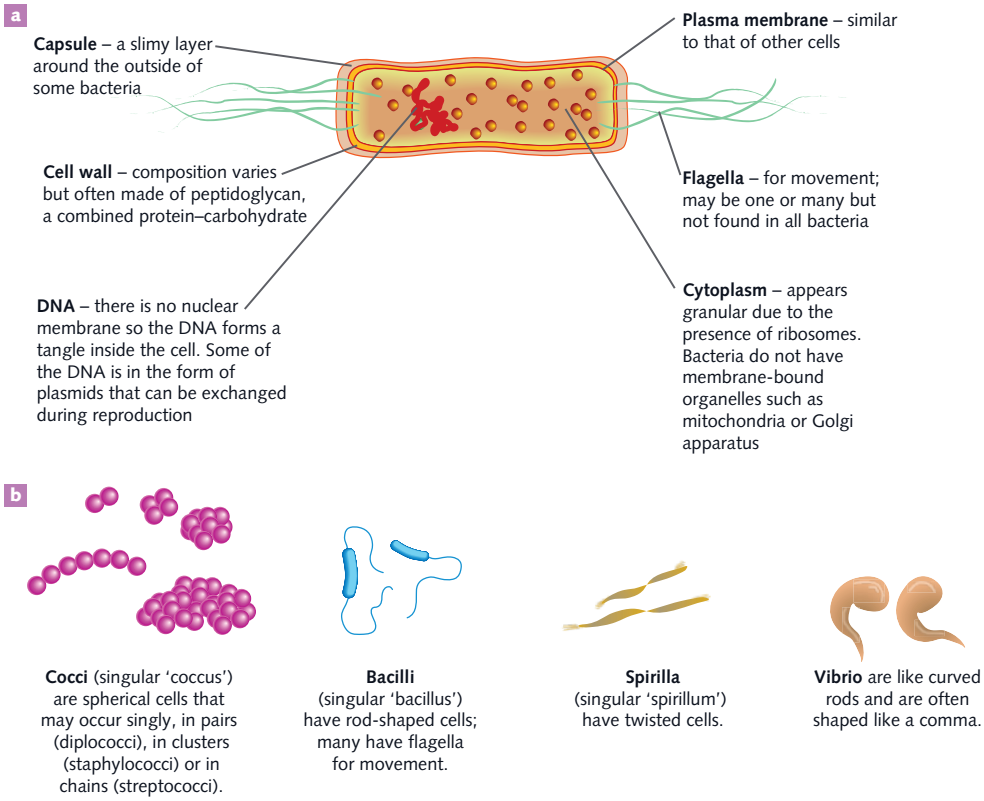

Figure 6.18 a The structure of a typical bacterial cell. **b** Bacteria are classified according to their cell shape.

Some bacteria have a **flagellum** (a hairlike appendage) or multiple flagella, which help them to move about (Figure 6.19). Some species have a slimy **bacterial capsule**, which they use to help the bacteria stick to surfaces such as teeth or mucous membranes. The capsule is a large, well-organised layer outside the cell wall. It usually increases the virulence of a species (the degree to which it causes disease) because it makes it harder for the body's immune system or antibiotics to recognise and attack the inner bacterium.

Bacteria are important pathogens of humans, plants and animals. Bacteria can be transmitted from one host to another in several ways: by direct contact, in food and water, and in droplets of moisture in the air. Biting insects, such as ticks and fleas, can also transfer bacteria on their biting parts.

Once inside a host, bacteria can divide rapidly. Different features of bacteria cause disease in different ways. Some bacteria damage host tissues directly, while others produce toxins (often their own metabolic wastes) that disrupt the functioning of cells nearby or even further away. For example, toxins produced by diphtheria bacteria in the throat affect tissues throughout the body. Many

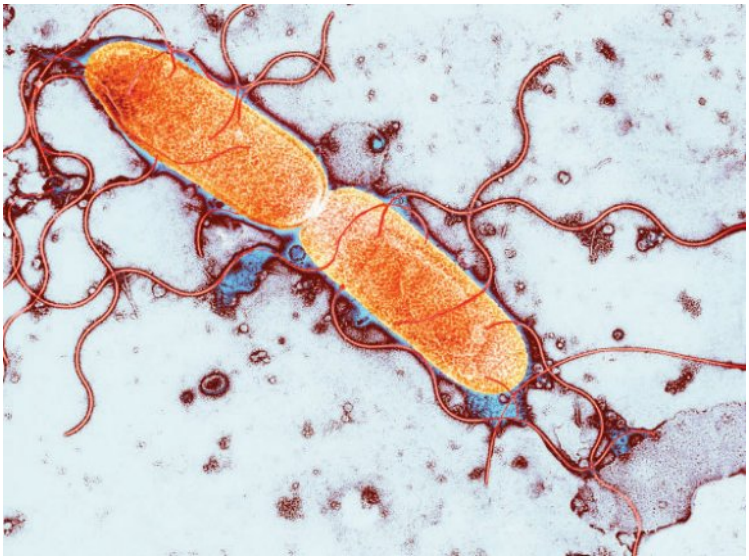

Figure 6.19 Transmission electron micrograph of *E. coli* dividing into two by binary fission. Note the flagella, which appear like hairs extending from the cells.

parts of the bacterial cell are highly pathogenic to the host. External molecules such as lipopolysaccharides (lipid–carbohydrate compounds) or peptidoglycans are such examples. These antigens can stimulate immune responses that are sometimes so strong that they damage host cells and tissues. Some bacterial strains interfere with the host's immune system, making the host susceptible to other pathogens.

Some bacteria produce exotoxins and endotoxins (Figure 6.20). Exotoxins are toxic substances produced by the bacterial cell and then released to act outside of the bacteria. The toxins damage the cells of the host organism and thus spread disease through the ongoing necrosis of affected cells. Endotoxins are lipid-based bacterial toxins located within bacterial cells and are released into the body's internal environment (plasma and interstitial fluid) upon lysis of gram-negative bacteria.

EXAM TIP
In biology, *exo* means outside or external and *endo* means inside or internal. Even if you don't know what a word means, you can sometimes work it out.

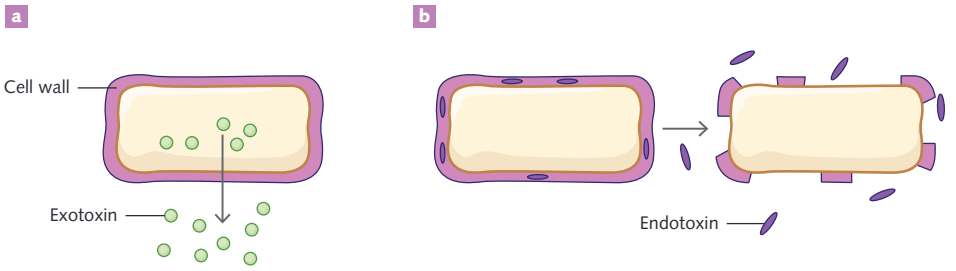

Figure 6.20 a Exotoxins are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are secreted or released, following lysis, into the surrounding medium. b Endotoxins are part of the outer portion of the cell wall of gram-negative bacteria. They are released when the bacteria die and the cell wall breaks apart.

The success of the treatment of any of these bacterial infections is determined by the effect of both the immune system and medications on bacterial cells. The most common treatment for bacterial infections are antibiotic medications. If the antibiotic works by rupturing the plasma membrane, there is then the added complication of whether that bacterial species will release toxins. So even though the discovery of antibiotics has been ground-breaking, treatments are often complicated because bacteria are very good at evolving and surviving assaults from human technologies and treatments.

Figure 6.21 A yellow fungus infection in a bearded dragon lizard. The fungus causes the lizard's scales to break off and leave swollen and painful ulcerated lesions.

Fungi

The fungal world includes large organisms such as mushrooms and toadstools, as well as tiny forms that can only be observed under a microscope. These microscopic **fungi** include unicellular yeasts and moulds. Fungi are eukaryotes that reproduce by spores and have cell walls made of chitin rather than cellulose. Microscopic fungi are generally larger than bacteria. Not all fungi cause disease, but some of them are pathogenic, causing disease in a wide range of organisms including plants and animals.

Most fungal diseases in animals are external, where they irritate and inflame the skin (Figure 6.21). A common example is

ringworm, a fungal skin infection of rabbits, dogs, cats, horses and humans. Tinea is another fungal skin disease of humans. Symptoms include a rash and itchy skin. Both diseases are easily transmitted from one individual to another. As they grow on the skin, fungi produce spores, and as the infected skin flakes off it carries these spores with them. If the spores come into contact with damaged or broken skin, they may cause new fungal infections. Spores are very long lived, an adaptation that improves transmission rates. They can remain alive for years in bedding, furniture and grooming tools, germinating when conditions are suitable.

Some fungal infections can be internal, particularly in people with suppressed immune systems, in which the fungal invasion represents a significant clinical problem.

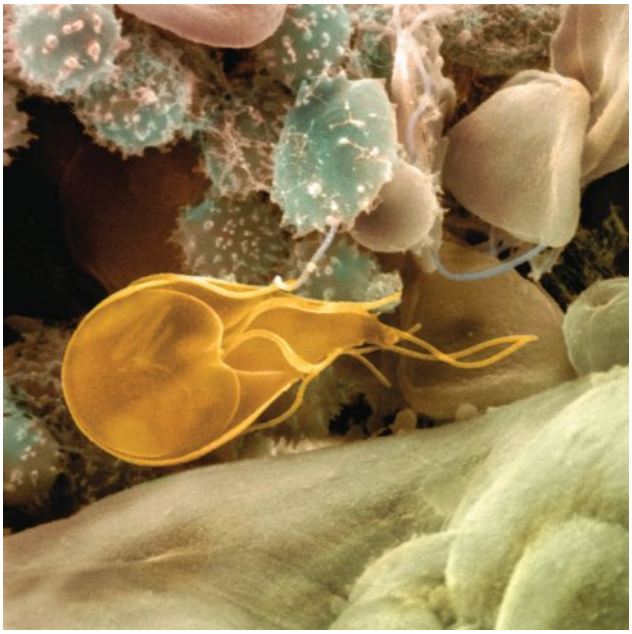

Figure 6.22 A scanning electron micrograph of *Giardia amba* (yellow) in the human small intestine. This flagellated protist contaminates drinking water, causing intestinal upsets.

Protists

Protists are unicellular, eukaryotic organisms. They reproduce both sexually and asexually. Of the 65 000 known species of protists, fewer than 24 species cause diseases in humans, but these few infect hundreds of millions of people each year. We still do not have effective preventatives against many of them and the drugs we have to treat them are limited in their effectiveness.

Examples of pathogenic protists include *Giardia lamblia* (Figure 6.22), *Trypanosoma* (which causes African sleeping sickness) and the protists that cause chlamydia, cryptosporidiosis, amoebic dysentery and malaria. Malaria has been plaguing humans for many thousands of years. It is caused by protists from the *Plasmodium* genus that are transmitted to the host by the bite of a female *Anopheles* mosquito. The *Plasmodium* protists infect host red blood cells, and reproduce inside them. Infected red blood cells eventually rupture, releasing more parasites and their metabolic wastes into the bloodstream. This toxic release induces the classic malarial headaches, chills and a burning fever. These symptoms eventually subside but can recur when more cells are lysed, releasing more parasites. If left untreated, the host may develop an enlarged liver and spleen or, in the case of cerebral malaria, brain injury that leads to death in severe cases.

KEY CONCEPTS

- Pathogens aecellular onn-elluar infectious agents that case dsease.
- Atigens re iacellular xtracellular components of protis, pptds, lipid or poysaccharides present n pathoens.
- Baceria are prokayotes; some are bene ficial totheir hosts and some arepthogenic.
- Baceria produce edotoxins orexotoxinsthat can also causedisese.
- Fugi are ekarytic organismthat are usually exteral pathogens that reproduce and spread through their spres.
- Proists aruicellular ekarytes, a small number of hich ar signi ficant pathogens ofhumans. Examplesof protist pathogens are *Giardia* amoebas, *Trypanosoma* and *Plasmodium* spcis.

Concept questions 6.4a

1 Define atien'.
2 Ho is anantigen different rom an allergen?
3 State three ways th a bacterial pathogen can har its ost.
4 Antiiotic can help fight bac ral disease butnot fungal dsease or protist athogeni ise se. Explain why.
5 Describe the advantaes toa bacterium of:
   a having a capsule
   b foring endospres.

HOT Chaenge

6 Mlrkills mor people tha any other infectious dises. Mlaria is vector-brne disease. The continent of Afica hasthe highes burdn of disse in the world, acording to te Wold Health Organization.
   a Ditnguish betwen malaria and *Plasmodium*.
   b What is a vecto-borne disease?
   c Wh is aariaso dif ficlt to contol?
   d What measures are used to control the pathogen and the vector?
   e Propose a hyothesis out why mainland Austaia is curety malria free.

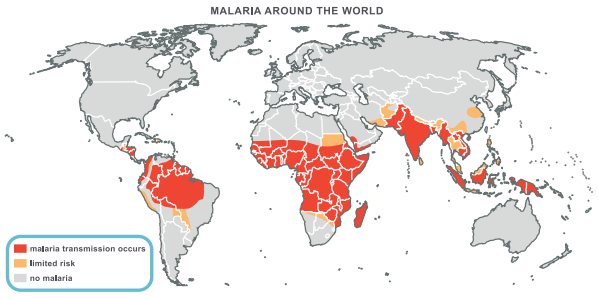

Figure 6.23 Malarial distribution in the world

CONNECT

The importance of these surface proteins in the development of various vaccines is discussed in detail in Chapter 7.

Viruses

A **virus** is a non-cellular agent composed of a protein coat and nucleic acid (Figure 6.24), either DNA or RNA (either single stranded or double stranded forms of both types of nucleic acid), but never both. A virus is often referred to as an **obligate parasite** because it cannot function and reproduce outside the host cell.

Viruses essentially vary in the:
» nucleic acid sequence within their core
» array of viral proteins incorporated into the phospholipid envelope.

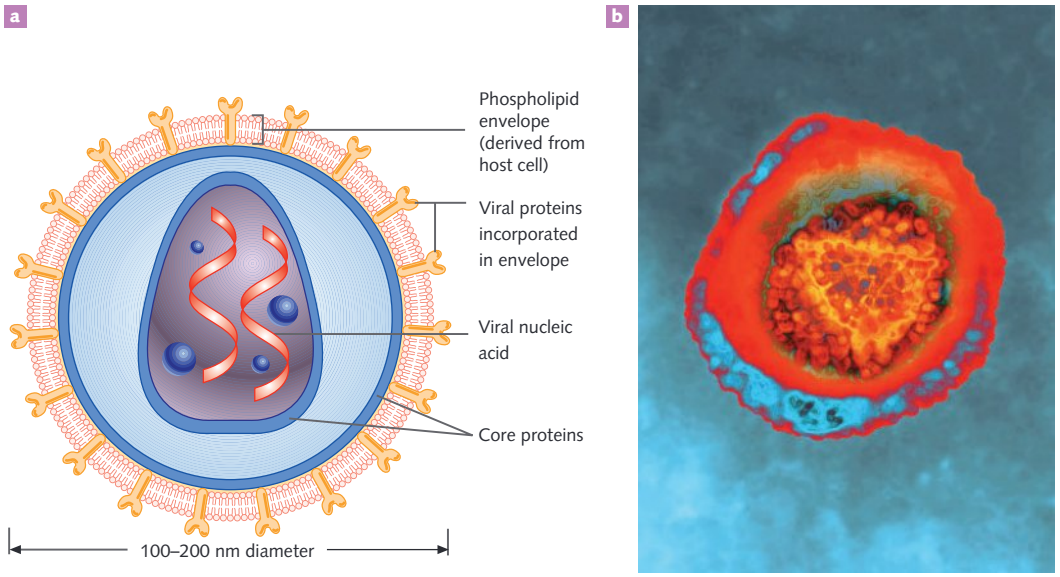

Figure 6.24 a Viruses consist of a nucleic acid core surrounded by a protein coat. **b** The virus that causes herpes in humans.

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The nucleic acid sequence determines the 'shape' of the viral proteins, which the body's immune system detects as being non-self. Some viruses, such as the variola virus that causes smallpox, remain relatively stable and therefore so do their surface proteins. Others mutate rapidly and thus change their surface proteins; for example, influenza and HIV. The immune response centres on identifying specific surface proteins as non-self and then mounting a response.

When a virus infects an organism, it injects its nucleic acid into a host cell. Once inside the host cell, viral proteins hijack normal functioning of the cell, and the viral nucleic acid directs the host cell to make multiple copies of the viral protein coat and nucleic acid. These then assemble into new viral particles and can bud from the host cell, becoming encapsulated by the host plasma membrane as they leave. Viruses can also replicate in vast numbers within the cells, becoming released when the host cell undergoes lysis due to the huge viral load. This releases many new viral particles, which can infect other cells within the host and can be **transmitted**, or transferred, to other host organisms. This life cycle is called a lytic cycle, and the viral genome stays separate from that of the host. Viral nucleic acids and proteins differ enough from those of the host that they can be identified as non-self antigens, stimulating an immune response to viral infection. Some viruses can incorporate their DNA into a host's chromosome and remain dormant, hiding from the immune system and being replicated along with the host's chromosome every time the cell divides.

Each virus is usually highly specific to the host cell or organism it can infect. For example, the SARS-CoV-2 virus specifically infects cells of the lung, causing the disease COVID-19. This is because the virus is able to recognise and bind to receptors that are expressed in respiratory tract epithelia. Even a small change in DNA sequence (such as might exist between closely related species) can result in an amino acid change in the target receptor protein, so that the host protein structure is different enough that the viral protein can no longer bind.

Bacteriophages: viruses of bacteria

Bacteria have their own group of viral pathogens, known as bacteriophages. Bacteriophage means 'bacteria eater' and these viruses lyse and destroy their bacterial host cells. Bacteriophages have a DNA genome surrounded by a protein shell. They inject their DNA into the host bacterium, which is then transcribed and translated to produce many copies of the bacteriophage genome and protein coat (Figure 6.25). These accumulate within the bacterium until the bacterium bursts and releases its contents. Some research efforts are focused on understanding whether bacteriophages (or phages for short) can be used as the basis of a therapy to kill bacterial pathogens that are resistant to multiple antibiotics.

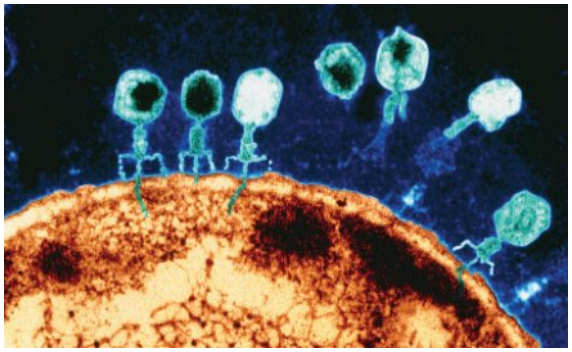

Figure 6.25 A coloured transmission electron micrograph of T-bacteriophage viruses attacking a bacterial cell of *Escherichia coli*. Seven virus particles are seen (blue), each with a head and a tail. Small blue strands of genetic material (DNA) are being injected into the bacterium.

Prions

A **prion** is a protein that has a different conformation (shape) from the normal conformation. The normal prion protein cellular form is denoted as PrP^{C}, and the disease-causing prion protein form such as scrapie is shown as PrP^{Sc}. When a PrP^{Sc} protein molecule encounters a normal PrP^{C} form, it causes a structural change that converts it to the harmful form. This now harmful prion can in turn convert other normal forms to harmful forms (Figure 6.26). When there are sufficient numbers of the harmful PrP^{Sc} form, they aggregate (clump together) to form filaments. These fibres kill brain cells (Figure 6.27), leaving holes in the brain tissue and affecting muscle coordination and brain function as a consequence.

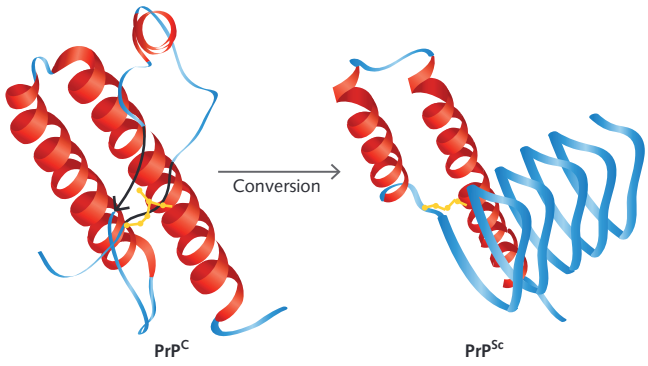

Figure 6.26 The process of converting a normal prion to an abnormal prion.

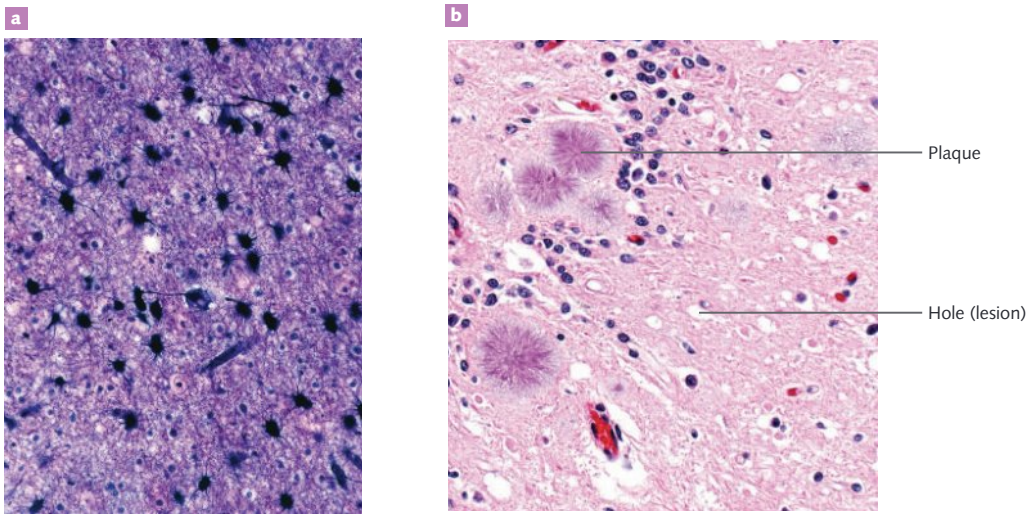

Figure 6.27 a Healthy brain tissue. **b** Brain tissue from a victim of Creutzfeldt–Jakob disease. Note the plaques, and the holes that give the brain tissue a spongy appearance.

Prions occur in different species. In cattle, prions cause mad cow disease, which is called bovine spongiform encephalopathy (Figure 6.28). In humans, a prion disease called Creutzfeldt–Jakob disease has been identified. Scrapie is a prion disease in sheep and goats. Prion diseases can be transmitted when infected flesh is consumed. The disease may remain dormant for many years before the symptoms appear.

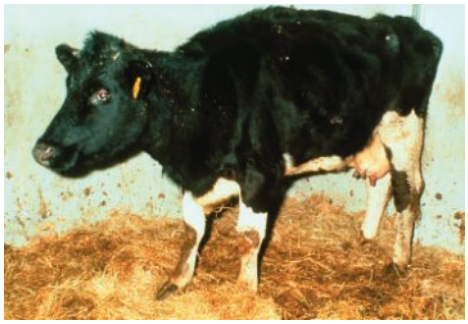

Figure 6.28 This cow is infected with bovine spongiform encephalitis (BSE), commonly known as mad cow disease, which is caused by a prion.

KEY CONCEPTS

- » iruses are n-cellular pa thogens which ae obligate parstes, because hey must inct a host cell to reproduce.
- » iruses consist of a ncleic acd DA or RNA, single or double stranded) surrounded a proein capsule. They use host machiner to eplicate.
- » Baceriophage are viral pathogens ecause they lyse and destroy therbacteral host cells.
- » Prions are inectiouson-celllar protein pathogens that cause ransmissble sponifomencephalopathies.
- » The pathogenc prionprotein form PrP ^{S} can convert the normacellular form (PrP ^{C} found n healthybrain tissue) to the PrP^{S} form that causes neurdegenerative dsea e.

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Concept questions 6.4b

1 Define oligate asite'.
2 Jusifythis stet: 'All viruss are pathogens'.
3 Virues infct nly speci fic hostellsExplin how this specificity come about.
4 Ouline thesteps involved for a vius to reproduce.
5 Research n the Internet to find four dseases that are lassi fied as tanmissible spongiform encephlopathies (TSEs).

HOT Chaenge

6 Reseachint Azhimer's disease has demonstrated that it may bea dobl prio disorder. Two proteins centrl to the ptholg o Alzheimer's disease act aspros.It is t believdtha Alzheimer's is nfectious by any common de fintion of th tem. Prion dseases seem to be transmitted to humans through contaminatd anmal sample such as blood, meat or nervoustise. nfeciou prion diseases are more commonin non-human secies.
   a Ouline hw alteed prin poein in infectious prion iseases such asCreutzfedt–Jakob disease affect t brain.
   b Alzhemer's disease ca begnetic or idiopathic (of unknown cause) i rigin but has not been shown to be infectius. Given he crrent issues with cross-species onamination of patogens not normally nfectious to hum, list five measures you think shoud be llowed wen eatng aimal tissues.

6.5 Allergens

WB 6.5 ALLERGENS PAGE 142

Some people's immune systems are excessively sensitive to certain substances, allergens, that cause allergic responses such as asthma, hay fever and anaphylaxis. Allergic reactions are rapid and most occur when IgE antibodies are produced. These antibodies bind to receptors on mast cells. The antibodies can bind specifically to antigens that are normally harmless. These antigens may be pollens, air pollutants, house dust, animal fur, fungal spores, cosmetics or other substances, which are known collectively as allergens. When the allergen–antibody complex is formed, the antibody attached to a receptor on a mast cell causes the mast cell to release its granules full of histamine, cytokines and other inflammatory molecules. This process is called **degranulation** (Figure 6.29). The abnormal amounts of histamine cause such effects as swollen bronchioles in lungs. The incidence of asthma and hay fever is around 15% in Australians and is increasing. These disorders are forms of allergies – exaggerated innate or adaptive immune system responses to usually harmless antigens.

These molecules bring immune cells to the affected area, causing localised inflammation. Histamine acts on smooth muscle to cause tightening of the airways, and symptoms also include excessive mucus production in the affected area. The inflammatory response is traditionally viewed as an innate immune system response.

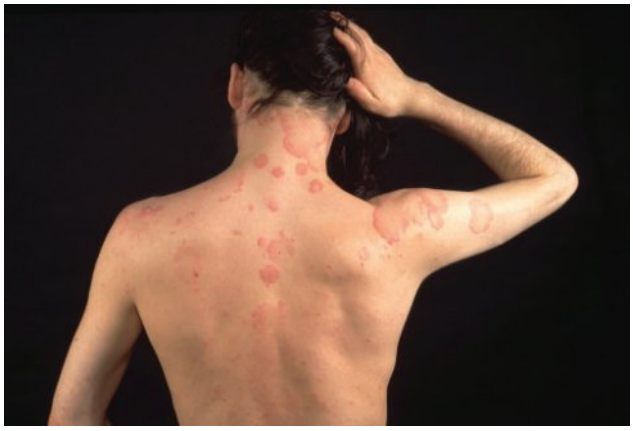

Figure 6.29 Mast cell response to allergen

Allergies begin with a **sensitisation** stage, in which IgE antibodies start being produced. These antibodies arise from an adaptive immune response, involving allergen phagocytosis and presentation to B lymphocytes and T_H cells in a secondary lymphoid organ – in a lymph node. Most allergens are not blood-borne and would therefore not cause a response in the spleen. It is not known why allergens cause an adaptive response in some people and not others, but it seems partly genetically determined and partly environmentally determined. For example, repeated exposure to the allergen can increase the risk of sensitisation.

Allergies can sometimes be treated by **desensitisation**, in which the affected individual is repeatedly exposed (usually by injection) to small doses of the allergen. This brings about a state of **immune tolerance**, stopping or reducing the production of antibodies to the allergen.

In most cases, allergies are simply annoying. However, they can be life-threatening if they result in **anaphylactic shock**. This is when inflammatory responses race through the body, leading to constriction of the airways and loss of fluid into body tissues from leaky capillaries. The loss of fluid into body tissues is due to high levels of histamines and results in a sudden drop in blood pressure, which may lead to a heart attack. Victims of anaphylactic shock need medical treatment urgently to counteract this exaggerated immune response.

6.6 Phagocytosis

One of the key actions of inflammation is to destroy invading pathogens before they can establish an infection.

Macrophages and neutrophils carry out phagocytosis in the same way that an amoeba engulfs food particles (Figure 6.30).

Figure 6.30 A scanning electron micrograph of macrophages with cytoplasmic extensions. These extensions engulf foreign particles.

Macrophages and neutrophils are stimulated into action when receptors on their cell surfaces detect either bacterial products or signalling molecules such as complement proteins or cytokines. On their cell surfaces there are also receptors that bind to receptors found on pathogens. The macrophages and neutrophils are attracted to the site of infection and move out of the capillaries and migrate towards the damaged cells. They are attracted by the increased concentration of the chemicals that triggered their activation.

The attracted cells then attach to the pathogen-infected cells in one of two ways.

» Unenhanced attachment is associated with the innate response. The macrophage's receptor binds to the receptor on the surface of the infected cells or pathogens.

» Enhanced attachment occurs between the receptors and specific immunoglobulins (IgG) that are on the surface of the pathogen-infected cells, opsonising them, and takes place as part of the adaptive immune response (p. 260).

The cellular response for both macrophages and neutrophils that follows is now similar. During phagocytosis, the pathogen is engulfed and destroyed within a membrane-bound vesicle called a **phagosome**. A lysosome fuses with the phagosome to form a **phagolysosome**, which becomes increasingly acidic, as seen in Figure 6.31. An array of digestive enzymes and antimicrobial compounds, often including a burst of highly reactive oxygen molecules, helps to destroy the invader.

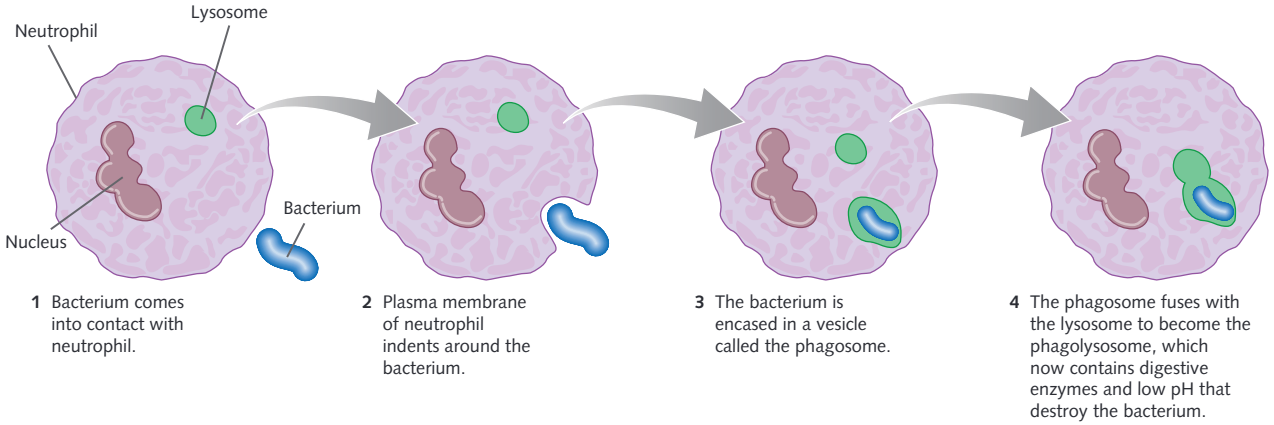

Figure 6.31 Phagocytosis and lysosomal degradation of a bacterium within a neutrophil

KEY CONCEPTS

» Anlergicreactio occurs in rspone to an allergen.

» Anlleren is a nrmaly hamless antigen that produces gE in larequntties, ausing an adaptive mmune respone.

» Phagocytosi(internalisatin)f a pathogen is followed by fsion of the phagosom to he lysosome. The resting phagolysosome contains digestive enzymes and an cidic environment to break downthe pathogen.

Concept questions 6.5/6.6

1 Describe the role f phagocytic leukocytes in the mmune sysem.

2 Arrange te olloingpoins irder, to illustrate the sequence of eventsthat would occur when a macrophage encounters a bcerium.
- » Lysosome fses wth vacuole.
- » Macrophage recognisebacterial srface molecules as non-slf.
- » Poweful enzymes diest bacterium.
- » Vacole forms aroundbaterium.
- » Macrophage nvelop bacterium ith its plasma membrne.

3 Whatcels relas histaminduring an allergic response?

4 What hapens ian allergc response in the immune system?

5 **a** Arallergies the sae as anaphylaxis?
b Many epleit allergiescanbe desensitised.
i What dos this mean?
ii Can pople withaaphylaxs be desensitised?

HOT Chaenge

6 Are llegie a sign ofa weak immue system? Discuss.

BRANCHING OUT

The war against bacterial infections

Penlin isa idely usedant biotic. It s a chemial that blcks th action of an enzyme called glycopeptide transpeptdase. Mny bacteria needhienzme to build cell wall s. Peilin ireversibly bids t the ative site of this enzyme so it cannotfunctn ad cell alls ca no longer be bui t. Bacteria equire cel wall to stop the from bursting. Without te abily to ild cell wall the bact eria sto iiding and the nfectin is eadicted.

Any baceria that use the enzye glycoeptide rapeide to bul cell wall will be sensitive to the action of penillin. Bacteri tha don't use glycopeptide transpeptidase are not suscetible topiilin.Scientsts have discovered antiiotics t trat infections caused bthese non-susceptible stans. There are now hundredsofantbotics available to trea infectionsby differen acteria.

Baceria reprodue aexually by binary fissi. If conditions re right, one bcteriangive rise to a billion cloned baceia in just 10 hours All sential genes codng for the proteins reqired for srival are fund na large circular chromosome int nucleid.Any mutations aring during chromsme replication are passed on to all clones. Other non-esntial genes are crid on small circular pieces of DNA ale plasmids. Tse non-essential genes often confer exta, ver useu fuction, including antibiotic resistance.

Sometime baceria from diffren species swap plasmd throug the process of conjugation. One bacterium bilds a tbe-like srucure called apilus that transfers pl asmids to a secon acterium.Throug mutations and plasmid tranfer bacteriaare ntatly evoling, acquiring new trats s they can survive in new environments.

The use of tibiics aplies an environmenta pressure to baceria populations. Those that survive because they contain a gene or ntbiotic resistance pass on the gene totheir clones when theydvide, ad heir clones are then alsoresstat. Several echanisms have evoed in acteria to provide ntibiotic resistance. Some make the antibiotic nacive by canging its hape or prpertiesthrs remove it from the cell, while others change the target site so the antiiotic can o lnge bind. This evolutionary process has re sulte in many acteria developing resistance to the action of antiiotics.

Staphylococcus aureus commonly knownas goldn stah, is a spciesof bacterium tat lives on our skin and in our nos. It isenerally ha less. However, if it entes ourbody through punctures in the skin, it can cause a range of nfections. Sm ar mild infetions sch s boils an schoolsores. Oters are severe, such as toxic shock syndrome, meniitis(brain) steoyelitis (bne, pnuoni (lungs),septic phlitis (veins) and endocarditis (heart valves). If the nfectiongets ino the bloodstream, it can spread to othe r orgns, ausing evre infectiosknownas sepsis. Sepsis can ead to ultiplergan falure and death.

At the peak f atibiotic dscovery and dveopment 15–20 new antibiotics were released onto the market every 10 years. As o June 019, approxmtly 2 new antibiotics wth thepotental to teat serious bacterial infections were in cnicaldeveopment. The succe rate for clini cl drugdevelopmnt s low;only one in five infectius disease drugs that enter the human tsting phase are apprved for patients.

Some companiesare turning awy from the eelopmentof new aiboics for various reasons, including:

- thehigh costo developing and testing a new drug
- thlmid ifetime of he rug if rsistance develops
- doctors ae startng oprscribe anibiotics more sparingly
- sales ma e limited cause antibitics are only pescribed for a sot duration to overcome the bacterial nfetion.

Questions

1 Explain how the antibiotic penicillin works at a cellular level.

2 **a** Figure 6.32a shows the *Staphylococcus aureus* sensitivity test. The grey-green background of the plate is a 'lawn' of densely growing bacterial cells. Discs soaked in antibiotics are placed on the lawn and rings of bacterial inhibition or death around the discs can give a measure of the susceptibility or resistance of the bacterial strain to the antibiotics. Use Figure 6.32a to decide which antibiotic(s) would be effective against *Staphylococcus aureus*.

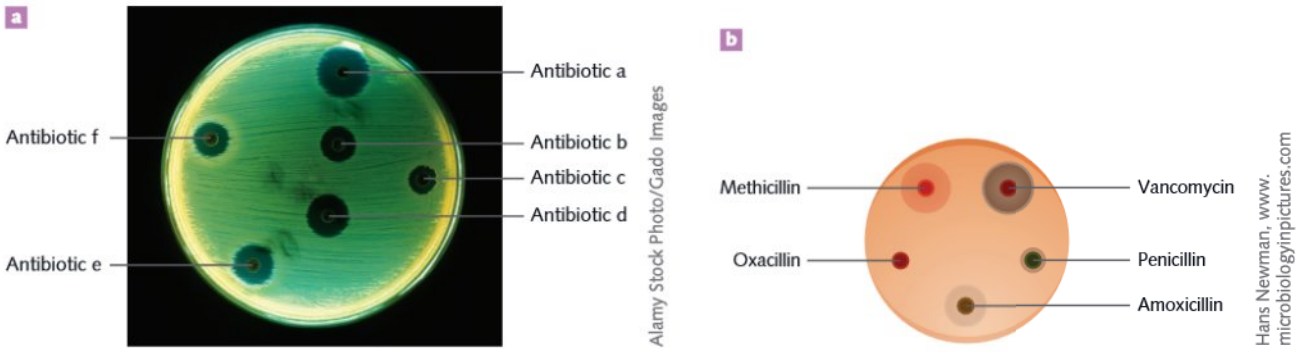

Figure 6.32 a *Staphylococcus aureus* sensitivity and b test for patient X

b A patient in a hospital, patient X, had a catheter inserted into his arm to deliver intravenous fluids. A golden staph infection emerged at the puncture site and the patient was given methicillin to treat the infection. Over time, the infection became worse and patient X's doctor realised she was dealing with a methicillin-resistant strain of *Staphylococcus aureus* (MRSA). She ordered an antibiotic sensitivity test to determine the best antibiotic to treat this MRSA infection. The results of this test are shown in Figure 6.32b. Use Figure 6.32b to decide which antibiotic would be most effective against MRSA for treatment of patient X.

3 Many companies are no longer developing new antibiotics. Antibiotics are complex to develop and new products cannot be sold freely on the open market. They instead must be stockpiled and kept in reserve as a 'drug of last resort'. This is not appealing to potential investors who are looking for a good financial return on their invested money.

a Identify the bioethical issue in this situation.

b Identify the perspectives of the drug company, an investor and a member of the public.

c What is your position on this situation? Develop a course of action that you would like to see undertaken and write a fully reasoned argument for its adoption.

Online key concepts
Chapter 6: Summary of key concepts

6 Summary of key concepts

6.1 Physical, chemical and microbiota barriers in animals – first line of defence

KEY CONCEPTS

p204

- Strucral,chemil ad biological features can act as barriers to pathogens as a firsline of defnce.
- The ki is a tough hsicl barrier made from kerinisd thlial cells that can prevent the entry of pathogns.
- When the arrier o he ski i brken,patelets quickly form apug, o scab,that upolds he barrier until the skn s repaird.
- Mucus traps pathogens that invade mucous membranes, d cilia beat themucus to a place where s can be pled. Urine and tears flush out micro-organisms an pistalsis keeps them moving through the gut.
- Chmcal barriers that preven pathogen colonisation ncludelow (idic) H onskin and inthe stomach, dgestive enzymes poduced n the gut, and lysozyme n tears, aliva and mucus.
- Sybotic rganisms form or mcrobiota, which take up space and use utriens. This reens colonisation by pathgens.

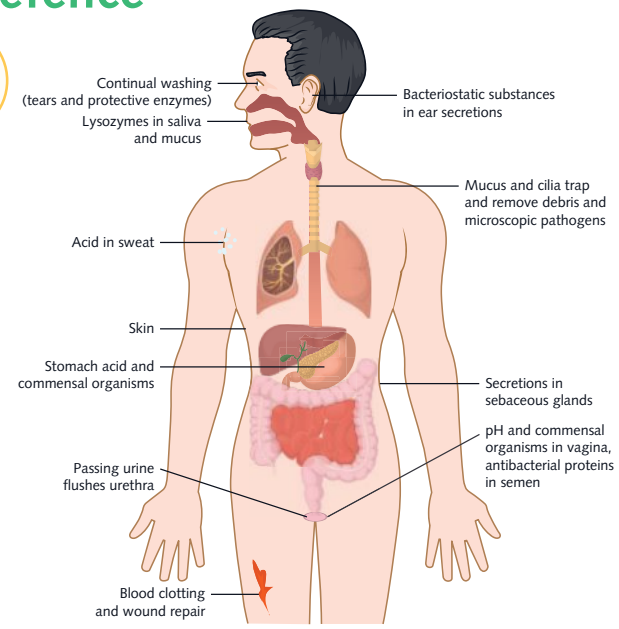

Figure 6.6 A summary of the physical and chemical barriers to pathogenic infections in a human. Peristalsis also helps by moving food and infectious agents through the digestive system.

6.2 Physical and chemical defences in plants

KEY CONCEPTS

p211

- lants have pysica nd hemical barriers to preventinvasion by pahoges These include thick bark, waxy cticles, hais andproduction of molecules such as dfensins that are toxic o pathogens.
- lants use PRRs (pattern re cogiion receptors) to detect PAMPs (pathoge-asscated molecular patterns) on nvding pathogens. They mun a raid innate immune response o invasion by pthogens.
- After ivasion by apathogen, plants attain a state of sysemicacquired resistance that makes them more prepared to fight nvsion by a broad range ofpathogens.

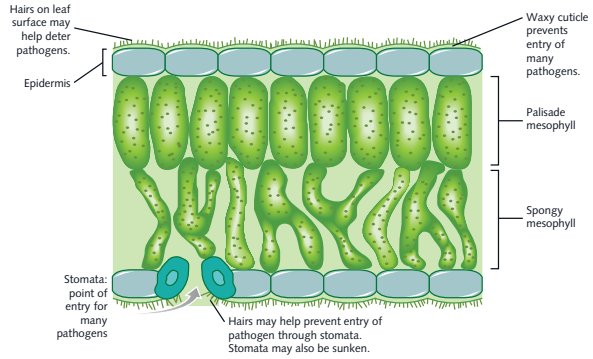

Figure 6.9 A cross-section of a typical dicotyledon leaf showing some barriers to pathogens found in plants

6.3 Innate immune response in animals– second line of defence

KEY CONCEPTS

p214

- »Innae immune responses are on-speci fic and are nborn features of the body.
- » Leukocyts whitelood cell) mediate the innate mmune response by engul fing and digestig foreign pathoens.
- »Immune lls such as ma crophages egi the immune response afte their recepto r stes in withforeign antiens.
- » Neutrhils are the type of phagocyte that are abundant n blood. Th redily migratrom caillaries to sites of nfecton, where they phagocytose pathogens and then slf-destruct to stop pthogen spread.
- » The complement sysem consists ofa number of small mlecues in he blood that hv important roles in inflammtion, ncluding osonisatin and deposition of the membrane attck comlex (MAC).
- »Interferons are produced by virusinfected cells and alert neighbourig cells to the dnger, cusing changes in gene expresson that make themmore ressant to viral nfection anreplcation.
- » llplants and anmals ount innate immune responses to pathogns.
- » Inflammton is chracterisedby pain, heat, redness and swl ng. In flammton is the key weaon of the innate mmune respone, detoyig invading pathogens before they can etabish n infection.
- » Chemotxis is the procs of cllular migration towards reions o higher concntrationsof chemokines. Each cytkine has a peci fic receptor tht is expressed by partcuar leukocte types.
- » Inflammto sinitiated when acrophges, dendritic cels or other lls detec antigenor signaling molecules and begin to produc cytokinsthat ring in and activate otherimmune cls.
- » Pain reduces voluntary movement toassist the repair proces.
- »Immune ll recruitmnt to sites of in flammton is assisted by hstamine, which is sereted by mast cells and ncreases the prmeabityof capillaries.
- » The reslutin of in flammton isa ighly active process that is necessry to limi ad repair tissue damage caused by te in flammatory response.
- » Th innate and dative immune rsponses rely on being ale to llself from on-self.

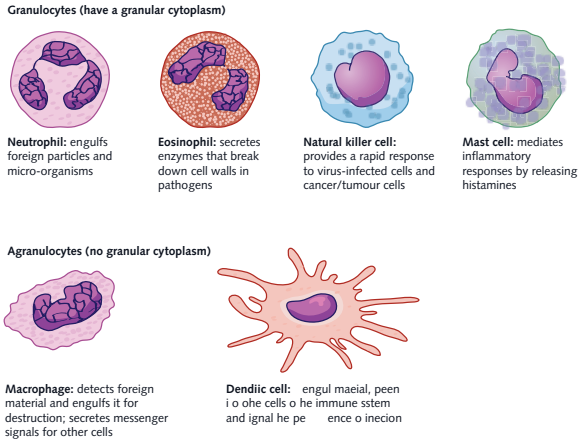

Figure 6.12 Dfferent types of hiteblood lls (eukocytes)

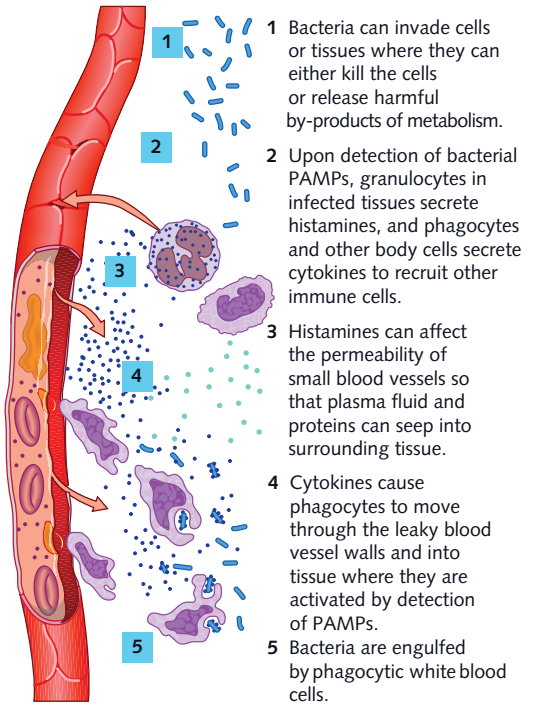

Figure 6.16 The steps that occur in acute inflammation after invasion by a bacterial pathogen

6.4 Antigens and pathogens

KEY CONCEPTS

p231

- Pathogens aecellular onn-elluar infectious agents that cause dsea e.
- Atigens re inracellular xtracellular copnents of proteins, pepid,lipids r polyaccharies presen in pathogens.
- Baceria are prokaryotes; some are bene ficial totheir hosts and some are pathognic.
- Baceria produce enotoxins orexotoxins that can also cause disease.
- Fugi are euarytic organis thatare usually external pathogens that reproduce and spread through thir spores.
- Proists arencellular ekarytes, a smal number of which are sgi ficant pathogens of hmans.Exampes of protist pathogens are *Giardia* amoebas, *Trypanosoma* and *Plasmodium* spcies.
- iruses areo-cellularpathogens,hich ar obligate parasites, because they must infect a hst cell toreproduce.
- iruses consist of a ncleic acid (DNA or RNA ingle o double stranded) surrounded by a potei apsule. They se hostachnery to replicate.
- Baceriophage are viral pathogens ecause they lse and destroy their bactrial hoscells.
- Prions are ifectiounn-celular protein pathogens that cause trasmisible spongifrm ncphalopathies.
- The pathogenc prionprotein form PrP ^{S} can convert the nrmal cellular form (PrP ^{C} found n healthy brain issue) to the PrP ^{S} form that causes neurdeenerative disease.

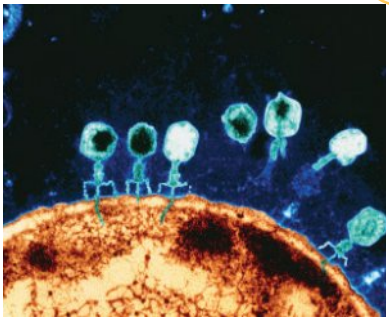

Figure 6.29 A coloured transmission electron micrograph of T-bacteriophage viruses attacking a bacterial cell of *Escherichia coli*. Seven virus particles are seen (blue), each with a head and a tail. Small blue strands of genetic material (DNA) are being injected into the bacterium.

6.5 Allergens & 6.6 Phagocytosis

KEY CONCEPTS

p232

- Anlergicreactio occurs in rspone to an allergen.
- Anlleren is a nrmaly hamless antigen that produces gE in larequntties, ausing an adaptive mmune respone.
- Phagocyosis is an example of a sgnal transduction pathwayivolvg a cel rcetor, sgnal transduction and lular resnse, in this cse to engulf the targetedforeig terial.
- Phagocytsis (ternalisation) f a pathogen is folowed b fusion of the phagosome to the ysosme. Th esultig phagolsosome contains dgestive enzymes and an acidic environment to break down the pathogen.

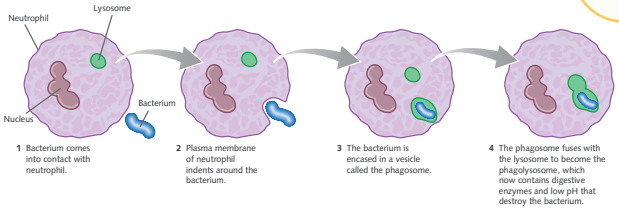

Figure 6.31 Phagocytosis and lysosomal degradation of a bacterium within a neutrophil

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WB
6.6.1
KEY TERMS –
SPOT THE ERRORS
PAGE 144

6 Chapter glossary

adaptive immune response an immune response directed against a specific antigen; it retains memory of that antigen so that, on subsequent exposure to the same antigen, it responds with a secondary response

allergen an antigen that is normally innocuous but can sometimes cause an over-reaction from the immune system known as an allergy

allergy an immune response characterised by IgE production to an innocuous substance

anaphylactic shock a severe allergic reaction that causes widespread swellig, including of the face and neck, which can lead to difficulty breathing and a life-threatening reaction

antibiotic a naturally or synthetically produced compound that is toxic to bacteria

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cells by various enzymes, including caspases

bacteria unicellular prokaryotes that can be pathogenic and therefore carry disease

bacterial capsule a polysaccharide layer surrounding some bacteria that makes them resistant to phagocytosis and thus more virulent

cellular pathogen a disease-causing pathogen that is made up of one or more living cells such as bacteria or fungi

chemokine a type of cytokine that induces chemotaxis

chemotaxis the movement of an organism or a cell along a chemical concentration gradient either towards or away from a chemokine

cilia slender hair-like structures projecting from a cell surface that beat against fluid

companion plant a plant that is grown with another plant because one species improves the growth of the other

complement a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

cytokine a signalling molecule that coordinates inflammation and immune responses and that leukocytes use to communicate with one another; includes interleukins and interferons

defensin a type of small antimicrobial peptide secreted by nearly all plants and animals

degranulation a cellular process in which the granules of neutrophils, mast cells, basophils or eosinophils are emptied into extracellular surroundings

dendritic cell a phagocyte with membranous extensions that engulf pathogens, process them and present them to other cells of the immune system

desensitisation a treatment to make a person more tolerant to a substance to which they are allergic

disease any condition that interferes with how an organism, or any part of it, functions

eosinophil a leukocyte that secretes powerful enzymes capable of rupturing multicellular organisms

first line of defence physical and chemical barriers that keep pathogens from entering the body of a living thing

flagellum a helical filament that rotates to give bacteria locomotion

fungus a heterotropic organism made up of one or many cells; has cell walls but is not a plant

granulocyte a white blood cell that has granules in the cytoplasm

histamine a chemical released by mast cells and basophils that increases blood flow and the permeability of capillaries

host the organism in which a parasite lives

immune system a complex network of cells, tissues and organs in the body that detect differences between self-molecules and foreign (non-self) organisms, and mounts an immune response that results in the formation of memory lymphocytes

immune tolerance tolerance of the presence of an antigen by the immune system so it does not mount an immune response to the antigen

infectious disease a disorder caused by bacteria, viruses, fungi and other organisms, that can often be transmitted to other members of a population

inflammation an innate response to infection or damage that causes pain, swelling, heat and redness

innate immune response a response to a pathogen that is not specific to the antigen, only that it has been identified as being non-self; the response does not generate antibodies or memory lymphocytes

interferon a type of cytokine produced by the cells of the immune system in response to challenges by foreign agents such as viruses, bacteria, parasites and tumour cells

keratin the tough, fibrous protein of the outer epidermis layer

leukocyte the general term for a white blood cell

lymphocyte a type of leukocyte involved in adaptive immune responses

lysis the process of a cell bursting

lysozyme an antibacterial enzyme found in tears, saliva and other body fluids

macrophage a large white blood cell that phagocytoses pathogens; originates as monocytes in circulation

mast cell a cell that is located in the tissues and releases granules containing histamines when activated

microbiome the bacteria, viruses and fungi that live in the gut plus their released metabolites and nucleic acids that exist in a specific environment

microbiota a community of micro-organisms, including fungi and bacteria, that live in or on another organism

microflora *see* microbiota

monocyte a white blood cell that circulates in the blood and matures into a macrophage when it moves from the blood into the tissues

mucous membrane a mucus-secreting membrane that lines the respiratory, excretory and reproductive tracts

natural killer cell a circulating leukocyte that kills body cells infected with a virus or transformed by cancer

necrosis cell death that results from tissue damage or infection when the plasma membrane is breached; results in inflammation

neutrophil a phagocytic leukocyte found in the blood and tissues

non-cellular pathogen a disease-causing pathogen that is not made of living cells; for example, viruses and prions

non-self antigen a molecule that is not recognised by the immune system as being part of the organism itself

non-specific response a response that is the same regardless of the type of antigen

obligate parasite a parasite that cannot complete its life cycle without a suitable host; without a host, the parasite cannot reproduce

opsonisation the process in which a pathogen is coated with antibodies and/or complement and marked for phagocytosis

pathogen an organism foreign to the body and capable of causing disease

phagocyte a cell that is capable of phagocytosis; includes macrophages, dendritic cells and neutrophils

phagocytosis a process by which phagocytes engulf a particle or cell

phagolysosome a membrane-bound vesicle formed from the fusion of a phagosome and lysosome

phagosome a membrane-bound vesicle formed around a particle during phagocytosis

platelet a cell fragment found in the blood involved in blood clotting

prion an infectious protein that can cause other unaffected prion proteins in the brain to take the affected form, causing transmissible spongiform encephalopathies

protist a unicellular eukaryotic organism

second line of defence non-specific immune responses including fever and inflammation

secondary metabolite an organic compound produced by bacteria, fungi or plants; its role is to sustain functional and homeostatic health of cells within organs by assisting cells to excrete wastes and toxic substances

self-antigen an antigen or a molecule that is a normal body component

sensitisation initial exposure to an allergen resulting in an adaptive immune response that generates IgE

sterile inflammation inflammation resulting from the detection of damage- or danger-associated molecular patterns released during tissue injury in the absence of infection

transmitted when an infection is passed from one person or organism to another

vasodilation widening of blood vessels, particularly arterioles

virus an obligate intracellular pathogen that can use the host cell's machinery to replicate itself; usually consists of a nucleic acid surrounded by a protein coat

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WB
6.6.2
EXAM MARKING
PAGE 146

6 Chapter review

Remembering

1 State two important differences between a bacterium and a virus. Give two examples of diseases that are caused by each of these pathogens.
2 State two diseases caused by fungi, and two diseases caused by protists.
3 Are viruses living organisms? If not, why not?
4 Identify the type of change (physical or chemical) that PRRs detect in host tissues after a pathogen has entered.
5 Outline one role of the bone marrow in the defence system.
6 Outline the advantage of keratinisation of skin cells.

Understanding

7 Describe the unique feature of a prion that distinguishes it from other non-cellular infectious agents.
8 Describe two changes to the structure of prion proteins that lead to Creutzfeldt–Jakob disease.
9 *Staphylococcus aureus* causes food poisoning by releasing a heat-stable toxin. Describe the effect of reheating food on the potential of this pathogen to cause food poisoning.
10 Defensins are peptides, so their synthesis requires significant amounts of nitrogen compounds, which are generally a scarce resource for plants. Yet, up to one-tenth of some seeds are defensins. Suggest an important advantage to a plant of putting very large quantities of such an 'expensive' chemical into their seeds.
11 Describe the events that follow the activation of complement.
12 Explain what is meant when we say the body can discriminate between 'self' and 'non-self'.

Applying

13 Eating diseased tissue that contains abnormal prion proteins can cause the brain to become infected. Predict a property you would expect prions to have, given that they manage to enter the bloodstream without being digested. Provide evidence to support your answer.
14 Cigarette smoke decreases ciliary beat frequency and reduces the number of ciliated cells in the airway epithelium. Predict the effect of smoking on the body's defences.
15 Both an infection in your foot and a sprained ankle cause the local area to swell, become red and painful, throb and feel hot. Explain why these two different events lead to the same response by the body.

Analysing

16 Figure 6.33 shows infection by a fungus responsible for rust in wheat and rye.

- **a** Identify the part of the plant the fungus probably gains access through.
- **b** Describe the damage the fungus causes to its host.
- **c** Predict, with reasons, whether antibiotics would be useful in controlling its spread. Design a controlled experiment to test your hypothesis.
- **d** Describe two methods that could be used to control the fungus.

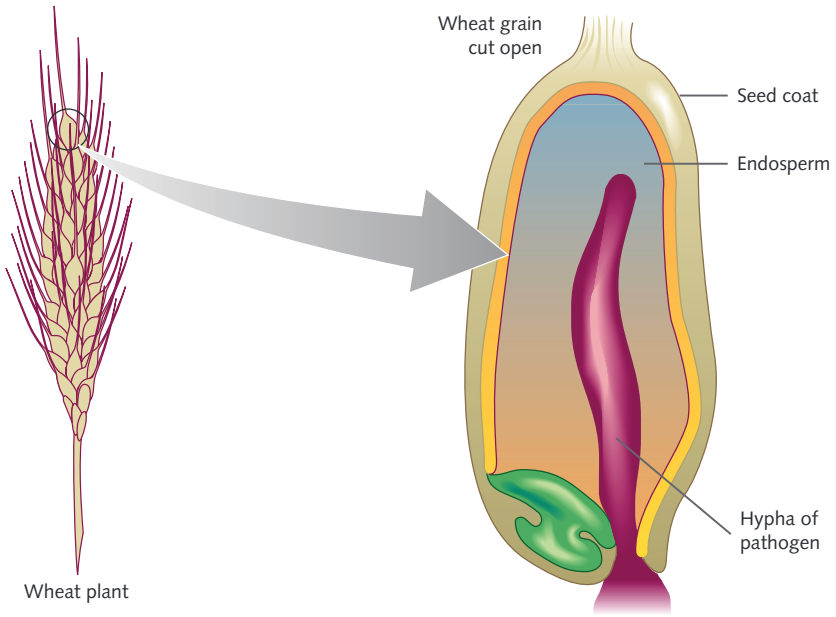

Figure 6.33 A wheat plant and magnified cut wheat grain, showing infection by a pathogen

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17 Figure 6.34 shows the number of cattle infected with the prion causing BSE (also known as mad cow disease) in the UK for the years 1985–2000. Since 1992, feedstuff containing animal neurological tissue, such as brain and spinal cord, has been banned.

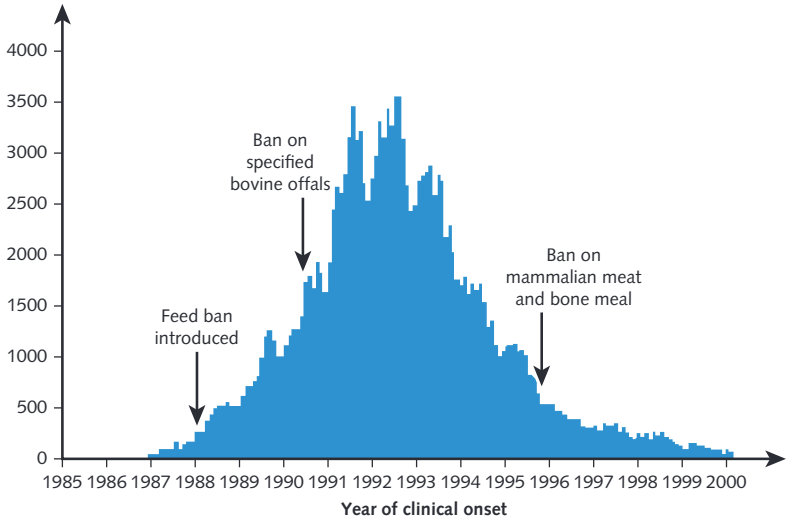

Figure 6.34 The number of cattle infected with BSE from 1985 to 2000

- **a** Describe the trend in numbers of BSE-infected cattle in the UK from 1985 to 1995.
- **b** Describe the action of a prion when it causes disease.
- **c** Suggest a reason for the decline in the incidence of BSE since 1994.
- **d** There are fears that the infectious agent causing BSE is now infecting humans, causing Creutzfeldt–Jakob disease. Describe measures that could reduce the transmission of this disease.

18 Consider the stages in the replication of a virus. Imagine you are a chemist trying to find antiviral medicines. Describe two points at which a virus would be susceptible to antiviral chemical therapies.

Evaluating

19 Given the increase in antibiotic resistance in recent years, discuss whether we should restrict the use of antibiotics to only those people with a life-threatening illness.

Creating

20 It has been said that we underestimate the effectiveness of our innate immune system because we do not usually become aware of the potential infections that it prevents. Design an investigation using mice and an immunosuppressant to test this idea.

21 When macrophages engulf bacteria, the phagosomes fuse with lysosomes to form phagolysosomes, where the bacteria are killed and broken down. One sample, the control, contains normal bacteria. The two other samples contain bacteria that can evade immune destruction, one by surviving inside the phagosome and the other by escaping into the cytoplasm from the phagosome.

Design an investigation to distinguish between these three samples, ensuring that you describe the results that you would expect from each sample of bacteria. If you use macrophages with red fluorescent labelled phagolysosomes and bacteria with green fluorescent proteins in their cytoplasm, you can see:

- disappearance of green bacteria as they are digested in the phagolysosome
- green bacteria in the cytoplasm when they escape the phagolysosome
- green bacteria remaining in the red fluorescently labelled phagolysosomes.

22 Prepare a flow chart to summarise the steps involved in inflammation.

Reflecting

23 Consider the observation that plant defensins have been shown to inhibit the growth of human cancer cells. Reflect upon why this might be.

Acquiring immunity

7

By the end of this chapter you will have covered the following material.

Key knowledge

Acquiring immunity

- the role of the lymphatic system in the immune response as a transport network and the role of lymph nodes as sites for antigen recognition by T and B lymphocytes pp. 249–253
- the characteristics and roles of the components of the adaptive immune response against both extracellular and intracellular threats, including the actions of B lymphocytes and their antibodies, helper T and cytotoxic T cells pp. 253–268
- the difference between natural and artificial immunity and active and passive strategies for acquiring immunity pp. 270–272

Key science skills

Analyse, evaluate and communicate scientific ideas

- discuss relevant biological information, ideas, concepts, theories and models and the connections between them pp. 268–269

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- Chapter 7 map (p. 246)

wutoue Key Oehb C:
- Chapter 7 flashcards (p. 248)

WeptoukC:
- The immune system explained (p. 249)
- The lymphatic system as part of the body's defence (p. 250)
- Types of immune responses (p. 254)
- Different levels of defence (p. 260)

wutoue WnhkCveecC:
- The lymphatic system's role in immunity (p. 250)
- Types of immune responses (p. 254)

Vœlen:
- The T and B cells of the immune system (p. 254)

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- Chapter 7: Summary of key concepts (p. 274)

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Chapter 7 map

7 Acquiring immunity

If you had chicken pox when you were young, you probably won't get it again because your third line of defence produces memory cells that remember how to fight a specific disease.

p 249

71 Adaptve mmune response – thrd ne of defence

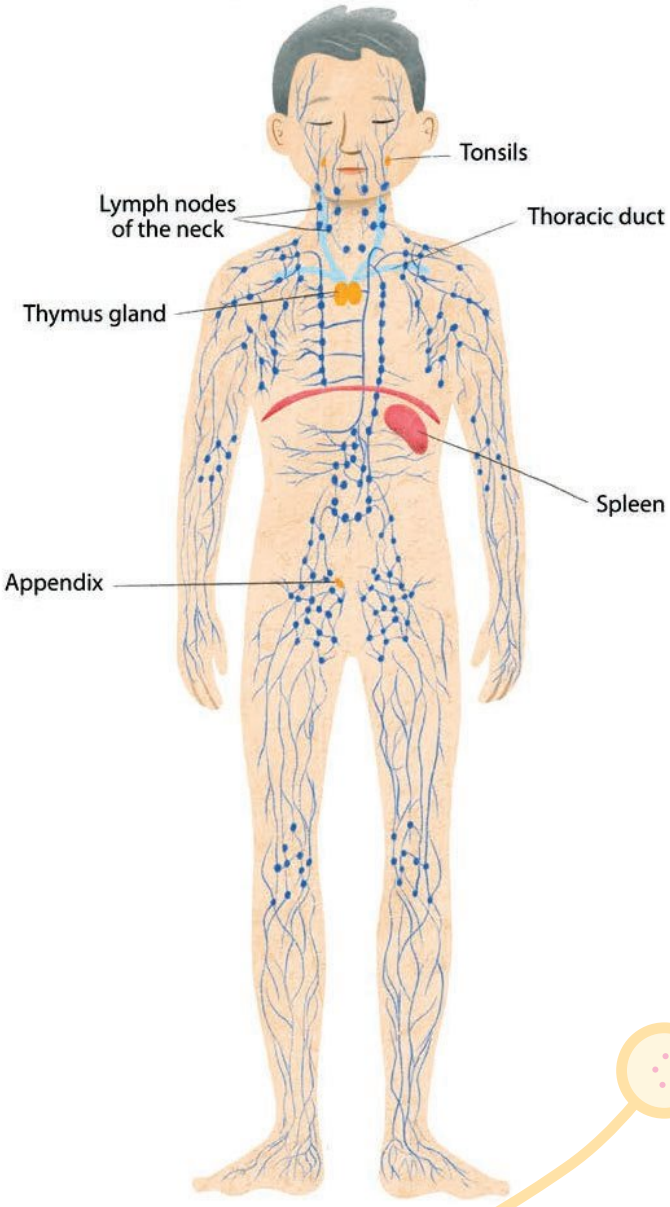
Your boy's immune resonse is sophstcated B and T ces are the key payers n the thrdine of defenc. B ces produce aniboies and T cls brng about potent antvra and anttumour responss. B and T lls can aso become memory ces that can rapdy mobse to protect aginst rinfetin.

p 260

72 Humora mmunty

Antbodes are Y-shaped moecues bound to the surface of B ces and secretedinto crclaio. They spread through the body n the boodstream and attack pathogens T heper ces can respond to the same anige, and actvate B ces

The lymph and immune systems



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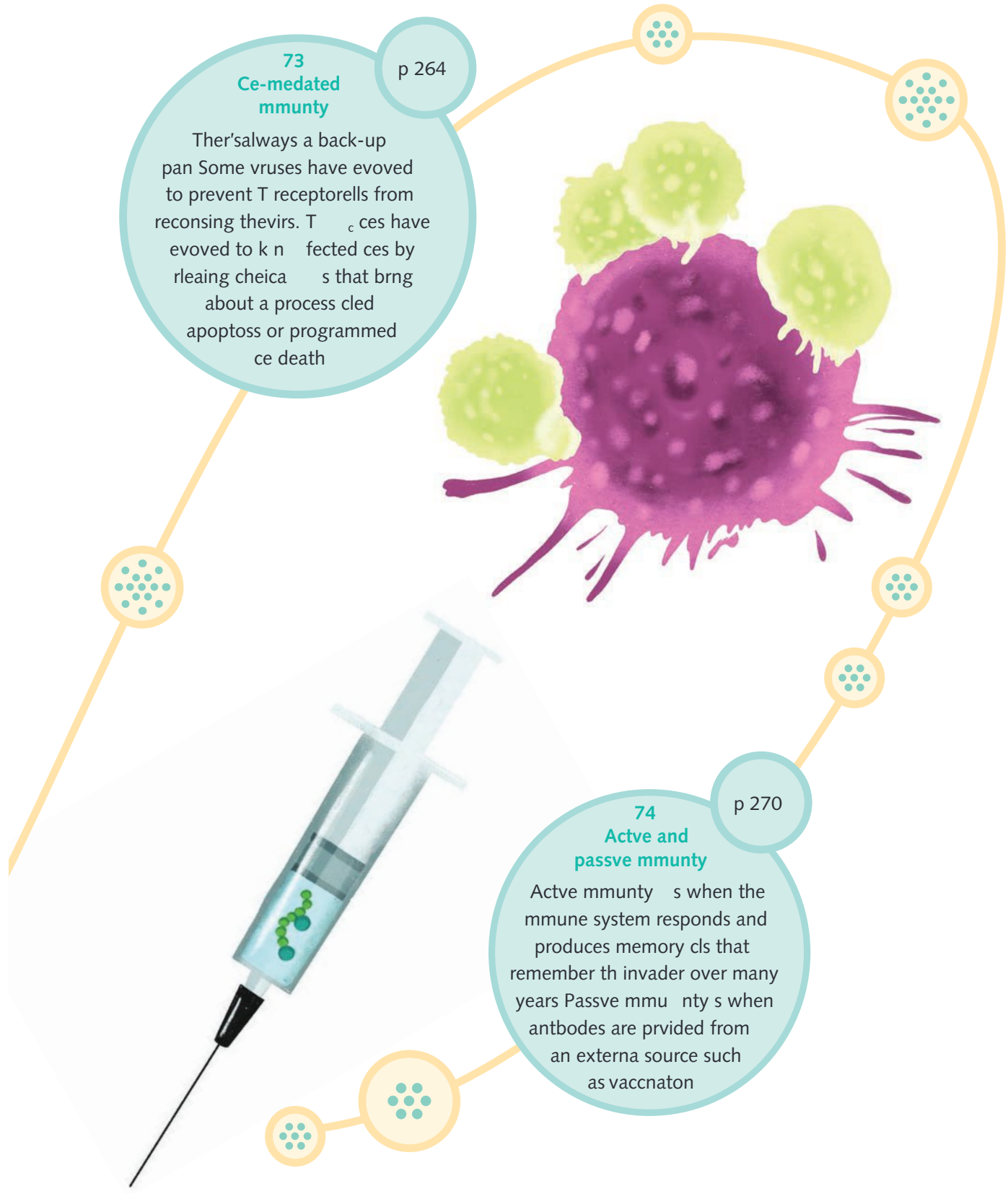

Your body is adapted to combat pathogens that it may encounter. Science has also produced ways to assist your body to defend itself.

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Chapter 7 Flashcards

Know your key terms

active immunity
agglutination
antibody
antigen-presenting cell (APC)
autoimmune disease
B cell
B cell receptor
B plasma cell
bone marrow
cell-mediated immunity
clonal selection
cytotoxic T cell (T_C cell)
epitope
helper T cell (T_H cell)
humoral immune response
immune
immunoglobulin (Ig)
interleukin
interstitial fluid
lymph
lymph node
lymphatic system
major histocompatability complex (MHC)
memory cell
MHC restriction
mucosal-associated lymphoid tissue (MALT)
neutralisation
passive immunity
primary lymphoid organ
primary response
regulatory T cell (T_{reg} cell)
secondary lymphoid organ
secondary response
self-tolerance
specific response
spleen
T cell receptor
thymus

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

WB
REMEMBER
PAGE 148

1 The innate immune response is non-specific.
2 PAMPs are pathogen-associated molecular patterns on the surface of pathogens.
3 DAMPs are damage- or danger-associated molecular patterns that are released from damaged or dying cells.
4 PRRs are pattern recognition receptors on cells of the innate immune system (e.g. macrophages) that recognise PAMPs and DAMPs.

In Chapter 6 you learned about the innate immune response. In this chapter you will learn about other components of the immune system, which work together to bring about the adaptive immune response. These are **specific responses** that target non-self antigens and pathogens when they have been detected by particular components of the immune system. Once the infection is over, some of the cells of the adaptive immune response (third line of defence) remain as specific **memory cells**. If you are exposed to the same non-self antigen or pathogen again, these cells rapidly respond, often overcoming the infection without you even knowing your body had been exposed to that specific pathogen again. Immunisations work to trigger your body to produce specific immune memory cells so next time you encounter the substance you were immunised against (e.g. the measles virus in Figure 7.1), your memory cells will be activated to prevent infection.

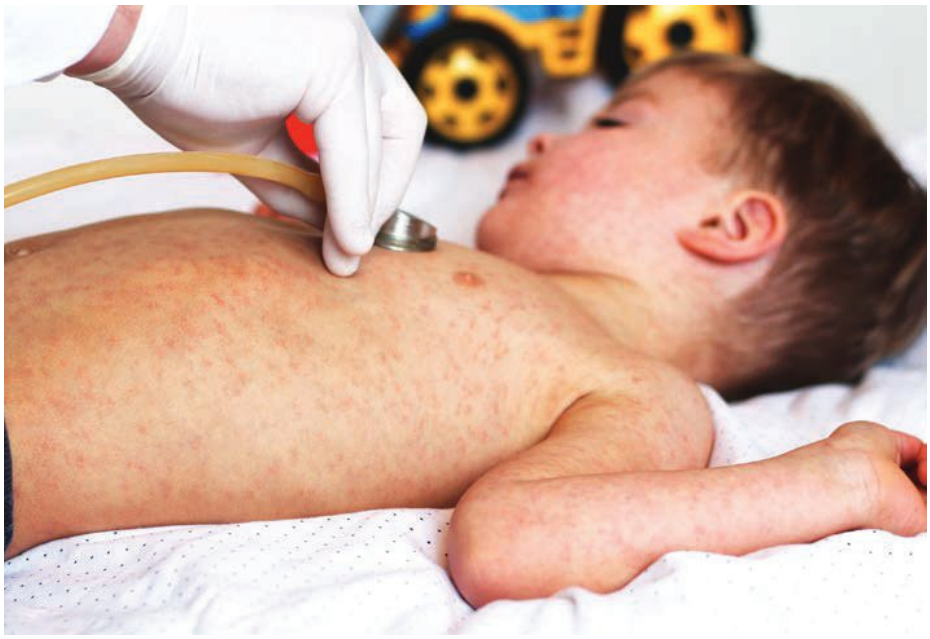

Figure 7.1 Measles is a viral infection that triggers an adaptive immune response and results in life-long protective immunity against the disease.

Wepouk
The immune system explained

7.1 Adaptive immune response – third line of defence

Spacesuits protect astronauts from the extremes of outer space, allowing them to survive in an environment too hostile for human existence. Filled with potential pathogens, Earth's atmosphere is also hostile, but the constant efforts of our immune system allow us to survive.

David Vetter was born in the United States in 1971 without an adaptive immune system, affected by a condition known as severe combined immunodeficiency (SCID). Without an immune system, his risk of catching a fatal infection was so high that he was raised from birth in a sterile isolator unit, or bubble, designed by NASA to keep all pathogens out. Not even his family was allowed into the bubble. At 5 years of age, David was able to walk outside for the first time using a special suit, also designed by NASA and based on their spacesuits. Although he lived more and more at home as he grew older, he died at the age of 12.

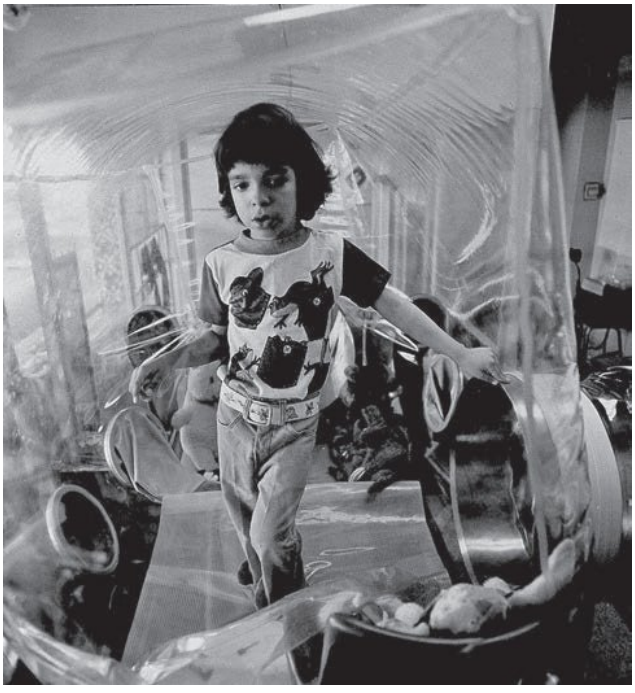

Figure 7.2 David Vetter was born without an immune system. He was raised from birth in a sterile isolator unit designed by NASA to protect him from pathogens.

Today, medical knowledge about SCID has improved and children with the disorder no longer have to be raised in such isolation. This rare disease demonstrates the critical role that the cells of the adaptive immune system, B and T lymphocytes, play in fighting pathogens.

Adaptive responses, which exist only in vertebrates, target pathogens only after they have been specifically identified by particular components of the immune system. They are termed 'adaptive' because they are capable of change in response to the experience of an antigen. These responses are highly specific because they attack only the pathogen that stimulated the response. Because of this specificity, the body requires some time to tailor its customised response, meaning that adaptive responses are not as rapid as innate responses. Adaptive responses occur in specialised structures in specific tissues and organs of the **lymphatic system**.

People surviving diseases such as smallpox and the bubonic plague seldom contract the disease again. This feature is called immunological memory, and we say the person has become **immune** to the effects of that pathogen.

WB
7.1.1 LYMPHATIC SYSTEM – AN ANALOGY PAGE 149

Lymphatic system

Under normal circumstances, when inflammation is not occurring, blood capillaries allow a small amount of plasma to leak out through their walls. This fluid that surrounds the body cells is called tissue fluid, or **interstitial fluid**. Although most tissue fluid returns to the capillaries, some, now called **lymph**, is drained away by lymph vessels of the lymphatic system. The lymphatic system transports fluid, wastes, immune cells and, unfortunately, pathogens around the body and back into the bloodstream. The lymphatic system (Figure 7.3) consists of lymphoid organs (Table 7.1) and transport vessels that carry lymph between these organs and back to the blood. Blood plasma, tissue fluid and lymph are essentially the same fluid with differing amounts of wastes and nutrients depending on where the fluid is in the body.

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Wepouk
The lymphatic system as part of the body's defence

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The lymphatic system's role in immunity

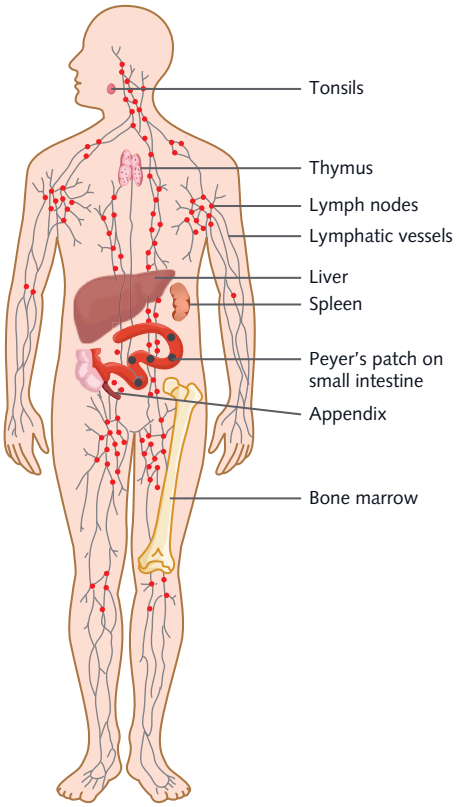


Figure 7.3 The location of organs and tissues involved in the lymphatic system in the human body

Primary lymphoid organs

The **primary lymphoid organs**, the **bone marrow** and **thymus**, are responsible for the production and development of the cells of the immune system. The bone marrow is the soft tissue in the centre of bones. It is where leukocytes are differentiated from stem cells. These stem cells produce approximately 200 billion blood cells every day. Some cancers of the bone marrow and leukocytes result in a person not having enough white blood cells for a sufficiently functional immune system. These patients are treated with bone marrow transplants. In this procedure, the recipient's stem cells are ablated (killed) and replaced with healthy donor bone marrow stem cells, which should repopulate the immune system with fully functional cells. In the time it takes for the donor bone marrow stem cells to fill the immune system with fully developed healthy cells, the individual remains highly susceptible to infections.

The thymus gland is located in the chest above the heart, and is where some immune cells mature. The thymus gland is relatively large in children, then shrinks (involutes) in adults. This accompanies a general reduction in the amount of new T lymphocytes that develop in the thymus as people grow older. Some immune cells are called T cells because they mature in the thymus gland. These cells are produced in the bone marrow, but at an early stage in their devel pment they migrate to the thymus, where they complete their developmental steps before maturng and entering circulation.

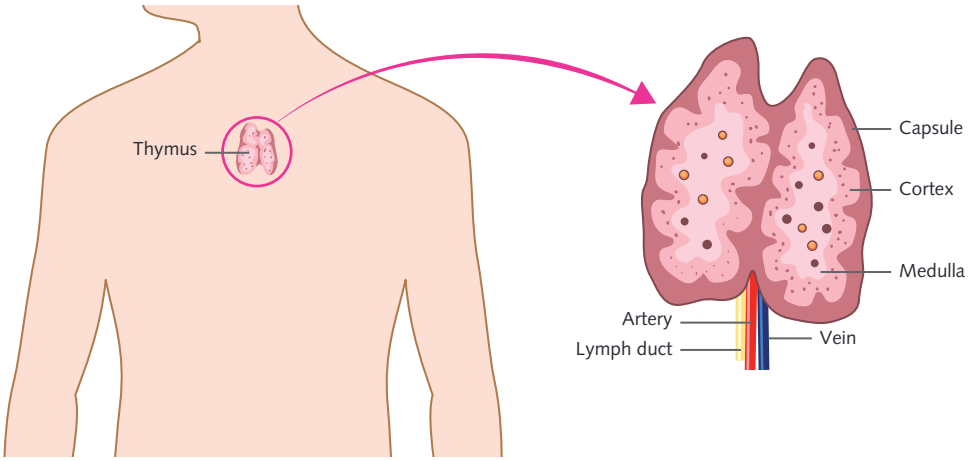


Figure 7.4 The thymus gand s the ste where some mmune ces mature

Secondary lymphoid organs

The **secondary lymphoid organs** hold mature immune cells and provide the environment for the initiation of the imune response. In the **spleen** B lymphocytes may be stimulated by the presence of an antigen, or by othe immune cells, to differentiate and proliferate into **B plasma cells**. Phagocytosis also ocurs in the spleen. As well as supprting the maturation of B lymphocytes, and acting as a source and storage site for seeral types of leukocyte, the spleen filters the blood to remove aged red blod cells. Because of the blood fitration that occurs there, and the cells that are present in well-organised structures within it, the spleen is an organ that is specialised for detecting and responding to systemic infections, in which the pathogen is present in the bloodstream.

Mature resting naïve B andT lymphocytes, as well as other whte blood cells in the circulation, move around the body through blood vessels nd the lymphatic system. Until they intercept their speci fic antigen, they are caled 'naïve'cells. Along the wy, they move through lymph nodes, scanning for antigens that they recognise through receptors that are particular to each individual lymphocyte. Within **lymph nodes** antigens are usually displayed to passing lymphocytes by macrophages that have collected them out of the circulation (mainly by complement receptors on the macrophages binding to opsonised antigens), or by dendritic cells that have picked them up in body tissues and migrate with them to lymph nodes for this purpose.

The secondary lymphoid organs also include **mucosal-associated lymphoid tissue (MALT)** – an extensive system of lymphoid tissue that initiates immune responses along mucosal areas such as the gastrointestial tract eyes and lungs.

Table 7.1 provides a summary of the lymphatic system.

Table 7.1 Components of the human lymphatic system

Organ or tissue		Location	Role
Primary lymphoid organs Responsible for the production and maturation of the cells of the immune system	Bone marrow	Central shaft of most bones, with a substantial amount in the thigh and pelvic bones	All blood cells develop from bone marrow stem cells. These stem cells can develop into all types of blood cells (are multipotent).
	Thymus	Inside the ib cag, made up of two pinkish grey lobes	Involved in the development of T cells (a type of lymphocyte) and shrinks (involutes) with age, beginning soon after birth
Secondary lymphoid organs Provide the environment for the initiation and progression of the immune response	Lymph nodes	Sall, bean-shaped structures in specific locations throughout the boy, including throat armpits groi, abdomen and chest	Filter the extracellular fluid (lymph) that drains from limbs and mucosal tissues, trapping foreign material, and are where lymphocytes can come across antigens and begin to respond to them.
	Speen	Lare, dark red ogan located ust above the stomach	Filters the blood, recognises and destroys old and faulty red blood cells, detects foreign invaders and produces antibodies. Generally the site for immune responses directed against blood-borne pathogens.
	Mucosal-associated lymphoid tissue (MALT)	Clusters of immune cells including lymphocytes found in association with the wet mucosal surfaces of the body, such as those of the respiratoy, digestive and female reproductive systems	Cells in these structures survey the mucosa for pathogens and protect the body from an enormous variety of invaders. Tonsils and adenoids are more complex examples of MALT. In the gastrointestinal tract, this is called gut-associated lymphoid tissue (GALT) and includes Peyer's patches, small clumps of white blood cells sitting in the wall of the intestine. Other examples include bronchus-associated lymphoid tissue (BALT) in the lungs and nasal-associated lymphoid tissue (NALT) in the nose.

Lymph nodes

Unlike the blood circulatory system, the lymphatic system has no pump. It relies on muscle contraction and one-way valves to move the lymph away fromthe tissues towards the heart. Lymph vessels coming from the tissues eventually join with the circulatory system by draining into the blodstream near the heart. Lying along the course of lmphatic vessels, sometimes in chans, are lymph nodes. Approximately 500–600 lymph nodes are distributed thoughout the body, with clusters in the armpis, groin, eck and hest, and abdomen. These collect and monitor material drained from the ars, lgs, oral and naal pasages, and gut, respectively. They range in size from a few millimetres to about 1–2 cm in diameter and are tightly packed with white blood cells (Figure 7.5). As lymph moves along the lymph vessels, the lymph nodes act as filters or traps for foreign particles and invading pathogens. When an antigen is present in a lymph node, white blood cells become activated, causing an influx of more white blood cells and enlargement of the node as an immune response begins to occur within.

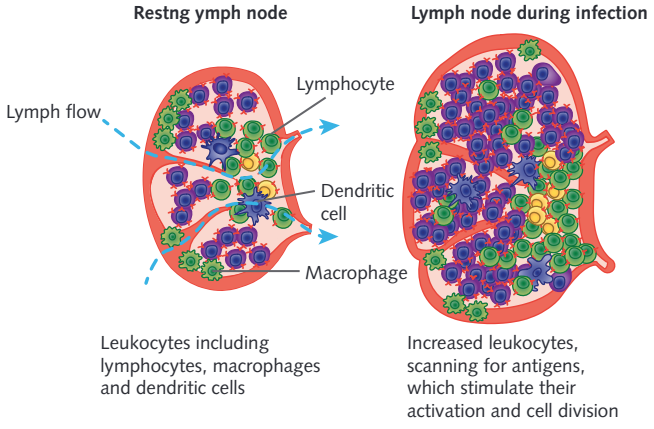


Figure 7.5 Lymph nodes aresites wh ere ymphocytes scan for antgens and can ntate responses when they come across a partcuar antgen

The lymphoid system, including the secondary lymphoid tissues, is a transport system linking innate and adaptive immune responses. When an infection occurs (for example, if a cut on the arm becomes infected), localised inflammation will occur at the cut site as part of an innate response. This inflammation causes chemotaxis of white blood cells into the area, including neutrophils, macrophages and dendritic cells. There, the phagocytes engulf foreign material, damaged cells and apoptotic debris, and secrete cytokines that stimulate further influx and activation of immune cells into the site (p. 221).

Dendritic cells, upon taking up the antigen, become activated and leave the inflamed site. They enter the lymphoid system and travel through lymphoid vessels to lymph nodes. Along the way, they change their surface receptor expression, down-regulating receptors that help detect and engulf antigens and up-regulating receptors that help present these to B and T lymphocytes of the adaptive immune system. When dendritic cells carrying antigens arrive in lymph nodes, where lymphocytes are concentrated, they also spread out their membrane to ensure a large surface area on which lymphocytes can scan for antigens. Dendritic cells are named for their small finger-like projections, or dendrites, that are on the surface of the cell that help them to take up, process and present antigens to lymphocytes efficiently. At this stage, they become **antigen-presenting cells (APCs)**.

KEY CONCEPTS

- » llblod cells are producedfom stem cells in the bone marrow Some leuoytes reside in lymphoid organs and oter crculate in te blood and lymphatic sysem. Othersareresient i the tissues.
- » Primary ymphoid rgans include the bone marrow and thmus, whercells o the immune system are produced and ature.
- » Seconarylymphoiorgans inlude lymph nodes, spleen and muosalasocaed lymphoid tissue (MALT), here immune resposes are initiated and carred ut.
- » Th lymphoid stm, including the secondary ymphidtisus, is a transpor system linking innate and adpive immune responses.

Concept questions 7.1a

1 Describe he ole of lymh noes in theimmune system.
2 Wher is an immune respnse likelyto be initiated when the pathgen:
   a enters through cut in the hand?
   b s a gastroinetinal pathogen that causes foo pioning'?
   c enters and circlate in the bloodstream?
3 How doesthe nnate immuneresponse differ from the adaptive immune espns, consiering the aim of both is to confer defence against ntigens? Give three examples.
4 What isan impotan distinction between the functions of primary lymphoid organs and secondary ymphid organs?

HOT Chaenge

5 Recent findng ndicate ha ntestnal mononuclear phagocytes, oprising denitic cells and macrophages, arecrucia or aintaning intestinal homeostasis. There are also about 10^4 commensl bactra in th gut.Commnsa bacteria derive food from the hot withut harming th host organism. Denritc cels mediate in flammatory responses and can becomeantigenpresting cells. Knowing that not al gut bcteria ar pathogen, why do you think that dnritc cells and marophages might be so vtl?

Cells of the adaptive immune system

All cells of the immune system, including B and T cells of the adaptive immune system and the many cell types of the innate immune system, are produced in the bone marrow from blood stem cells. Collectively they are called white blood cells, or leukocytes. Some reside in the lymphoid organs while others circulate in the blood and lymph, acting like a mobile surveillance squad. As they move around the body, they detect invading pathogens and initiate an immune response to clear the infection.

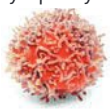
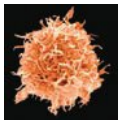
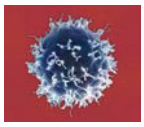
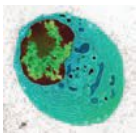
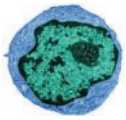
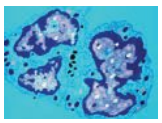
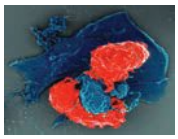
WB
7.1.2 CELLS OF THE ADAPTIVE IMMUNE SYSTEM PAGE 150

Wepouk
Types of immune responses

wutaue r nhkCveec
Types of immune responses

Vaten
The T and B cells of the immune system

Table 7.2 The cellular components of the immune system

Cell	Function
Dendritic cell 	An antigen-presenting cell that acts as a messenger between the innate and adaptive immune systems Engulfs material and presents it to lymphocytes Produces cytokines that direct immune responses
Lymphocyte 	A general term for a range of specialised leukocytes (white blood cells) that respond to specific antigens in the process of adaptive immunity. This is an example of Cytotoxic T cell.
B lymphocyte 	A white blood cell that is produced and matured in bone marrow and travels to the spleen and lymph nodes Produces specialised proteins called antibodies, which bind to specific foreign material on the surface of pathogens, thereby labelling it for engulfment and destruction by other white blood cells such as macrophages
Plasma cell 	A specific B lymphocyte that is differentiated to secrete very large amounts of specific antibodies Contains many Golgi apparatus and large amounts of rough endoplasmic reticulum to assist with production and secretion of antibodies
Memory B cell 	A specific B lymphocyte with receptors that are specific to one type of antigen When stimulated, rapidly differentiates into plasma cells and more memory B cells
T lymphocyte 	A white blood cell that originates in the bone marrow, then travels to the thymus where it matures Contributes to the adaptive immune system in a variety of ways
Cytotoxic T cell (also known as killer T cell) (T_C) 	A T cell that produces lethal chemicals, such as perforin and cytoxins, which are injected into the infected cell and destroy it along with the virus by initiating apoptosis
Helper T cell (T_H) 	A T cell that activates other cells of the immune system
Regulatory T cell (also known as suppressor T cell) (T_{reg}) 	A T cell that suppresses or turns off the activity of other cells once the threat has passed
Memory T cell 	A T cell that remembers a particular antigen to provide long-term immunity

Lymphocytes

Lymphocytes are cells of the adaptive immune system (Table 7.2). There are two major types: B and T lymphocytes (or B and T cells). Many B and T lymphocytes look so similar that scientists cannot tell them apart under the microscope. Special tests that measure surface proteins are required to distinguish between them.

B lymphocytes are a key cell type of the adaptive immune system. These cells go through a series of developmental stages in the bone marrow before they are released into the circulation, ready to respond to an infection. B lymphocytes (**B cells**) are responsible for the destruction of pathogens by producing specific proteins known as **antibodies** that bind to antigens and neutralise or opsonise them. Destroying virally infected and cancerous cells is the major role of **cytotoxic T cells (T_C cells)**. Helper T lymphocytes and regulatory T lymphocytes assist the other lymphocytes in performing their roles.

Helper T and regulatory T cells

Helper T cells (T_H cells) assist other cells of the immune system. They do this by secreting signalling molecules (including cytokines) that induce any activated B or T_C cell to divide and give rise to large numbers of clones that become the effector cells (which bring about the immune response) and memory cells. Cytokines can also stimulate macrophages to engulf invading cells more readily.

Regulatory T cells (T_{reg} cells) play an important role in modulatin the action of lymphocytes. T_{reg} cells may enhance or suppress the actions of other lymphocytes. They are also capable of suppressing the action of phgocytes. n this way, they help prevent the immune system overreacting to a stimulus. A T_{reg} cell deficiency causes a very severe **autoimmune disease** resulting from overactive lymphocytes.

Lymphocytes of the adaptive immune system differ from cells of the innate immune system because they are *specific*. This means that individual lymphocytes can each detect a particular invader, attacking only those that contain the specific molecular pattern or **epitope** matching the receptors on their surface. An antigen will usually have several epitopes, and each epitope will only be detected by the lymphocytes with a complementary receptor for that specific epitope (Figure 7.6). Even a small section of a molecule, such as a toxin, can generate an immune response. As even a small peptide length may be potentially antigenic, most protein antigens have several epitopes, each of which is recognised by a different lymphocyte and induces the production of a different antibody. Each different epitope is a specific chemical group or structure.

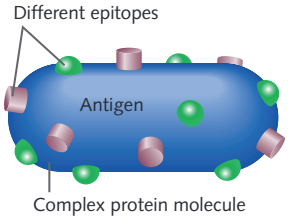


Figure 7.6 The distinction between an antigen and an epitope. A large antigen, such as a bacterium or a large protein complex, may have several different antigenic determinants, called epitopes. The different epitopes are specific chemical groups or structures.

Before a person is infected by a particular pathogen, they may only have a handful of lymphocytes that can detect the epitopes contained within the pathogen's antigenic components. However, once infected, an adaptive immune response is mounted, which results in cell division of those lymphocytes to produce an army of clones. These all have the same antigen receptor and collectively they can outnumber the pathogen and clear it from the system.

Determining self from non-self

For the immune system to function properly, cells of the immune system must be able to distinguish between cells of the body and foreign antigens. Our body cells identify themselves to the immune system as 'self' by marker proteins on the surface of the plasma membrane. As is the case with all proteins, the amino acid sequence of these markers is determined by the information coded in genes. The group of genes that determines these protein markers is called the **major histocompatibility complex (MHC)**. Because these MHC markers are determined by the genotype of an individual, they are unique to that person. There are many different alleles in the MHC gene locus, resulting in great variability between individuals. It is as if each cell of a person's body is tagged with a message that is read as 'self'. Any cell not displaying that particular marker is 'non-self' and treated as an antigen.

WB
7.1.3 INTRACELLULAR OR EXTRACELLULAR PATHOGENS PAGE 150

MHC markers

T cell receptors are present on the surface of T cells. These receptors do not bind directly with the antigen but rather with epitopes derived from the antigen protein that are displayed on the end of MHC marker molecules. MHC markers are the only molecules that can present the antigen to a T cell. The fact that the T cell receptor will only recognise the antigen when in association with the MHC marker molecule is termed **MHC restriction**.

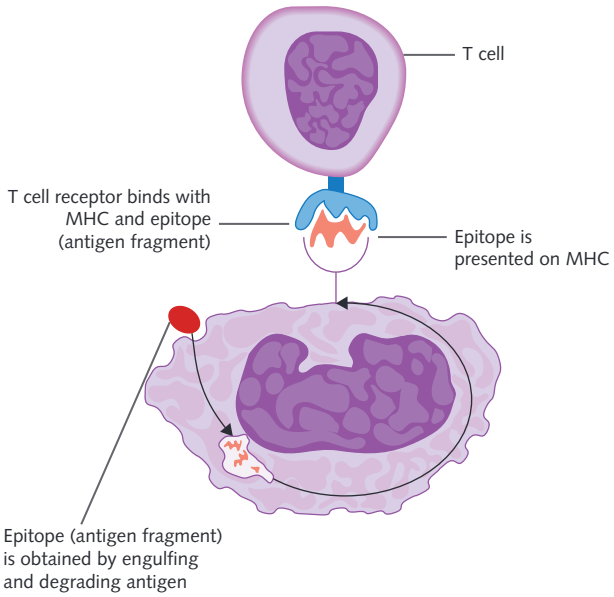


Figure 7.7 T cells must be activated to respond. T cell receptors will only recognise antigens presented by MHC markers.

There are two types of MHC proteins: MHC class I and MHC class II. Both types of MHC proteins contribute to the specific identity of the cell. MHC class I markers are found on all body cells that have a nucleus. MHC class II markers are found only on antigen-presenting cells (APCs): macrophages, dendritic cells and B lymphocytes.

MHC proteins contain a deep groove, which can hold a short peptide. Within a cell, antigens are broken down into small peptides. MHC proteins are synthesised inside the cell and pick up the antigen peptide lengths. These then sit inside the groove of the MHC. The MHC protein (bound to a peptide) travels to the cell surface where T cell receptors can then scan for their ability to bind to the MHC–antigen complex.

MHC class I and MHC class II proteins differ in the type of antigen that they can present, and the type of T cell that can recognise antigens bound to them (Figure 7.8a). MHC class I presents antigens that are found within the cell cytoplasm. These antigens are usually produced within the cell itself, and this method of antigen presentation allows the immune system to survey the intracellular activity of cells to detect virally infected or cancerous cells. Some pathogens are able to enter and divide within cells. Antigens from these pathogens are also presented this way. This process does not distinguish between antigens and normal proteins produced. Instead, a random sample of peptides from the breakdown of proteins within the cell is presented on the MHC. T cells can then bind to the MHC–antigen complex and trigger apoptosis of the cell if they recognise the presented peptide as non-self. As MHC class I proteins are found on all nucleated cells, this is how the immune system patrols the cells of the body to find any abnormal proteins within cells.

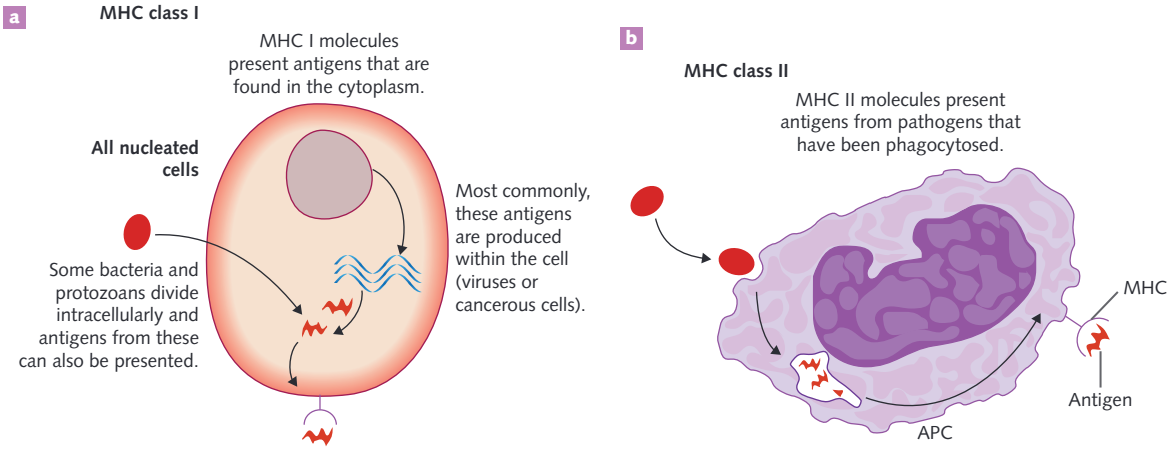


Figure 7.8 MHC class I and MHC class II proteins are cell-surface structures that present pieces of antigen to T lymphocytes. **a** Cytotoxic T lymphocytes recognise their specific antigens only if they are presented on MHC I molecules, and **b** helper T lymphocytes recognise their specific antigens only if presented on MHC class II molecules.

MHC class II proteins are only found on APCs and are used to present extracellular antigens (Figure 7.8b). APCs phagocytose pathogens following recognition by pattern recognition receptors or their opsonisation by complement or antibody, and then break them down in lysosomes. APCs then travel to the lymph nodes to present these antigens to T cells. Peptides that are derived from these antigens are presented on MHC class II proteins. APCs are usually macrophages and dendritic cells but B cells can also present antigens this way. APCs also express MHC class I molecules because they are nucleated cells.

Immune cell antigen receptors

Lymphocytes have surface receptors that distinguish self from non-self. **B cell receptors** and T cell receptors allow lymphocytes to identify foreign antigens. B cell receptors and T cell receptors recognise and bind to specific epitopes of the antigen. The binding of an antigen to a lymphocyte receptor is similar to that of a substrate binding to an enzyme. The molecules must have the correct shape (or conformation) and charge to be able to bind to each other.

Antibody molecules are glycoproteins whose function is to bind to antigens. Some forms are secreted into the circulation, and some contain hydrophobic protein sequences that anchor them to the plasma membrane. When antibodies are bound to the surface of B lymphocytes, they act as the B cell receptors. They have the ability to bind their specific antigens, and stimulate signal transduction pathways within the B cells. If paired with inflammatory cytokines or helped from helper T cells, this results in their activation and cell division. Antibodies also serve as effector molecules when secreted by B cells.

Receptor diversity

Each B or T cell carries a large number of identical copies of a receptor protein that will bind to a single, specific antigen. There are so many different receptors that about 10 million different epitopes can be recognised by all the B cell clones combined. This diversity means that, by chance, there will be a B and T cell receptor that is able to bind to almost any antigen that the body could encounter.

The genome does not encode for this number of different receptors. The particular type of receptor carried by a lymphocyte is determined during early embryonic development by random genetic recombination of the antibody or receptor genes. As a result of this genetic rearrangement, each B or T cell and all of its descendants will produce a unique receptor. This genetic lottery accounts for the huge diversity of lymphocyte receptors that are able to respond to the millions of different antigens that we experience in our lifetime. Whatever the antigen, there is a strong chance that there will be a lymphocyte receptor that can bind to it.

It is interesting to note that while receptor diversification is essentially a random process, the genetic pool from which each lymphocyte draws its receptor components has been shaped by the disease challenges our ancestors have faced during their evolution.

KEY CONCEPTS

- The adaptive immune response diffrs from the innate mmune response be ause it as speci fic recontion of antigens an displas memory.
- B lls, T $_{H}$ cels and T $_{C}$ cels are th main players of the adaptive immun system. he adaptive immune responseelies on thse cels detecting foreign antigens andditnguishing thmfrom self.
- T $_{eg}$ cels are Tells tha control the magntude and duration of mmune response o limit damageto bdy tissues.
- Atigens ae olecules that cn generate an immune response. Thepartiulr molecular structures on antigens that ae recognised by components of the mmune system a caledepitopes.
- The majo histocmtibiity comlex (MHC) is an mportant wyof dstiguishing sf from non-self.
- MHC rstriction refers to thea that T cells will only reconiseepitopes when they are presented on an MHC oleue.
- MH cas molecues prnt intracellular antigens and are presentn al nuclte cells (self-markers).
- MH cass I molecules pesent xtracellular antigens derived from phgocytosis andare present on antigen-presentin cells (maropgesdendritccells and B cells).
- Each cell anT cell ha an antigen eceptor that is speci fic for a unque eptope, sote population of lymphocytes has the capacity to detect a vas rnge of antigens.

Concept questions 7.1b

1 Define majr histooatibliy complex'.
2 What is the difference beteen an epitope and anantigen? Wat are epitopes made of chmially?
3 Describe the HC las I andclass II presentation pathwaysan identify the main differences betwee them.
4 Lst two mchanisms thatrevent T cells from moutin an immune resonse against a normal body componnt in the absence of any true nfection r injry.

HOT Chaenge

5 Lymphocytes ar esponsible or bth the induction and the expession of adptie immunity. The ymphocyes i adptive immuity are speci fic. There are two majr classes oflympocytes, B cells and T cel, wich can be furthe divided into T_H T_{eg} T_C memoy T, plasma B an emory B cells.
   a What are te speci fic funtions of each lymphocyte?
   b Compare th speci fic funtions of B a T cells.

Avoiding self-recognition

The random generation of receptors results in some receptors that will bind to self-molecules. An important step in lymphocyte development is the killing off or inactivation of those self-reactive lymphocytes, to ultimately protect the individual from immune attack of their own body cells and tissues.

A group of cells in the thymus expresses a wide range of proteins that are usually found elsewhere in the body. These proteins are not expressed to perform their normal function, but rather so that T cells can develop self-tolerance. In effect, these cells serve as a 'showroom' of the proteins that the body is capable of producing. Any T cell bearing a T cell receptor that recognises a peptide presented in the thymus undergoes apoptosis and is deleted from the collection.

A similar selection process may occur for B cells as they develop in the bone marrow and also as they mature in the spleen. This negative selection of self-reactive lymphocytes continues when they are mature, and those clones that carry receptors for molecules that already exist in the body are either inactivated or self-destruct by apoptosis. This process provides the adaptive immune system with the capacity to distinguish self from non-self. The result is **self-tolerance**, which means that ideally there are no mature lymphocytes that will react against self-molecules. All the lymphocytes in the collection are tolerant to normal components of the body; that is, they don't mount an immune response against such normal components. However, the process is not perfect. A very small number of lymphocytes that react against self-molecules usually survive the process. If there is a strong innate inflammatory response, and if the individual has a collection of alleles that allow it, those self-reactive lymphocytes may become activated and attack the body. This can result in autoimmune diseases.

The interaction between APCs and T cells is another mechanism for preventing responses against self-antigens. T cells can only recognise an antigen if it is loaded onto an MHC protein, which means they must interact with an APC. A T cell that recognises a complementary antigen on an MHC protein must receive appropriate signals from the cell presenting that antigen to become activated. If that APC has recognised a pathogen-associated molecular pattern (PAMP) or a damage- or danger-associated molecular pattern (DAMP), indicating infection or tissue damage, it will signal to the T cell that it should mount a response against the peptide presented on its MHC. This signal is usually in the form of cytokines, such as **interleukins**, and contact-dependent signals. Without this danger signal, a T cell recognising a peptide bound to an MHC protein will not mount a response against the peptide. This provides an additional safeguard that prevents T cells from mounting an immune response against the body's own cells and tissues.

Clonal selection

B and T cells originate as stem cells in a process that starts when we are embryos. By the time we are born, we have a large number of different types of B and T cells, each with a small number of clones that can recognise a specific antigen circulating throughout our blood and lymphatic systems.

A young lymphocyte is released from its 'training ground' into the bloodstream, in which it may encounter an antigen it recognises. Recognition of a specific antigen triggers an impressive response in the selected cell (clonal selection), causing it to divide rapidly, forming many copies or clones of itself and the specific antigen receptor it carries (clonal expansion). These clones can be one of two types of cells: effector cells or memory cells (Figure 7.9).

Random genetic rearrangements allow for a diverse range of lymphocyte receptors to be generated. **Clonal selection** and clonal expansion are responsible for the proliferation of lymphocyte clones with receptors that have bound to antigens.

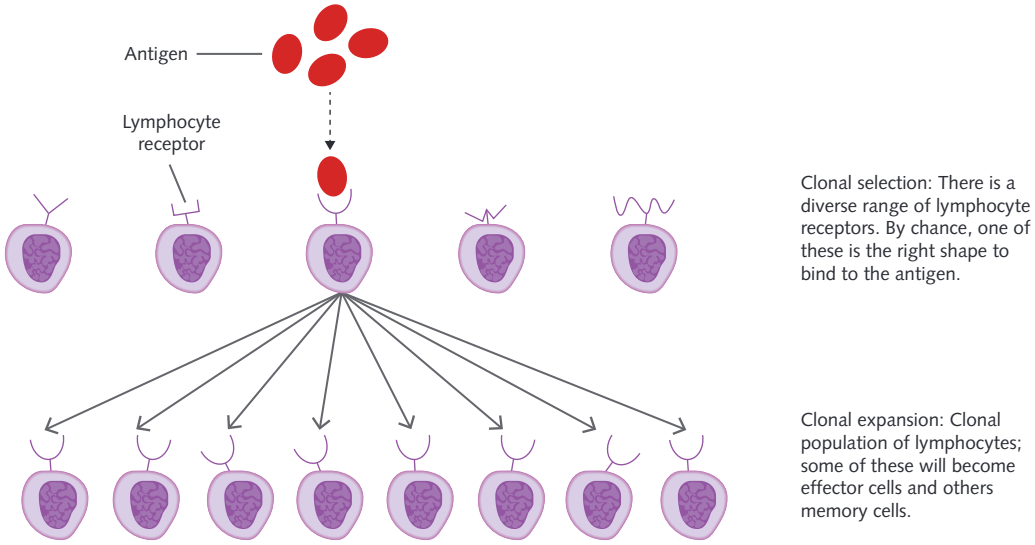


Figure 7.9 The rapid division of a particular lymphocyte clone, once it has bound to an antigen, is called clonal selection. Thus, the antigen itself selects which of the millions of different B or T cell clones becomes active.

KEY CONCEPTS

» Lymphoctes with antigen receptors that could reconiseself-componens aredelted or inactivated duringther devlopment.

» Drig ineton, nly the lympocytes bearing a receptor that canrecognise epitoes on the invading pathogen ar activatd an roliferate, in the process ofcloa sletion.

Concept questions 7.1c

1 Define selftolence'.
2 Slf-reognitio can ead to in flammtion and autoimmune isease. There are seeral ways in which th immune system void elf-cognition. Outline two methods used to manage the preferred outcome of slf-tolrance.
3 Define loe' i relatonto clona expansion.
4 Conal eection theory xplains the immune mecanism for gneratinga diversity of antibody specificity as partof adaptive immune system. The theory was postulted b Australian scientist Sir Macfarlane urnett in 1957. The first exeriental evdence was demonstrated byGustav Nossal and Joshua Lederberg i 1958. The eoy is still widely accepte. Bot T cells and B cels arecloned to mount a respone Using a flow chart,describe the steps nvolved inclonal selection theory.

HOT Chaenge

5 Azathopine is a drug that blcks the production of purine nulotides (adenin and guanine) in ymphocyes. redict the ef fect thatazathioprine would have on the procssof lnal selection.

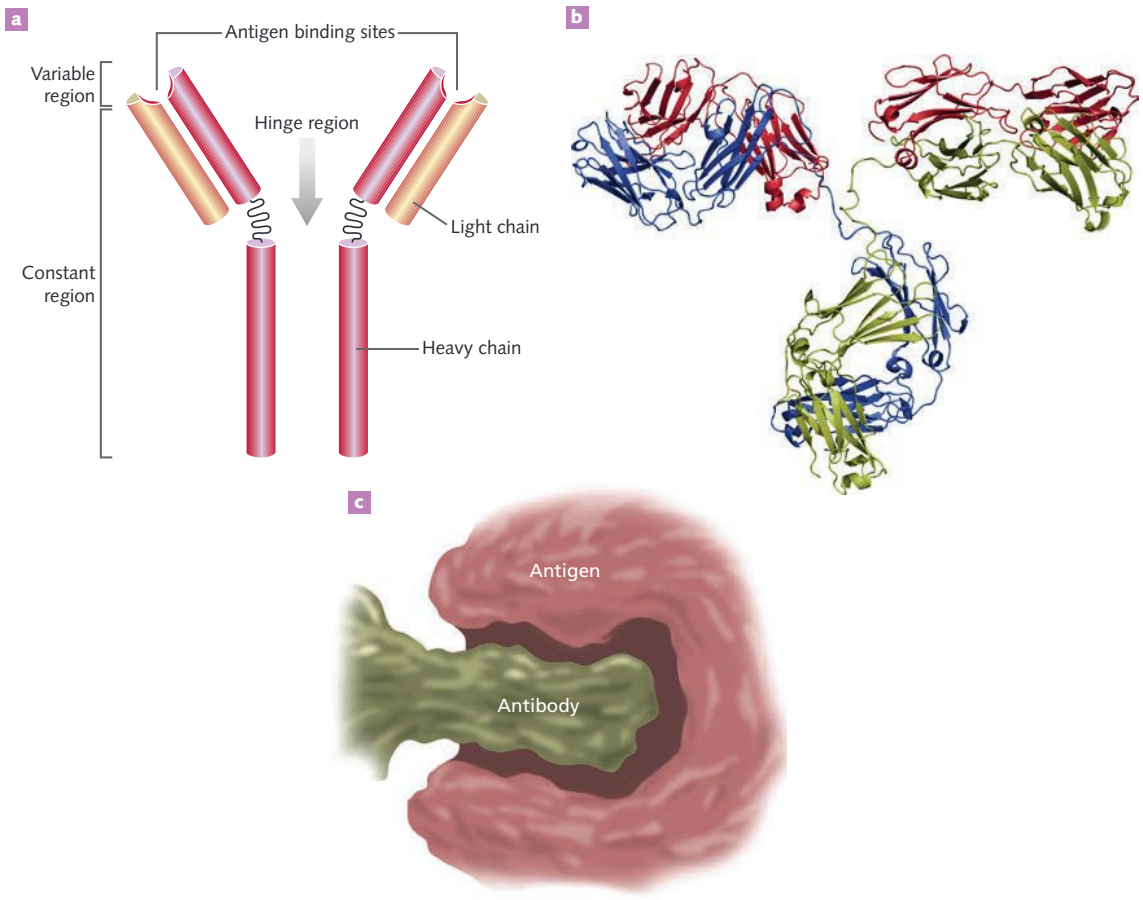
7.2 Humoral immunity

The **humoral immune response** is brought about by B cells, which produce an array of specific antibodies that tag foreign antigens for destruction. The word 'humoral' refers to the fact that the effects of this system are caused by the circulation of antibodies in 'humours', an antiquated concept that roughly means body fluids. You have already learned that antibodies bound to the surface of B cells act as the B cell receptor. Once activated, B cells divide rapidly (that is, they are clonally selected) and produce antibodies that circulate freely in the bloodstream and can lead to the destruction of pathogens.

Antibodies

Antibodies are also known as **immunoglobulins (Ig)**. They consist of four polypeptide chains: two heavy chains and two light chains that are arranged in the shape of a Y (Figure 7.10a). All antibodies have a constant region (most of the Y shape) and variable regions at the two tips of the Y, where there are two identical binding sites that are complementary to a specific antigen. The variability in this region is based on the different amino acid sequences that allow for differential binding to various antigens. This is the part of the antibody that results from genetic recombination during development. The binding sites work with a lock-and-key system of identification, similar to that of enzymes binding with their substrate (Figure 7.10c).

EXAM TIP
Make sure you can draw and label an antibody.



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7.2.1 ANTIBODIES PAGE 152

Wepouk
Different levels of defence

Figure 7.10 a The Y-shaped structure of an antibody. The hinge region gives antibodies great flexibility to improve binding to the antigen. **b** A ribbon diagram representation of the crystal structure of 1IGT, an antibody of the IgG family produced by plasma B cells. **c** The active sites on the antibody and antigen molecules are complementary; they fit together like a lock and key.

Once bound to an antigen, antibodies can lead to the destruction of pathogens in four ways, all of which may occur simultaneously (Figure 7.11).

» The binding of antibodies can cause **agglutination** of pathogens, meaning that they become stuck together in an antibody–pathogen net. In other words, the pathogens are immobilised and not able to spread. Being clumped together in one spot makes them more susceptible to destruction by phagocytosis.

» Bound antibodies are able to attract phagocytes, effectively 'tagging' pathogens for phagocytosis and destruction, a process known as opsonisation. Opsonisation by antibodies has similar results to opsonisation by complement but, in this case, opsonised antigens are recognised by receptors on cells that detect the constant region of the antibodies, called Fc receptors (FcR). FcR triggering can cause degranulation, phagocytosis, release of cytokines and chemokines and increased antigen presentation.

» Some antigens can act as toxins and cause cellular damage. In these cases, antibodies neutralise toxins by preventing them from binding to their target. This is known as **neutralisation**.

» Antibodies that are bound to antigens are potent activators of the complement cascade.

CONNECT

See Chapter 6, page 217 for details about the complement cascade and how complement, like antibodies, can also result in opsonisation.

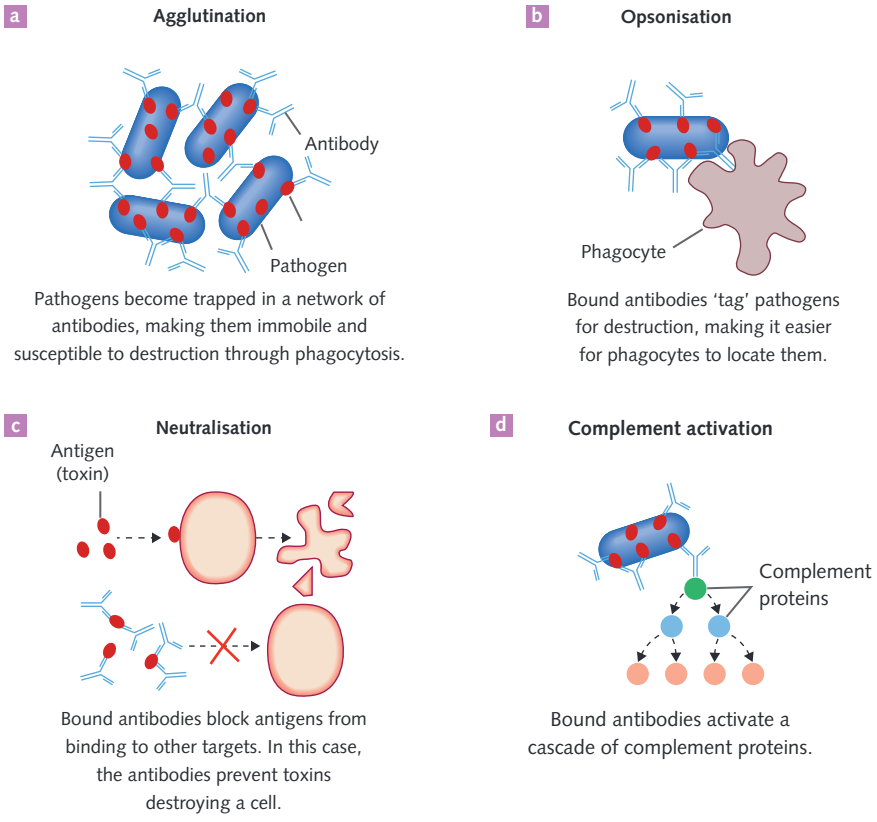

Figure 7.11 Antibodies can cause the destruction of pathogens in four ways: a agglutination, b opsonisation, c neutralisation and d complement activation.

B cells and antibody production

As with phagocytosis activation, B cell activation involves the three-step process of reception and transduction followed by cellular response. The role of B cells is to produce antibodies, and a B cell needs to be activated by an antigen. When a pathogen enters the body, it encounters a large number of B cells. These cells circulate through the blood and lymphatic system and congregate in lymph nodes where they

detect pathogens draining from the tissues or presented on APCs such as dendritic cells. B cells are also found in the spleen, where they can detect and respond to pathogens circulating in the bloodstream. Each B cell has a unique B cell receptor (antibody) on its surface that enables it to bind to a specific epitope. An invading pathogen will only activate B cells bearing a B cell receptor that is complementary to a particular epitope on the pathogen. In this way, the B cell is activated to produce an adaptive immune response. However, B cells are also APCs, and carry on their surface pattern recognition receptors that recognise foreign pathogens non-specifically. These cells engulf the pathogens they recognise and present pathogen components on MHC class II molecules for presentation to T_H cells. A T_H cell that bears a receptor that can specifically detect the same epitope, embedded in MHC class II, can become activated, and can provide direct assistance to the B cell in the form of cytokines (signalling molecules) and contact-dependent signals.

The result of this interaction between the T_H cells and the antigen-presenting B cells is that the B cell becomes activated and is clonally selected. It starts rapidly dividing to produce effector and memory B cells (Figure 7.12) in the process of clonal expansion, helped by the activated, antigen-specific T_H cell in this process. Following clonal selection and expansion, the B cell clones that have been activated will then be present in much greater numbers than others. This division occurs most effectively with the assistance of T_H cells that have been activated by the same antigen but can also proceed when the environment contains an abundance of inflammatory cytokines that indicate the presence of a foreign antigen.

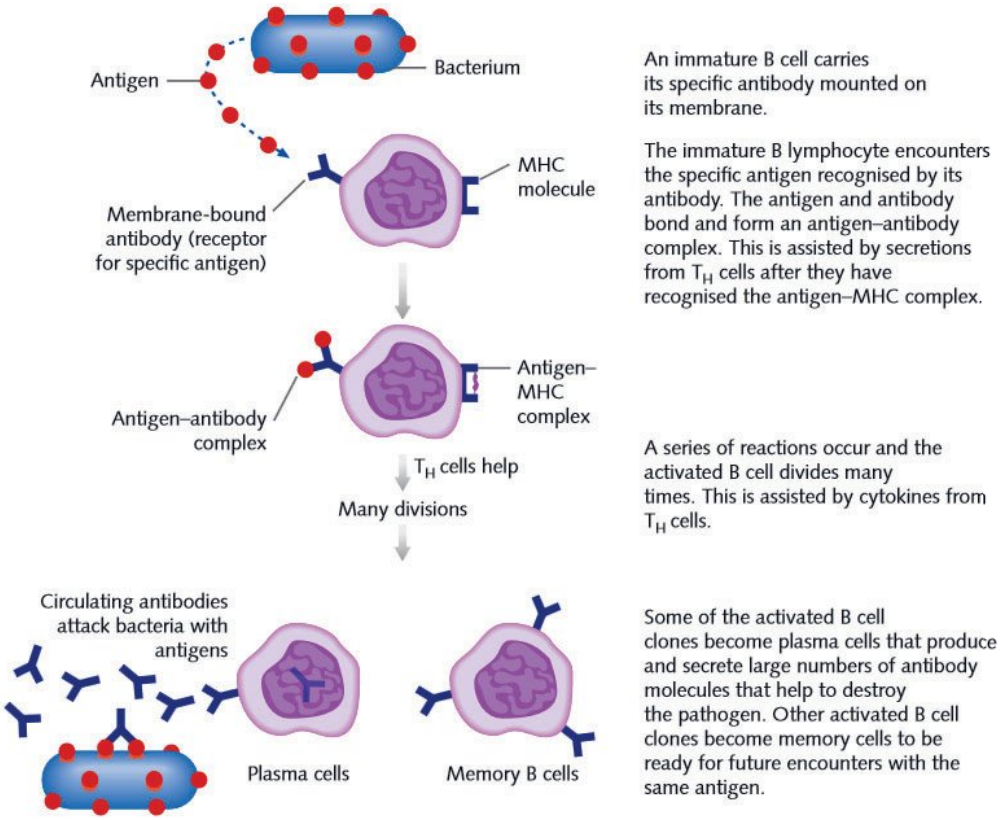

Figure 7.12 Example of an antibody-mediated immune response to a bacterial pathogen, summarising the steps of B cell activation. This response usually occurs in a lymph node or in the spleen.

The effector B cells that are produced after clonal selection of activated B cells are known as plasma cells. Plasma cells have differentiated to become highly specialised for antibody production (Figure 7.13), secreting up to 10 000 molecules of a specific antibody per second into the circulation. These antibodies generally provide protection for up to 28 days but the plasma cells that secrete them can last for years and even decades. Most antibodies will only attack one antigen, but a few will attack several different antigens if they are closely related and have similar structures, such as antigens located on the smallpox and cowpox viruses.

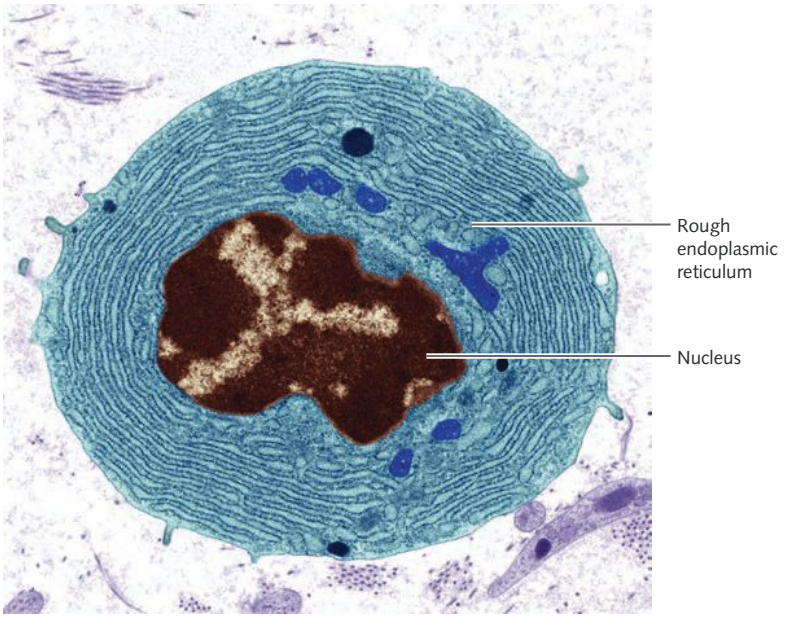

Figure 7.13 A transmission electron micrograph of a plasma cell. There is extensive rough endoplasmic reticulum to allow for the production of antibodies.

Memory B cells can persist within the body for months or even years, possibly almost a lifetime, not secreting antibodies but still carrying them on their plasma membrane. In this way, they can recognise the same antigen quickly should it reinvade the body of the host. Once activated by the specific antigen, the memory B cells rapidly divide and form plasma cells that produce large quantities of antibody, often attacking the pathogen before any symptoms of its presence arise. Memory B cells do not require T cell help to become activated and differentiate into plasma cells.

Figure 7.14 shows the speed of antibody production after initial and subsequent exposure to an antigen; for example, Rubulavirus, which causes mumps.

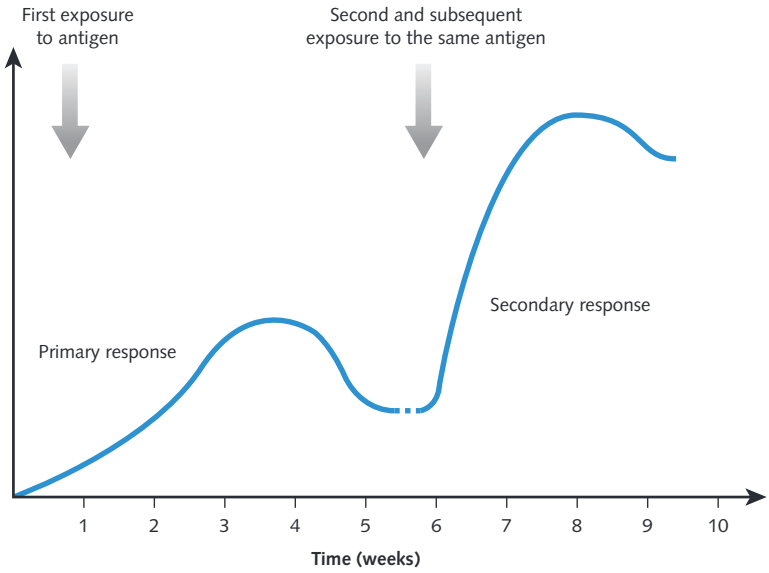

Figure 7.14 Antibody levels after an initial (primary) infection by an antigen and after a second exposure to the same antigen

When first exposed to the virus, the body produces antibodies, but there is a delay before enough are produced to neutralise the virus. This is why unvaccinated people develop symptoms of mumps when first exposed to the virus. When that person is later exposed to the virus again, memory cells recognising the virus quickly divide and form plasma cells, which produce antibodies that neutralise the virus while it is still in circulation before it can enter its target cells. You can see that the **secondary response** is faster (with a steeper response curve) and bigger than the **primary response** to that same antigen. This is why people are vaccinated against mumps. After an initial response to the vaccine, they become immune to future infections. Vaccination programs will be discussed further in Chapter 8.

KEY CONCEPTS

- Atiodies are present on the surace of B cells as the B ll receptors are screedino circulation by dffereniated cel called lasma cells.
- Atbodies functio by attaching to a speci fic antigen on the surface of a athogen which is then destroyed throughagltinatin opsonaion, neutralisation and compement atvation.
- Atiode exst in different forms hat have different specalised unctions.
- For cel ctivaton to ocur, the same antigen must activate T_H ce,llowing thm to provide contact-deped signaling for B cells and secrete cyt okine s t hat sim uat e B ell survival, activation and prifertion.

Concept questions 7.2

1 a What type of compund is an antibody?
b Whymight there be a constant region and a varabe rgion inan antibody?
c dentify the ining sites for antignson antibodies.
2 Antien–lympocyte bindin is ofen described as lock and key. Wha does this mean?
3 Complement cascde psonaion, neutralisation and agluination are four modes of antibody action. Ouline your undestanding of each mode.
4 Pasma B ells have n extensie ough endoplasmic retiulum, and my Golgi apparatuses and mitochdia. Relate the truture of plasma cells to their function.
5 B cel activation involves three main steps before cloal slection. Wht are these, and what is nvolved in each step?
6 How doprimary and econdary immune responses dffer? Elai the dfferencein erms of antibodies.

HOT Chaenge

7 Areplasa cells effctorcells? If so, why and what do they do?

WB
7.3.1 CELL-MEDIATED RESPONSE PAGE 153

7.3 Cell-mediated immunity

The response that results from the T_H cell detecting the antigen as presented on the MHC class 1 marker is **cell-mediated immunity**. The response involves the direct killing of virally infected and cancerous cells by T_C cells. Like B cells, T_C cells are able to distinguish self from non-self because of the various membrane-bound receptors, T cell receptors, that interact with antigens. You have already learned that T cells do not bind with antigens directly but bind with the antigens presented on the MHC proteins. MHC class I present antigens from inside the cell and thus flag virally infected or cancer cells.

Some viruses have evolved mechanisms to stop or reduce the expression of MHC class I on infected cells. This prevents T_C cells from recognising the infected cells as virally infected, allowing the virus to avoid destruction and continue to divide. In response, natural killer cells have evolved to destroy cells that have low levels of MHC class I on the surface. This is a clear example of how the immune system has influenced the evolution of pathogens and vice versa.

Like B cells, activated T_C cells (with the help of contact-dependent signals and cytokines from T_H cells) proliferate by dividing many times to form an army of clones. Some of these clones become effector cells,

while others remain as memory T_C cells and migrate in the lymph fluid and to the lymph nodes where they can be activated quickly upon a second encounter with the same pathogen.

T_C cells are highly effective killers; they can eliminate infected body cells or tumour cells by releasing powerful cytotoxins directly into the cell when they contact a cell that carries an unrecognised antigen (Figures 7.15 and 7.16). The cytotoxins, just as in natural killer cells, include the proteins perforin and granzymes, which work together to induce apoptosis in the target cell. Perforins can form pores in the target plasma membrane, and granzymes can enter through these pores and directly activate the process of apoptosis.

EXAM TIP
If you are asked to compare, you need to discuss both similarities and differences. For example, humoral and cell-mediated responses both involve lymphocytes, but a humoral response involves B cells producing antibodies, whereas a cell-mediated response involves cytotoxic T cells killing infected cells.

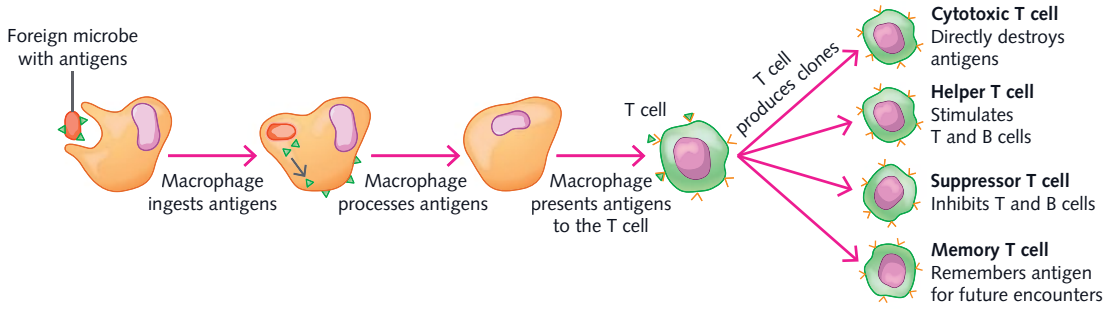

Figure 7.15 A cell-mediated response to a viral pathogen. This response will occur at the site of the infected cell.

The memory of cell-mediated immune responses can be demonstrated in experiments that use skin transplants (known as grafts) in mice. If a mouse is given a skin graft from a non-identical mouse, the graft will be rejected after about 14 days. If that same mouse later receives a second graft from the same donor mouse, the rejection only takes 4–5 days (Figure 7.17). This is because memory T_C cells formed after the first graft respond more rapidly when they encounter the foreign graft a second time.

T_C cells are the primary cause of transplant tissue rejection because they destroy the transplanted cells directly. Thus, patients receiving transplants must take high levels of immunosuppressant drugs to help counteract this response so that the new organ is not destroyed by the immune system. This is also the reason for HLA matching of organ transplant donors to recipients: HLA is the name of the human MHC markers, and the more closely related the donor's HLA markers are to the recipient's markers, the less likely it is that the T_C cells from each of the donor and recipient's immune systems will recognise the cells of the other as being foreign.

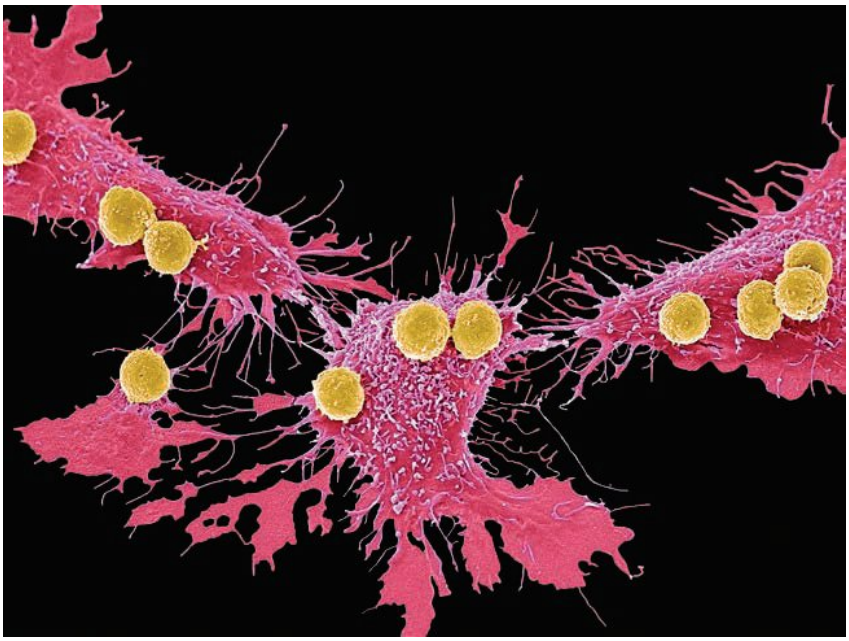

Figure 7.16 A scanning electron micrograph showing T_C cells (red) attacking a cancer cells (yellow)

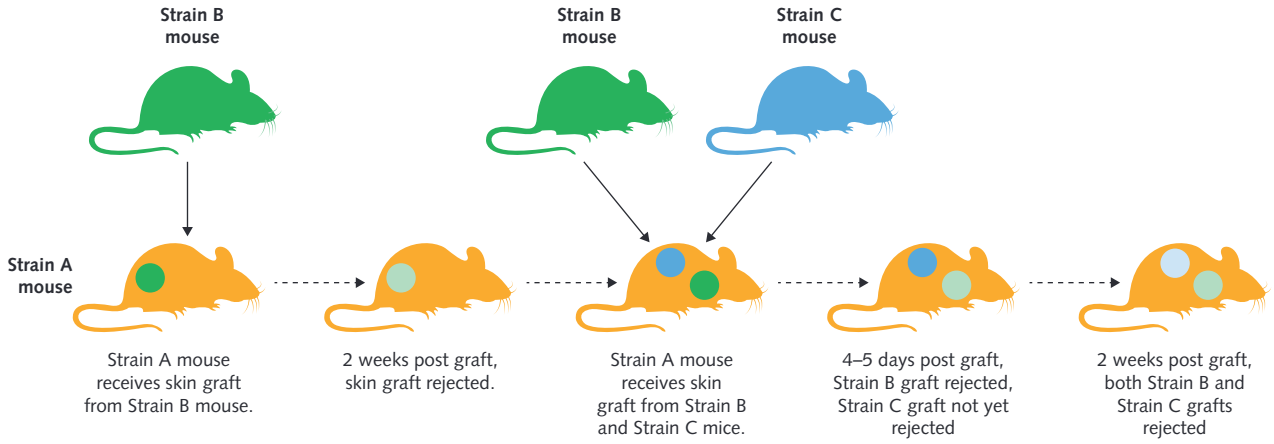

Figure 7.17 A graft-rejection experiment demonstrating that cell-mediated immunity displays memory

EXAM TIP
It is not a case of one type of pathogen causing a cytotoxic T cell response and another causing a B cell response. In most situations, both responses are initiated. A useful way of distinguishing their actions is that the humoral immune response is most effective against bloodborne antigens, whereas the cell-mediated response is most effective when the antigen is in the cell. Activation and participation by T_H cells ensures that each type of response is successful and carefully managed.

WB
7.3.2 ADAPTIVE IMMUNE RESPONSES PAGE 154

Humoral and cell-mediated responses work together

The immune system is a complex network of cells that rely on one another to function properly.

B and T cells share a number of features, which are summarised in Table 7.3. They both have a system for generating a diverse range of receptors for different antigens, and they rely on clonal selection to allow for proliferation of relevant clones. Both B and T cells form effector and memory cells.

Table 7.3 Three major groups of lymphocytes: B cells, T_H cells and T_C cells

	B cells	T_H cells	T_C cells
Development of self-tolerance	Occur in bone marrow	Occur in thymus	Occur in thymus
Antigen recognition	Recognise antigens not presented in MHC	Recognise antigen in MHC class II	Recognise antigen in MHC class I
Undergo clonal selection	Yes	Yes	Yes
Effector functions	Plasma cells produce antibodies, which attach to the antigen and mark it for destruction	Production of cytokines to aid B cell, T_C cell and macrophage functions	Releases cytotoxins directly into infected cell which induces apoptosis destroying infected cell and infecting virus
Formation of memory cells	Yes	Yes	Yes

The adaptive immune functions of lymphocytes are distinguished from the cells of the innate immune system by specific recognition of antigens and the ability to exhibit memory. The adaptive and innate systems are closely interlinked and do not operate in isolation. Communication between the cells of these systems is critical for the functioning of both. The following list and Figure 7.18 summarise some of the major connections.

» Antigen presentation by macrophages and dendritic cells allows T cells and B cells to recognise antigens.
» Full activity of T cells and B cells requires cytokine production by APCs that have recognised a PAMP or a DAMP.

» The binding of antibodies to pathogens can activate complement directly and promote phagocytosis by cells of the innate immune system.
» Phagocytosis is also promoted by cytokines produced by T_H cells.
» Following the destruction of cells by T_C cells, phagocytes play a role in 'cleaning up' the cell fragments produced.
» T_C cells release cytokines that promote destruction of phagocytosed antigens.

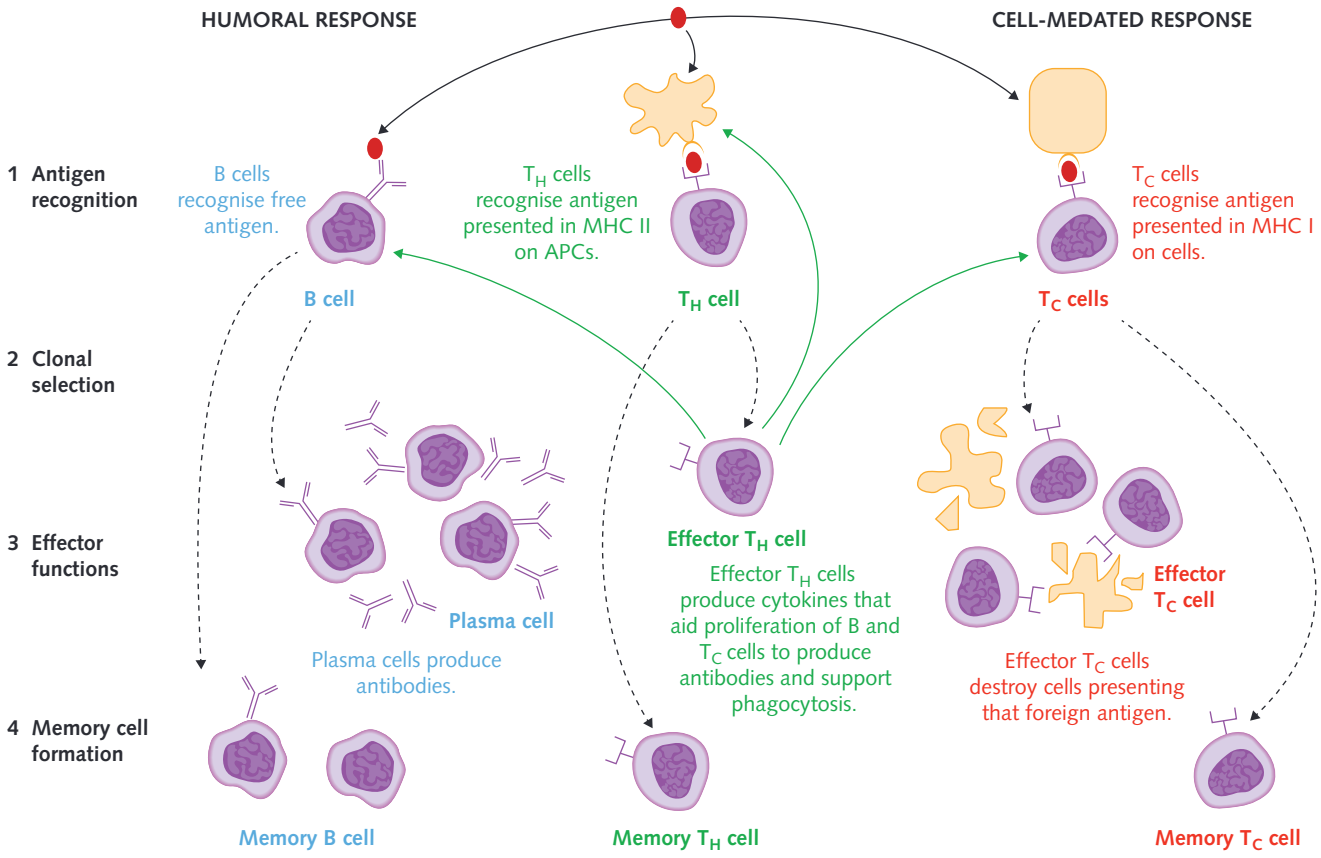

Figure 7.18 A summary of the actions and functions of the cells of the adaptive immune system

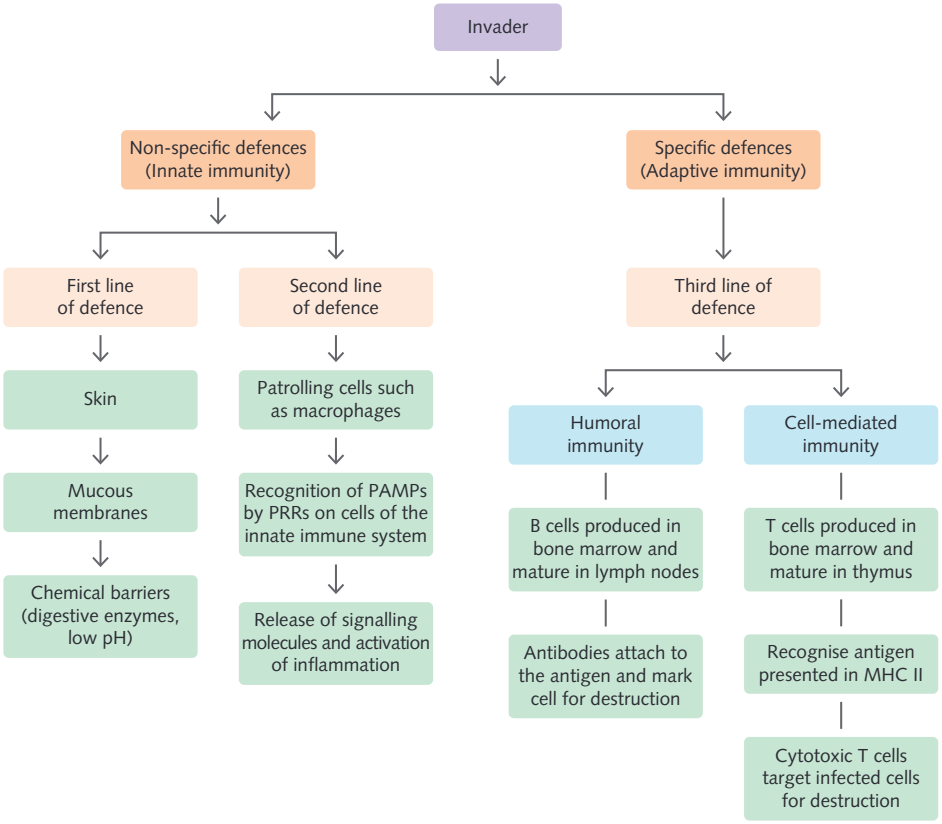

Figure 7.19 The three lines of defence in the human immune system

ACTIVITY 7.1

Adaptive immune analogies

Am

To deelop a set of aalgies fordi fferent parts of te immune system

Youll need

» Pen
» Paper

What to do

1 Workin with a parter,brai nstorm an anaogy for each of the parts of the mmune system sted beow. Be cretive and try to thnk of unusua deas For exampe an an aogy for vaccne s A vaccne s ke a tra exam n a tra exam exposure to quetions tains a student to perform better on the real exm.Sila ry, exposure to an ntign in a vaccne trans the body to respond more rapdy and effecivly to the real atig.'

» Atibody
» APC
» T_H ce
» T_C ce
» MH cls Imolecule
» MH cass I molecule
» Phagocyte
» Vacine
» lasma ll
» Cy okine
» Lymphocyte receptor

2 iscuss youristsin groups of three or fur. Howell does each anaogy work? Are ther e mtatons? Decde among the group on the anaogy that best fits each term
3 Present your grups list to the rest of theclas. Youcould vo te and have a prze for the best anaogy.

What dd you dscover?

Reflect on whether these anaoges have hep ed your understandng of the adaptve mmune system Make a st of thngs that have become cearer as a reult of his execse.

KEY CONCEPTS

» T_C cels scanpeptides presentd o MHC class I mlecles. When they etect foeign peptides, wth th helpof signals from T_H cel, they secrete granzymes andperin to kill the affted target cell.
» T_H cels scanpeptides presentd oMHC class II mlecles. They express contac-endent signalling mlecules an cytoknes to help activate T_C cels and B cel.
» Th innate and dative immune systems are nterinked with man connections at are essential for properimmune respones.

Concept questions 7.3

1 Wich of h following statements are true for T_H cels and hich are true for T_C ces?
   a Reconise ntigens presentedbyMHC class I moecues
   b Undergo lnal election
   c Destro cells by producng cytotoxic proteins
2 Describe ho the roles naral killer cells and T_C cels arediffernt.
3 The adaptive immunesystem s often described as haing memo. Expain wat this means, using T_C cels as an xmple.
4 Lst three ay in whih the innae and adaptive mmune systems comuniate.
5 Exlain how T_H cels help many oter cell of the immune system fight off invaders andmount an imune response.

HOT Chaenge

6 T_{eg} celsiiiate respones witin the immune response tomantain hoostasis. T_{eg} cels used to be known as suppressrcells. Te mechanism of acio is not ell understod and is an area of active reseach,eecially in the fight against cncer. But the outcome of thir act on contols the magnitude and durtion of the immune espone The following st is of some of theknown functions of T_{eg} ces Choose two and etermine why tes functions might be usful in homostasis and at what stages of the mmune response tey miht act.
   » Produtionoinhibitoy cytokines
   »Indution o vaious cell typs to synthesise nterlekin-10
   » Prodution of grazm B, which induces apoptsis of effetor cells
   » Reverse gnalling hrough dirct contact with dendritc cells
   » ialling through the production of mmunosuppresive adeosine

WB
7.4 ACTIVE AND PASSIVE IMMUNITY PAGE 156

7.4 Active and passive immunity

When the body is infected by a pathogen or stimulated with a vaccination, the memory T and B cells produced will be activated rapidly if that antigen is encountered again. This kind of immunity, which produces memory cells, is known as **active immunity** and generally lasts many years, although the immune system may need booster shots periodically to enhance its army of memory cells.

Passive immunity occurs when antibodies are provided from an external source (Table 7.4). These externally sourced antibodies will provide protection from the pathogen, but only for as long as those antibodies last. Because there are no plasma cells or memory B or T cells, the person will not be immune if they encounter the pathogen again.

Table 7.4 Examples of active and passive immunity

	Active immunity	Passive immunity
Natural	Exposure to a pathogen, resulting in memory cells	Transfer of antibodies from mother to foetus through the placenta Transfer of antibodies from mother to baby through breast milk
Artificial	Vaccination, resulting in the production of memory cells	Anti-venom Antibodies against particular pathogens (e.g. rabies) Mix of antibodies for immunodeficiency

Natural and artificial active immunity

Natural active immunity develops when an organism comes into contact with a pathogen and develops memory B and memory T cells to the antigens of that pathogen or its products. In the development of natural immunity, the contact with the pathogen occurs naturally. This is the typical way humans gain immunity to pathogenic organisms. Antibodies are made after exposure to infection, giving long-term immunity.

CONNECT
Vaccination is discussed in more detail in Chapter 8.

Artificial active immunity develops after a vaccination. Vaccinations introduce antigens (weakened or dead microbes or their fragments). The body produces specialised lymphocytes (B plasma cells) and antibodies. Memory B cells are produced. This type of immunity is long term because of the production of memory B cells.

Natural and artificial passive immunity

With natural passive immunity, the person does not produce their own antibodies, but receives them from another source. This means they have no memory B cells; hence, it is only short-term immunity. Natural passive immunity occurs when antibodies pass from a mother to the foetus through the placenta and to the baby during breastfeeding. These antibodies, which are mainly IgA antibodies, are essential for protecting a newborn or very young baby from pathogens. A baby is most vulnerable to infection two or three months after birth because its own immune system is not yet fully developed and the antibodies it received from its mother through the placenta have disappeared.

Artificial passive immunity also occurs when antibodies come from another source. In some cases, there is insufficient time for antibodies to be produced actively by the patient before death or serious injury occurs. In such instances, a dose of antibodies targeted to a specific antigen is administered directly to the patient. For example, the anti-venom given after a snake or spider bite is a solution of antibodies against the venom.

Solutions of antibodies can also be used to prevent the development of disease in someone who has been exposed to a pathogen. Rabies is a viral disease that is spread in the saliva of infected animals. Untreated, rabies is always fatal once symptoms start because the immune system cannot produce

a response quickly enough. However, the development of symptoms can be prevented by quickly administering antibodies against the rabies virus if somebody has been bitten by an infected animal.

Rarely, people are born with or develop a condition in which they cannot produce enough of their own antibodies. As a result, these people are highly susceptible to infections. A condition where the immune system does not function properly is called an immunodeficiency. A way of treating this type of immunodeficiency is to give the patient a mix of antibodies from healthy donors. This treatment is called IVIG (intravenous immunoglobulin). This will only provide protection for a short time, so these patients will need antibody infusions every month or so. This type of immunity is short term.

Preparing purified antibodies

Despite having many of the world's most venomous snakes, Australia has few deaths from snake bite. This is partly due to the availability of anti-venom treatment. Anti-venom is a solution of antibodies that are targeted against the venom.

In order to use antibodies for anti-venom or to protect against disease, solutions of antibodies need to be produced. One way to prepare a pure sample is to initially inject the specific antigen into a host such as a rabbit or a horse. This induces the animal to produce antibodies, which are secreted into their bloodstream. These are then extracted for use (Figure 7.20). This process is costly and time-consuming, and the purification of the sample is difficult.

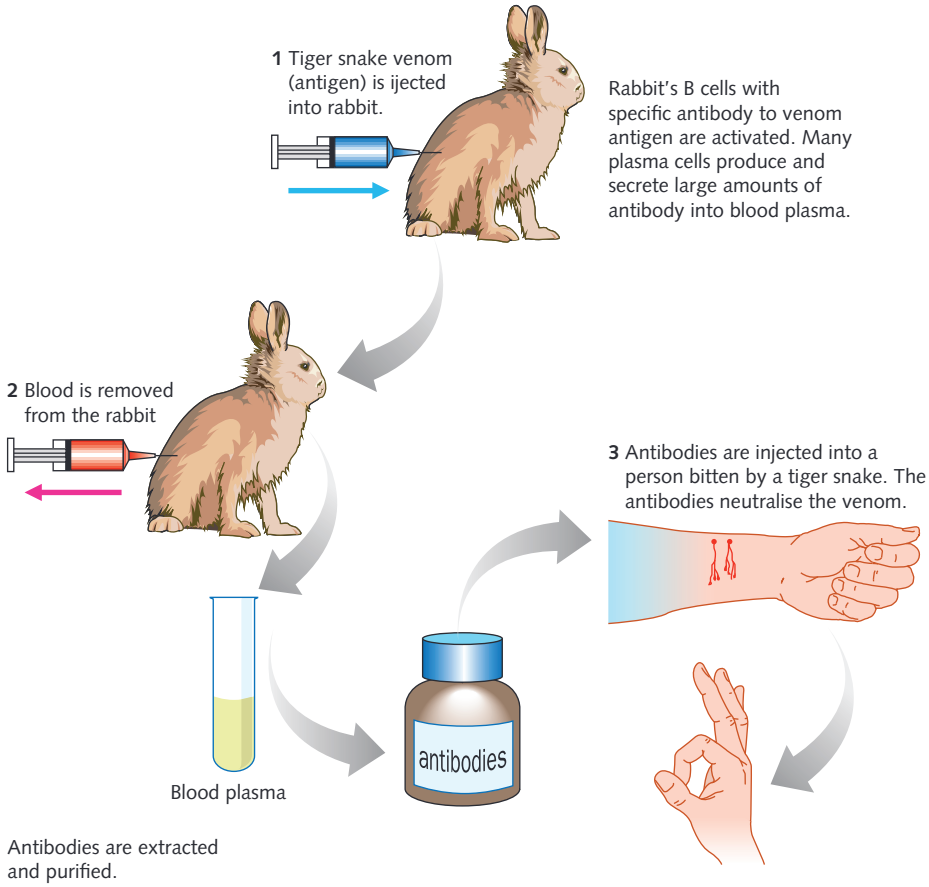

Figure 7.20 Anti-venom for a tiger snake bite can be produced by collecting antibodies from a rabbit that has been injected with small amounts of venom.

KEY CONCEPTS

- The protectio provided y antibdies may be passive or acive. Onlyactive imuniy provides long-term protectionagainst pthogens.
- Examples o pssie mmnity include transfer of antibodies from mother o baby and anti-venom to treat snaeite.
- Natualimmnity is when the bod produces its own antibodies or they are passed to a baby through the placenta or breat milk.
- Ar fitial imuity is whn vaccination occurs or antibodiescreated inaothr animl are injected into the bdy.

Concept questions 7.4

1 Describehowactive immnity is acquired through vaciaion.
2 Explain why brst milk can confer advantages to a babys immune system tha baby formula cannot.
3 Discuss why the roduction anti-venom is usualy costly.
4 Ouline whypasiv imunity asts only about 28 days.
5 Why d infections suchas rabies ned treating with antibodies from another sourc ather than relying sely on natural ative immune responses?

HOT Chaenge

6 gA antibodies in dult mammalsprotet the internal surfaces of hedigestve ystem, incuding the mouth, stomach nd ntestines, and the srfces of the lungs. gA antibodies are passed rm mother to child.
   a How are they passed?
   b What tye fimmuity is this?
   c What aspects of this process protect newborns?
   d Does this procesconferlifeong immunity?
   e Afterbirth, maintenance o omeostasis in the gut depends on ealthy iteractions between commensal gt mcrobiot and the immune system of the iividual. Research whether IgA antibodies ibreastmilk fo he mother initiate felon intstinal hmeostasis.

BRANCHING OUT

The lowing pres releasewas released on 2 January 2020.

Doherty Institute scientists first to grow and share Wuhan coronavirus

Sintists from The eter Doherty Instiute for nfectin and Immunity (Doherty Institute) in Melbourne have succesfully grown theWuhan cornavis ro a ptientsample hich ill provide expert international laboratories with crucial nforation tohelp combat the virus.

Ti is the firsttimethe virus ha bengrown in cellculture outside of China.

The Roya Melboure Hosital' D Jlian Druce, Virus Identi fication Laboratory Hed a the Doherty Institute, said tis was signi ficant breakthru s it wil allow accurate investigaion and diagnosis of the virus globally.

Chinese of ficial released the genome sequence o tisnoe cornavirus,which is helpful for diagnosis; however, haing tereal virus means now havethabilit toactuall validate ad verify all test methods, and compare their sestiities ad speci ficities t will be a gae chnger fordiagnosis,' Dr Druce said.

Th vis will beused a potive conrol materia forthe Ausralin network of public health laboratories, and also sipped to expert labortres workin closely with the Wrld Health Organization (WHO) in Europe.'

DrMike atto, Deputy Diretr f the Doherty Institute, sid that pssesion f a virus isolte extended what could be ahieedwith moleclar technology in the fight agaistthis virus.

The Dohry Institut-grown virus is expectedto beusd o geneate an antibody test, which allows detection of the virs i patienswo haven't displayed symptoms and er therefore unaware they had the virus.

An antibodes will enabl us to retrospectively test suspeted patients so we can gather a more accurate picture of how idespread e virus is, and consqently, amng ote things, the true mortality rate,' said Dr Catton.

It ll also assist in the assessmt ofefectiveness of trial vaccines.'

Thevirus was grown fom a atien sample thatarrived te Rya Mebourne Hospital's Victorian Infectious Diseases Reference aboratory VDRL) a te Dohety Institute on Friday, 24 January.

We'e planne forn incidet like this for may, manyyrs and that's really why we were able to get an answer so qicl,' said D Catton.

Dr Caton also credited t succes to Astrala' netwk of laoratories and public health authorities effectively working together.

We are vry pleased at how it has coe togeter and are adwe we able to respond quickly, which we will coninue do.'

The Peter Doherty Institute for Infection and Immunity

Questions

1 Uing his press release as n example, comment on the nterntonal cooperation thatis ncesary in science.
2 Elain wh the virus had toberow in cell cult ure. You may need to refer to Chaptr 6 to helpyou answer.
3 What did Dr Druce mean whn e said '… hvng the real virus mns we nohae the ability to actually validate and verfy all test method, an omare thir sensitivities and speci ficie …'?
4 'Th virwill be useas postivcontrol mateial …' What does this statement mean?
5 The grow virwill be used to generate an antibody te s. sing yourknowledgeof vial infectin nd antibodies, explain wha thi means.

Online key concepts
Chapter 7: Summary of key concepts

7 Summary of key concepts

7.1 Adaptive immune response – third line of defence

KEY CONCEPTS

p 249

- llblod cells are producedfom stem cells in the bone marrow. ome leukctes eside in lymphoid organs and others crclate n the lood andlymphatic system. Others are residen in the tissues.
- Primary ymphoid rgans include the bone marrow and thyms, where lls ofthe im mune system are produced and matre.
- Secondrylymphodorgns inclue lmph nodes, spleen and mucoal-sscitd lymphid tissue (MALT), where mmune responses initiated and carried out.
- Th lymphoid stm, including te scondary lymphoid tissues, is a transpor system linking inate and adaptive mmune responses.
- The adaptive immune response diffrs from the innate mmune response be ause it as speci fic recontion of antigens an displas memory.
- B lls, T_H cels and T_C cels are th main players of the adapive immune system. Theadaptive immune response rees on theseells detcting foregn antigens and dsiguishing the rom self.
- T_{eg} ces are cells tha control the manitude and duration of immune respons to li mt damage to bdy tssues.
- Atigens aremolecules that can generate an immune response. The particular molecular structures on antigens that are recognised by components ofthe immune sysem re alled epitopes.
- The majo histocmtibiity comlex (MHC) is an mportant wyof dstiguishing sf from non-self.
- MHC retriction refers to te ftthatT cells will only recognise epitopes when they are presented on an MHC mlcule.
- MH ca s molecues prnt intracellular antigenan are psenton all ncleated cells (self-markers).
- MH cass I molecules prsent extracelular antigensderived from phagocytosis and are present on antigen-presenin cells (macrphags denitic cll and B cells).
- Each B ll and cell hasan antigen rcepto that is speci fic for a unque epitope, so t epopulation of ymphocytes has the capacity to detect vast range of antigens.
- Lymphoctes with antigen receptors that could recog nse self-components are eleed or inctivated during their dvelopment.
- Drig ineton, nly the lymphocytes bearing a re ceptor that can econise epitopeson the invading pathogen ar activatd an roliferate, inthe roes of clonal selection.

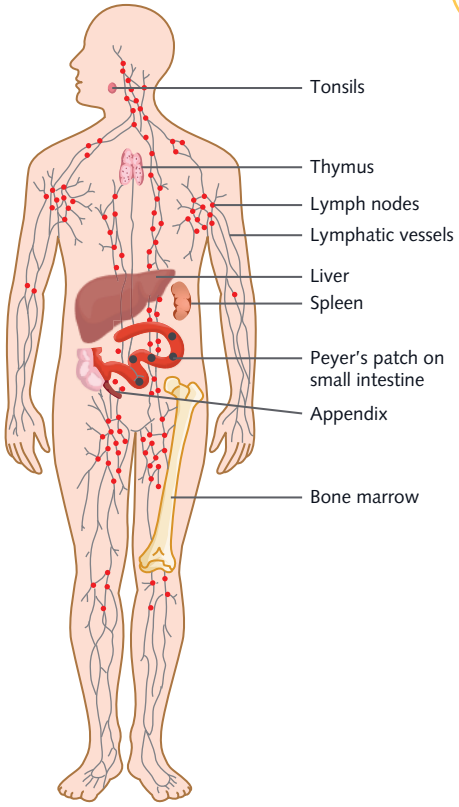

Figure 7.3 The location of organs and tissues involved in the lymphatic system in the human body

7.2 Humoral immunity

KEY CONCEPTS

p 260

- Atiodies are present on the surface of B cels as the cell receptors aresecreted into ciruationby diferentited cells called pasma ls.
- Atiodies functiontrogh agglutination, opsonisaion,eutralisat on and complement activaion.
- Atiode exst in different forms that have dfferent speialiefunctions.
- For B ll ctivatio to occur, the same antigen must activate T_H ces alowing them to proide contact-depnnt signalng for B cells and secrete ytokins thattimulates B cell surivl, activaton adproliferation.

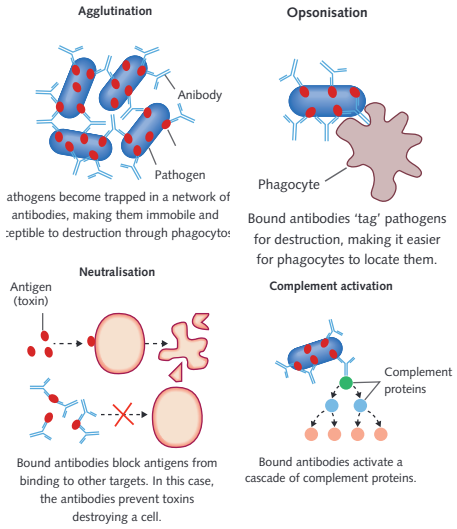

Figure 7.11 Antibodies can cause the destruction of pathogens in four ways: **a** agglutination, **b** opsonisation, **c** neutralisation and **d** complement activation.

7.3 Cell-mediated immunity

KEY CONCEPTS

p 264

- T_C cels scanpeptides presentd o MHC class I mlecles. When they etect foeign peptides, with the help of signals from T_H cel, they secrete granzymes and perorin kill the affecte target cell.
- T_H ces scan peptides presente d on MHCclssII olcules. They express contact-dpedentsinaling molecules and cytkines to elp ativate T $_C$ cels and Bells.
- Th innate and dative immune sysems are interlinked wth many connections thare essential for proper mmune responses.

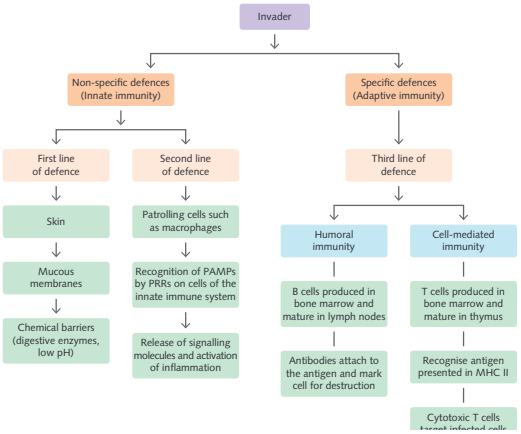

Figure 7.19 The three lines of defence in the human immune system

7.4 Active and passive immunity

KEY CONCEPTS

p 270

- The protectio provided y antibdies a be passive or active. Oly atve immuniy rovides lon-tem protection against pathoens.
- Examples o pssie mmnity include transfer of antibodies from mother to baby nd anti-venom o reat snakebite.
- Natualimmnity is when the bod produces ts own antibodies or they are passed to a baby through the paenta or breast milk.
- Ar fitial imuity is whn vaccination ocrs or antibodies created in anothe anmal ae injected into the body.

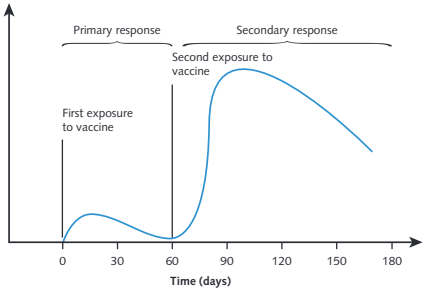

Figure 7.21 Response to vaccination against tetanus

WB
7.5.1
KEY TERMS
PAGE 158

7 Chapter glossary

active immunity when, after vaccination, memory cells are created that provide immunity against further exposure to antigens

agglutination when antigens or pathogens become stuck together because of antibody binding

antibody a Y-shaped protein that binds to foreign substances that invade the body; also called immunoglobulin

antigen-presenting cell (APC) a cell that displays peptides derived from processed antigens on major histocompatibility complex class II molecules for presentation to T_H cells; can be B cells, macrophages and dendritic cells

autoimmune disease a disease caused when a person's immune system mistakes self-cells and tissues as non-self and initiates an immune response against them

B cell a class of lymphocyte that, once activated, produces antibodies; also called a B lymphocyte

B cell receptor a surface-bound antibody that serves as a receptor so that B cells can detect antigens

B plasma cell a cell that originates in the bone marrow and produces large quantities of antibodies

bone marrow soft tissue found inside some bones that contains stem cells that produce cells of the immune system

cell-mediated immunity an immune response initiated by cells, which does not involve antibodies

clonal selection the process in which lymphocytes that have bound to an antigen divide rapidly and become more numerous than other clones

cytotoxic T cell (T_C cell) a class of lymphocyte that destroys virally infected or cancerous cells by secreting proteins that result in the extrinsic pathway of apoptosis; also called a cytotoxic T lymphocyte

epitope a small part of a larger molecule that binds to a receptor site such as B cell receptors and T cell receptors

helper T cell (T_H cell) a lymphocyte that assists cytotoxic T cells, B cells and macrophages by secreting cytokines and providing contact-dependent signalling; also called a helper T lymphocyte

humoral immune response an adaptive immune response mediated by antibodies

immune having resistance to infection by a specific pathogen

immunoglobulin (Ig) a Y-shaped protein produced by plasma cells that binds to a specific antigen; also called antibody

interleukin a subset of cytokines that assists with the coordination of cells involved in the immune response

interstitial fluid a fluid that lies between cells; also known as tissue fluid or extracellular fluid

lymph a colourless fluid that originates from tissue fluid

lymph node an immunological organ in which antigens are trapped or delivered by phagocytes to present to lymphocytes and initiate an adaptive immune response

lymphatic system a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and removing foreign matter

major histocompatibility complex (MHC) protein markers found on cell surfaces that are important in distinguishing self from non-self; MHC class I is found on all cells and MHC class II is found only on antigen-presenting cells

memory cell a long-lived lymphocyte capable of responding to a particular antigen when it is reintroduced; made from B cells and T cells

MHC restriction refers to the fact that T cells can only recognise antigens that are presented on MHC proteins

mucosal-associated lymphoid tissue (MALT) an extensive system of lymphoid tissue deposited all over the body; initiates immune responses along mucosal areas such as gastrointestinal tract, eyes and lungs

neutralisation the process by which antibodies prevent toxins from acting by binding to them and blocking them from binding to their targets

passive immunity immunity characterised by the transfer of antibodies from one individual to another; does not generate immunological memory

primary lymphoid organ the bone marrow and thymus; responsible for the production and maturation of immune cells

primary response the response generated when an antigen is encountered for the first time; contrasted with the secondary response

regulatory T cell (T_{reg} cell) a class of lymphocyte that helps to negatively regulate the immune response; also called a regulatory T lymphocyte

secondary lymphoid organ an organ that provides an environment for the initiation of the immune response; includes lymph nodes, spleen and mucosal-associated lymphoid tissue

secondary response the response generated when the body encounters a pathogen to which it has previously generated an immune response; involves reactivation of memory lymphocytes and occurs more rapidly and with greater magnitude than the primary response

self-tolerance the deletion or inactivation of lymphocyte clones that can bind to self-antigens to prevent an immune response to these antigens

specific response an adaptive immune response directed against a particular antigen that retains immunological memory of that antigen

spleen an abdominal organ that stores white blood cells

T cell receptor a protein receptor found on the surface of T cells; binds to antigens presented on major histocompatibility complex proteins

thymus the gland in the upper chest where T cells mature

WB
7.5.2
EXAM PRACTICE
PAGE 159

7 Chapter review

Remembering

1 a Identify two functions of MHC proteins.
b What is the name of MHC in humans?
c Why is it important for T_C cells to recognise MHC I?
2 Recall where the following cell types undergo their development.
a T cells
b B cells
3 The ability to distinguish between self and non-self antigens is crucial to the functioning of the immune system.
a Define 'self-antigen'.
b Outline how leukocytes learn to distinguish between self and non-self antigens.
c Discuss the problem that can occur if the immune system responds to self-antigens.
4 Draw a diagram comparing the amount and speed of antibody production in response to an antigen after the first and second exposures.
5 List the different ways that antibody binding can inhibit pathogens.
6 a Describe the role of T_H cells.
b Identify how they are able to perform this function.
7 List three ways that dysfunction of the immune system can cause disease, giving specific examples.
8 Identify whether the following statements are true or false.
a Immunodeficiency can be inherited or acquired.
b An autoimmune disease is one where the immune system attacks the body's own cells.
c People born without B cells can mount a fully functional adaptive immune response against a virus.
d Vaccination against measles is an example of natural active immunity.

Understanding

9 Describe what is meant by clonal selection, using B cells as an example.
10 Millions of different antibodies can be made by our B cells, even though our genome has only about 30 000 genes. Explain how this can occur.
11 Present an argument as to why passive immunity does not display memory.
12 Draw a diagram to illustrate one way that antibodies can be produced for commercial uses.
13 Distinguish between:
a natural and artificial active immunity
b natural and artificial passive immunity.

Applying

14 Liver, heart and kidney transplants are fairly common surgical procedures. However, recipients of these transplants face the problem of rejection of these organs.
a Explain why the immune system rejects these organs.
b Transplant patients are usually prescribed immunosuppressant drugs to prevent transplant rejection. Many immunosuppressant drugs work by interfering with DNA synthesis.

i Suggest a negative effect that these drugs may have on the health of the patient.
ii Explain how a drug that interferes with DNA synthesis can prevent transplant rejection.
iii A patient with kidney failure was successfully 'cured' with a kidney transplant from his identical twin brother. He was concerned that the doctor did not prescribe immunosuppressant drugs. Are the patient's fears warranted? Justify your response.

15 The Australian death adder (*Acanthophis antarcticus*) has one of the most dangerous bites in the world. The active component of the venom is an alpha-neurotoxin that binds to the receptor sites for acetylcholine (a neurotransmitter molecule). Paralysis of muscles results and death can occur when the muscles of the diaphragm become paralysed and breathing is prevented.

a What type of substance is acetylcholine?
b Describe the function of acetylcholine at a cellular level.
c Explain how the alpha-neurotoxin prevents acetylcholine from working.
d Fortunately, an anti-venom is available that, if injected quickly enough, prevents the paralysis. Anti-venom is prepared by injecting tiny amounts of snake venom into a horse over a long period of time. The amounts of venom injected are so small that the horse is unaffected; however, there is a response by the horse's immune system.
i Name the substances the horse would produce to counteract the snake venom in its body.
ii Name the cells in the horse that would be responsible for the formation of this substance.
iii Explain why small amounts of venom are injected into the horse over a long period of time.
iv Outline the steps involved in the formation of these substances.
v After 10–12 months, blood is extracted from the horse and the plasma can be injected into snakebite victims. Identify the term given to the use of horse plasma as a treatment for snakebite.
vi Explain how this is effective in treating the snakebite victim.

16 Immune thrombocytopenic purpura (ITP) is an autoimmune disease in which platelet counts drop very low. Patients may develop bruising, rashes and, in extreme cases, severe internal bleeding. Antibodies against platelet surface markers can often be found in the bloodstream of patients with ITP.

a Recall the role of platelets.
b Explain how the formation of anti-platelet antibodies may lead to the symptoms described.
c Platelets from blood donations can be given to patients as a transfusion. Predict whether or not these would be effective at preventing symptoms in patients with ITP. Justify your response.

17 Explain how the body's ability to distinguish between self and non-self is important in the development of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis.

18 HIV infection is caused by a viral pathogen.

a What are the initial symptoms in the primary stage?
b If left alone, a person can continue to be infectious as they move into a secondary stage. T_H cells are deleted from the immune response. What effect does this have on the adaptive T cell and B cell responses?
c T_C cells remain robust in their response, and it was found that up to 19% of T_C cells in infected patients were specific for HIV. Yet, the virus continues to spread in the bloodstream. Why does the viral progression continue if there is a good response from cytotoxic T cells?
d Sometimes a person might not realise they have the disease until it has progressed to AIDS. What does AIDS mean and how are opportunistic pathogens often involved that lead to the death of the individual rather than the HIV?

19 Figure 7.21 shows the response to two different doses of a vaccine against tetanus.

- **a** Explain the body's primary response.
- **b** Explain the trend shown following the second dose of antigen.
- **c** Copy the graph and add a second line that shows the expected response if the same person was exposed to a first dose of a vaccine against diphtheria at 60 days.

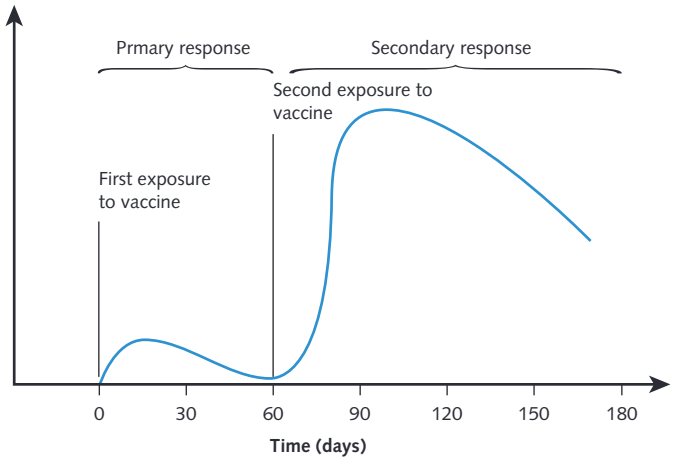

Figure 7.21 Response to vaccination against tetanus

Analysing

20 Compare the roles of the humoral and cell-mediated immune responses with regard to the type of pathogen targeted and how pathogen destruction is brought about.

21 Compare and contrast the MHC class I and MHC class II molecules.

22 Figure 7.22 is a graft-rejection experiment that builds on the one in Figure 7.17 on page 266. The aim of this experiment is to determine if the memory that the immune system exhibits with regard to graft rejection can be transferred between individuals.

- **a** Name the components of the immune system that are responsible for graft rejection.
- **b** Identify which part(s) of the blood (plasma, lymphocytes or both) would be expected to contain these components.

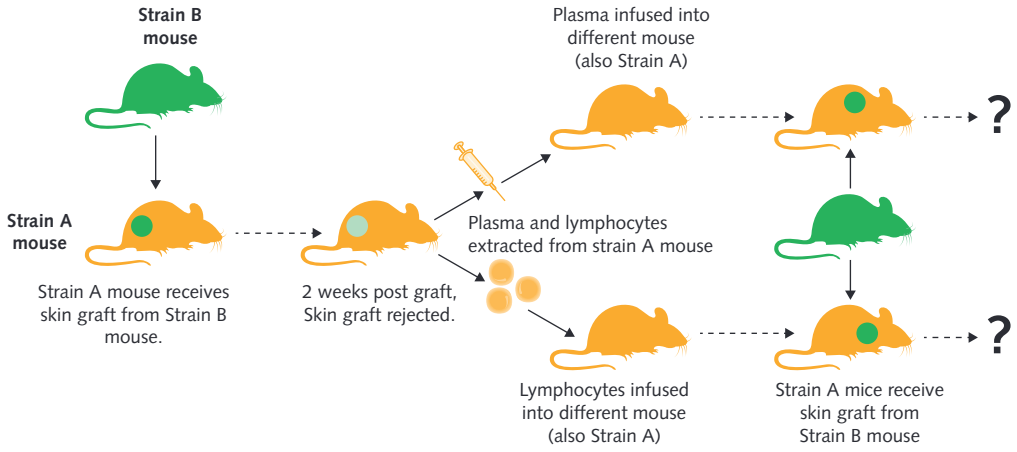

Figure 7.22 Experimental set-up to see if immune memory can be transferred between individuals

c Predict how long it will take each mouse (the one that has received the plasma and the one that has received the lymphocyte infusions) to reject the skin graft. Explain your reasoning.

d In this experiment, the infused lymphocytes are not rejected by the recipient's immune system. Explain why this is the case.

23 A mutation in a single gene on the X chromosome can prevent B cells from maturing. This causes the condition known as X-linked agammaglobulinaemia (XLA) in which patients produce extremely low levels of antibodies.

a Predict whether XLA is an immunodeficiency or an autoimmune condition.

b Draw a line graph to show the normal response to first and second exposure to a vaccine. Add a line to show the response to the same vaccine you would expect in somebody with XLA.

Evaluating

24 The adaptive immune system is sometimes described as more 'sophisticated' or 'important' than the innate immune system. Evaluate whether either or both of these adjectives is appropriate.

Creating

25 Draw a diagram that shows all the different defences encountered by an antigen, such as that associated with the influenza virus, when it enters the body. Indicate how these different defences communicate.

Disease challenges and strategies 8

By the end of this chapter you will have covered the following material.

Key knowledge

Disease challenges and strategies

- the emergence of new pathogens and re-emergence of known pathogens in a globally connected world, including the impact of European arrival on Aboriginal and Torres Strait Islander peoples pp. 285–290
- scientific and social strategies employed to identify and control the spread of pathogens, including identification of the pathogen and host, modes of transmission and measures to control transmission pp. 290–302
- vaccination programs and their role in maintaining herd immunity for a specific disease in a human population pp. 302–305
- the development of immunotherapy strategies, including the use of monoclonal antibodies for the treatment of autoimmune diseases and cancer pp. 306–310

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify independent, dependent and controlled variables in controlled experiments pp. 298–299; 300–301
- predict possible outcomes pp. 298–299; 300–301

Plan and conduct investigations

- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 298–299; 300–301

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 298–299; 300–301
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 298–299; 300–301
- demonstrate ethical conduct when undertaking and reporting investigations pp. 298–299; 300–301

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 298–299; 300–301
- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 298–299; 300–301
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 298–299; 300–301

Analyse and evaluate data and investigation methods

- process quantitative data using appropriate mathematical relationships and units, including calculations of ratios, percentages, percentage change and mean pp. 298–299; 300–301
- identify and analyse experimental data qualitatively, handling where appropriate concepts of: accuracy, precision, repeatability, reproducibility and validity of measurements; errors (random and systematic); and certainty in data, including effects of sample size in obtaining reliable data pp. 298–299; 300–301
- identify outliers, and contradictory or provisional data pp. 300–301

Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement pp. 300–301
- discuss relevant biological information, ideas, concepts, theories and models and the connections between them pp. 300–301
- analyse and explain how models and theories are used to organise and understand observed phenomena and concepts related to biology, identifying limitations of selected models/theories p. 296

Online Chapter Map
Chapter 8 map

8 Disease challenges and strategies

Sometimes, new pathogens such as SARS-CoV-2 emerge and old pathogens re-emerge to challenge living things. These pathogens can travel around the world so fast that they can quickly become in pandemic proportions.

p 290

82
Strateges empoyed to contro pathogen transmsson

n order to control apathogen, we must understand how t s transmtted Governments and heat h departments work together to contrl spre ad through a poultion and between popuato ns Canceng pubc events shuttng schoo s and enforing soil dstancng and quarntine are some of the ways to mtgate spre ad Vgant hygene and heath educaton are lso key n changng beha vours to ad nfecton contr.

p 285

81
Emergng and re-emergng pathogens

Sometmes the goba connectedness works aganst the human popuaton Human mob ty carres new and re-emeging pathogens around thewold in just one pane journey.In ths day and age we can earn from istor, partcuary the effects of whte coonsaton an d the dseases such as smapox scaret fever and meases that coonsts ntrodu ced to Aborgna and Torres SratIslander Peopes n Austraa

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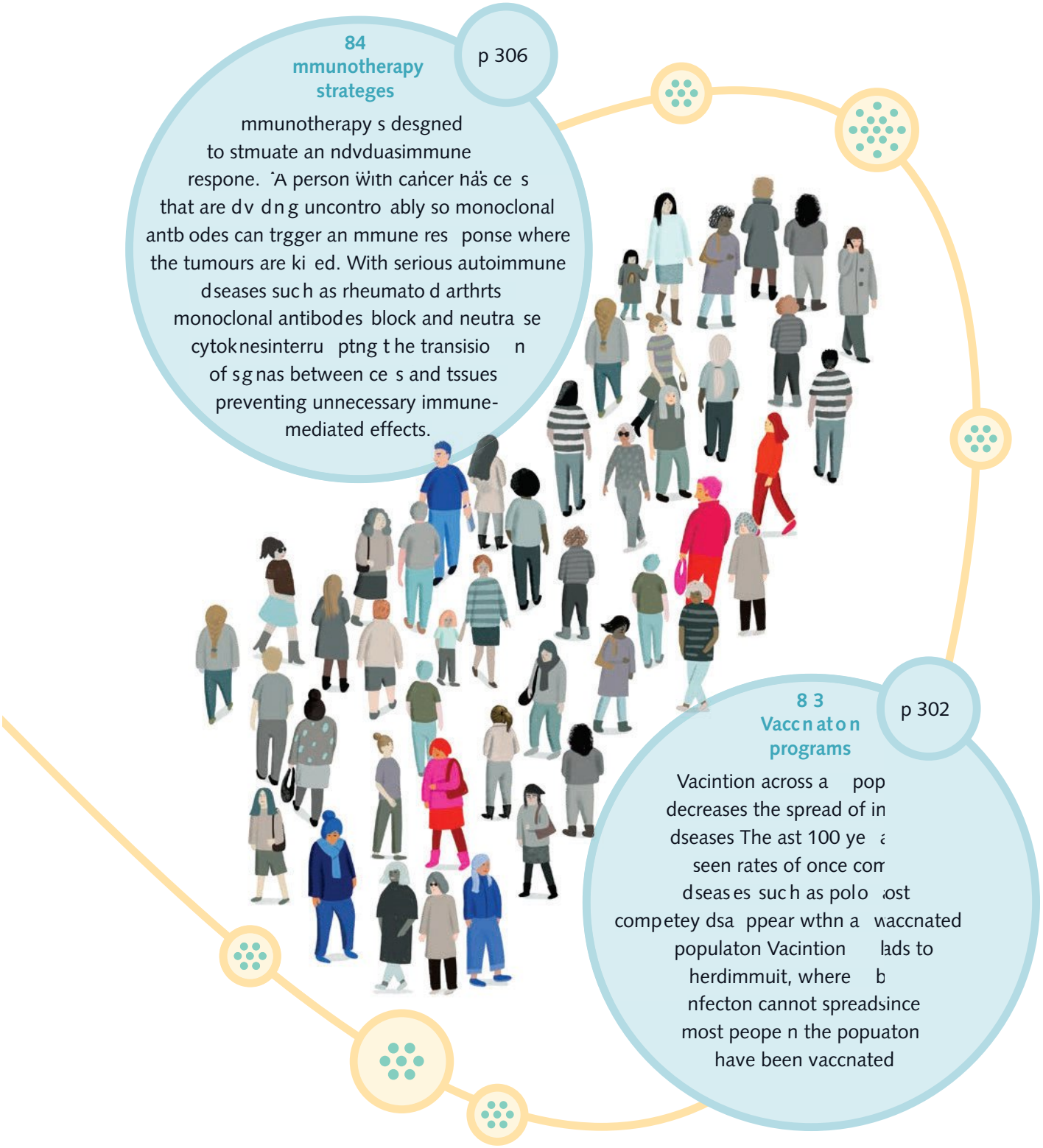

Understanding of new pathogens is increasing rapidly; so is our ability to react to them both on a social and scientific level. Communication with, and education of, the community concerned plays a large part in combatting new and re-emerging pathogens.

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Online Chapter Mapv
- Chapter 8 map (p. 282)

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- Play and say (p. 284)

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- Use the interactive lab simulator to investigate the spread of disease (p. 296)
- The flu vaccine explained (p. 302)
- How does herd immunity work? (p. 303)
- Watch a video of T cells killing cancer cells (p. 306)

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- The flu vaccine explained (p. 302)
- How does herd immunity work? (p. 303)

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- Chapter 8: Summary of key concepts (p. 314)

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Chapter 8 Flashcards

Know your key terms

autoantibody	herd immunity	latent	sporadic
autoimmune disease	hybridoma	monoclonal antibody	T-cell transfer therapy
cancer	immunisation	pandemic	tumour
carrier	immunotherapy	primary host	vaccination
endemic	infectivity	quarantine	virulence
epidemic	Koch's postulates	secondary host	zoonotic

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

WB
REMEMBER
PAGE 162

1 Diseases can be infectious or non-infectious.
2 Infectious diseases are caused by pathogens, which include viruses, bacteria and protozoa.
3 Antigens stimulate an immune reaction. Antibodies are produced in response to antigens.
4 Cytokines are cell signalling molecules that stimulate immune cells to move towards the site of infection or inflammation.

The term 'coronavirus' encompasses a large family of viruses that cause diseases, from the common cold to much more severe diseases such as SARS (severe acute respiratory syndrome), MERS (Middle East respiratory syndrome) and COVID-19 (coronavirus disease). Coronaviruses can be transmitted from animals to humans (**zoonotic**). SARS was found to be transmitted from bats to civet cats to humans, and MERS from dromedary camels to humans. It was later discovered that the viruses could also be transmitted from humans to humans. In December 2019, a novel coronavirus outbreak was detected in people who had been in a fish market in Wuhan, Hubei Province, China. It spread rapidly and led to shutdowns of whole cities, factories and airports, and travel bans from China in an effort to restrict contact with infected individuals. By the end of January 2020, deaths from coronavirus were recorded in the USA, Thailand and Japan, and cases were reported in the US, Nepal, France, Australia, Malaysia, Singapore, South Korea, Vietnam and Taiwan. By mid-February 2020, it had infected 45 171 people worldwide with more than 1360 deaths on mainland China alone. By end of February 2021, it had infected more than 114 million people, killed more than 2.5 million, and was continuing to spread uncontrollably around the world.

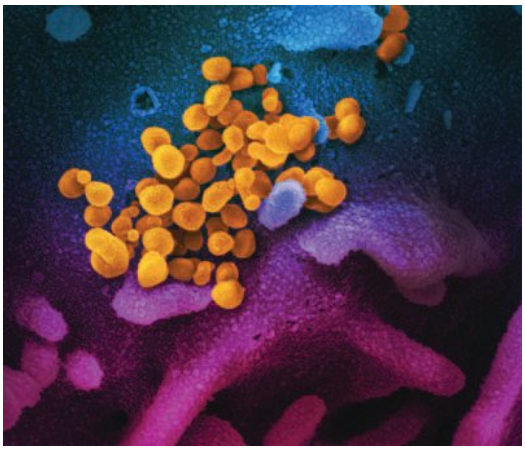


Figure 8.1 Researchers at Rocky Mountains Laboratories in the United States imaged samples of the virus and cells taken from a US patient infected with SARS-CoV-2, the pathogen that causes the disease COVID-19, using two different kinds of high-resolution microscopes – a scanning electron microscope and a transmission electron microscope. The virus has been artificially coloured yellow, and patient cells are purple and blue.

8.1 Emerging and re-emerging pathogens

Diseases that are caused by pathogens are:

- **sporadic** if they are only seen infrequently and in a small number of people; for example, the occasional cases of rabies that occur in western USA when people are in contact with infected rats
- **endemic** if they are consistently found in certain regions; for example, malaria, which is endemic to tropical regions
- **epidemic** if there is a rapid spread of the disease across a number of countries and a large number of people are affected; for example, the Ebola outbreak in 2018, which spread across the Democratic Republic of the Congo and Uganda
- **pandemic** if pathogens become worldwide threats to health; for example, the spread of Spanish flu in 1918 with deaths estimated at around 50 million people (1% of the world's population) and the COVID-19 pandemic starting in late 2019.

EXAM TIP
Make sure you know the difference between sporadic, endemic, epidemic and pandemic.

Emerging pathogens

Emerging pathogens are pathogens that were previously undetected, unrecognised or unknown; or they could be known pathogens that have spread to new locations. Emerging pathogens can be viruses, bacteria or protozoa. They cause infectious diseases in human populations and cause significant public health issues. Recently, emerging viral pathogens have caused significant deaths from diseases such as SARS, MERS, Ebola, chikungunya, swine influenza, avian influenza and COVID-19.

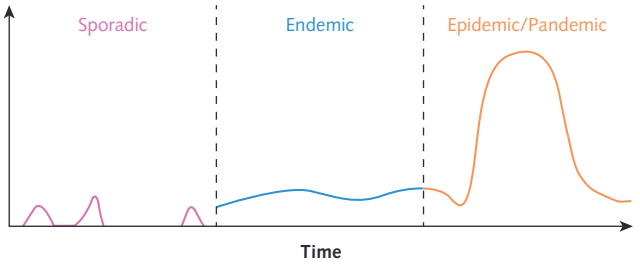


Figure 8.2 Within a population a disease may occur sporadically, at an endemic level, or at an epidemic/pandemic level.

Re-emerging pathogens

Re-emerging pathogens are pathogens that have caused disease in the past and are now reappearing. Since the number of infections has dropped, the impact of the disease on public health has diminished. The pathogen reappears and spreads into a population without immunity (naïve) and increases to epidemic proportions. Re-emerging pathogens cause diseases such as measles, pertussis, cholera, tuberculosis, malaria, gonorrhoea, syphilis, pneumococcal disease and influenza.

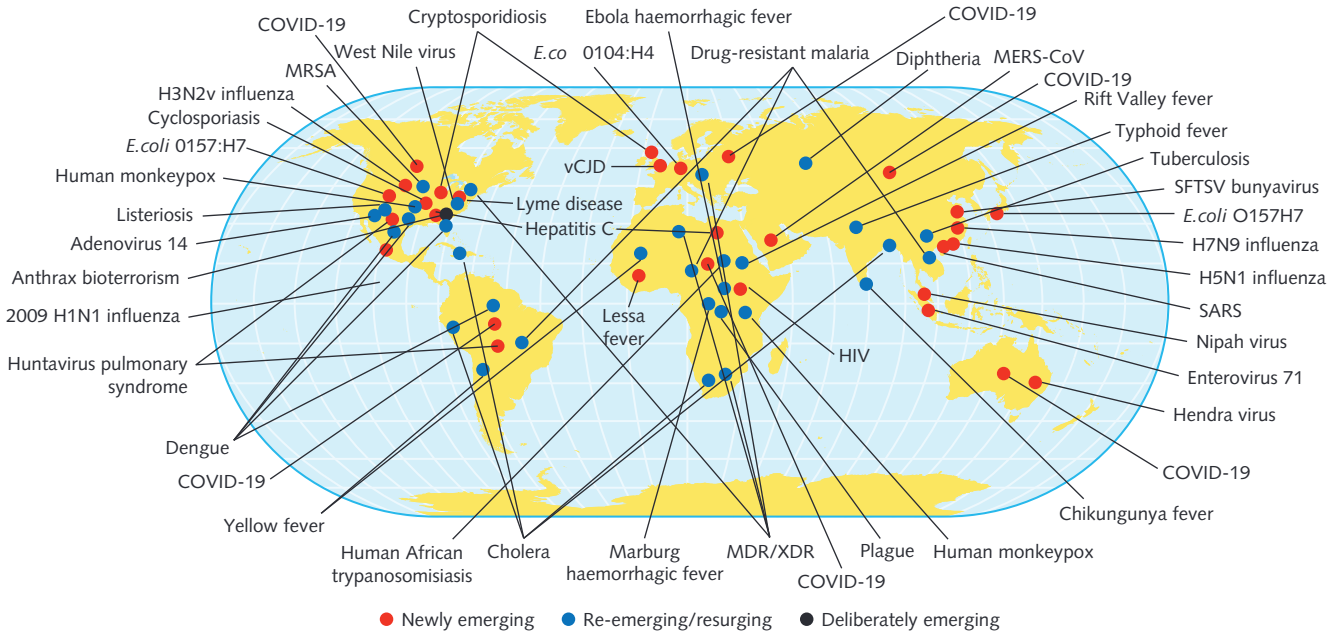


Figure 8.3 Examples of emerging and re-emerging pathogens causing infectious diseases around the world

WB
8.1.1 SPREAD OF PATHOGENS IN A GLOBALLY CONNECTED WORLD PAGE 163

Spread of pathogens in a globally connected world

On 31 December 2019, Chinese authorities alerted the World Health Organization (WHO) to a number of cases of pneumonia. The cause of the pneumonia was unknown. All 44 patients were connected in some way to the Huanan seafood market in Wuhan, China. On 1 January 2020, the seafood market was closed down for environmental sanitation and disinfection. On 9 January 2020, the first death was reported in China. On 13 January 2020, the first case of COVID-19 was reported in Thailand. The Thai patient had no connection with the Huanan seafood market. A similar story was reported in Japan on 16 January 2020. By mid-February, the coronavirus SARS-CoV-2 had spread across the world (Figure 8.4).

Pathogens can be spread by close contact of animals with humans, or humans with humans. In an era of rapid global movement and increasing population numbers, it is inevitable that the number of such contacts will increase. People are far more mobile than they were even 50 years ago, with interstate and international travel possible in under 24 hours. Planes, trains, ships and cars now move people between towns, cities and even continents in a matter of hours. When the incubation period of many infectious diseases is longer than 24 hours, a person can travel between Melbourne and London before they start showing any symptoms. In an effort to curb the import of highly infectious pathogens, the Australian *Biosecurity Act 2015* compels the operator of an international aircraft or vessel to report any unwell passenger who shows signs and symptoms of an infectious disease.

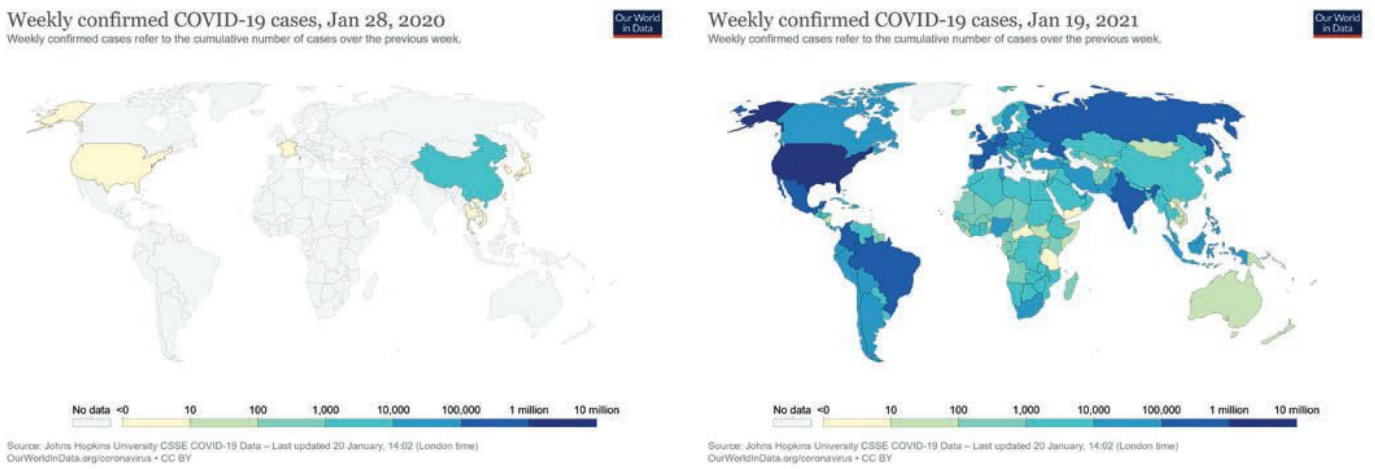


Figure 8.4 a **The number of reported cases of coronavirus (SARS-CoV-2) from Wuhan Province in China from 28 January 2020 to b across the world by 19 January 2021**

The rapid spread of SARS-CoV-2, the pathogen that causes the disease COVID-19, was a major priority for decision makers during the outbreak in 2020. Countries such as Australia shut their borders initally to non-Australian citizens returning from China. This prevented hundreds of thousands of international students returning to Australia for the start of the academic year. Following this, Australia only allowed Australian citizens and a few exceptions to return, and also to undergo quarantine for two weeks upon arrival. The cruise ship *Diamond Princess* spent many weeks held under quarantine off the coast of Yokohama, Japan, after a passenger from Hong Kong travelled for five days on the ship before disembarking in Hong Kong and presenting with COVID-19 six days later. All on board the *Diamond Princess* were immediately tested for SARS-CoV-2 and 10 were found to be positive for the virus. Two days later, 41 new cases were reported, then after three more days, 66 new cases were confirmed. Three thousand passengers and crew aboard the *Diamond Princess* were forbidden to leave the ship and were mainly confined to their cabins in an effort to halt the spread of the disease among those on board and at the port where the ship would eventually dock. However, the effectiveness of the quarantine measures on board was questionable because almost 300 people on board had contracted the virus by mid-February 2020.

Figure 8.5 **Efforts have been made to screen passengers arriving from international ports for symptoms of infectious disease.**

Although those on board were held in a situation of high risk, the virus was prevented from infecting people on land in Yokohoma. Arrival of a new infectious agent into a naïve population can have devastating consequences and is a pattern that has been repeated throughout the course of human history.

WB
8.1.2 IMPACT OF EUROPEAN ARRIVAL ON ABORIGINAL AND TORRES STRAIT ISLANDER PEOPLES PAGE 167

Impact of European arrival on Aboriginal and Torres Strait Islander peoples

For tens of thousands of years before Europeans arrived in Australia, Aboriginal and Torres Strait Islander peoples had a healthy lifestyle, a well-balanced diet with varied food sources, and an intricate knowledge and use of natural medicines. They collected and stored water and cultivated native plants by agricultural methods that produced bountiful amounts of food to sustain populations through the seasons. Indigenous people developed sophisticated means of herding and harvesting kangaroos and other native fauna, including eels (Figure 8.6) to be captured for food. Well-respected cultural traditions and spiritual rules meant that clans did not have to settle in one site to protect crops and they could move to plant and tend crops with the rotation of the seasons. There was no overcrowding for prolonged periods, which reduced accumulation and exposure to natural waste and prevented transmission of infectious diseases by the faecal–oral route.

Figure 8.6 UNESCO World Heritage Listed Budj Bim eel traps

Aboriginal and Torres Strait Islander peoples wore body coverings made from animal products and woven materials. The clothing only covered part of their body, which allowed their skin to receive ample sunlight, which helped keep surface bacteria in check. In such a geographically isolated country as Australia, the diseases present among Indigenous populations before the arrival of the Europeans were chronic diseases, such as heart and liver diseases, rather than acute infectious diseases capable of resulting in epidemic outbreaks.

The First Fleet arrived in Sydney in 1788, bringing with it up to 1500 convicts, marines, seamen, civil officers and free settlers. More ships arrived in the following months and years, bringing British settlers, supplies and more convicts. Also on these ships was a multitude of infectious pathogens that had not previously been present in the land now known as New South Wales.

These pathogens caused diseases that devastated the Indigenous populations. Diseases that were common among the early European settlers were measles, smallpox, influenza, scarlet fever, chicken pox, bronchitis and the common cold, and they were deadly for Indigenous people. Respiratory and sexually transmitted diseases spread rapidly, as the lands and waters used by Aboriginal people became inundated with European settlers and animals carrying these diseases. With no immunity developed from previous exposure to these diseases, local populations were decimated.

Within 14 months of settlement, Governor Arthur Phillip estimated that 50% of the local Indigenous population had been affected. The Wurundjeri people of the Yarra region, which is now modern Melbourne, were also severely affected by the new diseases, which caused about 60% of the deaths of Aboriginal people across the Port Phillip area. Aboriginal clans around the Melbourne area felt the severe impact of European colonisation even before Europeans reached Melbourne. An earlier smallpox epidemic had spread south from Sydney and killed up to a third of the population of the eastern Australian tribes.

Traditional food sources became restricted as the settlers arrived, with land being fenced off and vigorously defended by settlers. Settlers claimed and defended water wells and killed native animals for sport. Indigenous crops and gardens were ravaged and destroyed by introduced animals such as sheep, cows, rabbits, foxes, cats, dogs and birds. These crops had always been managed sustainably with a 'take

one, leave one approach' combined with regenerative fire stick burning. The crops could not cope with the European style of grazing and were unable to regenerate. They also could not compete with noxious weeds, which grew from seeds carried into the country on animals and ships.

Native animals fell victim to diseases that came with the introduced species and were also preyed upon by exotic animals and birds, thus reducing their populations and availability as a food source for Aboriginal people. Many Aboriginal people starved, and others were forced to adopt a European diet. Aboriginal people started living much closer together in communities in smaller areas to have access to the available food. With poor sanitation and overcrowding, dysentery spread through the populations. The European diet was also heavily based on flour, refined sugar, offal and poor-quality grains and meats, quite different from the traditional high protein diet, making Indigenous populations more susceptible to diseases.

In the winter of 1847, an influenza infection swept through an Aboriginal camp at the place where the Merri Creek and Yarra River met. This epidemic devastated the camp inhabitants, causing many deaths and much grief, which led to despair and sadness among the survivors. There was little desire to live in the camp anymore, adding a further force driving Indigenous people from their homes. Indigenous birth rates plummeted, partly due to the introduction of syphilis, and partly because Aboriginal and Torres Strait Islander peoples did not want to have babies if the babies could not be fed and kept safe.

Figure 8.7 **The Indigenous diet was well balanced with varied food sources.**

KEY CONCEPTS

- » iseases that are caused by pathogens can be sporai, endmic, pidemic o andemic.
- » Emrging pathogns re previously unrecognised pathogens or pathogens that have sprad o new locations.
- » Re-emerging pathogens are pathogens that have cause disase in the past and are now reappaing.
- » Rpd global movement f humans, animals and other olgic mateia means it is inevitable that the spread of pathogens as inceased.
- » Pathogens brought into Astral a by European sttlers, such as he viruses thatcaued measles, smallpox and the commo old, wee deady or Indigenus peoples who had not been exposed to these pathogens before.

Concept questions 8.1

1 Tetaus is a sporaic dieas.Malaia is usually casi fied as anendmic iseas in Africa. In 2003, SARS (severe acte respiratory syndrome) was casi fied as an pdemcin Asia. The Spanish in fluenza outbreak of 1918 as classi fied as a panemic What is thedifference between ech classi fication?

2 Over te last50 years, about 40 pathogens have beenclssi fied as ausin emrgig diseases. The dsease include OVID-19, Zika irus disease, MERS, Ebla and swine flu State to mincriteria that may cause WH toclassify a pathogen as causing emerging dsease.

3 Re-eerging diseases are those that have previously beenclssi fied as conrlle, eradicatd or sporadic and therefore not aublcheath issue.Hw do the following factors ontribute to the re-eergence of disease?
   - **a** Antiiotc resistance
   - **b** Travel
   - **c** Destuctio of habitat
   - **d** Mutatons in pathogens

4 SA S avian flu, wine flu and COVID-19 (as of December 2020) arbelieved to have undergone cross-seciatio from animls tohumans. What is cross-seciation henconsdering disease?

5 n the ainstreammedia the folloing expressions are used whe disussng isease.
   - **a** RavageIndigenouspoulations
   - **b** Commnicable disease
   - **c** Fatten the curve
   - **d** Schols are Petri dish

   What dos mains reamediareally mean in each case?

HOT Chaenge

6 Lst thre impacts that Europea hd on Aboriginal and TorresStrait Ilner peoples. Discuss how each of thse impact contributed to the spread of emergig diseases amgst the Aboriginal and Torres Strait Isadr populations.

WB
8.2 STRATEGIES FOR CONTROLLING PATHOGEN TRANSMISSION PAGE 170

8.2 Strategies for controlling pathogen transmission

As the world was reeling from World War I, many nations faced a new serious threat. Soldiers in crowded quarters at the major staging and hospital camp in Étapes, France, and at least a dozen military camps in the USA, were succumbing to a deadly infectious respiratory disease, which physicians recognised as the flu. Reports of the infections were suppressed to maintain morale, but this allowed time for the outbreaks to spread. Once it reached the neutral country of Spain, the press could report on its devastation of populations, giving the perception that the infection began there. With military personnel travelling to new areas and returning home from war zones, and with more modern systems for civilian transportation, the 1918–19 Spanish influenza outbreak spread rapidly throughout the world. Strikingly, this strain killed healthy, young people along with the frail, and it killed up to 100 million people, many more people than the war itself. Despite intensive efforts to stop the disease spreading to Australia, the Spanish influenza outbreak killed about 12 000 Australians.

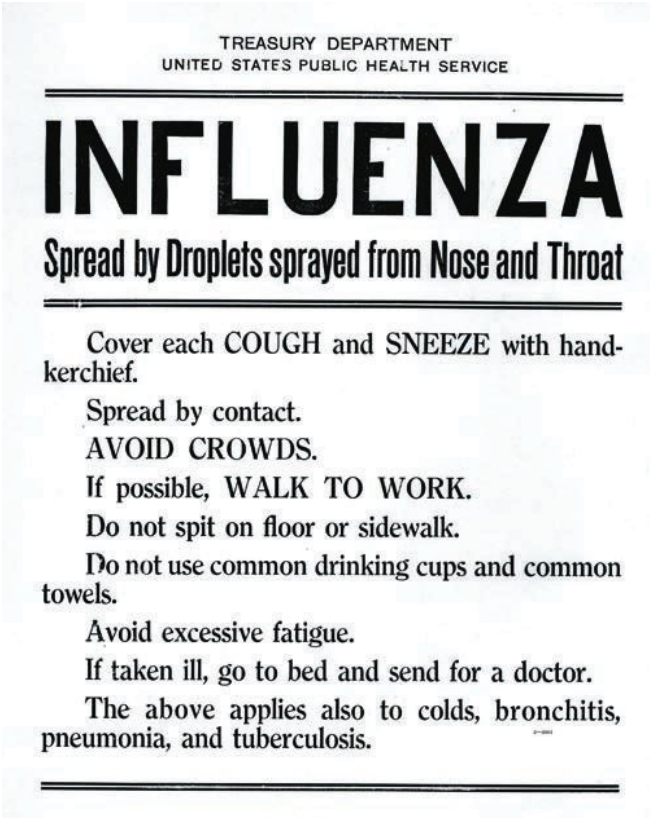
Figure 8.8 Military personnel returning home in cramped conditions from World War I spread the Spanish influenza among themselves and to naïve populations.

Several factors contributed to the rapid spread and made the outbreak so devastating. People were exhausted, stressed and poorly nourished as a result of the war, making them more susceptible to infection. The transportation of soldiers to many different countries around the world helped the rapid spread of the disease. War had disrupted normal healthcare programs, leaving countries unprepared and unable to effectively quarantine those infected. Additionally, Spanish influenza was deadlier than regular seasonal influenza, primarily killing otherwise healthy adults 20-40 years old. Even people with strong immune systems were susceptible. Many patients infected with influenza succumbed to the secondary deadly bacterial infection pneumonia, and many experienced a 'cytokine storm' – a massive systemic flood of inflammatory cytokines that overwhelmed the body and proved to be fatal.

Spanish influenza is an excellent example of how factors relating to the host organism, the pathogen, modes of transmission and the environment must all be considered when implementing measures to control the transmission of the pathogen. The hosts in which the Spanish flu strain flourished were highly susceptible adults affected by war. The pathogen, an avian-derived influenza strain, likely mutated to produce a deadly second and third wave of infections, following a relatively mild early first wave. The high concentration of individuals housed in training and staging camps and hospitals, and the global displacement and mobilisation of civilians and troops, produced ideal conditions for disease spread. The wartime environment was directly to blame for the extent of this pandemic.

Many important lessons were learned from the Spanish influenza pandemic. Today, major investments are made throughout the world to monitor and investigate new disease outbreaks. The primary goal of investigating a disease outbreak is to limit its spread and to prevent any more outbreaks (Figure 8.9). Once a country has identified a new outbreak, they must initiate immediate measures to control its spread, and begin to investigate its source, the nature of the infectious agent, and its pattern of transmission. The results of these investigations determine the implementation of longer-term control measures.

Figure 8.10 shows just how difficult it is to control viral spread in large populations. This figure shows the timeline



TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

INFLUENZA

Spread by Droplets sprayed from Nose and Throat

Cover each COUGH and SNEEZE with handkerchief.

Spread by contact.

AVOID CROWDS.

If possible, WALK TO WORK.

Do not spit on floor or sidewalk.

Do not use common drinking cups and common towels.

Avoid excessive fatigue.

If taken ill, go to bed and send for a doctor.

The above applies also to colds, bronchitis, pneumonia, and tuberculosis.

Figure 8.9 Control measures used to contain the spread of the 1918 influenza pandemic in the USA

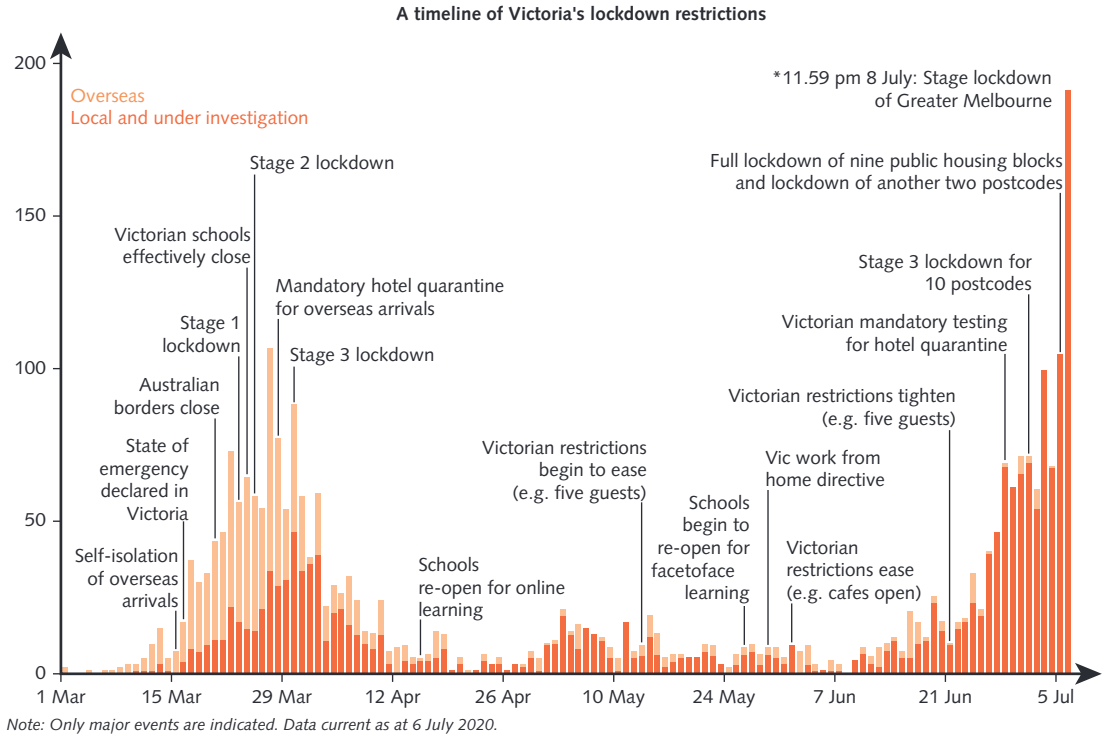


Figure 8.10 A timeline of Victoria's lockdown restrictions to manage COVID-19, March–July 2020

of new daily cases of COVID-19 in Victoria from March to July 2020 by source of infection as well as the lockdown restrictions imposed by the Victorian government. In the initial stages of infection, most of the cases were brought in from overseas. All arrivals into Victoria were quarantined in hotels. Victorian schools were closed in mid-March and Stage 3 lockdown was imposed on 30 March.

From mid-May, case numbers fell and restrictions began to ease, schools reopened for Term 2, cafes opened with restricted numbers and five guests were allowed to visit homes. Community transmission rose from this point to be the major mode of transmission of the virus. From mid-June, it was apparent that the daily number of new cases was increasing, particularly in 10 hotspot postcodes. These postcodes were locked down in early July along with nine public housing blocks. By 8 July, new cases went into triple digits each day and Victoria was well into a second wave of the virus. All metropolitan Melbourne locked down along with one regional shire on 8 July 2020 and came out of lockdown on 28 October 2020 after 112 days.

Identify the host

A host is the organism in which the disease-causing pathogen resides to gain nutrition and shelter, and a place for reproduction. Some pathogens can have many different hosts.

Ebola is a fatal haemorrhagic disease caused by viruses in the genus *Ebolavirus* (EBOV). An outbreak of Ebola virus occurred in West Africa in 2014 and there were more than 28 600 reported cases and 11 325 deaths. Death is due to multiple organ failure and tissue death. It is thought that the Ebola virus' natural **primary host** was the fruit bat and that the virus spread to **secondary hosts** such as gorillas, chimpanzees, antelopes and pigs when they either ate or were bitten by infected bats. Humans in close contact with the bodies (blood, organs and body fluids) of infected animals can become infected with the virus. It is thought that the 2014 outbreak started when a young boy from Guinea was infected by bats. Human-to-human transmission occurs with close contact with infected humans through broken skin, mucus or other bodily fluids. Corpses of Ebola victims have high viral loads and the local custom of funerals and burials accompanied by ceremonial washing and touching of deceased persons was a large factor in the spread of the virus.

Identify the pathogen

Researchers must be able to decide whether a pathogen is the cause of a disease. They do this by applying a set of criteria known as **Koch's postulates**. Koch's postulates enable researchers to isolate a suspected pathogen and demonstrate that this pathogen causes the disease under investigation. The postulates are as follows.

1. The suspected pathogen must be present in large numbers in all organisms suffering from the disease, but not in healthy organisms.
2. The suspected pathogen must be isolated from the infected organism and grown in pure culture.
3. The suspected pathogen should cause the disease when introduced into a healthy susceptible host.
4. The suspected pathogen must be recoverable from the inoculated organism from step 3, re-cultured and compared to the pathogen from step 2. If they are the same, then it is concluded that this pathogen has caused the disease.

Koch's postulates need to be modified when investigating viral diseases. Viruses cannot reproduce outside a living cell so they need to be grown in a cell culture (step 2). This can cause unintentional genetic alterations that change the way the disease presents in a new host (step 3). More modern techniques have now replaced Koch's postulates.

Ebola virus is usually diagnosed by its early symptoms of fever and headache, which can take up to three days before they are at detectable levels. These symptoms could be attributed to other diseases such as malaria. By the time the person develops more definitive symptoms, they may have spread the virus to other people.

It is important to give a diagnostic test for the early stages of Ebola infection as soon as possible. Polymerase chain reaction (PCR) (Chapter 3, p. 93) is the most commonly used diagnostic method. PCR can detect the presence of low levels of the virus in small amounts of blood. The ability to detect the virus increases as the amount of virus increases during an active infection.

Another diagnostic method is based on detecting antibodies that the body produces as a result of Ebola infection (Chapter 7, p. 260). This can be used to confirm a patient's exposure to the virus and then measures can be taken to reduce their risk of infecting others.

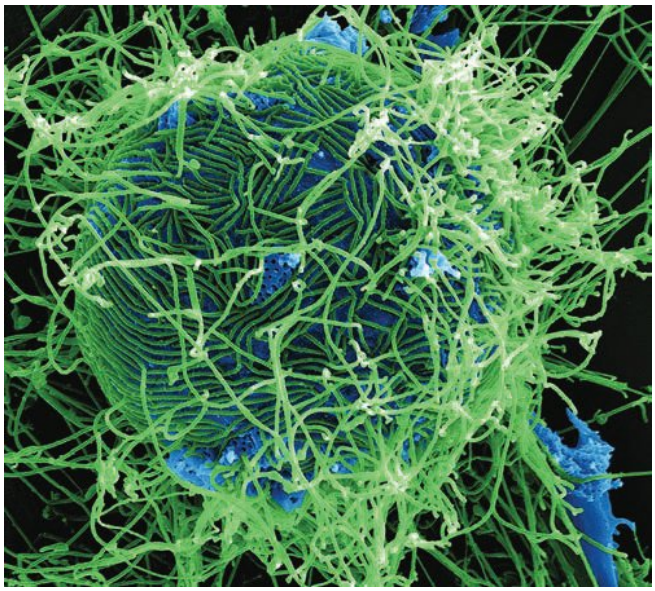


Figure 8.11 A cell (blue) infected with the Ebola virus (green)

Modes of disease transmission

The mode of transmission of a pathogen strongly affects its ability to spread within a population. To limit the spread of infectious diseases, it is crucial to understand how the pathogen is transmitted.

Geographic area

Some of the transmission methods restrict the spread of the disease to certain climates or geographic areas. Malaria is a disease caused by protists from the *Plasmodium* genus and transmitted between human hosts by the *Anopheles* mosquito, which acts as a vector or **carrier** of the pathogen. Malaria is found only in areas of South America, Africa and Asia that are near to the equator, which are areas where the *Anopheles* mosquito can live. By contrast, influenza can spread through most populations of the world because its mechanism of spread by droplet transmission (coughs and sneezes) does not depend on a vector or specific environmental conditions.

Groups within a population

Some modes of transmission mean that infections are more likely to spread in certain groups within a population. These are groups that have behaviours or risk factors that promote the spread of infection. Hepatitis C is a chronic viral infection that can cause cirrhosis (scarring) of the liver or liver cancer. It is spread by body fluid contact, particularly through contact with infected blood. Consequently, a high risk group is people who share needles and syringes to inject drugs. Historically, another group at risk of acquiring hepatitis C infection were people who require regular transfusions of blood products, such as those with haemophilia. Before 1990, blood donated for transfusion was not screened for viruses and hepatitis C transmission occurred when some patients received transfusions from infected individuals. Today, extensive screening means that the risk of acquiring infections this way is very low.

Infectivity

The transmission of disease is also influenced by a pathogen's **infectivity**, which is its ability to spread from one host to another host. Diseases with high infectivity, such as influenza, spread readily through a population. Infectivity is different from **virulence**, which is the capacity of a pathogen to cause severe disease within its host. For example, rabies kills all people who are infected with the virus once symptoms begin, making it an extremely virulent disease. Some pathogens with a high level of virulence may have low infectivity, and vice versa.

Once an individual has been infected with a pathogen, there are several possible outcomes (Figure 8.12). An infected host may be able to transmit the infection at several stages, including before they develop symptoms and when they are symptomatic. The different stages of infection can vary with each pathogen and can influence how an infection spreads within a population.

Latent infection

The persistence of a pathogen within its hosts can also contribute to the spread of the disease. Some pathogens may persist in carriers who can transmit the infection to others, but do not show symptoms themselves. This is the case with people who are HIV positive because their white blood cells are infected with HIV, but they show no symptoms. In some other diseases, such as tuberculosis (TB), the pathogen can survive within its host for

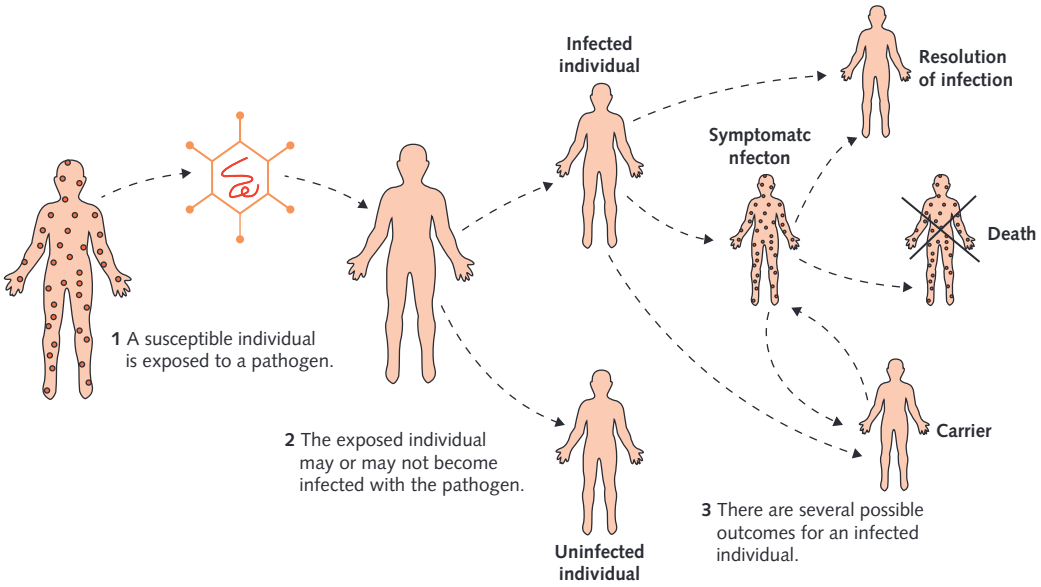


Figure 8.12 Exposure to a pathogen can have several different outcomes for an individual.

a long time before causing symptoms. TB is caused by the bacterium *Mycobacterium tuberculosis* and is spread by droplet infection. Individuals become infected by inhaling the bacterium, which settles in their lungs. At this stage, most patients enter a **latent** phase of infection during which they are asymptomatic (not showing symptoms) and not contagious. In about 10% of those infected, the disease can reactivate years or even decades later and cause symptoms. A period of latent infection such as this may be advantageous for a pathogen, allowing for its spread within a population over a longer time or into new populations as individuals move to new areas.

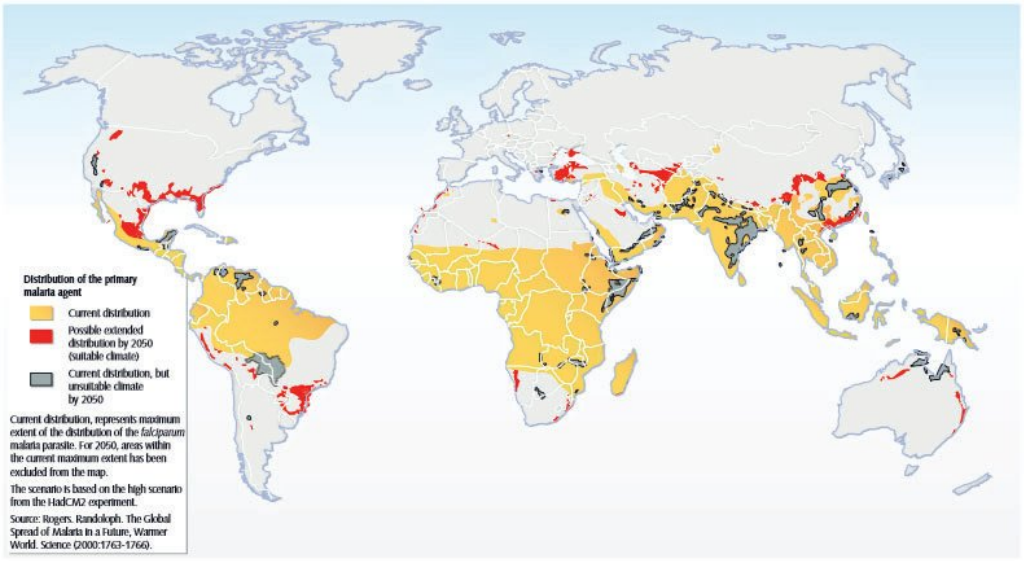
Environmental factors

A wide variety of environmental factors, including infrastructure and climate, can affect the spread of disease. The design and quality of infrastructure, such as water supply, roads and sewerage systems, can have a profound impact on disease transmission.

Communication and collaboration between scientists, doctors and politicians within and between regions, countries and continents are vital to help reduce spread, encourage and provide vaccination programs and aid in development of new drugs. Effective treatment of TB can prevent the spread of the disease but requires therapy with many drugs over a long time. In turn, this requires collaboration between drug manufacturers, supply networks, public health officials and healthcare workers. The spread of TB after the fragmentation of the Union of Soviet Socialist Republics (USSR) is an example of how infrastructure breakdown can result in the spread of infectious disease. Previously well-coordinated treatment programs became fragmented and countries of the former USSR could no longer ensure regular, constant supply of antitubercular medications. The fact that many patients received partial, but not complete, courses of treatment, led to very high levels of TB strains that are resistant to conventional drugs in countries of the former Soviet Union.

Some diseases, such as dengue fever, are particularly prone to transmission in urban environments. Dengue fever is a mosquito-borne viral illness that can cause fevers, muscle aches, headaches and rashes, and, in severe cases, death. The vector for dengue fever, the mosquito *Aedes aegypti*, is well adapted to living in urban environments and breeds in artificial water pools such as water tanks or buckets. This, along with a high population density in urban areas, contributes to the spread of this disease.

Changes in global temperature are predicted to have profound effects on ecosystems worldwide and to impact on human health in several ways. Models are used to make predictions about the possible spread of diseases under new conditions. Models help to predict changes within the already complex set of factors



UNEP/GRID-Arendal/Hugo Ahlenius

Figure 8.13 This map shows the predicted change in distribution by 2050 of *Plasmodium falciparum* malaria, as a result of climate change, based on modelling data.

that influence disease transmission. As global climates change, the geographic distribution of pathogens and vectors, and the diseases they carry, can change. Increases in temperature and changes in rainfall are likely to result in the spread of disease-carrying vectors, such as malaria-harbouring mosquitoes, into previously uninhabitable regions. Figure 8.13 shows the predicted changes in the distribution of malaria as a result of climate change.

Extreme climate events, such as tsunamis, floods and droughts, can also promote the spread of disease. In these situations, displacement of populations and the breakdown of sanitation, food and health infrastructures are major contributing factors. For example, outbreaks of diarrhoeal illnesses such as cholera, typhoid and hepatitis A can occur with both floods and droughts (Figure 8.14). During flooding, water supplies can become contaminated with human waste containing bacteria or eggs of parasites. In drought, the lack of fresh water can lead to breakdown in hygiene practices and promote disease spread.

Hosts

It is not only the characteristics of the pathogen and the environment that affect the spread of disease. The characteristics of the infected population and individual hosts are important too. A person's behaviour may increase their risk of becoming infected. For example, the risk of becoming infected by a blood-borne virus is increased by injecting drugs and getting tattooed. Risk of infection may also vary with age, sex or socioeconomic status. Once exposed to a pathogen, some hosts, such as the very young, elderly or chronically ill, may be more susceptible to contracting infections because their immune system may be less able to combat the pathogen.

The transmission of disease is complex and can be affected by a wide range of different factors. The large number of factors, as well as their interrelatedness, can make understanding the transmission of a particular disease difficult. Figure 8.15 shows how these factors are connected. Other factors also may affect transmission of each specific disease.

Getty Images/Casmir Oduor

Figure 8.14 An increase in diarrhoeal illness in Kenya was observed after extensive flooding in 1998.

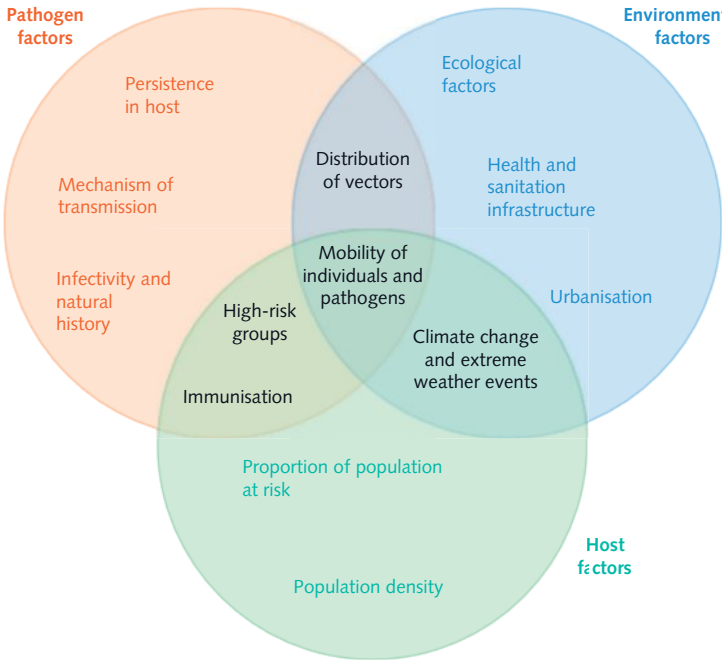


Figure 8.15 The transmission of disease is affected by a wide range of factors, many of which are interrelated.

ACTIVITY 8.1

Modelling disease spread

Modes are tols emloyed by epdemoogsts to preict the mpact of dfferent facto rs on dsease spre ad The dsease aboratory smuator ao ws you to modfy varous dse ase characterstcs (nf ectvty, mortity ra te and duraton of nfecton) popuaton de nsty and vaccnaton status and observe ther mpact on the spread ofdiseae.

Am

To eplore te impact of sever a varabes on dse ase transmsson

Use the interactive lab simulator to investigate the spread of disease.

What to do

1 Access the weink and carry out your own nvestgaton Ch oose a dsease and the smuaton parameters and then run the dsease What parame ters ead to a quck spread of dsease? How coud you change some parameters to sow the spread ofdisease?

2 The isk of another pandeic spreding to Austli is very ral Using what you hve leared i thisatiit, discuss how th is rsk s dfferent from 100 years ago ad idntify the pathoen, host and nvironmntal factors that cntribute t ths isk.

Controlling transmission of pathogens

Government organisations play a key role in controlling the transmission of pathogens. Transmission can be prevented by improving access to vaccinations (p. 302) and contraceptives, and by enabling access to health care, screening, counselling and education. In the case of epidemics or pandemics, governments can also limit freedom of movement in an attempt to control the outbreak.

Scientists who study disease transmission (epidemiologists) can design interventions to halt disease spread. The implementation of several interventions, such as hand hygiene and vaccination programs, has had an enormous impact on our control of communicable diseases.

Improved public education

Childcare centres and kindergartens have been educating young children about their health for many years. They have handwashing policies that enable young children to learn the technique and value of handwashing. Primary and secondary school children learn about the hazards of sharing drink bottles, the importance of healthy eating practices, drug education and sexual health.

The Australian Department of Health has devised the National Framework for Communicable Disease Control. Part of this framework is public education. In a country as diverse as Australia, this framework takes into account peoples' different sets of beliefs, values and attitudes, along with social and cultural norms and perceptions. Any health communication aimed at educating the public needs to tailor its message to maximise its success in raising awareness of health risks, improving health literacy and adopting good health behaviours.

Figure 8.16 A public health message on COVID-19

Public health care

Public health legislation mandates that anyone suffering from a notifiable disease must report it to their state health agency. The notifiable disease list is extensive and includes bloodborne diseases (e.g. hepatitis), gastrointestinal diseases (e.g. cholera), sexually transmitted diseases (e.g. chlamydia) and vaccine preventable diseases (e.g. diphtheria). In the event of an infectious disease outbreak, it is important to screen people to determine whether they have been exposed to the pathogen. This enables early intervention and quarantine to prevent further infection. Screening can be coupled with counselling, ensuring test results remain confidential or protecting people suffering from certain diseases from discrimination.

Physical distancing

A key strategy in the fight against COVID-19 has been the roll-out of public education campaigns promoting social distancing, which means maintaining a distance of 1.5 metres between individuals and a maximum of one person per 4 m^2 inside a building. Other strategies include working from home where possible, cancelling mass gatherings, and enforcing lockdowns in which people's movements are tightly restricted.

Face masks

The outbreak of COVID-19 in 2019 saw the sale of P2-grade face masks spike to the point where it was nearly impossible to source one. In July 2020, the wearing of face masks became mandatory for Victorians as part of further restrictions brought into play to reduce the spread of COVID-19 within the community.

Hand hygiene

Before the mid-19th century, the transmission of infection was not well understood. In hospitals, surgeons did not wash their hands and death rates from post-operative infection were extremely high. In fact, the contamination of a surgeon's clothes with bodily fluids was considered a sign of experience. A British surgeon, Joseph Lister, having read Louis Pasteur's theory that micro-organisms cause disease, hypothesised that preventing their entry may stop disease. Lister experimented with the use of carbolic acid to clean wounds and instruments, as well as handwashing, as a way of maintaining a sterile environment and preventing infection. These strategies proved successful in lowering post-operative infection rates and Lister's practices gained favour with other surgeons. Today, regular handwashing and the use of sterile equipment are considered key elements in effective health care.

SOUTHERN Biological Stress-tested by Southern Biological

INVESTIGATION 8.1

Effectiveness of alcohol-based antiseptic

Alcool-based had rubis widely usd in hospitals as an altern aive to soap ad water. To ue the hanrub, squirt a small amoun into th palm of your hand and rub your hands togeth so that the lquidcoveryour hands. The alcohol rapidly evaporats,leaving yourhands dry.

Am

To detemine whether hadwashing wih alcohol-based hand rub or handwshig with soapand wateris more effective n reduing the number of bacteria on hands

Materas

- lcool-based hand rub
- iuid soap (ot aibacterial handwash)
- inkwith water
- Paper owel
- 2 serile gar plates
- lear tape or Para film

What are the rsks n dong this investigation?	How can you manage these rsks to stay safe?
Micro-organisms will grow on the agar patesDo no	t open lates once they are securely tape. Dispose of plates appropriately after autoclaving.
Lqud soap or acoho-based ha nd rub may be rrtatng to peope wth senstve skn	f you know you cannot use on e of these product,inform your teacher or arrange to use the aternatve

Method

1. Wite a research question and a hypohsis bfore einning th investigation (Chapter 1, pp. 6 & 8).
2. Youll conduc this ivsigaion in pair. One person will use an alcohol-based hand rub an he other will use soap and water to wah their hands.
3. Lael your gar plate onthe underside with your name the date and your teatent.Lael one plate 'Before wahig' and th other 'Ater washing'.
4. Remove te lid fro the pae labele 'Before wasing' and press the palm of one hand firly on the gar, covering as much ofthe plat s ossib Replace the lid.
5. Fllwing teuidlines, wash yr hands itheither alcohol-based han rub or soap and water.
6. Without ouchin anything, reeat stp 4 using theopposi hand on th pate labelled 'After washing'.
7. Pace th plates upsidedown (agar ontop), seal with clear tape or Para fim an incubate them at 25°C fr 24 hours.

Resuts

1. Count the number f colonies on ech agar plate before and after hanwshing. Copy th results table into your ogbook, extending he table to show the nmber of pairs in yourclass. Record our result s n the table. Cmbine all class data to increas tesample size.

Resuts of nvestgaton c omparng acoho-based hand rub to soap and water

Par	Alcohol-based hand rub			Soap and water		
	Number of colnies before wasing	Number of clnies after wasing	Percentage redc ion in number of coonies	Number of colnies before wasing	Number of clnies after wasing	Percentage redc ion in number of coonies
1						
2						

Anayss of resuts

1 Clulate the mean percenae reduction in number of clnies for each teatment.

Dscusson

1 Compare the mean percetage reduction between the tw o treatmns. Is thre any difference?Do your results support your hpothesis?
2 dentify some tential sourcs of eror inth eperimental design.
3 Explain why youhave calculated the percentage redution in number of clonies, rather than comparing the number of coloies reaining for each treatment.
4 Do yo thinkthat it would be better to use the ame hand or the pposie hand for the control plate? Justify your response.
5 n hospitals,it is not just te ability of the treatm ent to reduce the number of acteria on hands that in fluences the trasmissio ofinectio Make a list of oherfactors that might in fluence whethe alcohol-based hand rubs or soap and water are mor effectve in eucig osptal-acquired infections.

Concuson

Draw a onlusion hat is cosistent wit h the vidence yo obtanedduin this inestigation and is relevant to the question undr inetigation.

Quarantine

You have already seen how the mobility of individuals can facilitate the rapid spread of infections such as SARS and COVID-19 around the world. **Quarantine** is a practice used to stop individuals who have been exposed to infectious diseases from carrying that disease into healthy populations. Potentially exposed individuals are kept from entering a healthy population until the incubation period of the disease has passed, and they can demonstrate that they are not infected.

From the mid-19th century, people arriving on ships to Australia from ports where certain infections were present were subject to quarantine. Luggage was fumigated and non-immune individuals were detained at quarantine stations, such as at Point Nepean in Victoria (Figure 8.17). People who contracted the disease were given appropriate treatment.

Figure 8.17 At one time, all immigrants to Australia had to pass through the Point Nepean Quarantine facility.

Australia has quarantine laws that give the government the power to exclude, detain and segregate people to prevent or control the spread of disease. The Australian government used these laws in 2020 to detain more than 200 Australian nationals who had recently been evacuated from Hubei province in China, the epicentre of the COVID-19 outbreak. They were flown to the Christmas Island detention centre, 2700 km off the coast of Western Australia, where they were placed in quarantine for 14 days, the incubation period of the virus. No cases of disease were reported during the quarantine period and everyone returned to their homes after two weeks.

SOUTHERN Biological Developed by Southern Biological

INVESTIGATION 8.2

Infectious disease transmission

nfectiou diseases can spread through seveal ways, such as di rect contat with an infecte person, ndirect contact via surfaces or objects, and irborne droplets when infected peop e sneee, cough orlaugh. Tetransmission of disease through droplets depends on ow close the infected person and potentialhos are becauset droplets disperse and settle quickly. The pathogens responsible for t ommon cold, in fluenza and OVID-19 re tyically transmittedthrough droplets in the air.

Local ealth deartments, WHO and he US Centers for Diseas e Control and Pevention a resposble for monitoring nfectiou disease oubreaks One of thei r resoibiltis is to identify the first person in population wh has the disease (paient zero) and then track routs o transmission. ver the ast 00 years, hese organisations, along with vaccine developmentand saitatin improeent, have effectiely fouhtthe pread of disease. Many of the infectious diseases that havehitoricall een resposil for devastating epidemicshav now been reduced or even eradicated.

Am

Tosiulae infctous diseasetranmision and identify patient zero

Time reuirement

45minutes

Materas

Per studet:

- latc pipette
- Screw-cap val contaning a soution representing a body flud
- Permanent marker
- Index card
- ispsabe gloves

Per lss:

- Phenol red indicator
- 4 × 96wll-plas lbelled 0, 1, 2 or 3

What are the rsks n dong this investigation?	How can you manage these rsks to stay safe?
The vials may contain sodium hydroxid, which can cause severe skin burns and eye damae.	Wear appropriate personal protectve equpment at a tmes ncudng eye protecion and g loves Wash skin immediately if contact does occur.
Disposable gloves can cause aleric rectios in snitive people.	Use a type ofglove that has n allergy ris andis uitable to use with the chemicals n ths nvestgaton

Method

1. Cllect an indx ad,plastic pipette a scew-ap vial containing solution.
2. There are fouwll plaes lbelled 0, 1, 2and 3. Locate your in dvdual ells on the caslwples. These will be ablled with the number crresponig to your vial.
3. Usng a ls ic ipette, transfer 5 drops of the flud from yorval into yur well n Plate 0.
4. Slect a partner for your first exchange. Record thei ame and vial numbe on your index card.
5. Usng you pati pipette, transfr5 drops from orial to your ptrsvial. Return any remaining liquid in your pipette to yor vil. Repce the val cap nd mx the solutio by nverting it several times.
6. Usng a lsic ipette, tranfr 5 drops of liquid fr om yourval into your coresondig wll on Plate 1.
7. Repeat steps 3–6 for the scond and thirdexchne and depsit your liquidinto your wells on Plates 2 and 3, respectively. Select a different partner for each roun and complete each step before proceeding to the next exchange.
8. Afterll exchanges hav been made, your teacher l add one drop of phenl re d to yourvl whch i an indicator sltion tht willdetrmine fyour vialas beome ‘infected’. Vials that turnred or pn are positive for the pathogen (infectin). Vials hat turn yellow are negative, wh chinicates thayur vial did not become infected.
9. Report whether yr vial teed poitive. If so, share the names of the partners you exchanged fluds wt.

10 Based on yorindvidual results and the data from your cl assmates, try ad idntify whch val the infection spread fro. Your teacheill add a drop of pheno red to each of th wells inhe wll plate. You may be able to trace the spread of infection to eoriginal source by obse ringwhichsample r positiv in each round.

11 Copy th resut tabl into your logbook and complete the tabl e to help you idntify the sourc of infection. Once you hav listed e positive vials andwh thy exchanged with, circle the numbers fte partners whose vials tested positive.

Resuts

Postve vas and exchange partners

Positive vial numbers	1st exchange partner number	2nd exchange partner number	3rd exchange partner number

1 Who waspatient zero?
2 After the three rounds of exchanges,hw many vls tesed positive? Calculae what prcentage of your class this represents.
3 Construct a grah showing how many s tudents wer infected after ech round.

Dscusson

1 f theclass re dvided into three groups of 10 at the st art of this nvestiation and alloe t exchange only within their grup, wha would the ransmision o te disease look like?
2 Did you kno whi vial were infected during the procedure?
3 Do yo thin an inividual who does notshow anysignsof a isease s apabe of transmitting the disease to others?
4 What is he importanc of idetifyig pt ent zero in epidemics?
5 How doe ths imlation differ from the spead of disease in th e real world; frexample, the spreado OVID-19? Explain.
6 Lst the appropriate measuethat individua s shoud takeolimit the sprea of iseases.

Takin it further

Researchpas infctiou diese epidemics our esearch should include:

» origins of the disease
» how the disase i transmitted
» tpcal icubaion period
» symptoms of te disease
»impac i.e. t tol,cultura hifts, historical context)
» posble accines and treatments
» prevetative easures.

KEY CONCEPTS

» Factorsrelating o the host, pathogen, mode of trasission and eviroment all contribute to the measures taken to contol the transmission of the pathogen.
»In an outbra, t is importat to identify the pathogen cauing te diease.
» The trnmissio of disase-causng pathogens is complex and can be affected bymany factors relating to pathoens, the environment an the host.
» Measures to cotrol ranmssion ilude public healh, euation god ygiee, social distancing and quaranine.

Concept questions 8.2

1 Robert Koh deelopd hs postulates in 1884 in relation to tuerculoss nd hoera, two infectious dseases that were rampant in th human population. Althou still ued, och's postulates are not useful for studing disease caused by viruses or in asymptoatic arriers. Explain why.
2 Other ways of studying inectious disease epideiloy is to record symptom, caustive agent, mode of trnsmision, nectvity, incubation rates and presence ofantibody to he casative agent in the ptent.Summarie the valueof investigating two of thee in studingdisease.
3 Ho isvrulnce differet from infectivity of a pathogen?
4 Tyhoid Mary was a omestic heper who worked in many housheols during the late19th and early 20th centres. Se infected 5 peole with typhoid fever but never showed s ptoms herself. How would Mary be classified in eationto hisdisease, and what doe thi classi ficaion mean?
5 When halth department nd epidemiologists study the trnsmision f ifectious diseases through communtie, they consider many factors so that they can track and control he disease. List 8 of these factors and provide a hort description of each.
6 Cosider the goernment's response to the COVID-19 case number in ictori (Figure 8.10) and account for the second wave that stated in June 2020.

HOT Chaenge

7 Researchand wite abrif explanation of the quarntine measures usd or two of the following.
   a Bubonc plague
   b Tubecuosis
   c Leprosy
   d Yllow fever
   e Ebola
   f COVID-19

Weolink
The flu vaccine explained

Onne Wyrkm heet
The flu vaccine explained

8.3 Vaccination programs

Vaccination programs have great potential for limiting or even eliminating human infection, as exemplified by vaccination programs targeting the *Variola major* virus, which causes smallpox. Smallpox is estimated to have been a major problem to human health as far back as 12 000 years ago, and probably even earlier than this. Evidence of smallpox has been found in Egyptian mummies dating back to 3000 BCE. As civilisations and trade expanded across the globe, so did smallpox. Smallpox was recorded in China in the fourth century, and in India and Asia from the seventh century. The Crusaders brought it to Europe on their return from the holy wars in the 11th century. As European colonisation spread through Africa and the Americas, smallpox arrived in Australia in the 18th century with the colonists from the United Kingdom.

Smallpox is spread by direct and prolonged face-to-face contact. It has also been known to spread through contact with body fluids, clothes and bedding from infected people. Smallpox has an incubation period of 7–17 days, causes high fever, aches and pains, and culminates in a rash. The raised, pus-filled blisters can take 3–5 weeks to subside. After the infection, large pitted scars can form on the face and body. Smallpox can also lead to blindness if blisters form near the eyes.

Research into protecting people from the ravages of smallpox began with Edward Jenner and his studies on cowpox, which led to the development of what is now widely accepted as **vaccination**. In the 1790s, Jenner observed that people who had been infected with cowpox did not develop smallpox if exposed to the *Variola* virus. Within his farming community, cowpox was prevalent among those who worked with animals. Jenner speculated that a bout of cowpox would produce immunity to smallpox. To test this, he inserted pus from an infected milkmaid into a cut on the arm of a local boy. A little while later, Jenner exposed the boy to smallpox and the young boy was found to be immune. He called his method vaccination, but it wasn't until 30 years after Jenner's death in 1823 that smallpox vaccination was made compulsory in parts of the United Kingdom.

The Global Smallpox Eradication Program was initiated by the WHO in 1959. The aim of the program was to eradicate smallpox from all humans in the world. Initially, travellers from various

countries were vaccinated, as well as local populations. By the mid-1960s, the program was still not particularly effective at eliminating the disease and, in some countries, containment and monitoring those infected proved to be a more effective strategy. Increasing availability of international air travel hindered efforts to keep countries free of the disease, even though it had previously been eradicated. Despite a range of setbacks, including scepticism about the feasibility of the vaccination, as well as inadequate technical and material resources (qualified staff to administer the vaccine and quantity of vaccine required per population), the disease was officially declared eradicated by the WHO in 1980.

Figure 8.18 In 1901, one of these boys was vaccinated against smallpox and one was not vaccinated.

Vaccination leading to **immunisation** is a highly effective public health intervention that has substantially reduced worldwide morbidity and mortality from infectious diseases. In Australia, children are routinely vaccinated against many infectious diseases, including hepatitis B, diphtheria, pertussis (whooping cough), tetanus, measles, mumps, rubella and poliomyelitis. Groups that are at high risk of infection, such as the elderly or chronically ill, may also need additional vaccinations. As new vaccines are developed, immunisation programs against more diseases are being introduced. Figure 8.19 shows the rates of infection of *Haemophilus influenzae* and meningococcal C after the introduction of vaccines against these pathogens.

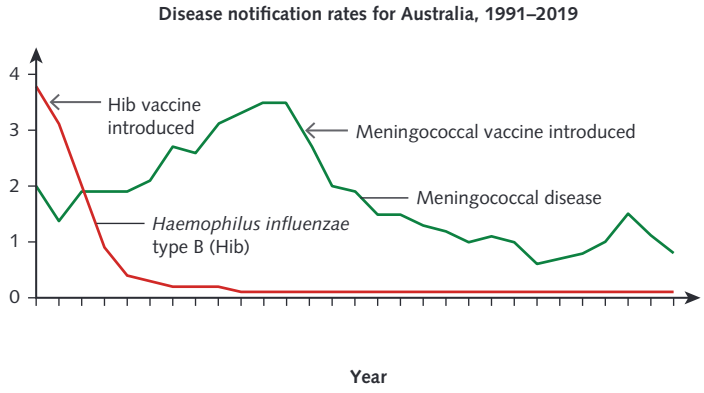


Figure 8.19 Rates of *Haemophus n fluenzae* type B (Hib) and meningococcal infection since the introduction of vaccines against these organisms.

EXAM TIP

When you are presented with a graph, take the time to interpret the graph and work out what it is telling you. Once you are confident that you know what the graph is telling you, then answer the question.

WB
8.3.1
HERD IMMUNITY
PAGE 171

Herd immunity

Not all individuals within a population need to be vaccinated to prevent the spread of a disease. If a large enough proportion of the population is immune to a disease, then there are too few susceptible individuals to sustain disease spread. This effect is known as **herd immunity** and is demonstrated in Figure 8.20. Only infected individuals (orange) can spread the disease to those they have contact with. When there are enough immune individuals (blue), the chance of an infected individual contacting a susceptible individual (black) is so low that the disease cannot spread. For herd immunity to prevent the spread of disease,

n.
Weolink
How does herd immunity work?
Onne Wyrkm heet
How does herd immunity work?

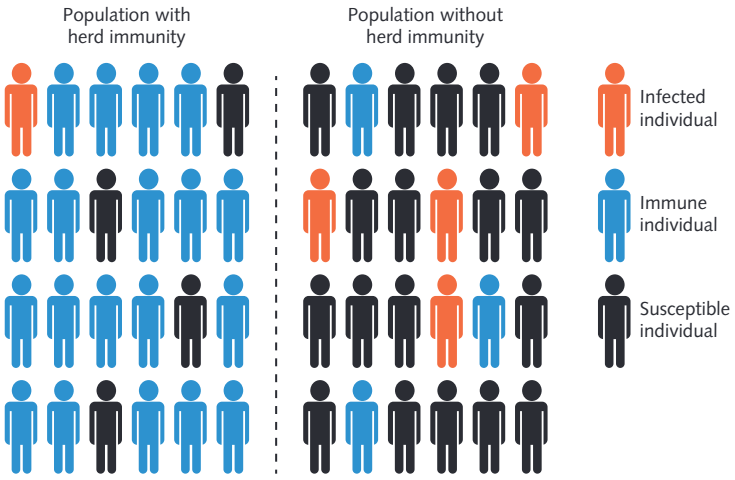


Figure 8.20 Herd immunity occurs when a large enough proportion of a population is immune to a disease. Disease cannot spread because there are too few susceptible individuals.

a high proportion of the population needs to be immune. The exact proportion depends on the virulence and infectivity of a specific disease. For measles, 95% of people must be vaccinated for herd immunity, but for polio, it is 80%. Some people have health conditions that mean that they cannot be immunised and so they rely on herd immunity for protection from infection.

Some people object to immunisation of children. The reasons for this vary, but a major concern raised by these groups is the safety of vaccines. While vaccines can have some side effects, these are usually mild (such as pain or swelling at the site of injection) and serious reactions are very rare. Overall, vaccines are far safer than the diseases they protect against.

One of the conditions erroneously linked to vaccination is autism. In 1998, a small, unsubstantiated report suggested that the measles, mumps and rubella (MMR) vaccine could cause autism. The claim was later discredited, and further research has not shown any link. Despite the lack of scientific evidence, vaccination rates dropped markedly, and measles infections rose after publication of this report.

WB
8.3.2 VACCINES PAGE 173

In some cases, levels of immunity within the population have dropped so low that herd immunity is no longer sustained. In 2013, several measles outbreaks in the United Kingdom were linked to low vaccination rates after the MMR scaremongering. The risk of potentially devastating infectious diseases re-emerging is substantial if high vaccination rates are not maintained. To combat this risk, several states in Australia have legislation requiring children to be vaccinated before they can be enrolled in childcare or school.

The Victorian government implemented a 'No Jab, No Play' policy in 2017, which requires parents to supply the childcare provider, kindergarten or school with a current immunisation record as well as a statement to show their child is up to date with their immunisations. The introduction of this policy was coupled with extensive media and social media coverage, as well as online support through the Department of Education website.

Samoa experienced a measles epidemic in 2019. In a country with a population of 200 000, there were 5700 reported cases and 83 deaths. Thirty-five of those deaths were children under 4 years of age. In 2015, the vaccination rate for measles was at 84%. In 2018, only 31% of children under five had been vaccinated. The decrease in rates was put down to the deaths of two infants following vaccination. Further investigation ruled out any negative effects from the vaccine, with the deaths thought to be due to other medications that were incorrectly administered.

The Samoan government declared a state of emergency on 17 November 2019, closing schools and cancelling all Christmas celebrations. Unvaccinated families were ordered to display a red flag at the front of their house to alert others and to enable mass vaccinations to occur. On 7 December 2019, the state of emergency was lifted, and the government declared that 90% of the population was now vaccinated.

KEY CONCEPTS

- Vaciation i ahighl effetive public health ntervetion that has utantial reduced worldwide moidity anmortaityfrominfectious diseases.
- Her immnity occur when a large enugh proportion of the ppuatin is immun e to a dsease (sually due to a succesfulvacinationprogram), and there are too few suscetibleidivduals to usain disease spread.

Concept questions 8.3

1 Ditnguish between vaccinaio and immunisation.

2 Mesls is anoti fialedisee. I can lead to severe medicalproem, ncludingnehalitis, brain damage and dath. In stralia, measles devasted th Indigenouspoulatio when t was introduced during European sttleent.A measles vaccine was devlopd in 1963 ndis givn in Australia to young baies ad chldren in the MMR vaccine as part of a nainwide acinationproram.Worldwide, there are about 2llion cases ev ery year.Why s measles re-emerging?

3 Before there wasa chicke pxvaccination, parents would oldchicken px partiesor their children.
- **a** What were the prents doing?
- **b** Why were the parnts oing this?
- **c** Wha did the discover of a vaccination do to these prties?

4 Her imunity fo meales requires 95% of people to be vcciated, bt o poio tis 80. Explain what is meant by hrd mmunity and why the percentage of the poplation that needs to be accinated for speci fic dseases is different.

5 The *Vaccinia* virus that isthe acive ingredient of the smalpoxvacine is aver loe relative of, and may be the same secies as, the cowpox virus that Jenner used fo his first sallpx vaccne However, it is not the *Variola* vrus that cses smallox. How could the *Vaccinia* vacine proue alielong immunity in humans agains smallpox?

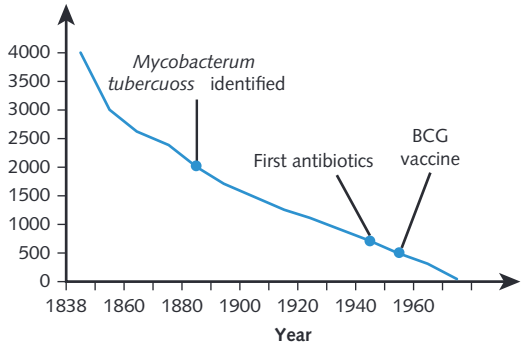


Figure 8.21 The decline in respiratory tuberculosis rates in England and Wales since 1838

6 What is he 'No Jb, o Play policy? What is your opiion abou ths policy?

HOT Chaenge

7 **a** nterpret th epidemiology of TBthat is shown in Fgure .21.
- **b** Compare thedaa in igures .21 and 8.22.
  - **i** The daain Fiure821 implies that TB rates dropped to 0 p ilion in abou 1975 in Enland an Wales. De the data in Figure 8.22 corelte wit that inference?
  - **ii** f the BCG acination haseen vailable since about 120, why oesFigure .21 show it to be in us since 1954?
  - **iii** Has TB been eadicated as of2016 in England and Wales?
  - **v** BC is not part of the reular vaccination program forminos in Aralia. xplain why.
  - **v** n Austalia, 1440 new cases of TB were recorde in 218 It s a noti fialedisese. What dos this mean?
  - **vi** n Austalia the incidence of TB before 2018 was extemely rare because of a 27-year eraic tion program. Where are the new cases comng from?

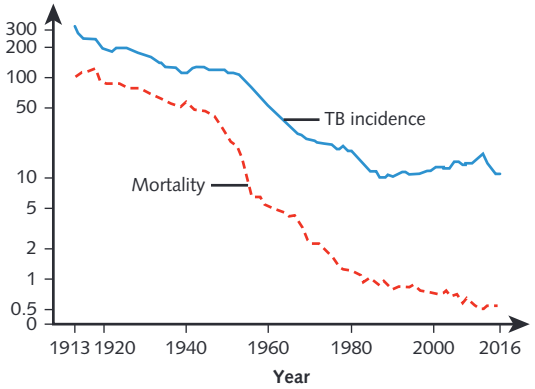


Figure 8.22 TB incidence and mortality rates per 100 000 people per year in England and Wales, 1913–2016

8.4 Immunotherapy strategies

Immunotherapy is a treatment given to a person suffering from cancer or an autoimmune disease to boost or suppress their immune system. Boosting or suppressing the immune system has to be undertaken with extreme care. It can have unwanted side effects, such as fungal infections and lymphomas, and trigger life-threatening complications such as autoimmune damage to normal tissue.

Cancer

Cancer is a disease that is caused by uncontrolled cell division. Cancer arises when a cell escapes the normal immune response that limits its growth and survival. This is due to mutations in the cell's genome that change the expression or function of genes. Because apoptosis is the main way of removing new potentially cancer-causing mutations, the most important factor in the initiation of cancer is the ability of mutated cells to evade apoptosis and continue to survive and divide.

Cancerous cells arise from normal body cells, and only a relatively small number of molecular changes are needed to cause disease, so cancers can be hard to treat specifically without inducing harmful side effects for other cells or tissues. Some small molecules can target specific gene mutations that commonly occur in cancers or can reduce the activity or expression of signalling pathways that cancers rely on to grow and spread.

Immune checkpoint inhibitors

T cells are one of the immune cells that our immune system uses to fight cancers. T cells have proteins on their surface that activate the cells to start their immune response. These cells also have other proteins that turn off the immune response when the threat is over. These proteins are called checkpoints. Cancer cells can produce their own proteins that can turn T cells off so they do not attack cancer cells. Checkpoint inhibitors are drugs that stop the proteins on the cancer cells from turning off T cells, thereby allowing the T cells to attack the cancer cells.

Weolink
Watch a video of T cells killing cancer cells.

T-cell transfer therapy and cancer treatment

T-cell transfer therapy is a technique that boosts the natural ability of a person's T cells (Chapter 7, p. 251) to fight cancer. Scientists remove T cells from a cancerous **tumour**. They select the most active against the cancer, alter them in the laboratory to make them more effective and then grow them in large batches *in vitro* (in glass). These super-powered T cells are reintroduced into the patient by intravenous injection and then target and kill cancerous cells. (See weblink.)

8.4.1 MONOCLONAL ANTIBODIES AND THE TREATMENT OF CANCER PAGE 175

Monoclonal antibodies and cancer treatment

Antibodies that detect antigens unique to cancer cells can be used as anticancer drugs. The body can be stimulated to produce these antibodies or they can be produced in a laboratory.

One way to produce antibodies is to culture B cells in a laboratory and collect the antibodies they produce. The problem is that B cells, like most mammalian cells, do not live for very long in culture and so do not mass produce the specific antibody for any length of time. This can be overcome by fusing the B cell clone that produces the antibody of interest with cells extracted from a plasma cell tumour, creating a **hybridoma**. The hybridoma can produce antibodies coupled with the property of tumour cells to divide repeatedly *in vitro* (the cells have now become 'immortalised'). Each hybrid cell produces many clones of itself, and each clone produces the same antibody. These antibodies are termed **monoclonal antibodies** because they are produced by clones of the same hybrid cell and are thus identical. Hybridomas have revolutionised the production of antibodies.

Monoclonal antibodies can be designed to trigger an immune response to attack cancer cells (Figure 8.23), block signals that cause cancer cells to divide, or transport toxic molecules or radioisotopes to cancer cells. An example is the medication trastuzumab, which is a preparation of monoclonal antibodies that can bind to cells of some types of breast cancer, blocking their growth and promoting their destruction by the immune system. Ideally, the monoclonal antibody will bind to an antigen that is unique to the cancer and not present on normal body cells. This means there are no unwanted side effects of the monoclonal antibody. However, sometimes antibodies bind to antigens that are also present on other cells of the body, resulting in various side effects depending on the target cells.

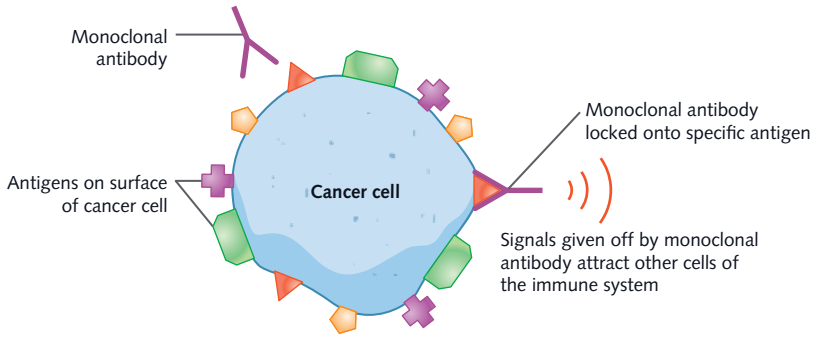


Figure 8.23 **Monoclonal antibodies lock onto the antigens on the surface of the cancer cell. This can signal other immune system cells to attack the cancer cell.**

Table 8.1 **Monoclonal antibodies and specific cancer treatments**

Cancer type	Mechanism of action
Non-Hodgkin lymphoma	Binds to the protein CD20 on the surface of cancer cells and activates the immune system to destroy them
Advanced melanoma	Binds to and blocks CTLA4, a negative regulator of the immune system, to keep immune cells stimulated
Breast cancer	Binds to the growth factor receptor HER2 to block the signal and stop cancer cells growing
Bowel, breast and some other cancers	Binds to the growth factor VEGF to inhibit binding to its receptor

Autoimmune disease

The human immune system is a complex mechanism with many regulatory checks in place, but it can malfunction. **Autoimmune diseases** can occur when the immune system recognises a normal component of the body as a foreign antigen and mounts a response. This results when cells of the immune system fail to distinguish between self and non-self or when the immune system overreacts to substances that are not pathogenic. The immune system is carefully tuned to remove or suppress any B or T cells that may respond to antigens in the person's own body, but sometimes this system of self-tolerance fails, and an autoimmune disease develops. There are many different types of autoimmune diseases and almost any part of the body can be affected. The effects the autoimmune disease has on the body depends on which self-antigen (known as an auto-antigen) the body is reacting to.

Autoimmune diseases caused by autoantibodies

Some autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE, or lupus) are the result of the action of **autoantibodies**. The production of autoantibodies is a malfunction of the adaptive humoral response. Autoantibodies are produced by the immune system and target the body's own components, including proteins, nucleic acids, lipids and carbohydrates. The autoantigen is usually specific at the onset of disease, but as more tissues are damaged by the uncontrolled inflammation, more antigens are released, activating other clones of auto-reactive B cells. In rheumatoid arthritis, autoantibodies target components in cartilage and bone in the joints, causing inflammation, bone destruction, pain and joint malformation. The autoantibodies bind to the tissue lining the joints and work to destroy the cells, much in the way that antibodies work to neutralise the effects of pathogens by targeting specific antigens (Figure 8.24). In lupus, autoantigens are varied and can include ubiquitous (universally utilised) components such as DNA, which means that any organ or tissue can potentially come under antibody-mediated autoimmune attack.

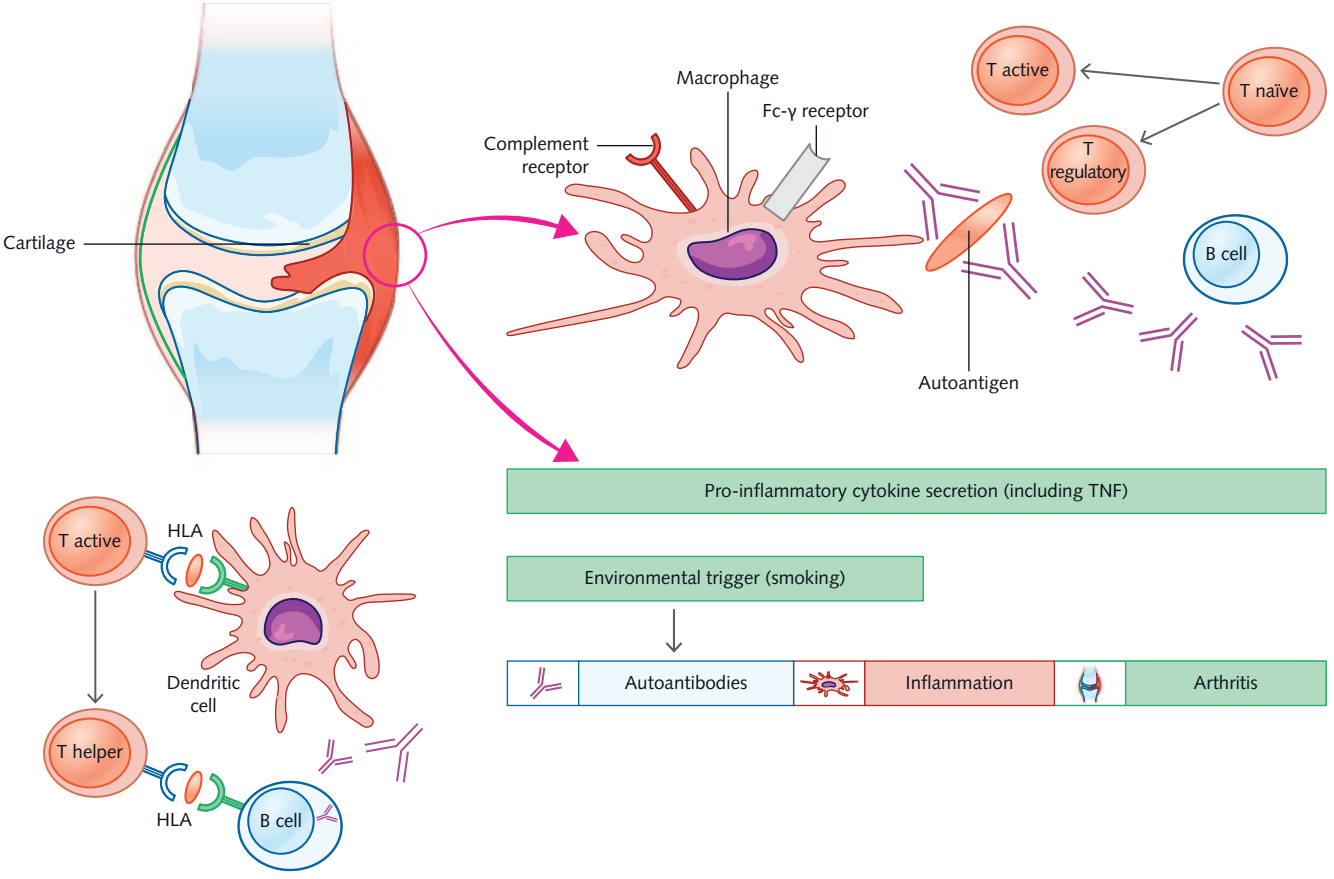


Figure 8.24 The action of autoantibodies in rheumatoid arthritis of the knee joint

Autoimmune diseases caused by T cells

Autoimmune conditions also arise when T cells target the body's own cells and mark them for attack. Conditions such as Crohn's disease and multiple sclerosis develop in this manner. Multiple sclerosis results when reactive T cells attack the myelin sheath of neurons and form plaques that lead to neurological decline. It presents as less or spasmodic control of movement. Crohn's disease is the result of T cells targeting cells in the bowel. Inflammation in the bowel leads to blockages and extreme pain, and sometimes resection (removal) of entire segments of the bowel is required to prevent sepsis, or widely disseminated bacterial infection.

Type 1 diabetes is the result of a coordinated autoimmune attack on beta cells (insulin-producing cells) in the pancreas. Both helper T cells and cytotoxic T cells mistakenly target beta cells for destruction, so the person cannot produce insulin. The action of the T cells is often the result of a person's genetic predisposition and becomes apparent when the person is young. This is the reason type 1 diabetes is also known as juvenile diabetes.

Monoclonal antibodies and treatment of autoimmune diseases

8.4.2
CANCER AND
AUTOIMMUNE
DISEASES
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Monoclonal antibodies are effective against some autoimmune diseases. Cytokines are signalling proteins that coordinate immune responses (Chapter 7). The expression and release of cytokines usually occurs as a cellular response to a signal, such as the presence of a foreign invader during infection or injury. Because cytokines have such powerful effects on cells of the immune system, and therefore the whole organism, several signals are often needed before a cytokine can be secreted or released. There are also many natural mechanisms to degrade cytokines and counteract their functions so that immune responses last only as long as needed to clear the danger. Other types of cytokines are anti-inflammatory, and these often start acting at the same time as the pro-inflammatory cytokines, to limit the extent of the inflammatory response. These factors ensure that immune cell activity only occurs when it is really needed, preventing unnecessary immune-mediated effects such as asthma, allergy and autoimmune diseases.

Monoclonal antibodies can be made that block and neutralise cytokines, interrupting the transmission of signals between cells and tissues. Tumour necrosis factor (TNF) is a cytokine that is involved in the very early phase of inflammation. However, TNF is a strong mediator of harmful inflammation in many conditions, such as inflammatory bowel disease and rheumatoid arthritis. Monoclonal antibodies that neutralise TNF are used in patients with these diseases, limiting the actions of this pro-inflammatory cytokine. A clinical trial in patients with the autoimmune disease lupus showed effectiveness of a monoclonal antibody that binds to the interferon-alpha receptor, preventing the whole family of interferons from binding to it, reducing inflammation in this disease.

KEY CONCEPTS

» Cance arises en a cel arts didng uncontrollably and escapesdetectin by theimmune system.
» An auoimmune ondiion is whe the immune system starts ttckigself-cells.
»Immunotherapy is a treatment tha orks to stimulate th immune system to either suppressor enhance its respons in those uffering from ither autoimmune dseases or cncer.
» Atbodie speci fic to an antigen can be produced throug stmulationf B cells or they can be produced n a aboratory and are know a monoclonal aniodies.
» Moncona antibodies lock onto the antigen on the surface of the canr cel, casing the immune system to attack it.
» Moncona atibodies bock a d eutralise cytokines, nterruting the ransmisio of signas between cells andtisu limiting the ctions of pro-in flammatory cytkies.

Concept questions 8.4

1 What two typesof conitions can immunotherapy be used to treat?
2 Cytkines ae ivoled in theimmune response. They can beati-in flammatory a d pro-in flammatry. What doesthis mean?
3 Monconal ntibodies are commonly manufactured using a hbrdoma.
  a Explain how ahybidomais made.
  b Describ to stuaions i whch monoclonal antibodies may help s v person's life.
  c Explain what property o tumour cells makes them useful for fusingo B cells or onolonal antibody prodution.
4 What has gone wong in the body when an autoimmune iseasedevelops?
5 Whatfaulty steps of the daptive humoral response areinoled in th productin of utoantibodies in dseases such as heumatoid arthritis?

HOT Chaenge

6 Fgure .25 sumarises an in flammatory response to cancer ls.
  a Would the cytokins inFigure 8.25 be anti-n flammatory o pro-in flammatory?Explain.
  b Effecto T cels re signaled by cytokines to mount a response to a antigen.
  Lst three ssible types offfector T cells nvlved.
  ii What is te role of e dditic cell in this example?
  iii What is te role of the macrophage?
  v The final cl i laelled as an exhted T cell. What do yu think might be he next step in progresion of disease?

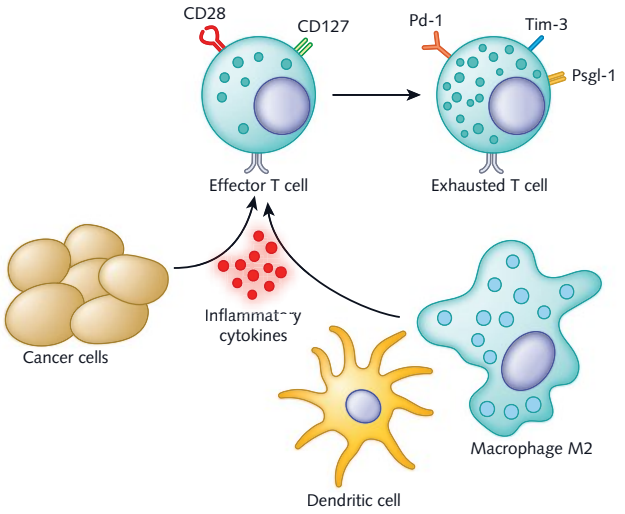

Figure 8.25 A cytokine pathway showing the outcome of T-cell exhaustion

BRANCHING OUT

Tracking influenza to prevent a pandemic

n fluenza (the flu) is a contagius disease o the respirat ory tract caused y nfection with in fluenza irs. Woldwide, approximately 5 millio peoplesuffer with the flu each year. In Aralia, 1500 dathsare associated with flu nfetions each er. ulnerable pepl iclude the very young, the elde rly and thse with u nderling halth ssues. n fluenza symptomsinclude fever and muce ach, which equire bed rest.

Humanin fluenz virus infets cells ofthe respiatoysystem.Onceinside he host, a protein on the virus surface clled hamagglutinin attches o sialic acid reidues loated on the tip of glycoproteins that protrude from the host cesplasma membrae (Fiur 8.26). Haemaggtnin is the key requred togain entry i the host cell. Once the virus has at ached, eceptor-me iaed endocytosiss triggered so the human cell engulfs the virus. Inside the human ce, the virs trics theell ino eplicating viral component s irus capsidsasemle, enclosig a set of virus genes. Hundreds f viruses start to bud fom the host cll.Bu as theytry to eave, their haemagglutinin proteins get stuck to the hoscel's plasma membran. A second proteinon he surfac of thevirs, an enzyme called neuraminidase, acts ke cissors to cut the virus ee rom the cell. The vus the infects anther cell in the respiratory system or leaves the hot in mucus drop as the infected personcughs, sneezeso tals. I susceptible host is infected, they will become sick.

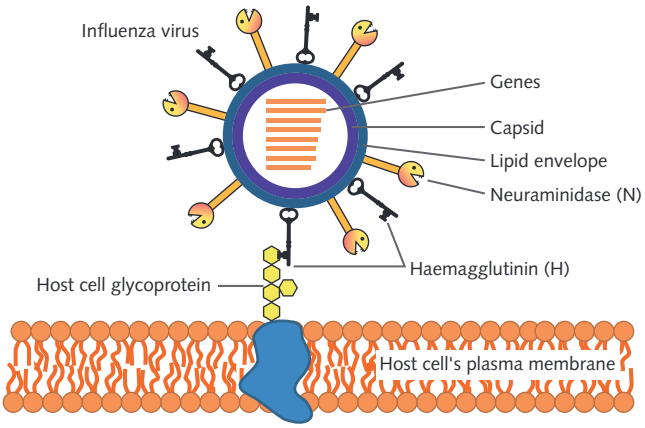

Figure 8.26 Influenza virus uses haemagglutinin (H) protein to enter a host cell. A second protein, an enzyme called neuraminidase (N), is required to release budding viruses from the host cell's plasma membrane.

Three types of influenza can infect humans. Type A and type Bca cause eidemics, while type C only causes md nfectins. Tye A vir ses can infect a number of diff erent species and are fthr divided into subtypes based on their heagglutin and neuramnidase surface proteins There are 18 different haem aggtinins ad 11 different neuramindases.Theserteins vary slightly in structure as a reslt of mutations that hve arisn nthe haemagglutinin and neraminidase gees. Haemagglutinin and neuraminid ase proteins are the antigens that trigger an immune response t te virus. If these surface proteins change, the new flu virus may not berecognised by our immune system

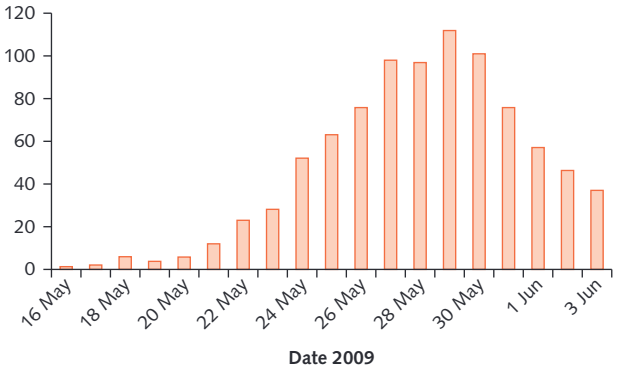

Figure 8.27 Confirmed cases of influenza A/H1N1 in Victoria each day during the 2009 pandemic

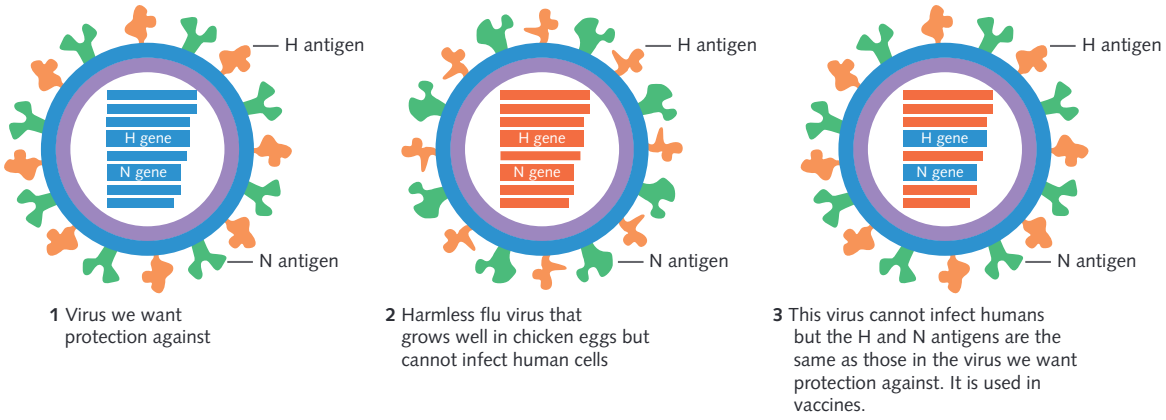

Figure 8.28 To make a vaccine against a new strain of influenza virus (virus 1), the genes for haemagglutinin (H) and neuraminidase (N) are taken from that virus and put into a virus that cannot infect humans (virus 2). This results in a new virus strain (virus 3), that is harmless to humans but produces the H and N antigens to which an immune response is required. Virus 3 is infected into chicken eggs so it replicates. Proteins expressed by this virus are used in the vaccine.

and can cause sious illness before an mun responseevelops. Melbourne is generally the firstcity in the southern hemisphere to succumb to ne strains of in fluena. Figure 8.27 shows the peak outbreaks of influenza infction during the 2009 H1N1 pandemic.

The WHO tracks influenza outbeasglobally. The WHO identi fies the emergene and locationsof new outbreaks, deterines the type of in fluenz crcuating, and easures the impact ithas b te number of people hospitalised or kld. The most severe strans can be contrlled b producing a vaccine. Information about in fluenza in the northern hemisphere can be used o prepare vaccines against flu stains for when teyarrive in te southern hemisphere. Fgure8.28 shows how flu vaccines are prepared.

When a vccin is injcted intoyour arm, your body moun ts an immune respone againstt haemagglutinin and neuranidase antigensof the viru se in that vaccine. As a resu t, you produce memoy cellshat will provide a faster and arge immune respose if you encounter that sae antigen agai n Youll ridly prodce ntibodies that control the nfecton. You pobably wi not experience any flu symptoms.

Questions

1 Construct a flow chart to show the sequence of events that occur when you areinfcted with in fluenz. On your flow chart, show both vaccinaed and unaccinated responses.

2 Ther i incrasing pressure to make anul vacciations against in fluenza mandatory fo healthcare workers. The Peter MaCallum Cancer Centre has had mandatory in fluenza vacintion progam sinc 2009 However, there are no puntive consequences for staff who rfus to participate.
Do yo think that annual in fluenza vaciationsshould be mandatory for everyn? What ethical issues do you see? Use the tical cocept inTabl 8.2 o assst you n deciding on this issue.

Table 8.2 Ethca concepts used as a fram ework for anaysn g ssues n boogy

Ethca concepts	Des cription	Considerations
ntegrty	Honest reorting of all information and communication of results	s the informat ion honest? s information required by the pubc to make a decso n open to public scrutiny?
Justce	Far conideraion of competing claims	Can everyone have equal access? How can discrimination be avoided?
Beneficence	A duty to do more good than harm to consider the welfare of the research participant	Who will benefit from this technology? How will they benefit? (Can include physical psycho logical economic or social bene fits) How many will benefit?
Non-maleficence	The duty not to cause harm	Wh mi ght be harmed by this technology? How are they harmed? (Can include physical psych ological economic or social harms) How many are harmed? s t possbe to m nimise the harm?
Respect	liing tings have intrinsic value and can make their own decisions	Are people able to make their own decision based on their own criteria? Are people with diminished capacity protected where necessary?

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Chapter 8: Summary of key concepts

8 Summary of key concepts

8.1 Emerging and re-emerging pathogens

KEY CONCEPTS

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- » iseases that are caused by pathogens can be spoadic, ndei , epidemic or pandei.
- » Emrging pathogen ae previously unrecognised pathogens or pathogens that have spread to ew lctions.
- » Re-eerging pathogens are pathogens that have caused dsease in the past and are now reappering.
- » Rpd global movement f humans, animals and othrbologcal mateial means it is nevtable that the spread of pathogens has ncreased.
- » Pathogens brought into Atralia by Europe an stlers, such as the viruse tha caused measles, smallpox and the commo old, wee deady or Indigenus peoples who had not been exposed to these pathogens before.

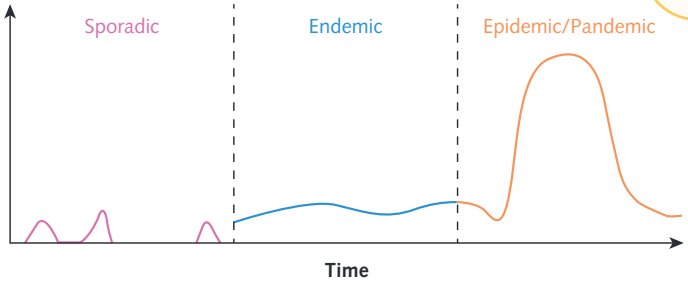

Figure 8.2 Within a population a disease may occur sporadically, at an endemic level, or at an epidemic/pandemic level.

8.2 Strategies for controlling pathogen transmission

KEY CONCEPTS

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- » Factorsrelatingto the ost, pathogen, mode of trasmission ad envirment ll contribute to the measures taken tocontrol he transmission of the pathogen.
- »In an outbra, t is importat to identify the pathogen cauing te diease.
- » The trnmissio of disase-causng pathogens is complex and can be affected bymany factors relating to pathoens, the environment an the host.
- » Measures to cotrol ranmssion ilude public healh, euation god ygiee, social distancing and quaatine.

Figure 8.14 An increase in diarrhoeal illness in Kenya was observed after extensive flooding in 1998.

8.3 Vaccination programs

KEY CONCEPTS

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- Vaciation i ahighl effetive pblic health inte rvetion that hassstantialy reduced worldwide moidity anmortaityfrominfectious diseases.
- Her immnity occur when a large enugh proportion of the opultion is immune to a dsease (sually due to a succesfulvacinationprogram), and there a tofw ssceptibe idividuals t sustain disease spread.

Figure 8.18 In 1901, one of these boys was vaccinated against smallpox and one was not vaccinated.

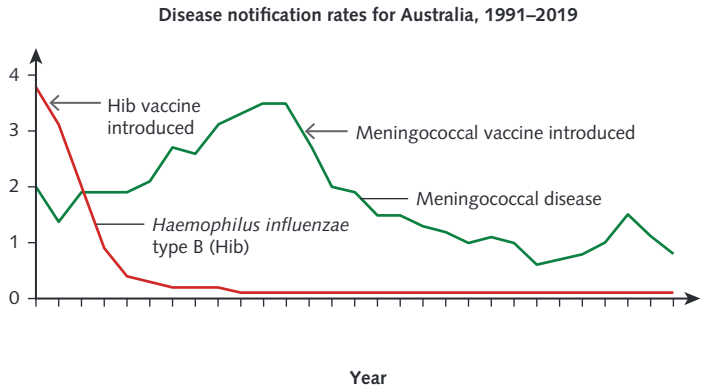

Figure 8.19 Rates of *Haemophus n fluenzae* type B (Hib) and meningococcal infection since the introduction of vaccines against these organisms.

8.4 Immunotherapy strategies

KEY CONCEPTS

p 306

- Cance arises en a cel arts didng uncontrollably and escapesdetectin by theimmune system.
- An auoimmune ondiion is whe the immune system starts ttckigself-cells.
- Immunotherapy is a treatment tha orks to stimulate th immune system to either suppressor enhance its respons in those uffering from ither autoimmune dseases or cncer.
- Atbodis speci fic to an antigen can be produced throug stmulatioof B cells or they cn be produced in a aboratory and are knownas monolonal antibodies.
- Moncona antibodies lock onto the antigen on the surface of the canr cel, casing the immune system to attac it.
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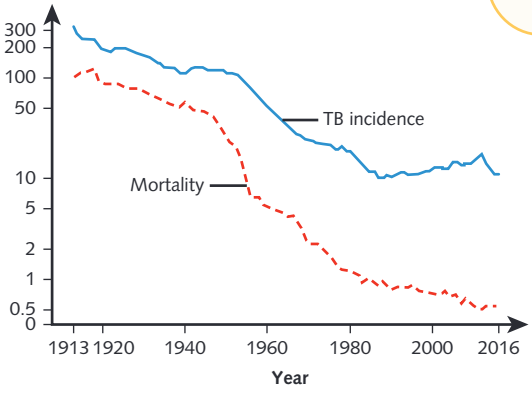

Figure 8.22 TB incidence and mortality rates per 100 000 people per year in England and Wales, 1913–2016

8 Chapter glossary

autoantibody an antibody produced by a person's immune system that is directed against the person's own proteins

autoimmune disease a disease caused when a person's immune system mistakes self-cells and tissues as non-self and initiates an immune response against them

cancer uncontrolled abnormal division of cells that are not kept in check by the immune system and invade other areas of the body

carrier a person who does not show symptoms of a disease but can transmit the infection to others

endemic restricted or native to a certain locality

epidemic the rapid spread of a disease across a number of countries

herd immunity when unvaccinated individuals are protected against a disease because a large number of people (between 60–95% depending on the disease) have been vaccinated, thereby making it unlikely that unvaccinated people will come in to contact with anyone suffering from the disease

hybridoma a cell involved in the production of large amounts of monoclonal antibodies

immunisation the process of making a person immune to a disease through vaccination

immunotherapy boosting the ability of a person's own immune system to fight cancer

infectivity the ability of a pathogen to spread from one host to another host

Koch's postulates a set of criteria to determine the causative agent of a disease

latent not active

monoclonal antibody a laboratory-produced molecule that serves as a substitute antibody to fight cancerous cells

pandemic the spread of a disease across the world

primary host an organism in which a pathogen reproduces

quarantine restricting the mobility of person or persons to a certain area so they reduce contact with other people in order to stop the spread of a pathogen

secondary host an organism in which the immature pathogen becomes mature

sporadic seen infrequently in a small number of people

T-cell transfer therapy a therapy that boosts the ability of T cells to fight cancer cells

tumour a mass of abnormally growing cells

vaccination the administration of a vaccine to protect someone from a disease

virulence the capacity of a pathogen to cause severe disease within its host

zoonotic describes a disease that can be transmitted from animals to humans

WB
8.5.1
EXAM PRACTICE
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8 Chapter review

Remembering

1 The following terms are associated with disease: virulence, infectivity, asymptomatic, symptomatic. Define each term and state why it is of concern.

2 Herd immunity is a useful concept in epidemiology. What does it mean? Use an example to discuss how it works.

3 European settlers introduced diseases that decimated Aboriginal populations in Australia, Maori populations in New Zealand and native American populations in North and South America. The Black Death was introduced into Europe through trade with the Orient in the 1300s and led to the death of more than 50 million people. The origins of the COVID-19 pandemic is currently under investigation.
   a What aspects of diseases can make them very hard to control?
   b Is it reasonable to ban travel and trade to prevent these horrific outcomes? Why?
   c What do governments do today to arrest the movement of diseases around the world?

Understanding

4 The following steps show the WHO protocol for the preparation and use of blood products in the treatment of Ebola virus disease. This disease is characterised by significant blood loss.
   1 A patient recovers from Ebola virus disease.
   2 The same patient is disease-free for 28 days.
   3 Blood is taken from the patient and screened for transmissible diseases.
   4 Plasma is separated from the whole blood.
   5 Plasma is transfused into another person with early signs of Ebola virus disease.

   (Source: *Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease*, http://www.who.int)
   a Explain why this protocol produces an effective treatment for Ebola virus disease.
   b Does this protocol involve the use of a vaccine?
   c What postulate does this relate to and how is it different?

5 Refer to Figure 8.15 on page 296. List the three main factors that affect the transmission of disease. Describe how these three factors interlink to affect disease transmission.

6 What are the main steps leading to the development of an autoimmune disease? How are immunotherapies being used in rheumatoid arthritis to limit symptoms in this debilitating condition?

Applying

7 Public health institutions regulate food handling to try to stop outbreaks and the passage of diseases such as hepatitis, typhoid, salmonellosis and shigellosis. All of these diseases can kill. Some countries have cultural practices around eating that are designed to prevent the spread of these diseases. However, cultural practices can also facilitate the progress of disease in a community. Research the cultural practice that leads to the spread of each of the following diseases. Describe public health measures that have been put in place to control outbreaks of:
   a norovirus
   b Creuzfeldt–Jakob disease
   c kuru.

8 The Australian Government Department of Health website lists and defines Australian national notifiable diseases. The department states that incidences of the diseases on the list are to be notified nationally and provided to the Commonwealth's National Notifiable Diseases Surveillance System. The earliest date on this website is 2004.
   a What do you think happened before 2004?
   b Why are notifiable diseases a government concern?

9 Lack of fresh water in communities is a public health problem. Explain why.

10 What is the difference between 'social distancing' and 'quarantine'?

11 Malaria kills hundreds of thousands of people worldwide every year. Discuss historical and current measures used to control this disease.

Analysing

12 In the UK, at the beginning of the COVID-19 pandemic, the UK government considered herd immunity to be a possible pathway towards mass immunity in the UK population by allowing large numbers of people to contract the disease. The UK government then rejected the proposition. Why do you think this occurred?

13 Some herpes viruses are known to cause cancer. Gardasil immunisation is given to teenagers in Australia as part of the national vaccine program.
   a What cancer is Gardasil protecting against?
   b Initially the vaccine was only administered to females. Why do you think that was?
   c The vaccine is now administered to both males and females. Why do you think that is?

14 Figure 8.29 shows a model of disease transmission.

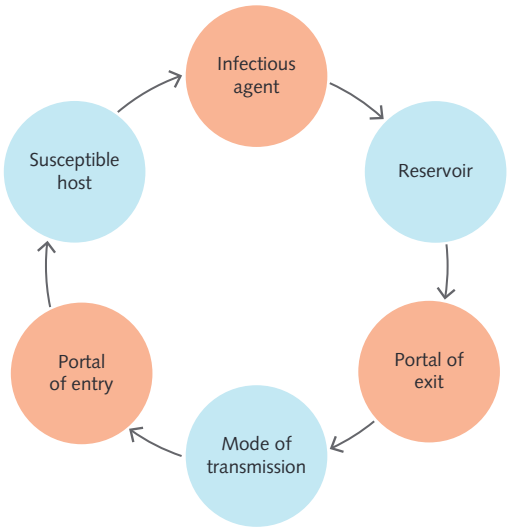


Figure 8.29 A model of disease transmission

   a What is:
      i a portal of entry?
      ii a susceptible host?
      iii an infectious agent?
      iv a reservoir?
      v a portal of exit?
   b A pathogen was identified as being non-adapted to dry conditions and as having the gastrointestinal tract as the portals of entry and exit. Is the mode of transmission most likely to be respiratory droplets, skin to skin or food?

15 Some pathogens mutate during the course of disease transmission in a community. Virulence can increase, decrease or stay the same. In the 1918 Spanish influenza pandemic, what was the pattern within communities?

16 In Australia, the operators of what modes of transport are compelled to report notifiable diseases under the *Biosecurity Act 2015*? Explain why this is.

17 Why isn't malaria found in Australia?

18 When a host is infected with a pathogen, why do they not show symptoms immediately?

19 Foodborne illness can kill (Figure 8.30). Some diseases tracked by health departments are caused by active pathogens in the host. Others may be caused by exotoxins, which are proteinaceous (made of or consisting of protein) particles. Why do we have strict rules around food preparation in Australia?

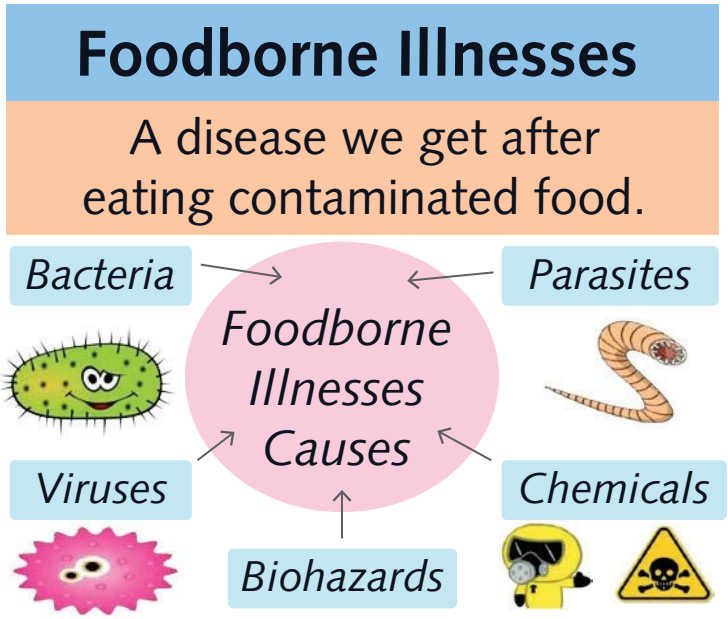


Figure 8.30 A poster explaining that foodborne illnesses are caused by consuming contaminated food or drink

20 Melanomas are characterised by uncontrolled cell division caused by mutations that continue to occur once the tumour has developed. Scientists have discovered that vaccines produced from antigens extracted from the patient's own melanoma cells can be useful in treating melanoma. When injected, the vaccines stimulate an immune response. What can be inferred from the scientists' discovery? Choose one alternative.

A Cancer cells carry unique antigens.
B Self-antigens are not present on cancer cells.
C The melanoma patient has a dysfunctional immune system.
D The body cannot mount an immune response against cancer cells.

21 Choose one alternative. The melanoma vaccine stimulates:

A T cells, which produce antibodies.
B cytotoxic T cells, which activate B cells.
C cell division to produce more lymphocytes.
D production of B cells, which destroy melanoma cells.

22 Even though Australia is an island and Melbourne is not near the northern coastline, each year Melbourne is the first place in Australia to register new patients with notifiable diseases. What might be some of the reasons for this?

23 Why does the influenza vaccine have to be redeveloped each year?

Evaluating

24 Influenza can be a deadly disease and has led to world pandemics. The US Centers for Disease Control and Prevention estimates that as many as 56 000 people die from the flu or a flu-like illness worldwide every year. In Australia, it is believed to cause 1500–3000 deaths, 18 000 hospitalisations and 300 000 visits to the GP each year. This is a huge load on health budgets. In 2019, more than 2.1 million vaccines were administered.

- **a** If there is a vaccine available, why isn't it mandatory to receive it?
- **b** Which parts of the community are most susceptible to the flu?
- **c** Consider your own experience in relation to influenza and other diseases. Do you think the Australian population has become complacent about influenza and did this affect community responses to COVID-19 in the initial stages of that pandemic?
- **d** List four factors that contribute to an individual's response to a disease in the community.

25 Several types of immunotherapies are used against cancer, including monoclonal antibodies, cancer vaccines, oncolytic virus therapy, T-cell therapy and non-specific immunotherapies. The therapies are still being developed, do not work for all types of cancers, vary in efficacy, and inhibit one cancer receptor pathway only. The pharmaceutical company Merck has been researching and developing the drug Pembrolizumab for more than 15 years. Pembrolizumab was accidentally discovered when researchers were looking for a way to use drugs to dampen down the immune response in patients with autoimmune disease. There were gaps in time in the research when the company went through two mergers and acquisitions and then in 2009 research was nearly cancelled. In 2013, research in lung cancer patients continued and in 2015 the drug went to market for a very limited use at about $100 000 per treatment and billions of dollars in research and development spent by the company. Since then, the market for use has increased as other cancers, through the use of drug trials in cancer patients, have been shown to respond to the drug.

- **a** Make a list of the cancers currently approved for treatment with this immunotherapy drug.
- **b** What does 'approved' mean?
- **c** Why aren't all cancer patients given this type of therapy?
- **d** Pembrolizumab (tradename Keytruda) is known as a humanised monoclonal antibody. What does this mean?
- **e** Pembrolizumab has a positive statistical effect in patients administered the drug in terms of efficacy, depending on the type of cancer with varying rates of destroying cancer cells with the treatment. Overall survival rates are still being researched. Why do you think that is?
- **f** The science that the drug is based on was recognised in 2018 when the Nobel Prize in Physiology or Medicine was awarded to James Allison and Tasuku Honjo. In the 1990s, they respectively made discoveries involving CTLA-4 and PD-1, which are two proteins that can act as a brake or check on tumour-fighting T cells. If you inhibit that process, you can reawaken the T cells to do their job. Keytruda is what is known as a checkpoint inhibitor.
  - **i** What type of material is a checkpoint inhibitor?
  - **ii** What is the function of a checkpoint inhibitor?
  - **iii** Where do checkpoint inhibitors operate in your body?
  - **iv** Why are checkpoint inhibitors important?
  - **v** Keytruda blocks the interaction between programmed cell death receptor-1 (PD-1) and the molecules to which it specifically binds. What is programmed death?
  - **vi** Does this process only affect tumour cells? If not, what else can it affect? Is that a problem?

Unit 4, Area of Study 1 review

Multiple choice

Question 1 ©VCAA 2013 Q39 ADAPTED HARD

Doctors are concerned about the overprescription of antibiotics. Many antibiotics have become ineffective against certain types of bacteria. This is due to

A antibiotics being less concentrated than they were 20 years ago.
B mutations occurring in the bacteria due to exposure to antibiotics.
C the antibiotic acting as a selection pressure selecting antibiotic-resistant phenotypes.
D selectively bred, antibiotic-resistant bacteria being introduced into the population.

Question 2 ©VCAA 2012 EXAM 1 Q9 ADAPTED HARD

Major histocompatibility complex (MHC) class 1 molecules

A are responsible for releasing antigens from the cell nucleus.
B display foreign antigens to cytotoxic T cells.
C produce antigen-specific antibodies.
D are found on all cells in the human body.

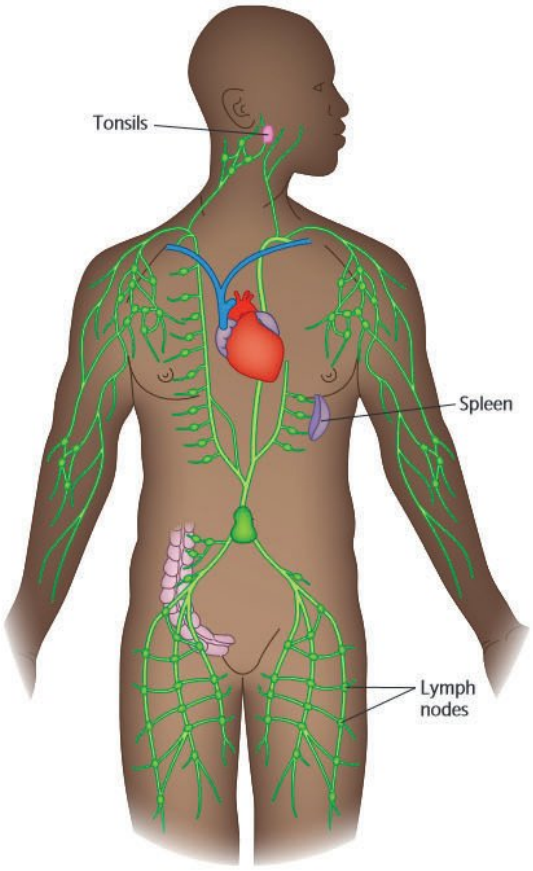
Question 3 ©VCAA 2014 Q14 EASY

An example of 'self' material in an adult human female is

A pollen inhaled from flowers in her garden.
B sperm cells present in her reproductive tract.
C cells lining the inside of her arteries.
D bacteria inside the alveoli cells in her lungs.

Question 4 ©VCAA 2017 Q23 MEDIUM

The following diagram represents the lymphatic system, which includes the lymph nodes, spleen and tonsils.



The human lymphatic system

In these particular organs

A allergies trigger an initial response.
B clotting factors are inactivated to help seal a wound.
C red blood cells identify non-self antigens.
D B cells detect pathogens presented on dendritic cells.

Question 5 ©VCAA VCAA 2014 Q15 ADAPTED EASY

The first line of defence against pathogens includes the

A production of antibodies.
B release of tears from tear ducts in the eyes.
C release of histamine from mast cells.
D ingestion of viruses by phagocytes.

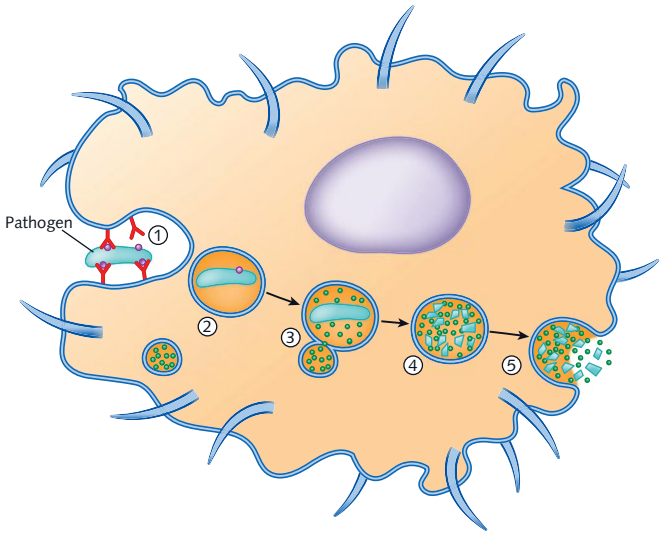
Question 6 ©VCAA 2018 Q23 ADAPTED MEDUM

Chickenpox is caused by a virus. Once you have caught chickenpox and recovered, you will not develop symptoms of chickenpox again even if you are exposed to the virus. This is because you have

A natural passive immunity.
B natural active immunity.
C artificial passive immunity.
D artificial active immunity.

Question 7 ©VCAA 2019 Q20 ADAPTED MEDUM

The following diagram shows the process of phagocytosis. This process is vital for immunity against extracellular infections.



What is happening at position 4?

A Enzymes that break down the micro-organism are released into the vesicle.
B Antibodies are added to the vesicle to kill the micro-organism.
C Enzymes are digesting the micro-organism.
D Intracellular microbes are attacking the micro-organism.

Question 8 ©VCAA 2016 Q20 ADAPTED EASY

Higher organisms have evolved the inflammatory response as a defence mechanism against infection and injury. If you step on a nail, you notice that the site around the wound goes red. This response

A is specific to the form of foreign body.
B is part of the adaptive immune system.
C involves activation of the complement system.
D involves lymphocyte production.

Question 9 ©VCAA 2014 Q16 ADAPTED EASY

An example of a specific response by the human immune system is

A production of cytotoxic C cells.
B epithelial cells producing defensins.
C cytokines being produced by macrophages.
D the engulfing of non-self material by a phagocyte.

Question 10 ©VCAA 2017 Q37 ADAPTED MEDUM

Mosquitoes are responsible for the transmission of the viral disease yellow fever. An outbreak of yellow fever occurred in an area of Brazil in January 2017. The outbreak then spread to other areas within Brazil.

Which one of the following is a correct statement about this outbreak of yellow fever?

A This outbreak of yellow fever is defined as an epidemic.
B Bathing in the large bodies of still waters in the areas with yellow fever helped reduce the number of individuals affected.
C This outbreak of yellow fever occurred in populations with high vaccination rates for yellow fever.
D Infected individuals who travelled to other areas of Brazil did not increase the spread of the disease.

Question 11 ©VCAA 2016 Q24 ADAPTED MEDUM

A protein called circumsporozoite protein (CSP) is being studied by scientists in their search for a malaria vaccine. CSP is secreted by the malaria parasite and is present on its surface. For the vaccination to work, the scientists want CSP to act as an

A antibody.
B allergen.
C antigen.
D antibiotic.

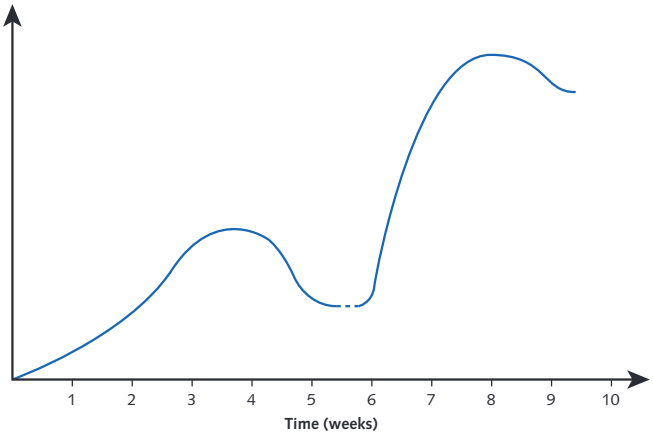
Question 12 ©VCAA 2016 Q23 ADAPTED MEDUM

When bitten by a redback spider, a builder was treated with the injection of an antivenom serum. The treating doctor explained to him that the injection would not protect the builder against any future redback spider bites. This is because antivenom serum is used to achieve

A active and natural immunity.
B passive and artificial immunity.
C active and artificial immunity.
D passive and natural immunity.

Question 13 ©VCAA EASY

The following graph shows the relative concentration of antibody in the blood after exposure to an antigen.



From this graph you can conclude that

A the highest level of antibody in the blood occurred in week 4.
B at week 6, the person was exposed to the same antigen for a second time.
C at week 1, the person had not been exposed to the antigen.
D antibody production was more rapid at week 2 than at week 6.

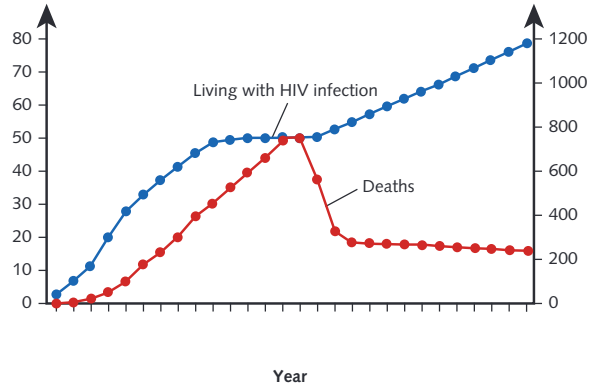
Question 14 ©VCAA 2018 Q24 ADAPTED MEDUM

Monoclonal antibodies can be produced and used to treat different types of cancers. Which one of the following statements about monoclonal antibodies is correct?

A Monoclonal antibodies are lipid molecules.
B Monoclonal antibodies pass through the plasma membrane of a cancer cell and attach to an antigen within the cell.
C Monoclonal antibodies are produced by fusing a B cell clone with cells extracted from a plasma cell tumour.
D Monoclonal antibodies produced to treat bowel cancer will be identical to monoclonal antibodies produced to treat throat cancer.

Question 15 ©VCAA 2018 Q29 ADAPTED MEDUM

The following graph shows the death rates from AIDS (acquired immune deficiency syndrome) and the number of people infected with the human immunodeficiency virus (HIV) in the period 1981–2008.



Based on the information in the graph, you can conclude that

A a vaccination program for HIV was introduced in 1995 across many countries within a targeted population.
B more people were living with HIV infection in 1986 than were dying from the infection in 1995.
C HIV infection killed more people in 2005 than in 1985.
D before 1995, many people who were infected by HIV went on to develop AIDs, which led to their deaths.

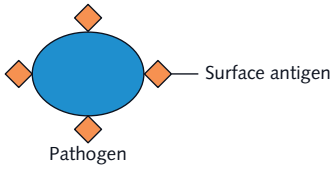
Short answer

Question 1 ©VCAA 2014 Q4 ADAPTED

a The term 'pathogen' is not a classification of any organism group. How is pathogen defined?

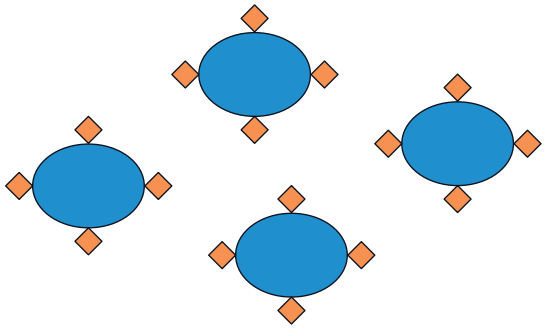
1 mark

The following diagram shows a generalised pathogen with antigens on its surface. The immune system responds to antigens by making antibodies.



b Draw an antibody that would be effective against the pathogen shown in the diagram above. Label the different parts of the antibody. 2 marks

Antibodies can work by forming antigen–antibody complexes. The following diagram shows four pathogens.



c i Illustrate on the diagram above how the antigen–antibody complex forms. Use at least four antibodies in your drawing.

2 marks

ii What is the purpose of the antigen–antibody complex?

2 marks

Question 2 ©VCAA 2018 Q3 ADAPTED

Many organisms, such as bacteria, fungi and viruses, can infect plant species. Plants do not have an immune system comparable to that evolved by animals. However, they have evolved physical barriers to stop invading pathogens from causing significant damage.

a Describe two physical barriers that a plant may have to protect itself from an invading pathogen.

2 marks

b Humans have a complex immune response to invading pathogens. State two physical barriers that the human body has to prevent pathogens from entering.

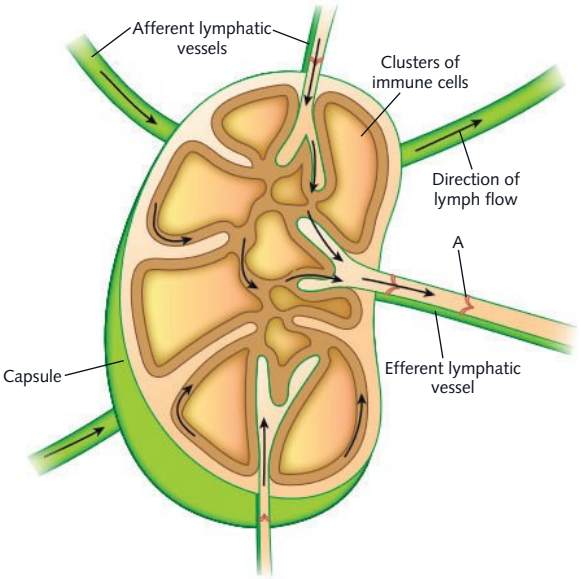
2 marks

c Once a pathogen has gained entry to the internal environment of a human, it can encounter macrophages and dendritic cells. Describe how these help defend the human body.

2 marks

Question 3 ©VCAA 2015 PART B Q5 ADAPTED

The following diagram represents a human lymph node.



a A substance enters the afferent lymphatic vessels and exits at the efferent lymphatic vessel. Name this substance.

1 mark

b Describe two roles of lymph nodes in the immune response.

2 marks

c One cell found within these clusters of immune cells in the lymph node has a large nucleus and extensive rough endoplasmic reticulum. Name this cell and explain its role in the adaptive immune system.

2 marks

Genetic changes in a population over time

9

By the end of this chapter you will have covered the following material.

Key knowledge

Genetic changes in a population over time

- causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow; and mutations as the source of new alleles pp. 329–340; 343–346
- biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity pp. 341–343
- manipulation of gene pools through selective breeding programs pp. 346–349
- consequences of bacterial resistance and viral antigenic drift and shift in terms of ongoing challenges for treatment strategies and vaccination against pathogens pp. 349–357

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- identify, research and construct aims and questions for investigation pp. 339–340; 352–354
- identify independent, dependent and controlled variables in controlled experiments pp. 339–340; 352–354
- formulate hypotheses to focus investigation pp. 339–340
- predict possible outcomes pp. 339–340

Plan and conduct investigations

- design and conduct investigations; select and use methods appropriate to the investigation, including consideration of sampling technique and size, equipment and procedures, taking into account potential sources of error and uncertanty; determine the type and amount of qualitative and/or quantitative data to be generated or collated pp. 339–340; 352–354
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 339–340; 352–354

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 339–340; 352–354
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 339–340; 352–354
- demonstrate ethical conduct when undertaking and reporting investigations pp. 339–340; 352–354

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 339–340
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 339–340; 352–354
- plot graphs involving two variables that show linear and non-linear relationships pp. 339–340

Analyse and evaluate data and investigation methods

- process quantitative data using appropriate mathematical relationships and units, including calculations of ratios, percentages, percentage change and mean pp. 339–340
- identify and analyse experimental data qualitatively, handling where appropriate concepts of: accuracy, precision, repeatability, reproducibility and validity of measurements; errors (random and systematic); and certainty in data, including effects of sample size in obtaining reliable data pp. 339–340; 352–354
- identify outliers, and contradictory or provisional data pp. 339–340; 352–354
- repeat experiments to ensure findings are robust pp. 339–340

Construct evidence-based arguments and draw conclusions

- evaluate data to determine the degree to which the evidence supports or refutes the initial prediction or hypothesis pp. 339–340
- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 339–340
- identify, describe and explain the limitations of conclusions, including identification of further evidence required pp. 339–340

Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement pp. 339–340; 352–354
- discuss relevant biological information, ideas, concepts, theories and models and the connections between them pp. 339–340; 352–354

Online Chapter Map
Chapter 9 map

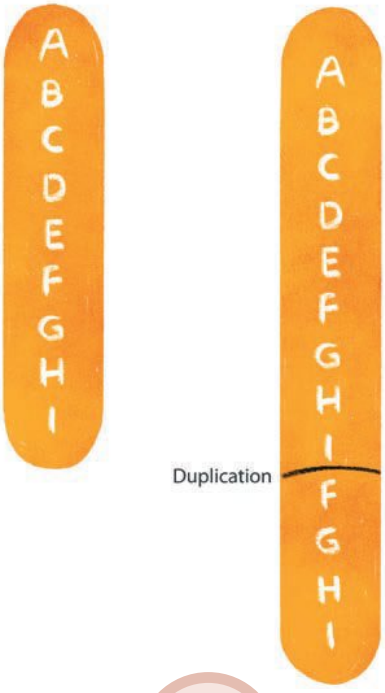
9 Genetic changes in a population over time

The gene pool of the Australian population today is very different from that of 100 years ago. New alleles have been introduced into the population by gene flow and genetic drift. New alleles have been made by mutation.

p 329

91 Mutatons are the source of new aees

Mutatons are permanent changes n DNA due to mstakes durng relictin. They are the source of new aees and ther effect can be neutra harmfu or advantageous for a speces They are the raw matera for natura seecton



p 333

92 Chromosoma rearrangements

Sometmes whoe chromosomes are rearranged Bts of chromosomes break off and flp aroun, jin to an other chromosoe, or become dupcat ed so there are many copes of the same gene Some genes mutate to become competey new genes for new protin.

p 336

93 Changng aee frequences n popuatons

The gene poo s made up of a the aees n a popuaton Aee frequenies can change because of gene flow, when mgrants enter the popuaton and nterbree; because of geneic dif, when some aees are not passed on and are ost from the gene po; or when the popuaton sze changes dramatcay.

9780170452533

p 343

94 Natura seecton

The aee frequenies of gene poos are ltered by the process of natura seecton n a popuaton wth varaton the ndvduas wth favourabe trats (a nd aees) that overcome slecion pressurewill urvive and reprodce, pssi ng those trats onto ther offspin. Ths eads to a change n the gene poo of a popuaton over tme

p 346

95 Human manpuaton of gene poos

Throughouhistry, humans have seected pants and anm as wth the most desrabe trats for br eedng n ths way, humans have seectvey bred modern igh-yedng cro p varetes fromild speces hundreds of dog breeds ith attracive features and horses for strength and speed Humans are a lso reducng t he capacty o f wd s peces to ada pt to changng con dtons

p 349

96 Natura seecton and consequences for dsease

A consequence of natura seecton s the emergence of aniioic resistanc in bacte ra and superbugs whch are nolonger contrled by antbotcs New vrus strans arse as a reult of muttions and gentic rearrangemens; new and updated vaccnes are as mportant as ever.

Populations do not stay stable. Mutations introduce new alleles into populations. Natural selection acts on this variability to select the most well-adapted individuals to reproduce and pass on their alleles to a new generation. This has been going on for a very long time.

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Online Chapter Mapv
- Chapter 9 map (p. 326)

Onne.e:Ker, m v
- Flashcards (p. 328)

Weonkm v
- Natural selection (p. 344)
- Human impacts on Brazilian parrots (p. 348)
- Learn how influenza spreads (p. 355)

Onne Wyrkm heetmv
- Natural selection (p. 344)
- Parrot gene pool (p. 348)

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- Chapter 9: Summary of key concepts (p. 360)

Online . e: Ker, m
Chapter 9 Flashcards

Know your key terms

amino acid sequence
antibiotic resistance
antibiotic resistance gene
antigenic drift
antigenic shift
artificial selection
beneficial mutation
block mutation
bottleneck effect
broad spectrum
conserved
deleterious mutation
deletion mutation
double-strand break
duplication
extinct
fitness
fixed
founder effect
gene duplication
gene flow
gene pool
gene sequence
genetic drift
genotype
germline
heritable
horizontal gene transfer
insertion mutation
inversion mutation
missense mutation
monoculture
multidrug resistance
mutagen
mutation
natural selection
neutral mutation
nonsense mutation
phenotype
point mutation
population
population genetics
selection pressure
selective breeding
silent mutation
somatic
species
subspecies
substitution mutation
synonymous mutation
translocation

Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

WB
REMEMBER
PAGE 181

1. A gene is a unit of inheritance. At the molecular level, a gene is a sequence of DNA that codes for a protein. Alternative forms of the same gene are called alleles.
2. Homologous chromosomes are matching chromosomes that have the same genes located at the same positions.
3. Genotype is an organism's genetic composition. Phenotype is how those genes are expressed in that organism.
4. Complementary base pairing in DNA is the pairing of the nitrogenous base adenine with thymine (or uracil in RNA) and cytosine with guanine.
5. Dominant alleles are represented by a capital letter whereas recessive alleles are represented by a lower-case letter.
6. Gene expression refers to the transfer of the DNA code in a gene, via transcription and translation, to ribosomes in the cytosol to produce a functional gene product, protein.
7. Natural active immunity develops when an organism comes into contact with a pathogen and develops memory B and memory T lymphocytes to the antigens of that pathogen or its products.
8. Antigens (weakened or dead microbes or their fragments) are introduced in vaccines. The body produces specialised lymphocytes (B plasma cells) and antibodies.
9. Herd immunity is developed when a large enough number of people in a population are vaccinated so that it halts the transmission of the disease to non-vaccinated people.

The peppered moth, *Biston betularia*, is widespread in the UK. Historically, the standard moth form, *typica*, was white, liberally speckled with black spots (Figure 9.1a). The dark *carbonaria* form (Figure 9.1b) was much rarer. During the 1800s, British cities and the countryside were transformed by the Industrial Revolution. Hundreds of coal-powered factories produced large quantities of airborne soot and other pollutants. By 1895, 95% of moths in industrial regions, such as Manchester, were the black *carbonaria* form. Lepidopterist J.W. Tutt proposed a link between the Industrial Revolution and changes in the moth population. The typical light-coloured tree trunks had become blackened by soot and now presented a new environment for the moth population. The black moths were better camouflaged against the black tree trunks than the common white speckled form. Consequently, the white speckled moths were easier for birds to see and were preyed on more than the black moths. Over time, black moths came to dominate the population.

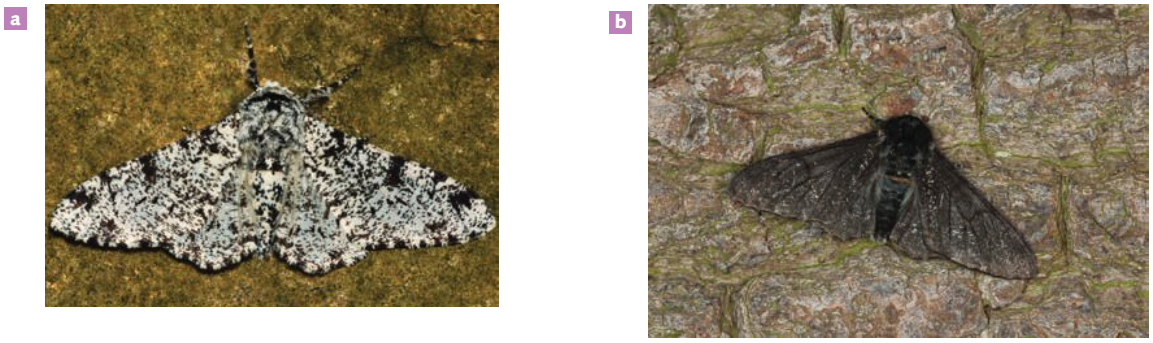

Figure 9.1 The peppered moth, *Bston betuara* , has a a white speckled *typca* form and b dark *carbonara* form.

In 1950, clean air legislation was passed, and tree trunks are no longer blackened with soot. Dark-coloured moths are again suffering greater predation on the naturally white tree trunks and they have become less common. Both dark and white forms continue to exist in the population.

Individuals in any population, such as the peppered moths, express a range of different **phenotypes**, or observable characteristics. This is because members of a population have variation in **genotypes**, or genetic make-up, that results in variation in their phenotypes. This genetic variation is **heritable**; it can be passed to the next generation and under certain circumstances may give an individual an advantage in survival and reproduction compared to the rest of the population. In the peppered moth example, a **mutation** (genetic change) in genotype produced a dark-coloured form in this population. This dark phenotype conferred a survival advantage in the changed environment. The genotypic variation may also give a disadvantage or have no effect at all. Either way, genetic mutation introduces new alleles (alternative forms of a gene) and, therefore, new variation into populations.

9.1 Mutations – the source of new alleles

Phenotypic variation is crucial for a population to survive when changes occur in the environment. Consequently, genetic variation is essential for the survival of populations. New alleles generally come from existing alleles through mutation. Mutations are rare and barely noticeable in a large population but they are essential because they are the ultimate source of variation within a population.

CONNECT

Translation of proteins is discussed in Chapter 3.

Mutations are changes to DNA. A spontaneous mutation may arise from a mistake when DNA is copied during cell division, induced by physical or chemical agents called **mutagens**. Mutations may also arise through the action of biological agents, such as viruses that insert their genetic sequences into the host's DNA. Mutations that occur in genes sometimes result in changes to the translated proteins they code for. These changes may be subtle, or they may be severe with potentially catastrophic effects for the survival of the organism. Occasionally, the mutation enhances the function of the protein or makes the organism better suited to the environment.

The effect of a mutation also depends on whether it has occurred in non-reproductive (body, or **somatic**) cells or in reproductive (gametes, or **germline**) cells (Figure 9.2).

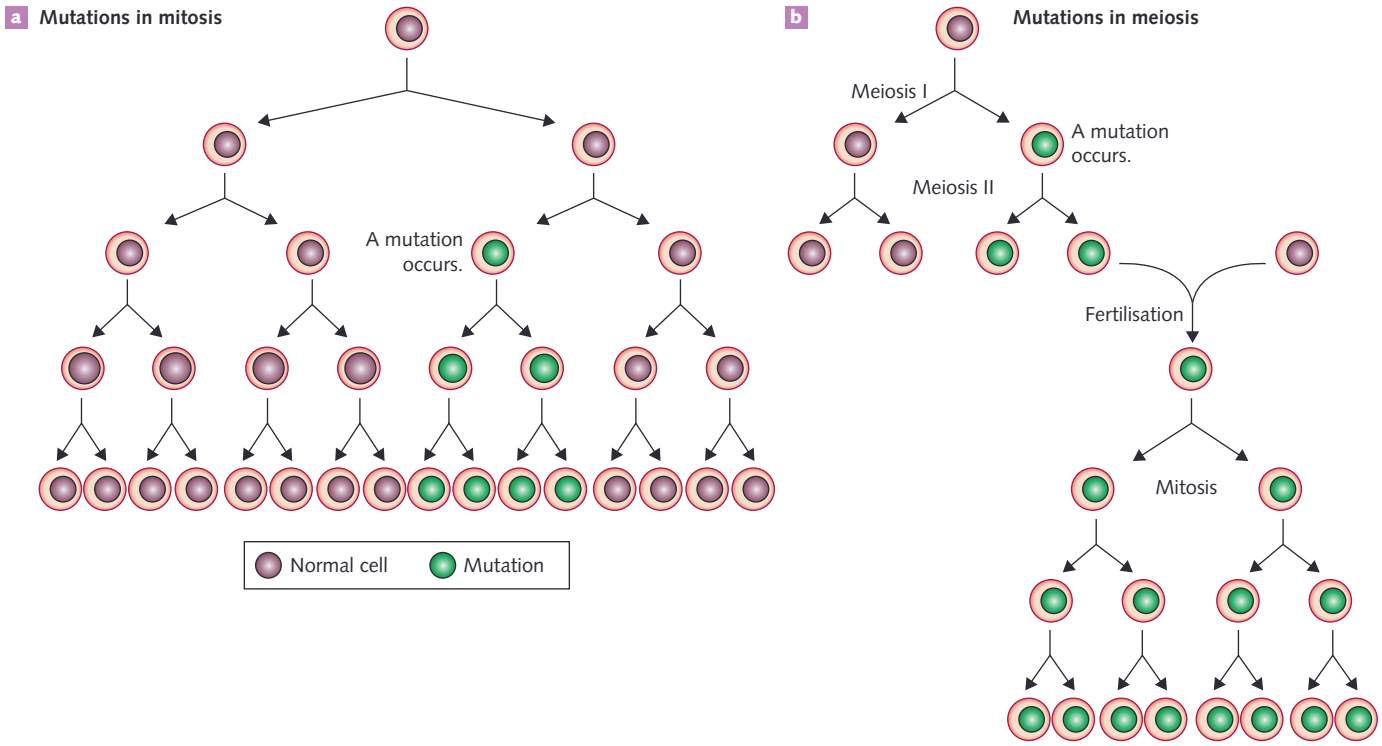

Figure 9.2 a Mutations in somatic cells affect only the cell in which it occurred and all its daughter cells. b Mutations in germline cells affect all body cells of the individual who inherits them.

A mutation in a somatic cell occurs only in the affected body cell and the daughter cells produced from it by mitosis. All other cells of that organism lack the mutation. Cancer is one possible outcome of mutations in somatic cells. The mutations accumulate in particular genes or regions of the DNA that accelerate the rate of cell division, abolish the cell's ability to undergo apoptosis or increase the rate of mutations within the cell.

Mutations that occur in germline cells affect gametes and can be inherited or passed on to the next generation so that they are incorporated into every cell of the offspring. Often, the germline mutation results in developmental abnormalities that cause the affected embryo or foetus to spontaneously abort. If carried through to birth, the germline mutation may result in congenital disorders in the offspring with varying severity. Occasionally, a gene mutation changes or enhances the function of the encoded protein, which, if circumstances suit, enhances the survival of the organism. If the mutation is consistently passed on from one generation to the next, a new allele has entered the population.

Point mutations

WB
9.1.1
POINT MUTATIONS
PAGE 182

The simplest form of mutation is a **point mutation** in which just a single nucleotide within the original DNA sequence is affected. If the point mutation occurs in a gene, the mutated **gene sequence** can be transcribed and translated into a protein that may be the same as that encoded by the original form of the gene, or it may be altered. When the protein is altered, the mutation may have a subtle or a dramatic effect on its structure and function.

Substitution

A substitution occurs when one nucleotide is replaced by another (e.g. adenine substituted by guanine). **Substitution mutations** have a number of possible effects on the translated protein.

A **silent mutation**, also referred to as a **synonymous mutation**, occurs when the substituted base results in a nucleotide triplet, or codon, that codes for the same amino acid as the original. For example, AGA and AGG both specify for the addition of an arginine amino acid in the polypeptide chain (Figure 9.3). Therefore, the protein encoded by the mutated gene is identical to that encoded by the original gene. Silent mutations are possible because there is a level of redundancy in the genetic code. Recall that the genetic code consists of 64 codons that code for 20 amino acids plus the instructions to start and stop translation. Therefore, any individual amino acid can be encoded by more than one codon.

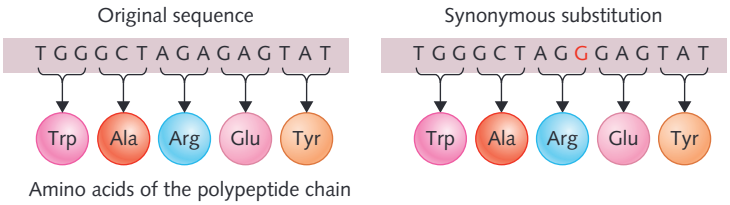

Figure 9.3 A silent mutation

A **missense mutation** arises when a single nucleotide substitution changes the amino acid. For example, substitution in an AGA codon to generate an AGC codon results in a serine amino acid being added to the polypeptide instead of the original arginine (Figure 9.4).

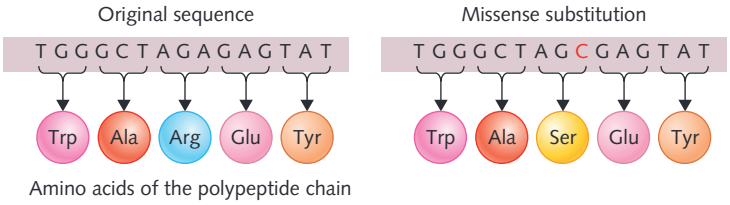

Figure 9.4 A missense mutation in the gene sequence leads to one amino acid being substituted for another in the polypeptide chain.

A **nonsense mutation** occurs when a single nucleotide substitution creates a new stop codon within the original gene sequence; for example, substitution in a GAG codon to generate a TAG codon (Figure 9.5). This leads to early termination of translation of the transcribed gene sequence because the remaining sequence downstream of the new stop codon is not translated. This results in an incomplete polypeptide.

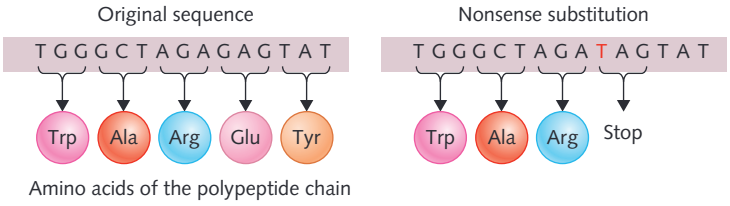

Figure 9.5 A nonsense mutation in the gene sequence results in premature termination of translation.

Insertions and deletions

An **insertion mutation** occurs when one or more nucleotides are added at a site within the original gene sequence. A **deletion mutation** occurs when nucleotides are lost from a site within the original gene sequence. The effect of the insertion or deletion is frequently a frameshift mutation, in which the reading frame for the corresponding amino acids has been shifted away from the original and all the codons downstream of the mutation are affected. The consequence for the translated protein is that the amino acids downstream of the mutation bear no resemblance to those of the original polypeptide (Figure 9.6). It is also probable that a new stop codon will be introduced in a different position from that in the original gene sequence. Under such circumstances, even a single nucleotide insertion or deletion can have a profound effect on the corresponding protein.

WB
9.1.2
CYSTIC FIBROSIS MUTATION
PAGE 184

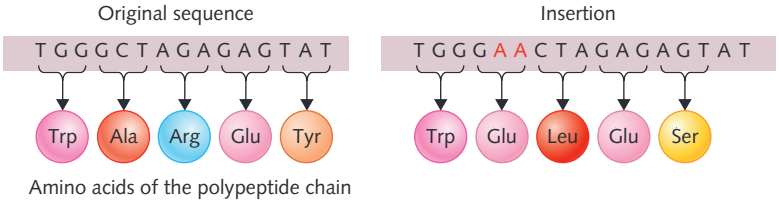

Figure 9.6 An insertion in the gene sequence results in a frameshift mutation. Here, two adenines have been inserted.

Mutations affect protein structure and function

A protein's function ultimately depends on its shape, which is determined by the protein's primary structure. Although mutations affect protein primary structure directly, it is how they influence protein folding that ultimately impacts on an organism's survival.

CONNECT
Revise protein structure on page 57

Some regions of a protein's three-dimensional structure are much more sensitive to alteration than others. Changes to the amino acids lining the active site of an enzyme can profoundly influence its function. Even a single missense substitution that leads to the loss of an enzyme's function is likely to be harmful. The critical amino acids involved in binding substrates or carrying out reactions in the active site of enzymes are often **conserved**. These amino acids are retained in the protein over time and across different species, whereas other amino acids have become substituted. For example, the **amino acid sequences** and three-dimensional structures in the active site of a family of enzymes called the aspartyl proteases are consistent across diverse eukaryotic organisms from fungi to mammals, even though the details of the rest of the proteins vary considerably (Figure 9.7).

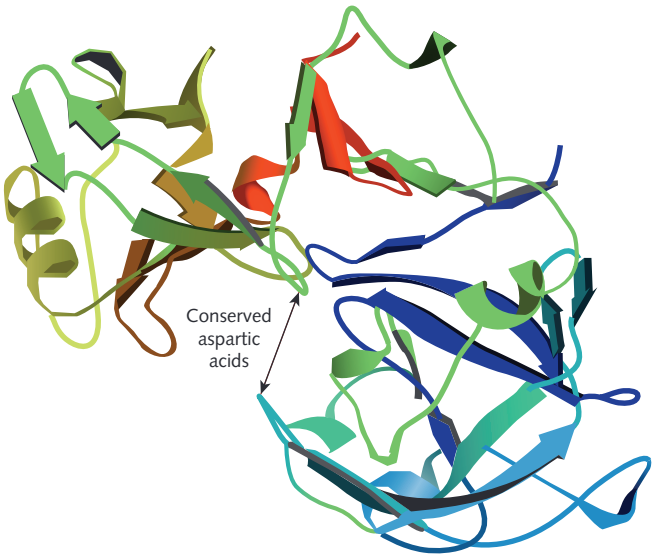

Figure 9.7 Human pepsin, an example of an aspartyl protease. The aspartic acid residues in the active site are conserved in the aspartyl proteases of fungi and animals.

Effects of mutations on survival

A protein's function depends on its structure. Mutations that change a protein's structure can affect protein function, impacting on the organism's survival. Therefore, mutations can also be classified according to how they affect the protein's function and expression and whether the organism's survival is unchanged, changed for the worse, or changed for the better.

Neutral mutations

Silent mutations do not change the protein product, so the organism's survival is unaffected. This is a **neutral mutation**. Missense substitutions are sometimes also neutral mutations, provided that the original amino acid is swapped with another that has similar properties. For example, in the ABCA1 gene, which codes for a protein involved in cholesterol transport, a missense substitution in a single GAA codon generates a GAC codon. This causes the amino acid glutamic acid to be swapped for an aspartic acid. Both amino acids are negatively charged and reside on the surface of the protein where they interact with surrounding water, so the properties and function of the protein remain essentially the same.

Deleterious mutations

Living organisms are very complex and random mutations that disrupt the function of an encoded protein can affect the whole organism, undermining the organism's overall ability to carry out its basic processes and survive. Such mutations are referred to as **deleterious mutations**. Most mutations are deleterious.

Nonsense mutations are typically deleterious because they result in the production of an incomplete protein that is non-functional. However, these deleterious mutations may persist if the individual who carries them also has a copy of the normal allele that encodes for the functional version of the protein. The deleterious mutation is thus masked within the phenotype of the organism. If the organism only has two non-functional alleles for a particular gene, the condition usually results in the death of the organism before they have the opportunity to reproduce and pass the alleles onto any offspring.

Beneficial mutations

Occasionally, gene mutations generate a new allele that benefits the survival of the organism. The type of **beneficial mutation** can vary; it could be a missense mutation that changes the function of the original protein, or it could be a nonsense mutation that eliminates a protein that may have been harmful to the organism in some circumstances.

Many mutations produce recessive alleles that can be masked by the effects of the original allele, which becomes the allele for the dominant phenotype. Each human may carry several hundred mutations, most of which will never be noticed, particularly if they have children with partners who are not closely related.

Conversely, recessive alleles are an important source of variation within populations. This was the case with the peppered moth in the United Kingdom. Before the Industrial Revolution, the dominant *carbonaria* forms were extremely rare. During the Industrial Revolution, alleles coding for the recessive white (*typica*) trait survived mainly in heterozygous members of the population at a low level. Only the extremely rare homozygous individuals experienced the selective pressure of increased predation.

Sexual reproduction has been key to producing populations with variation, through the random mixing and assortment of traits from one generation to the next through meiosis and chance fusion of gametes.

KEY CONCEPTS

- Mutations are canes in NA. Mutations occur spontaneously or are caused by mutagens. The potetial effect o a mutation depends on whether the mutation ocur in sotio germline cells.
- Point mtations can caue changes in a DNA sequence by subtttion, inserton or delein f a nucleotide.
- Substitutions cn be slen muations, which cause no chang in the encodedpotein, or they can be missense or nonsensemutations which alter the structure and function of t encoded protein.
- Insrtionsordeletions can cuse frameshift mutations that affec the amino acid seqence downstream, sevrely afecting the ecded protein.

Concept questions 9.1

1 Mutations can be beneficil, harmful neutral. What doesthis mean?
2 What is the difference beteesomatic cells and gerlineells?
3 Describe tee possible efecs a mutation could have on an oraism's rvival.
4 A frequent effect of aframshft mutation is to produce a stop con earlier tan normal. What effect would his have on the structure and function of the encoded prtein?
5 Lst the types of poit mutations in the order of potetial severity of their effects from least to most severe.

HOT Chaenge

6 Varaion in species is a funtion of mutations and can e vital fr speces' survival. Greater varation ocurswithin speces that engage in sexual reproducton. Ho could se xual reproduction produce greater vriation?

9.2 Chromosomal rearrangements

In addition to gene mutations, genetic variation can be driven by wholesale changes to the chromosomes. Alterations to chromosomes contrast with single point mutations because they can affect many genes simultaneously. Rearrangements of whole segments of chromosomes are described as **block mutations**. Some of the variations that occur with chromosomes, such as chromosome number, are natural in certain situations and are therefore integral to the functioning and continuity of the species. Others arise because of anomalies that occur during cell division or the formation of the gametes.

WB
9.2 CHROMOSOMAL REARRANGEMENTS PAGE 185

Block mutations

Block mutations lead to changes in chromosome structure. A block mutation may occur within a single chromosome or between different chromosomes. In the process, a segment of a chromosome containing multiple genes may be lost, duplicated, switched in orientation, or swapped between chromosomes.

Deletions

A chromosome may undergo **double-strand breaks** at two positions and the section in between may drop out, removing all its genes with it. If the two ends then re-join, a shorter chromosome results with a segment missing. This is called a chromosome deletion (Figure 9.8a). It can have a profound effect on the development of an organism because it leads to an absence of certain genes. All but the shortest deletions are usually fatal and the few that are not fatal are associated with adverse effects.

Inversions

Another kind of chromosomal rearrangement occurs if a chromosome breaks in two places and the middle segment rotates 180° before being re-joined within the chromosome. This reverses the normal sequence of genes (Figure 9.8b) and is called **inversion**. The effects of inversions are usually less dramatic than other types of chromosomal changes because genes have been neither gained nor lost and the genes within the inverted segment can still function normally. However, the inversion may disrupt a gene through which it occurs or cause two different genes to be fused together. Also, if the chromosomes do not align properly for meiosis, the affected individual may have reduced fertility.

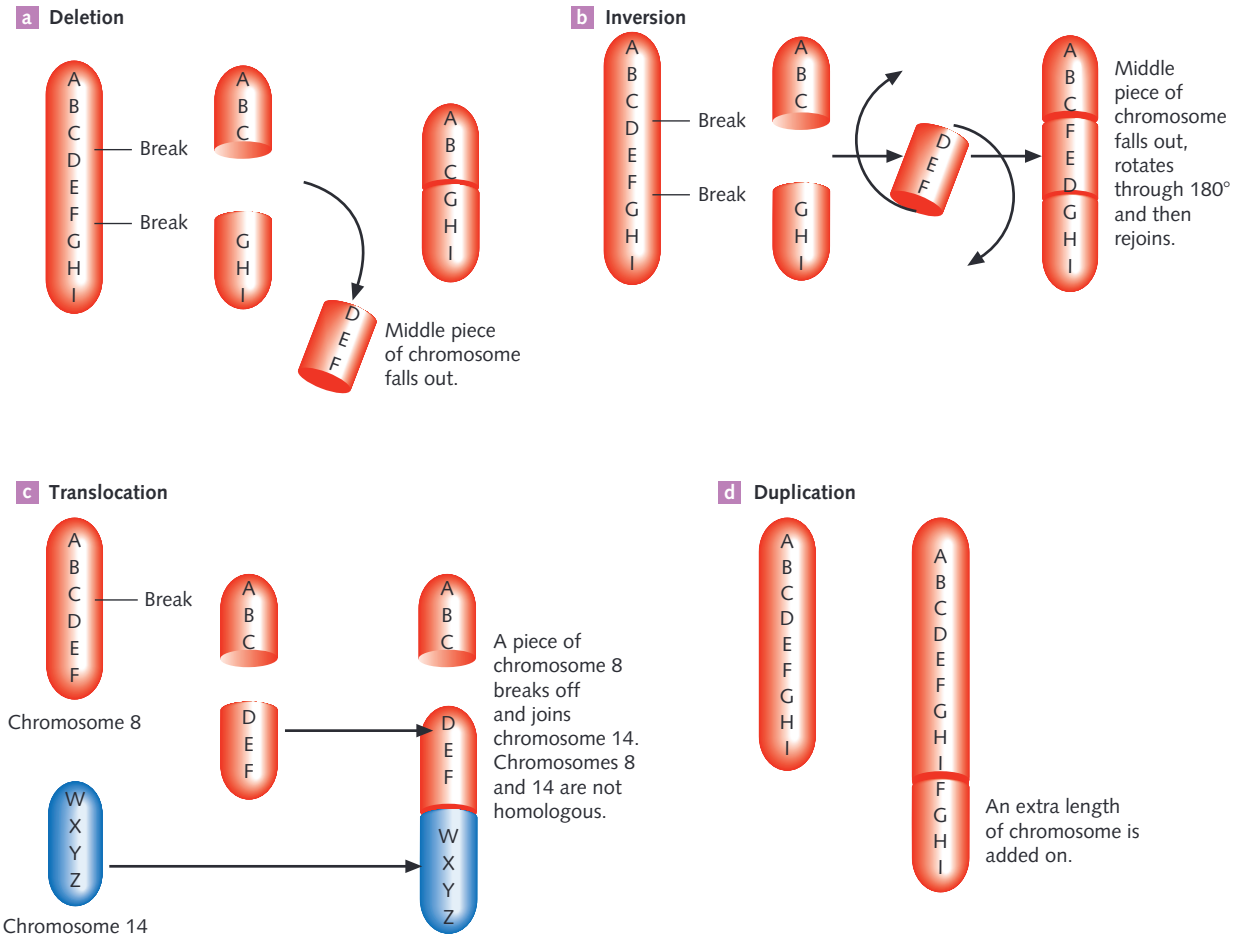

Figure 9.8 Abnormalities caused by chromosomal or 'block' mutations may arise by **a** deletion, **b** inversion, **c** translocation or **d** duplication.

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Translocations

Sometimes a section of one chromosome breaks off and attaches to another chromosome. This is known as **translocation** (Figure 9.8c). In humans, translocation can occur between chromosomes 8 and 14. Normal control over the genes in that segment is lost, often resulting in a form of cancer.

Duplications

A **duplication** occurs when an extra copy is made of a section of chromosome and inserted into the same chromosome or another chromosome (Figure 9.8d). Gene sequences can be replicated many, sometimes thousands, of times. Like other chromosomal abnormalities that change the number of copies of particular genes, duplications are frequently harmful. However, on occasions, they can be advantageous. The various genes that control the different haemoglobins produced in human red blood cells are thought to have arisen by duplications.

Acquiring new genes

A combination of block and point mutations generate entirely new genes coding for novel proteins. New genes arise primarily through **gene duplication** and subsequent point mutation. This contrasts with the appearance of new alleles, which arise by point mutation in an existing gene. The gene duplication event is normally the result of chromosomal duplication. Following gene duplication, one of the gene copies usually retains its original function. The fate of the other copy can vary (Figure 9.9). In some cases, the copy fortuitously mutates into a new gene, and the consequent new protein has an innovative property or function that benefits the organism.

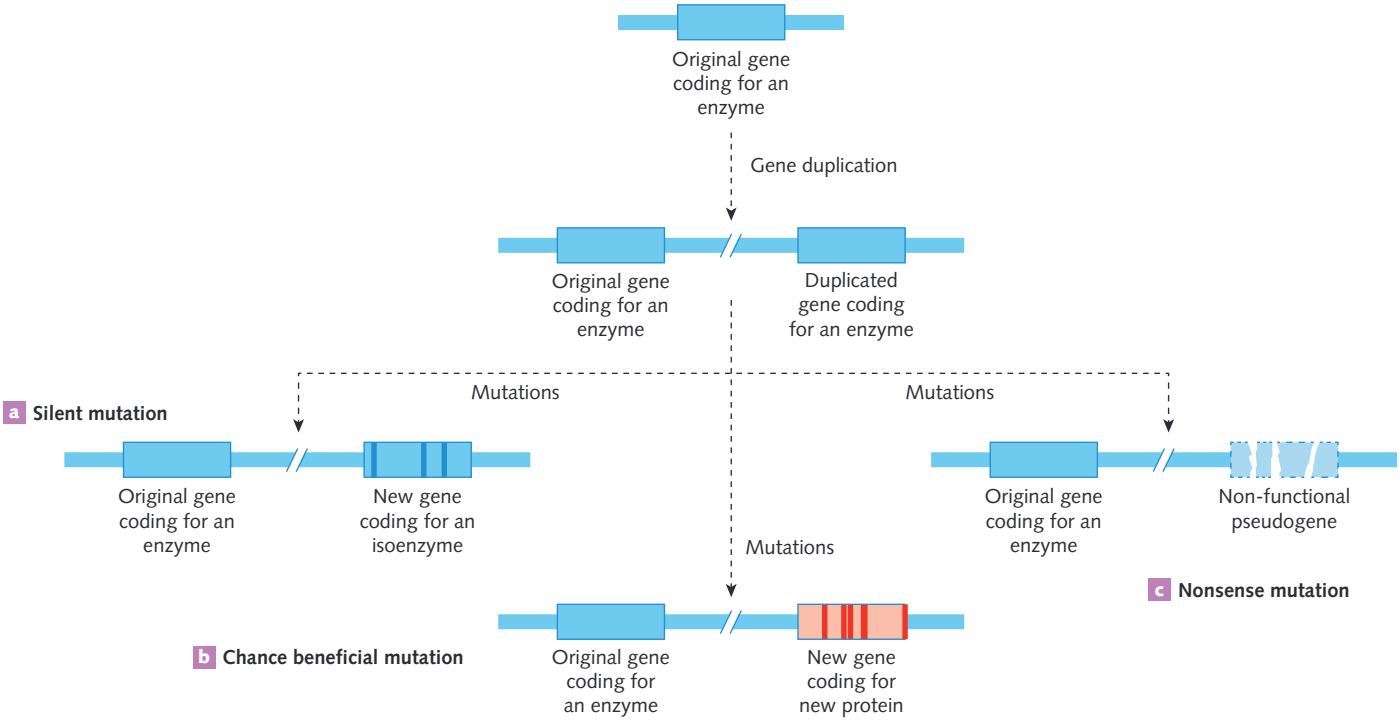


Figure 9.9 An example of duplication and subsequent mutation of a gene coding for an enzyme. a Silent mutations result in two genes for enzymes with the same function, so-called 'isoenzymes'. b Chance beneficial mutations may result in a new gene for a protein with a novel function. c Nonsense mutations in a duplicated gene may result in a non-functional pseudogene.

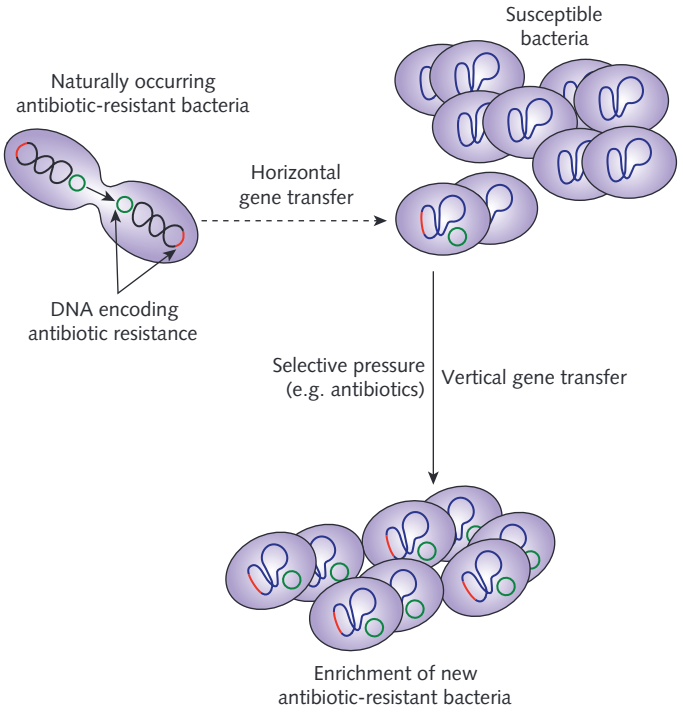


Figure 9.10 **New species and strains of antibiotic-resistant bacteria are sometimes generated by horizontal gene transfer.**

Gaining new genes by horizontal gene transfer

Genes for proteins with novel properties can also arise by **horizontal gene transfer**. This is the process by which an organism obtains a new gene directly from another organism, even another species, rather than by mutation or inheritance from a parent.

Horizontal gene transfer is common in prokaryotes. Horizontal transfer mechanisms include acquiring naked pieces of DNA from their environment, new genes through infection by a virus (bacteriophage), or new genes directly from another bacterium through cell-to-cell transfer (conjugation). Conjugation enables genes that enhance survival to spread through a population of bacteria in a relatively short time. It is believed to be one reason for the emergence of new strains of multi-drug resistant bacteria (Figure 9.10).

In contrast, vertical gene transfer is when genetic material is transferred from parent to offspring.

KEY CONCEPTS

- Mutations can causechanges in chromosome structure and chromosome number.
- Deetins, ivrsins, ransloations and duplications mayinolveparticular segments of a chromosome and may change the numbeof lleles infected cells.
- Mutations ca b silent nonsense, bene ficl or disadvantaeous.
- New genes may b acquired bygene duplication and subsequentpoint utation o byhorizontal gene tranfer.

Concept questions 9.2

1 What ar homologous chromosomes?
2 When would chromsomal rearrangements ccur in the prodction of a nw organism?
3 Drawa diagram to show thefour main types of mutations that affect whole segments of chromosoms. hichwould have the most severe effect on an organism?
4 Contrast how ae allele and a new gene may be ntrodued into poplation.
5 Contrast t cquisition of a new ene by horizontal gene transfer with thatygee duplication.

HOT Chaenge

6 Most eukaryotic orgaim sow iplidy. Polyploidy s relatvely cmmon in flowein plans butis lethal n human. How candiploidy mask mtations in the genome?

9.3 Changing allele frequencies in populations

Mutations introduce new variations into a population, and so can many external influences. The collection of alleles within a population is shaped by the movement of individuals and by environmental events that can sometimes rapidly and considerably change the composition of populations. The study of allele frequencies in populations and how they change over time in response to various environmental circumstances is called **population genetics**.

Gene pools

Genes are the means of transmitting phenotypes from one generation to the next. Many genes exist in different forms as alleles, and the characteristics of individuals are largely determined by the alleles they inherit. Sometimes there may only be one version of a gene (one possible allele), or there may be many versions or alleles of a gene (many possible alleles). The variation in alleles carried by different individuals leads to most of the variation in a population. The total collection of all alleles for all genes within a population is referred to as a **gene pool** (Figure 9.11). In biological terms, a **population** is a group of individuals of the same **species** that live in the same geographical area and readily interbreed to produce fertile offspring, so they share the same gene pool.

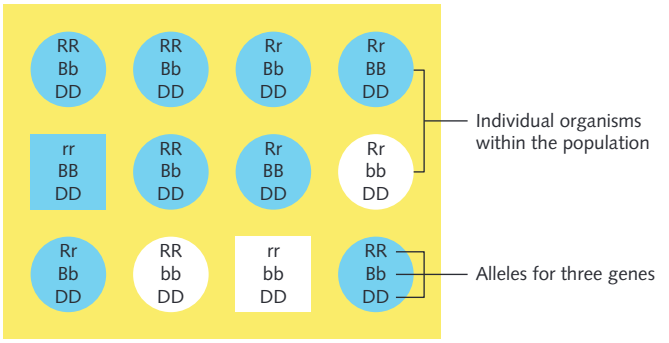


Figure 9.11 A simple example of a gene pool depicting three genes. For the third gene, the allele D is fixed in the population.

The range of variation possible in a population is restricted by the genes and the alleles available in its gene pool. For example, frill-necked lizards do not carry genes for characteristics related to wings or hard-shelled eggs or for the enzymes required to synthesise chlorophyll or to digest cellulose. However, all frill-necked lizards carry genes for a tail, rudimentary teeth, scales and four legs.

Many genes may have only one possible allele in a gene pool. Such genes do not contribute to any variation and the incumbent allele is said to be **fixed** in the population (Figure 9.11). Scientists believe that 80–85% of human genes are fixed in this way.

For variation to occur in phenotypes, more than one allele of a gene must exist. These alleles occur because of mutations that have generated different versions of the gene. The frequency of different alleles is not usually constant and can be affected by further mutation of an allele, immigration of individuals into the population, emigration of individuals out of the population and the reproduction rate of various individuals in the population.

Migration and gene flow

In a biological sense, populations are defined by their reproductive and genetic isolation. Few populations are completely isolated from each other, and generally some migration takes place both into and out of the population. **Gene flow** is the transfer of alleles that results from emigration and immigration of individuals between different populations. The flow of genes occurs if the migrants to a new population breed. Immigrants may add new alleles to the gene pool, and emigrants may completely remove some alleles or significantly change the frequency of others (Figure 9.12).

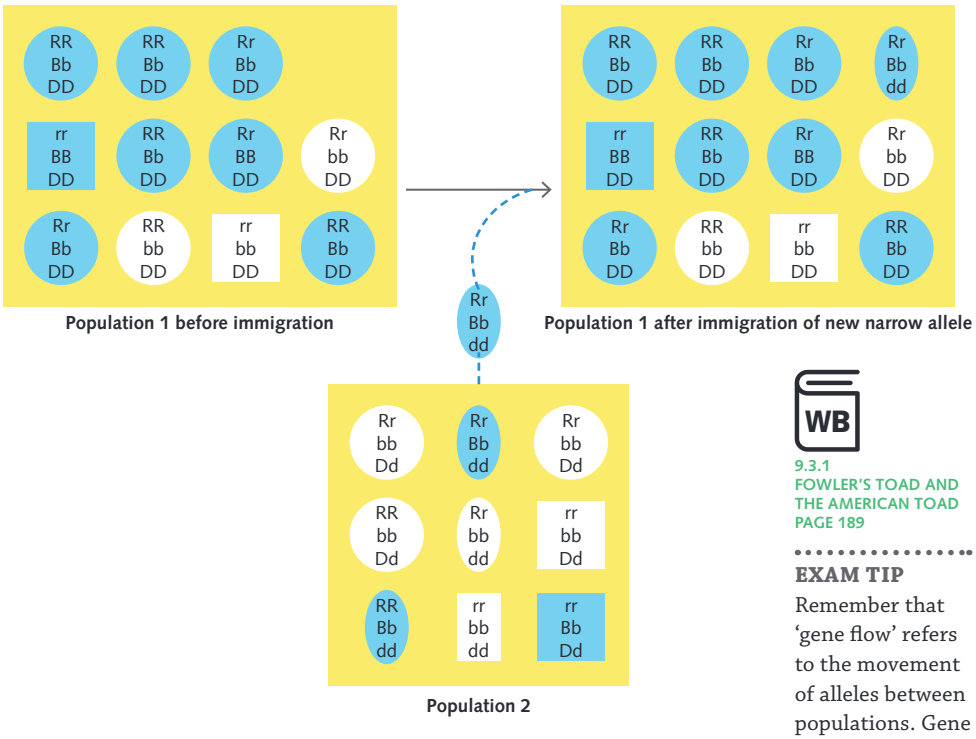


Figure 9.12 A new allele d enters Population 1.

WB
9.3.1
FOWLER'S TOAD AND THE AMERICAN TOAD
PAGE 189

EXAM TIP

Remember that 'gene flow' refers to the movement of alleles between populations. Gene flow is not simply another term for the migration of individuals.

Humans are polymorphic for the ABO blood types; that is, there are several different blood types. Some alleles are present in Indigenous Australians at different frequencies from other populations in the world. Indigenous Australians have largely been isolated for at least the last 50 000 years, except for some gene flow from Asia and New Guinea in the northern regions of Australia. Most Indigenous Australians do not have the B allele of the ABO blood group that results in either the B or the AB blood type. The B allele occurs at a frequency of up to 10% in European populations and up to 20% in Asian populations. The overall frequency of the B allele is increasing within the Indigenous Australian population as a result of migration from Asia and Europe into Australia and the gene flow between these populations. The most common allele in the Australian population and the world today is the O allele. The A allele is the most ancient allele and evolved before the human species diverged from its hominin ancestors.

Genetic drift

The term **genetic drift** applies generally to random changes in small populations. Every reproductive event involves chance. Each of us inherited half our alleles from our mother and half from our father. Which half of their alleles our parents passed on to us was a matter of chance. It depended on the random assortment of chromosomes and recombination during meiosis and whichever gametes met at fertilisation. In large populations, this randomness in inheritance of alleles is not noticeable overall and the allele frequency of the gene pool tends to remain fairly stable. But if a population is small, there is a chance that some alleles present in a parental group will not be passed on at all. These alleles may be permanently lost from the gene pool (Figure 9.13). Alleles may be easy to lose, but they are virtually impossible to replace.

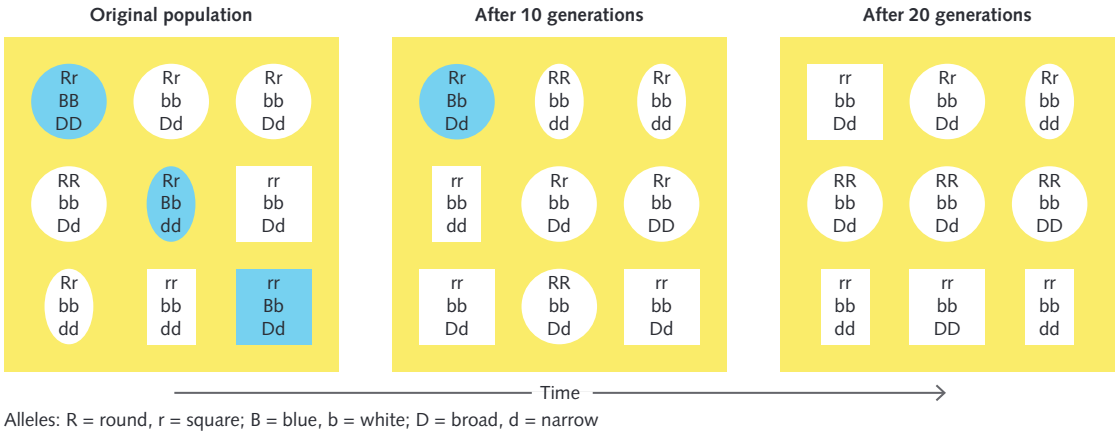


Figure 9.13 Allele b for colour has become fixed in the population as a result of genetic drift.

Genetic drift can occur in a small population or when a large population is suddenly reduced by a catastrophic event. This can give rise to a **bottleneck effect**. When a small group of individuals migrates and establishes a population in a new location, the **founder effect** may occur.

SOUTHERN Biological Developed by Southern Biological

INVESTIGATION 9.1

Natural selection

Am

To eplore how htcin viabiity of brine shrimp (*Artemia* specis) is affeted b difeent aline level environments

Time reuirement

45 inutes

Materas

- Brineshrimp eggs (cysts)
- .5, 1.% an 2.0% solutions of salt water
- iilled water
- 4 Ptri dishes
- ine brush
- 4Microsce slides
- 4 Strips of ouble-sided tape
- Stere microscope
- Permanent marker
- Graduate cylinder

What are the rsks n dong th is investigation?	How can you manage these rsks to stay safe?
Brne shrmp may cause an aeric recton.	Wear lab coats, sa fety glasses and gloves wash hands thoroughly at the end of the activit. nform your teacher if you have an allerg.

Method

Preparing eggs or hatching (Day 1)

1. Usng a permanentmrer, labe fou Ptr dshs:0%, 0.5%, 1.0%, 2.0%.
2. Form your hothesis; for example, f the alinity of the htching solution is 2%hen more ysts will hatch and thrive.
3. Usng the graduate cylinder, pur 1 ml of the appropriately labelled Petri dish.
4. Cllect fur microsope slides. Measure nd cu four 1.5 cm stri ps of dobl-sided tape and gently adhere one of them to each of he micrcoe slides.
5. Lghly touch the fine brush to th side of he dis ontaining the br ne shrimp eggs Collect 20–30 egs on the brush. Do not llect too many eggs causeyou will be rquired to count them.
6. To adhere the eggs to h double-de tape, lightly press the brush onto the tape on the firstmicroscoe lide. Repeat his step for t remainng thre miroscope slides.
7. Usng a icroscope, count the number of eggs on the firstlie. Recor this inoration inthe esults table.
8. Once the eggs have ben couned pace this sideino the0 alt soluion etri dish. Place the slide with the tape sde facing up.
9. Count the eggs oeach slie and place them in the respec tive saltsolutions. opy Reults table 1 into your logbook and record the eggcount nformation(at 0 hours).
10. Pace the Ptri dishes der a light bank for 24 hours at oom temperature.

Data clection (days 2 and 3)

1. After 24 hurs,examine the contents of each Peri dish under the stereomicroscoe. You should ee that some brine shrimp have hatched adare swiminginthe salt solution. ecord the number of eggs, the number of dead or partally hatched eggs and the umber of swimmi ng rine hrmp infomtion in your rults table.
2. After 48 hurs,examine the contents f the Petri dishes again and record your bservatios in the results table. Clulate thehathg viability of each dish at 48 hours by dvdng the number o shrim swimming y the initial number of egs in th Petri dish. Rud p your calculations to the nearst hunredth and add this information to the class esultstable.
3. Draw a bar graph that shos the sample eansfom the class results.

Resuts

Results table 1 Hatchng vabty of brne shr mp at varyng eves of santy

NaCl (%)	0 h	24 h			48 h			
	No. eggs	No. eggs	No. dead or partay hatched	No swmmng	No eggs	N. dead or partay hatched	No swmmng	Hatchng vabty (%)
0								
0.5								
1								
2								

Results table 2 Cass resuts of hatchng vabty of brne shrmp n varyng eves of santy

		Santy (%)			
		2	1.5	0.5	0
Hatching viability	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
Calculation	Mean hatching viability				

Draw a graph of yor resuts, enuringyouinclude labels.

Dscusson

1 Describe o conditions tht erecontrlled in this experiment.
2 Wich Peri dish ha the higes tching viabiliy? Which had e lowest? Suggetpossible reasons for these results.
3 Was your hyothesis suported? Explain why.
4 Based on yu ndiidual dataand t class data, is there enough evidence to cnclude tht environments of different slnties affec teatchig viability of brine shrimp?

Takin it further

What othe onditions may affect thatching viability of brine shimp?Design n exprient to investigate another enironmenal factor that my ipt atching viability.

Bottleneck effect

WB
9.3.2 BOTTLENECK EFFECT PAGE 190

Sometimes a catastrophic event or a period of adverse conditions drastically reduces the size of a population. In this scenario, certain alleles may be lost through chance (Figure 9.14). If a portion of the population survives the catastrophe, the original population's gene pool cannot be recovered. The expanded population can only carry the alleles that existed in the population that survived the event. Therefore, the gene pool will now carry an indication of the bottleneck that occurred long after the population has recovered.

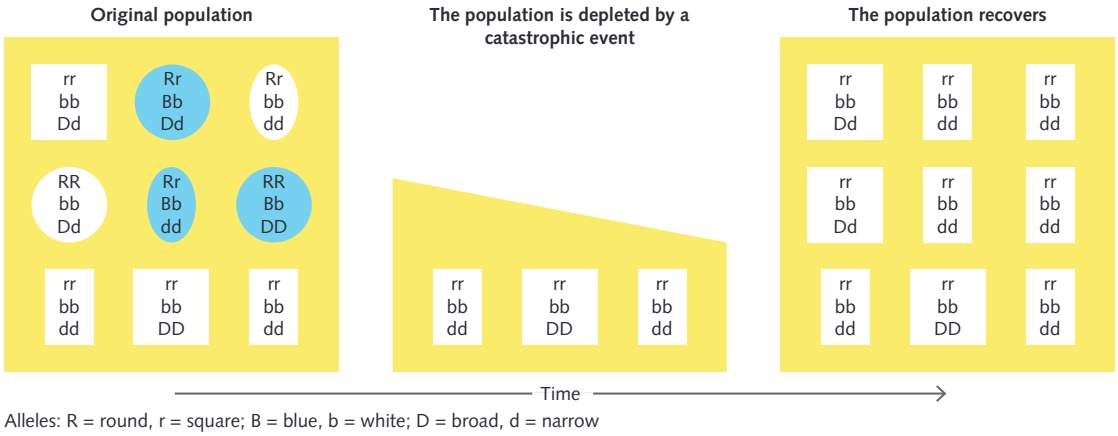


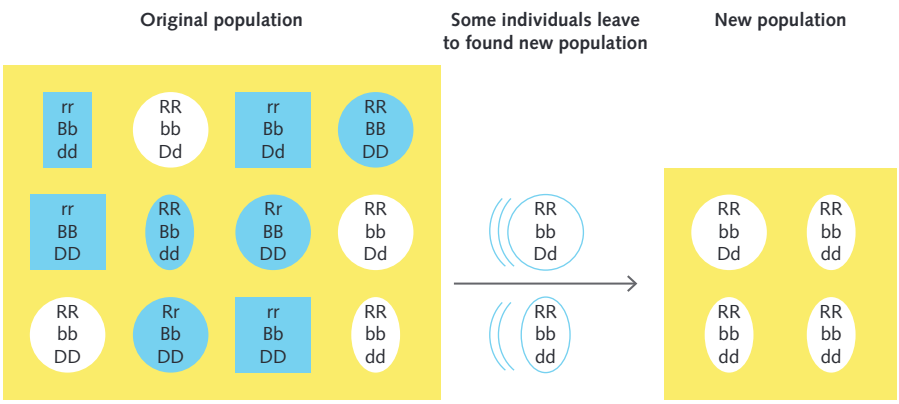
Figure 9.14 When the population recovers from a bottleneck effect, the gene pool is less diverse than that of the original population.

Cheetahs are an endangered species that have survived a genetic bottleneck (Figure 9.15). In a declining population, parents mated with their own offspring, and the resulting generations were left with strikingly similar alleles. One of these is a mutated allele with negative effects on fertility. Typically, a male cheetah's sperm count is low and 70% of the sperm are abnormal. Other shared alleles result in lowered resistance to disease. Infections that are seldom life-threatening to other cat species can be lethal in cheetahs. Today, there are only about 7000 cheetahs left in the world.

Figure 9.15 Cheetahs survived a severe bottleneck that increased the frequency of some mutated alleles.

Founder effect

The founder effect is a particular example of gene flow. A few individuals who move to a new area and become isolated from a larger population might not carry all the alleles that were present in the original population (Figure 9.16). This means that the isolated population has less genetic diversity than the original population and recessive alleles that may be deleterious have a higher chance of meeting during fertilisation than they did in the original population.



Alleles: R = round, r = square; B = blue, b = white; D = broad, d = narrow

Figure 9.16 **The founder effect occurs when individuals migrate to an isolated area and form a new population with allele frequencies different from the original population.**

This effect has been observed in human populations when small groups of people with particular religious or ethnic backgrounds have settled somewhere new and mixed very little with other populations. About 200 people originally settled the Amish community of the USA, and at least one of the settlers had a recessive allele for Ellis–van Creveld syndrome. This syndrome has been relatively common among Amish people of this region ever since. Ellis–van Creveld syndrome includes symptoms of dwarfism, polydactyly (extra toes or fingers, Figure 9.17) and sometimes a hole in the heart.

Figure 9.17 **Examples of polydactyly, one of the symptoms of Ellis–van Creveld syndrome**

KEY CONCEPTS

- Geneti drift is thechge in allle frequency in a poultion due to the random assortment and slection of certaingenes uring meiosis and ferlisaion.
- The bttleneck effect occurs when an event causes a arge redction in the gne poolo a population, decresing eneic ivrsity in subseuen generations.
- Gene flow reslts from the tranfr of alleles into or out of a gene pool because o the migration of ndvduals betwen opuations.
- The founder effect occurswhen a small number of ndidus, crrying a restrict umber of alleles, form a new ppuaton with reducd enetic diversity compared withth orignal population.

Concept questions 9.3

1 Recll the elationship between genotype and phenotype.
2 Ditnguish between a ge d an allele.
3 Ouline wh variations av t be inheritable for them to be elevant t eolutionay change.
4 Define the lowing terms and describe examples of where these processes may have occurred.
   a Founder effect
   b Genti drift

5 Define poultion and 'ne pool'. Describe the mecanisms that can leadto changes i the gene pool of a ppuaion.

HOT Chaenge

6 a Otline how gene flow can affe allelefrequency.
b How canmallppulatins hve low allele frequency?
c For a poplatio that s in eticequilibrium, the sum of the freuencis ofl he alleles is 100%. The conitions t maintin ts quilibrium are no mutatins, no gene flo large oultion, random maing and nonaural election.
s tis a rlisicsituatio i a population?
ii Cold a poulton in gnetiquilibrium be acheved through the founder effect?
iii What are some possible efcts of achieving a geneic euiibrium in appultion if this was possible?
v Howmight a ottleneck effect change the genetic eilbrium of population?
v Aeles that are remove d from a poplation are not easy toreplace. What might be some of the processes that could restore more genetic dversity to population?

9.4 Natural selection

CONNECT
Evolution is discussed in Chapter 10.

Gene pools can change as a result of mutations, migration and chance events. Environmental factors also play a role in altering gene pools. **Natural selection** is the process whereby individuals with certain heritable traits survive and reproduce more successfully than other individuals, leading to changes in the gene pool of a population. Natural selection is the mechanism that drives evolution. Through natural selection, favourable traits are selected for, inherited and become more common in subsequent generations. The capacity of an individual to survive and reproduce is sometimes referred to as its **fitness**.

Selection pressures

WB 9.4.1 SELECTION PRESSURES PAGE 193

Selection pressures are environmental or ecological factors that promote the survival of some individuals in a population over others. When conditions are favourable, most members of a population prosper. However, if environmental conditions become adverse, individuals of the population compete to survive and reproduce. Those individuals unable to endure the environmental challenge (the selection pressure) tend to die young, leaving no or very few offspring. In effect, the selection pressure weeds out the inadequate members of the population and their traits. Those individuals that endure the environmental challenge have a selective advantage; they prevail in the 'struggle for survival' and are prone to reproduce and leave relatively more offspring. The next generation predominantly inherits the characteristics of the survivors. The characteristics of the subsequent generation are better attuned to survive the selection pressure. If the selection pressure persists for many generations, the population becomes adjusted, or 'adapted', to cope with it.

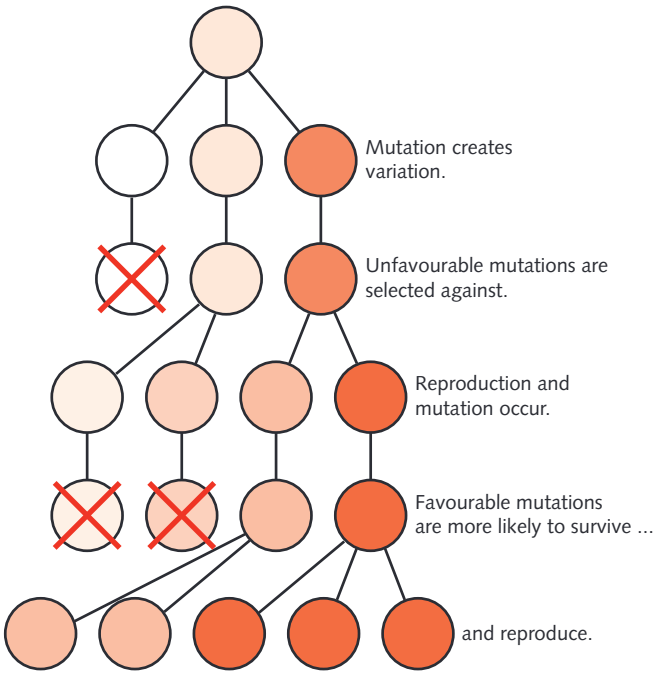
Selection pressures include predator–prey interactions, such as the case of the peppered moth outlined at the beginning of this chapter. Other examples are competition between species for food or territory; competition within species for food, water, territory, mates or breeding sites; and the differing susceptibility of members of a population to an environmental stressor, such as heat, poisons or disease.

Principles of natural selection

WB 9.4.2 PRINCIPLES OF NATURAL SELECTION PAGE 194

Selection pressures act on phenotypes but they lead to changes in the gene pool. The processes of natural selection and genetics allow us to make some key propositions.

1 Individuals differ from one another; that is, individuals within populations show variation.
2 Many of these variations are caused by mutations that create new alleles. All the alleles are heritable.
3 In general, more offspring are born than can survive to maturity so only some organisms survive to reproduce.
4 Some individuals have traits that make them more suited than others to their environment; those individuals with a selective advantage are better able to reproduce and pass on their alleles to the next generation. Natural selection is thus represented in Figure 9.18.



Weolink
Natural selection

Onne Wyrkm heet
Natural selection

Figure 9.18 **A diagrammatic representation of natural selection over successive generations. In this example, the darker traits confer an advantage over lighter traits.**

WB
9.4.3
EXTINCTION
PAGE 195

Vulnerability and extinction

A large population with a diverse gene pool is relatively well equipped to respond to selection pressures. However, this is not always the case. Populations that have arisen through founder or bottleneck effects, even if they grow large in number, tend to have a gene pool with limited variation. This may have a couple of detrimental consequences for the population.

First, certain recessive alleles with potentially harmful phenotypes become concentrated in the gene pool. The result is that the potentially harmful condition occurs more frequently in the population; for example, reduced fertility among the cheetah population.

Second, a restricted gene pool means the population has less genetic and phenotypic reserves to respond to selection pressures. When environmental conditions become unfavourable, the population may suffer a substantial reduction in numbers as many of the individuals are incapable of surviving. This results in a bottleneck effect. If the population lacks the phenotypes required to survive an adverse environmental change, the whole population could die out. If all the members of a population have died out, the population is said to have become **extinct**.

Experimental evolution

One of the approaches biologists use to study the impacts of selection pressures is to experimentally replicate the effects on populations and their gene pools. Biologists can manipulate and measure the distribution of phenotypes and alleles in the populations. Such experiments are typically conducted on populations of small, rapidly reproducing organisms whose life cycles are well understood and are easy to maintain in the laboratory. Organisms suited to studies of experimental evolution include bacteria, microscopic algae and shrimp species.

Experiments using the shrimp species *Americamysis bahia* (Figure 9.19) show the consequences when populations become small or have a limited genetic diversity. Initial small populations with high diversity grew to much larger sizes than comparable initial populations with low diversity (Figure 9.20a). An added effect was that the diversity of the larger population continued to increase

while that of the smaller, low-diversity population stagnated or even decreased. In the process of applying a bottleneck effect, about 20% of the low-diversity populations died out before the biologists had the chance to trial them in experiments, possibly because the population accumulated alleles unfavourable for long-term survival through inbreeding. When a selection pressure was applied, in this case fresh water that is normally harsh to a species that prefers saline conditions, all of the high-diversity populations survived. By contrast, more than two-thirds of the low-diversity populations went extinct, with all the members of those populations dying out (Figure 9.20b). Therefore, it can be demonstrated experimentally that populations with low genetic diversity are at greater risk of extinction when conditions become unfavourable.

Figure 9.19 Scientists use the mysid shrimp *Americamysis baha* to study the effects of natural selection.

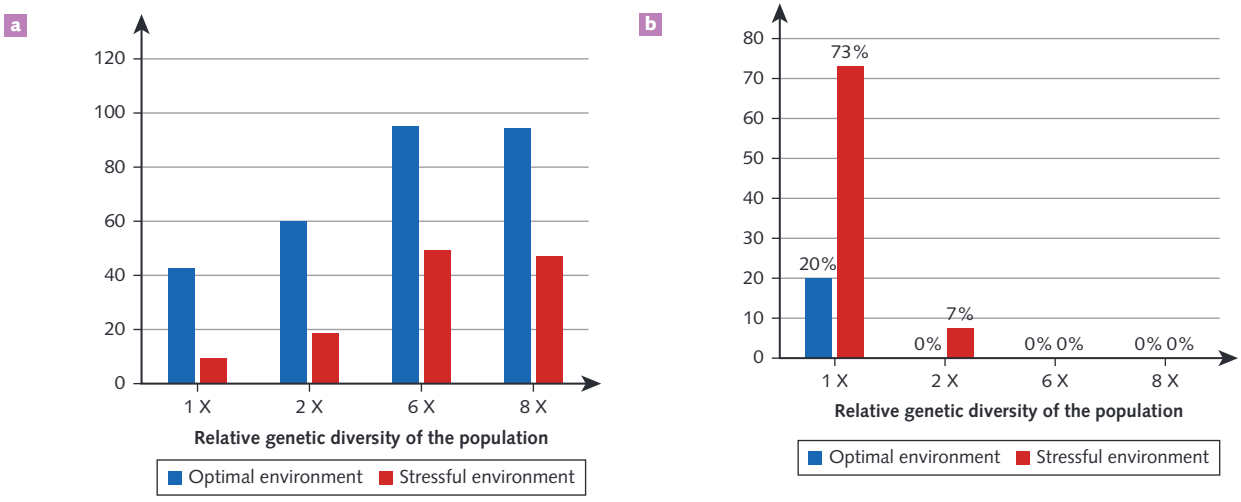


Figure 9.20 a The average population of mysid shrimps after 3 weeks of growth in saline (optimal) or freshwater (stressful) environments. Each average represents 15 replicate populations that each started with 12 animals. The 'relative genetic diversity' was determined using genetic markers. The 8X population is 8 times more diverse than the 1X population. **b** Results from the same experiment showing the proportion of the 15 starting populations of each condition that became extinct.

Multiple selection pressures

Another aspect of natural selection is that, if conditions change dramatically, two or more selection pressures may be acting simultaneously. For example, a population may be coping with drought stress. Individuals would be suffering from the heat and competing for the little available water and food. The population may become further stressed if a disease is introduced to it. As the selection pressures increase, there are fewer individuals in the population with the required phenotypes to cope with all the stressors at the same time. Multiple and extreme selection pressures can drive even large, diverse populations to extinction.

KEY CONCEPTS

- Selection pressures favourthe survival of some members of a oulation ovr othr t ose individuals wth a eective advantage re beter able to survive and reprouce.
- Natural eectio s the driving force that causes poplations to adapt to cangng environmental crcumsances.
- Poultios wth lowgenticdiversity are more vunerable to xtiction.
- Experimentl evoltion enables theeffects of selection pressures on poultions to be tested nd measured.
- There may b mltileselection pessures acting on a poultion at the ame time.

Concept questions 9.4

1 Lst as many examples of selection pressures as you can.
2 denify the roe fvariton n atural selection.
3 Summaise the ke rinciple o ntral selection.
4 Define etncion'. an an alee be extinct'? What is th leding cause of xtinction in species?

HOT Chaenge

5 itns' is a ter applid o species i terms of natural slec ion. What des 'survival of the fittet' mean?

9.5 Human manipulation of gene pools

Humans have a long history of manipulating gene pools, either intentionally or unintentionally. The classic case is that of **selective breeding**, in which humans are the agents of selection, choosing parents with the most desirable traits (to humans) to breed the next generation. The result is the domestication of species suited to human subsistence. Humans have also had a substantial effect on populations of wild species.

WB
9.5.1 ARTIFICIAL SELECTION: ANIMAL AND PLANT BREEDING PAGE 196

Artificial selection: animal and plant breeding

Principles of natural selection can be applied to breeding programs for domesticated animals and plants. The processes for breeding have been understood and practised for centuries. Such breeding programs built on the observation that there was variation in the population. Parental stock with certain desirable traits were selected and mated, and it was understood that these traits were often passed on to the offspring. Over time, the new traits could be established in later populations. This process is called **artificial selection**, also called selective breeding, and relies upon human intervention to determine which traits are selected for.

Many of the familiar forms of domesticated plants and animals have arisen as a result of selective breeding. This process relies on human intervention to determine which animals are allowed to breed, removing alleles that produce undesirable traits from the gene pool and increasing the frequency of alleles that produce desirable traits.

Figure 9.21 Belgian blue cattle have been selectively bred to fix an allele for abnormal muscle growth that appeared in the 19th century.

Which traits are considered desirable in an organism depends on the use of the organism and the preference of the breeder. The Belgian blue breed of cattle contains an allele that promotes abnormal muscle growth (Figure 9.21). Farmers have only allowed the cows and bulls with the highest muscle mass to breed, producing more profitable offspring.

Dog breeds have been subjected to intensive selective breeding programs, producing forms that are aesthetically pleasing to breeders but which sometimes come with a number of considerable health problems for the dogs. This is because their acquired form may not be the best for optimal functioning, and because selective breeding processes may cause deleterious recessive alleles to become homozygous. Once alleles have been lost from a population through natural selection or selective breeding, the traits they confer are permanently lost with them. Figure 9.22 shows the dramatic changes to English bulldog skulls that are a result of selective breeding.

Figure 9.22 Skulls of English bulldogs showing the effects of selective breeding: **a** the original English bulldog, with a functional skull in 1860, **b** 1867 and **c** 1906, showing a very exaggerated skull. The skull has changed dramatically in a short span of time.

Many of the food crops that are now commercially and domestically grown have been selectively bred to favour traits that make them better foods or better products for the producers; for example, bananas (Figure 9.23). Traits that have been selected for include yield, fruit size and longevity, the timing of grain or fruit maturation, and resistance to diseases.

Figure 9.23a Wild bananas with large seeds and **b** selectively bred commercial bananas

While artificial selection generates species that are valuable to humans, it also risks decreasing the fitness of domesticated breeds to natural selection pressures. The risk is exacerbated by combining artificial selection with large-scale propagation of relatively uniform populations, described as a **monoculture**. Populations with low diversity are more susceptible to being eliminated when confronted by natural selection pressures.

A historical example took place during the Great Famine of Ireland commencing in 1845. The Irish population of about 4 million people was heavily dependent on potatoes as a staple food crop. However, the potato crop had low genetic diversity across the country. The summer of 1845 was relatively cool and wet, ideal conditions for the infestation of the water mould *Phytophthora infestans* in the country's potato crops (Figure 9.24). About half the potato crops across Ireland were lost that year, and severe losses continued for several years after. During those years, approximately a million people starved to death and another million were forced to emigrate.

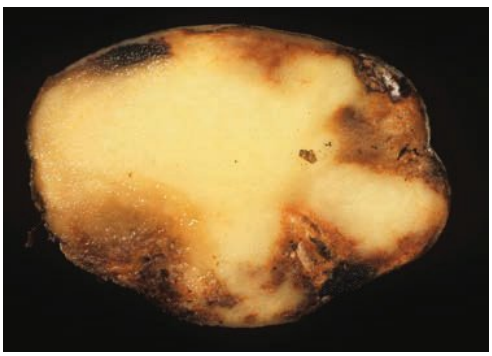


Figure 9.24 A potato infected with *Phytophthora infestans*

Figure 9.25 **The northern elephant seal has experienced a human-induced bottleneck effect.**

Human effects on wild populations

Human actions have also affected gene pools in wild populations of animals and plants. Hunting for food or animal parts or culling to protect domesticated species has reduced the sizes of many animal populations.

Centuries of commercial hunting severely depleted populations of marine mammals, such as seals and whales. For example, the northern elephant seal (*Mirounga angustirostris*, Figure 9.25), which lived in the eastern Pacific Ocean along the west coast of North America, was declared extinct in the late 19th century. However, a handful survived on a remote island off Mexico and the population recovered during the 20th century, spreading, and repopulating much of its original home range. Numbers now exceed 250000. The rebound is encouraging, but the species has been subjected to a human-induced bottleneck effect that severely reduced its level of genetic variation. The pattern has been repeated many times where populations have been depleted by human hunting.

Humans impose many selection pressures on wild species. Land clearing diminishes the habitat and resources available to resident wild populations. Introducing non-native invasive species, such as rats, foxes and feral cats, increases competition and predation of wild populations. Chemical and plastics pollution and the geographical spread of parasites and pathogens add further pressures on survival.

Many human-induced stressors are augmenting existing selection pressures in the natural environment. At the same time, humans are decreasing the size and variability of many wild populations, reducing their capacity to adapt. The risks of wild species extinctions are consequently amplified. For example, it has been estimated that amphibian species are now becoming extinct more than 200 times faster than background rates of extinction. Many small populations of animals with low diversity have already been driven extinct, including the Caspian, Balinese and Javan **subspecies** of tiger (*Panthera tigris*), the West African black rhinoceros (*Diceros bicornis longipes*), the Pinta Island tortoise (*Chelonoidis nigra abingdonii*), the Iberian ibex (*Capra pyrenaica pyrenaica*), and the Tasmanian tiger (*Thylacinus cynocephalus*).

Weolink
Human impacts on Brazilian parrots
Onne Wyrkm heet
Parrot gene pool

KEY CONCEPTS

» Selectie breeing, or arti ficial slection, occurs when humans selctively breed organsms fo dsired traits.
» In selctiv beeding, the fqueny of alleles encoding for deiredtraits increases and the fquency of other alleles at te locus decreaes, and this educes genetic diversity.
» Human ctivitieshave resulted indecreased genetic dversity in ny id species, aking them vulnerable to exintion.

Concept questions 9.5

1 Explain the effet of slective breeding on the gene pool of appuation.
2 Give anexampleof selective breeding other than those described ere.
3 Describe the elationships btween the genetic dvesity ofapoulation, the number and intensity of the eection pressues acng on ,nd the likelihood of the ppuationbecoig extinct.
4 The foration of monoculure through large-scale breeding of dmetic animals ca be a problem. The artificial slection of caracteristics based on culture and tastehas led o hysica and funtional issues with some dog beeds. Labradors are pron t ip dysplasia, dachshunds are prone to both hip nd elbo ysplasia, and pugs can suffer frm brathing dif ficties and conjuncitis because of te contrction of their nasopharyx rea. Deeerious alleles can become fixedwihin apoulation and ths pose erious health concerns and malities.
   a What des ' fixed' meanin this sense?
   b f the fixed eleerio llele becomes homozygous recessvein the p opuain, what does that mean for any ofspring?
5 a What are h selection ressures acting on the norther elephat seal?
   b Explain how hman-induced stressors are augmnting xisting selection pressures in the naturl evironment. Provide o example.

9780170452533

HOT chaenge

6 Mlrkills hundreds of thouands of people every ye.It is endemic to eqatoria egions of Africa. Usaly, f a dieae killsall the hosts, it dies off. Mlria is caused by a number of the *Plasmodium* species and the moe f tranmission is the infective *Anopheles* mosqito.Maarial plasmodia undergo a ife cycletat incudes sending some time in human blood. I African natios here maria is endemic, the genetic diorderickl cell anaemia is also prevalent.Te sckl cel(red blood cell) is marked by defective hamolobin Th sicke cell blocks blood flow tovtal rgns. eole wh inherit two copies of thelel or icke cll anemia usually die quite youg. But people who are heterogous for the allele (carrier) survivan have children. They seem to have an advantag i malaria-prone areas because of the ower concentratio of oxygen in their blood caused by the icle ell trait. Baed on your understanding of fitness, electiveadantage, selective pressures and natrl election, write a hypothesis that seeks to answer thequestion:'hy oes malaria remain endemc in some areasof the orlwhere sickle cell anaemia occurs,whn maia is a killer?'.

9.6 Natural selection and consequences for disease

Antibiotics are chemicals that are toxic to bacteria. Almost as soon as antibiotic production was industrialised in the 1940s, it was clear that their therapeutic use could be compromised by pathogenic bacteria developing resistance to them. Throughout the 20th century, each time a new antibiotic was introduced, **antibiotic resistance** eventually followed. The repeated pattern has resulted in a global increase in antibiotic resistance (Figure 9.26).

When a bacterium becomes resistant to two or more antibiotics it is described as **multidrug-resistant** or is called a 'superbug'. One of the most pervasive multidrug-resistant bacteria is methicillin-resistant *Staphylococcus aureus* (MRSA), sometimes referred to as 'golden staph'. Some strains of MRSA are resistant to at least six different types of antibiotics. Such MRSA strains are also insensitive to disinfectants and they are a major source of hospital-acquired infections. The rise of multidrug-resistant pathogenic bacteria is a serious concern because fewer antibiotics are available to treat the infections the pathogens cause.

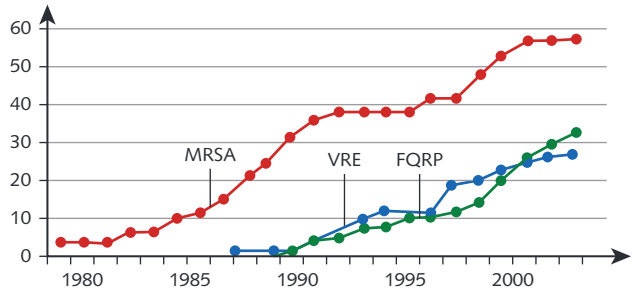


Figure 9.26 The increasing prevalence of some antibiotic resistant bacteria over time. MRSA = methicillin-resistant *Staphyococcus aureus* ('golden staph'); VRE = vancomycin-resistant *Enterococcus*; FQRP = fluoroquinolone-resistant *Pseudomonas aerugnosa* . Based on USA data sourced from Centers for Disease Control and Prevention (2013).

Natural selection explains antibiotic resistance

Bacteria have many different mechanisms for rendering antibiotics ineffective. These include:

- modifying the chemical structure of the antibiotic
- producing cellular transporters that remove the antibiotic from the cell
- adjusting the bacterium's own physiology to evade the antibiotic.

In all cases, the effects are carried out by proteins. These proteins are not essential to the normal survival of the bacterium but they may be expressed under certain circumstances, such as when the bacterium encounters a particular toxin in its environment. Proteins that mediate antibiotic resistance are encoded by genes collectively referred to as **antibiotic resistance genes**. The antibiotic resistance genes are typically found on bacterial plasmids. Multidrug-resistant bacteria have two or more antibiotic resistance genes.

A key observation for understanding how bacteria develop drug resistance is that the capacity for resistance is already present in the population, even when the bacteria have not been exposed to a therapeutic dose of the antibiotic. New genes for such antibiotic properties arise by a combination of gene duplication and mutation. Once the gene has entered the gene pool, a few members of the population will have the antibiotic-resistant phenotype.

WB 9.6.1 NATURAL SELECTION EXPLAINS ANTIBIOTIC RESISTANCE PAGE 198

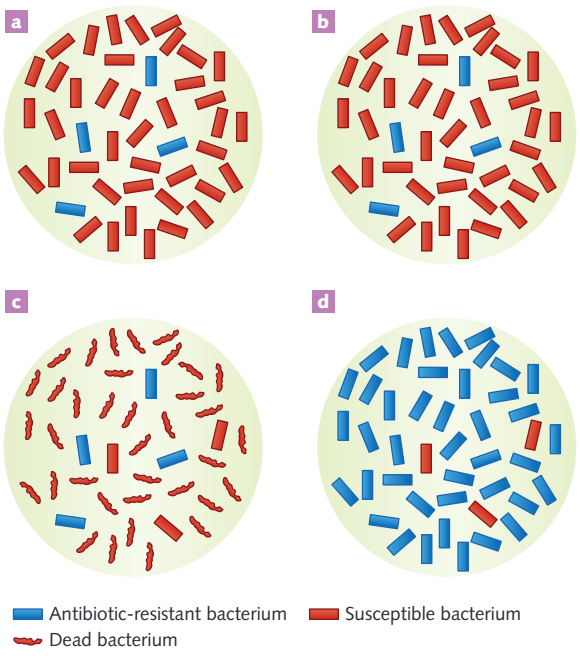


Figure 9.27 a An initial population of bacteria includes a few antibiotic-resistant individuals. **b** On exposure to the antibiotic, a new selection pressure is introduced. **c** Many of the susceptible bacteria die off; the survivors tend to be those with antibiotic resistance. **d** Under continuing selection pressure, these bacteria continue to reproduce until, eventually, the population thrives again.

Figure 9.28 In some countries, antibiotics are widely used as growth promoters in agriculture.

If only a low proportion of bacteria in the population are antibiotic resistant, and the dose of antibiotic is sufficient, then the whole population will die off. In such a case, the therapeutic dose of antibiotic successfully clears the infection. However, antibiotic resistance may arise if the dose of antibiotic is not enough to kill all of the bacteria. The dose may temporarily inhibit bacterial growth but it does not exterminate the population. This level of antibiotic acts as a selection pressure that favours the survival of the bacteria that have the resistant phenotype (Figure 9.27). In time, under continuing exposure to the antibiotic, the population recovers and almost all are equipped for antibiotic resistance. Successive exposures to the antibiotic have no detrimental effects on the bacterial population.

Once a large proportion of the population of bacteria has an antibiotic-resistance gene, members of the population can acquire a second antibiotic-resistance gene from an unrelated bacterium through horizontal gene transfer (p. 336). Combinations of subsequent exposures to different antibiotics, natural selection and horizontal gene transfer result in multidrug-resistant bacteria.

Emergence of a global health threat

The growing scale of multidrug-resistant bacteria poses a major threat to global health, officially recognised by the World Health Organization (WHO). Multidrug-resistant pathogenic bacteria increase the risk of medical complications, extended hospitalisations, and fatalities caused by infections. In Australia, approximately 1600 deaths per year are the result of antibiotic-resistant bacteria.

There are many selection pressures acting to increase the prevalence of multidrug resistance. One of these is the over-prescription of antibiotics, especially when patients have a viral rather than a bacterial infection. This is exacerbated when patients who are appropriately prescribed antibiotics do not finish their course. In many countries, antibiotics are available without prescription. This situation allows indiscriminate use of antibiotics by the public. Many of the over-the-counter antibiotics are **broad-spectrum** antibiotics, which are effective against several different bacterial species. They are frequently purchased to treat short-term and self-resolving illnesses, such as upper respiratory tract or gastrointestinal infections.

Another selection pressure occurs when antibiotics are used in agriculture, farming and aquaculture. Approximately three-quarters of the antibiotics consumed in the USA are used in these sectors. The most common use is for cattle, pigs and poultry as a feed supplement, where they act as growth promoters. The antibiotics inhibit gut microbes, stimulate the animals' immune systems and provide some protection against disease for animals kept in close quarters (Figure 9.28). The occurrence of antibiotic-resistant bacteria in farm animals continues to rise, with the highest rates in low- to middle-income countries in Asia, Africa and South America where livestock farming has increased rapidly in the last two decades.

Excess antibiotics escape into the environment where they continue to exert selection pressure on environmental microbes. Antibiotic-resistant populations of bacteria have been detected in soil, freshwater and marine samples far from the likely sources of commercial antibiotics. For example, in a population of dolphins in southeastern USA (Figure 9.29), the prevalence of erythromycin-resistant bacteria growing around the blowholes and in the gastrointestinal tracts of the dolphins has increased during the last decade. It is thought that the source of the erythromycin is sewage in Florida.

Figure 9.29 **Antibiotics are leaking into the environment, resulting in antibiotic-resistant bacteria growing in wild animal populations.**

Multiple strategies are required to counter multidrug-resistant bacteria. These include:

- prohibiting the sale of over-the-counter antibiotics
- delaying the prescription of therapeutic antibiotics
- tighter management and use of prescribed antibiotics
- enforcing stricter regulation of antibiotics in agriculture
- more rigorous surveillance and reporting of multidrug-resistant bacteria
- educational programs for both the public and practitioners.

It is also important that new antibiotics (naturally occurring or chemically synthesised) are discovered and developed. However, pharmaceutical companies have become reluctant to develop new antibiotics because they are less profitable than other drugs. The situation may be alleviated by governmental policies and incentives.

Lessons from nature

The environmental context for antibiotic resistance is the perpetual competition for resources between different species of fungi and microbes. One species can eliminate its competitors by producing an antibiotic that is toxic to the other species. Many antibiotics have been isolated from naturally occurring sources. For example, penicillin was isolated from the *Penicillium* fungus. The target species may improve its chances of survival by developing resistance to the antibiotic. The first species may in time produce another antibiotic to which the second species eventually develops resistance, and so on it goes. Human activity is now replicating this natural 'arms race'.

Bacteria are ideally suited to develop antibiotic resistance for several reasons.

- They are vastly numerous. A single teaspoon could hold literally trillions of bacteria.
- Their small size makes them highly mobile and prone to encountering new and different environments with corresponding new and different selection pressures.
- Bacteria have relatively compact genomes and reproduce rapidly with consequently increased levels of mutation.
- Short generational times and the capacity for horizontal gene transfer enable bacteria to rapidly transmit beneficial genes from generation to generation and between members of the same generation.

INVESTIGATION 9.2

Antibiotic resistance

Antiiotics are molecules that are producd by bacteria and fngias adefence against other microbes. The discovery of penllin and ote antibiotics in the20th century provid ed uswith a way to teat diseaseand se lives. Antibiotics have beendeveloped fr use against a broad range of path ogeic icroes; each has its strength and weaknesses. Unfortunately, ovruse hasled to ntibiotcresistanc. N antbioi works against all bacteria and it is important to know whch tibiotic to useto treat diffret bacerial diseases.

Am

To investite antbioti effectivenss against common bacteria

Time reuirement

45 inutes

Materas

- *Escherichia coli* brothculture
- *Staphylococcus epidermidis* broth clture
- 4 nurient aar plates icky tape
- leach or 7% alcohol
- 2 serle lstic pipettes
- 2disposable spreaders
- 2 Mastrg atibiotic discs
- Forceps
- Meauring ruler callipers
- S
- Permanent marker
- Bunsen burner
- Contaminated waste bag
- ispsabe gloves

What are the rsks n dong th is investigation?	How can you manage these rsks to stay safe?
Although lab strains are usuly harles s bacteria may cause disease so assume them to be pathogeni.	Wear lab coas, safety glasses and goves wash hands thoroughly at the end of the activit. Decontaminate benches before and after the actvty. Food sps wth beach
Micro-organisms will grow on the agar patesDo not op	en plates once they are securely tape. Dispose of plates appropriately after autoclaving.
Disposable gloves can cause al eric rectios in snitive people.	Use a type of gove that has no aergyrisk an is uitble to use with the chemicals n ths nvestgaton

Method

Noe: to use asepti tecnique, wipe your ench down with 70% alcool o bleach and keep your work near the Bunsen burner to take advantage of the updraught the flamell create to waftpotential contaminants away frmyour materials.

1. Lael the bottom of yur for agar plates with your name and the dat. L beltwo plates *Ecoli* and t o plates *S. epidermidis* La el one plate of each type bcteia with'E' for experiment and abel the other 'C' for contrl Figue 9.30).
2. Usng a strie plastic pipette, transfer a drop of the *E. coli* bactrial broth onto the surface of the agar on your two *E. coli* pates.
3. Wokig n clos roximity of the unsen burner, use a spreader to spread the baceial broh evenly overthe plats. If you are using a lass sprader, pass it through the flame of the Bunsen burner before each ue.

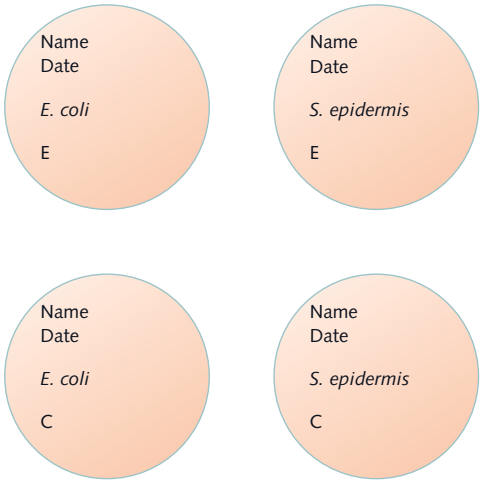


Figure 9.30 The four agar plates

4 Replace th lids on the plae immeiately to avoid contmntion.
5 Repeat steps 2–4 for *S. epidermidis* usng a new ipette and spreader.
6 Wit 10–15 minutes beoe applyig the Mastring to ensure the baceria has a chance to grow.
7 Fame your forcepsand let them coo efore picking up the Masting. Place theMastrng in the middle of one of your experimnt plates and puh (ery gently) with the forceps to helpit st yin lace. Each lobe of he Mastring is impregnated wth adifferent tibiotic; use the code on the packet to dffereniate them.
8 Repeat step 7 for your othe experiment plate. Ensure you flame the forceps betwee eah pplication.
9 Sea all our pats with sicky tape and incubate them for 24 hours at 37°C, upside down so that the agar is at the to.
10 Wipe your bench down wth70%alcoholor bleach and clean your hands thoroughly.
11 Dispose f allmaterils safey in a contaminated-waste bag.
12 The nextday, observe the plates for the presence or ab sence of growth near each disc an measure the diameter of any zone of nhbition.
13 Copy th resutstables into your ogbook and record yor results.

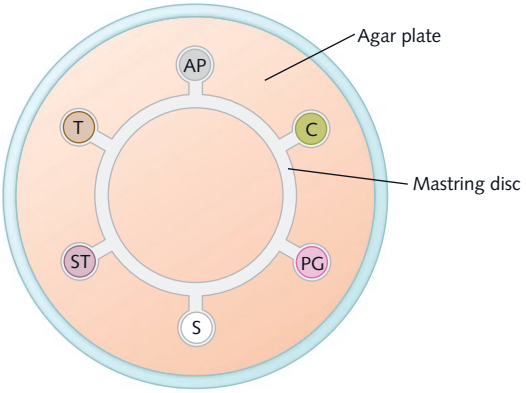


Figure 9.31 The Mastring antibiotic disc; AP, ampicillin; C, chloramphenicol; PG, penicillin; S, streptomycin; ST, sulphatriad; T, tetracycline

Resuts

1 Draw a belled diagram of what you see on each plate.
2 Coplete Rsult tale 1 wit the result of your experiment.

Results table 1 Expermenta resuts

Antbotc	Dameter of zone of nhbton (mm)	
	Escherichia coli	*Staphy ococcus epdermds*
Ampicillin		
Chloramphenicol		
Streptomycin		
Sulphatriad		
Penicillin		
Tetracycline		

3 Clulate the ea class diamtro zone of iniiion for each antibitic andcopy and complete Results table 2.

Results table 2 Cass means

Antbotc	Mean dameter of zo ne of nhbton (mm)	
	Escherichia coli	*Staphy ococcus epdermds*
Ampicillin		
Chloramphenicol		
Streptomycin		
Sulphatriad		
Penicillin		
Tetracycline		

Dscusson

1 Explain th function o h contrl plate How cul control plate be helpful if there is no growth on the experient plate?
2 What were fou variables that yu kept contant in his experiment? How did you control them?
3 What is a zoe inhibition? How wer they created in your experiment?
4 Wh i i importan to pool daa frm the class results and find the mean zneof inhibition for each aibiotic?
5 Wich tibiotic had the greatest zoneof inhibiton? xplain why this might be.
6 Did youridiidua results differ rom the clas s reslts? If so, suggest posible reasons.
7 Wich tibiotic woud be most suitabl to treat an infection by *Staphylococcus epidermidis*?
8 Wich tibiotic would you use if you were unure of te pathogen in an infection? Explain your ans er.
9 Did your esults showany sigsofantibiotic resistance?
10 Discuss he impactstht antibiotic eistanc has on medical treatment.
11 Why have ntibiotis become a less effetive treatment for infection in recent years?

EXAM TIP
In a written exam question, do not simply describe the control as a 'control'. It helps to describe how the result for the control is compared to that of the treatment and how the difference explains the effect of the treatment.

Concuson

Wite a conclusion fo this nvetigation.

Takin it further

Test the efficacy of atual ntiotics o imilar bacteria.

Viruses

Viruses share many attributes with bacteria. They are extremely small and highly mobile, have compact genomes, reproduce quickly in large numbers and have relatively high mutation rates. However, viruses must infect hosts to reproduce. Transmission between susceptible hosts is important for the survival and replication of viruses.

Once your body is exposed to a viral infection, the adaptive immune response helps clear the infection and also prepares it for future infection from the same virus. Memory B and T cells and circulating antibodies recognise the antigens of previous viral pathogens. Viruses bearing those antigens are doomed by the immune system if they re-infect the host. The virus's capacity to replicate stalls when the population of susceptible hosts is exhausted, and transmission is restricted.

Antigenic drift

During a pandemic, a viral infection migrates across geographically dispersed hosts. Substitution mutations accumulate in the population of viruses as they replicate, some of them altering the antigens that would otherwise be recognisable by the adaptive immune system. If the antigen is altered enough, the result is a new strain of the virus capable of evading the adaptive immune system and re-infecting the host, even if the host was previously infected or vaccinated against the original strain of the virus. This is described as **antigenic drift**.

Antigenic drift helps to explain seasonal influenza (flu) epidemics. The influenza virus is an RNA virus that bears the antigenic surface glycoproteins haemagglutinin and neuraminidase (Figure 9.32). Haemagglutinin helps the virus attach to a susceptible host cell on infection. Neuraminidase helps the newly replicated viruses detach from an infected host cell.

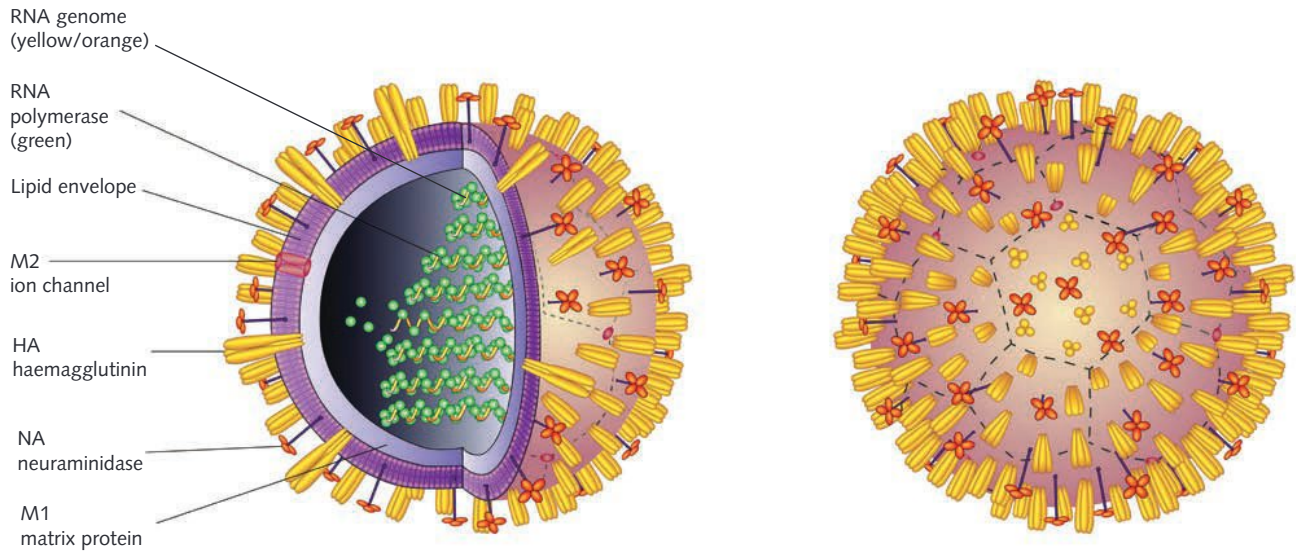


Figure 9.32 A cutaway diagram of an influenza virus

There are four types of influenza virus: A, B, C and D. The four types infect different groups of host animals. Humans are susceptible to types A, B and C. Only type A is known to have caused global pandemics in human populations. Type A influenza viruses are subdivided into different subtypes identified by their particular haemagglutinin (H) and neuraminidase (N) proteins. For example, the subtypes H1N1 and H1N2 have the same haemagglutinin but different neuraminidase proteins. The different proteins reflect the different genetic variants of each subtype. More than 130 subtypes of influenza type A have been detected in nature.

As influenza spreads across global human populations, substitution mutations occur in the replicating virus, creating new strains. A particular subtype (e.g. H3N2) may be prevalent in Australia during a given

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Learn how influenza spreads

year. By the time the epidemic has run its course, a new strain of the H3N2 subtype will have emerged elsewhere in the world and migrated to Australia, causing a new epidemic the following year. For this reason, specific strains of flu virus are distinguished according to when and where the strain was first isolated (Table 9.1). Each new strain emerges because of antigenic drift.

Table 9.1 Examples of influenza virus strains

Strain name	Influenza virus type	Influenza virus subtype	Location first identified	Year first identified
A/Nanchang/933/1995 (H3N2)	A	H3N2	Nanchung, China	1995
A/Sydney/5/1997 (H3N2)	A	H3N2	Sydney, Australia	1997
A/Panama/2007/1999 (H3N2)	A	H3N2	Republic of Panama	1999

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9.6.2 VIRAL ANTIGENIC DRIFT AND SHIFT PAGE 200

Antigenic shift

Normally a single subtype of influenza infects an individual host. However, on rare occasions a single host may be infected by two different subtypes of influenza. When this happens, the genetic material of the two subtypes is shuffled into a new combination in the replicating viruses. The result is the sudden appearance of a new subtype of the virus. Its antigens are substantially different from those of the original viruses. The adaptive immune system of hosts that were previously infected by or vaccinated against the original viruses will not recognise the new antigens. This is referred to as **antigenic shift**.

Antigenic shift typically occurs when different host animals are living close together. Each reservoir of host animals is infected by different subtypes of the virus, increasing the chances that the two different subtypes will meet in a common host animal. New influenza subtypes have appeared in rural environments where humans live closely with aquatic birds and pigs. Sometimes, the subtype infecting birds jumps directly to a human host bringing the two subtypes together. On other occasions, a susceptible third species may act as a 'mixing pot' that brings the two subtypes together.

If the new subtype of virus is highly contagious in humans, it can lead to a global pandemic. A 2009 pandemic of influenza originated in pig farms was thus informally referred to as 'swine flu'. The deadliest global influenza pandemic in history was the 1918 Spanish influenza outbreak, during which at least 50 million people died of both the flu and opportunistic secondary infections of flu victims. Figure 9.33 summarises the difference between antigenic shift and antigenic drift.

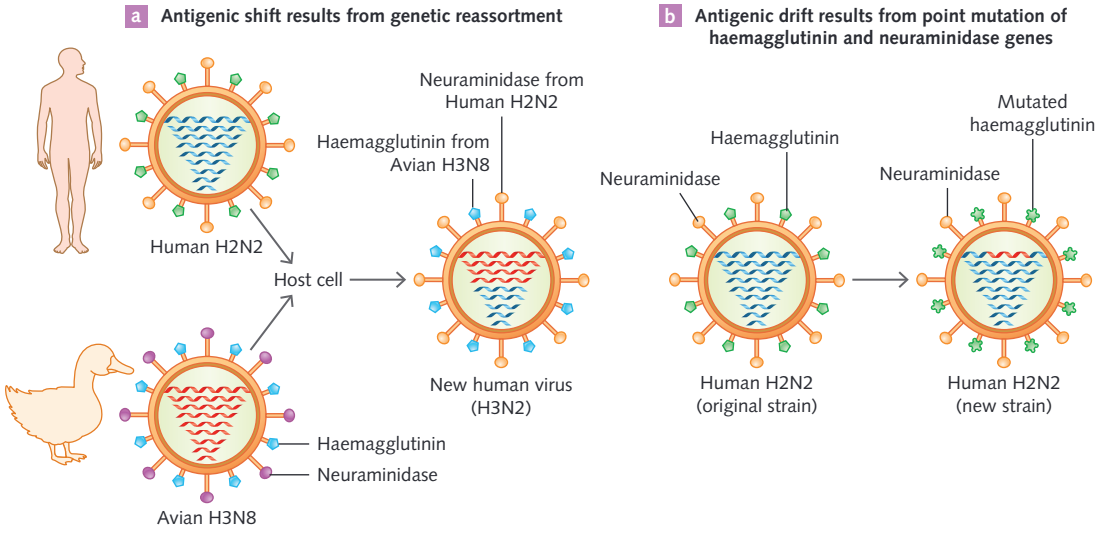

Figure 9.33 a This antigenic shift example models the emergence of the 1968 H3N2 subtype of influenza virus. b Antigenic drift occurs when an existing virus steadily mutates into a new strain.

If an influenza outbreak of a new virus subtype occurs that appears to have a high mortality rate, policy makers may act to limit the spread by restricting contact between people. This may involve shutting down public transport within the affected area as well as travel into and out of the affected area. It may also require closing down communal hubs, such as schools and sports venues where large numbers of people normally congregate. Antiviral drugs (e.g. Relenza) may be prescribed to treat infected individuals. However, if drug stocks are limited, the supply may be reserved for personnel involved in health or emergency services. Once an outbreak has begun, it is difficult to contain. The preferred option is preventing the outbreak in the first place; for example, through vaccinations.

The value of vaccination

Annual vaccination is the chief preventative measure against influenza. Current guidelines recommend vaccinations for people over the age of six months, including pregnant women. Building herd immunity in the population reduces the spread of the virus to vulnerable people, such as children under the age of six months.

The vaccination normally protects against three or four subtypes of influenza; however, the formulation may change from year to year. Influenza surveillance is conducted at a network of centres in more than 100 countries, including at the Victorian Infectious Diseases Reference Laboratory in Melbourne. The network monitors the emergence and spread of influenza subtypes, how contagious they are, and the severity of their disease symptoms. The WHO convenes a meeting of the network twice a year, one each for the northern and southern hemisphere flu seasons, to assess the data and determine which subtypes should be the focus of the upcoming season's vaccine. Recommendations must be made six months in advance of the flu season to allow time for production of the updated vaccine.

KEY CONCEPTS

- Theglobalrise i anibiotic-rsistant bacteria is explained byt principe ofnatural selection. Natural slection ad horizontal gen transfer contribute to the emergence of multirug-resiant bacteria.
- Maagin the rise of multidrug-resistant bacteria reqires measures t regulate ntibiotic use and devlop appeline ofnw ntibiotics.
- Atigeni drift andanigenic shift account for the emergence of new trains and subtypes of viruses.
- Prevnting outbreaks of ne virus strains or subtypes s bestachieved b acciation; managing an outbreak reqires measuresat limi cntact within the poplation andreulating accessto antiviral drugs.

Concept questions 9.6

1 Describe thechracteriticsof bacteria and viruses that nable them to adpt readil to changing enironmens.
2 Ouline how the processof natural selection most kl led to the emergence of fluoroqinlone-resistant *Pseudomonas aeruginosa*.
3 Lst the ways thathma actvity is causing the wolwide inrea n antibiotic-resistant bacteria.
4 Explain he difference betweenantigenic drift and antigeicshift. Wht is te difference in the eative amont f antigenic change between the two?
5 How doemultidg anibiotic resistance arise?

HOT Chaenge

6 About 90% of aatibiotic dose may be excreted from the body andend up in the sewers of towns and cies. *Escherichia coli* s a natur inhbitant of the human gut and is excrted in large umbers into the sewer system whn people ethe toilet. Run-off from farms ca include large amouts f antibiotics and *E. coli* Mlidrugresistance has been found in *E coli* and his hs led to the use ofthe tem 'superbug'.
   a Lst the steps tat could lead t drug-resistant *E coli* n the human poulation throg natral selection.
   b What is a superbug?
   c Antiiotics o ntdiscriminate between bene ficl and harmfulbcteri. Why could this be a problem for us?

BRANCHING OUT

Wildlife reservoirs of antibiotic-resistant bacteria

Pgs are no nativeoAustralia. They wre introdued as livstock y early European settlers in the late 19th century but soon escaped intthewild. Oing to hei hardy disposition ad cpaciy to roam long distances, pigs flourished n Austalia onditions (igre 934). Feral pigs are a pest damagin native ecosystms throuhhabitat destruction, predtion, resourc ompetiion and dis ease trasmissio. eralpigs are dif ficlt to control and a burden to stralian agrcultur, costing the secr abou $100 million per year.

Figure 9.34 It is estimated that today there are up to 24 million feral pigs in Australia covering more than one-third of the continent.

One poplationof erapigs nhbitsthe emi-arid Kimerey eginoWestern Australia. This population is isolated from other fral pi ppulations to the east and souh.The andisreatvely arid sparely ppulated by humans and used chiefly for beef cttl gazing. The cttl xperience minimal co ntactwith humans so vterinrncare is limited, but the catle and teferal pigs cmpete for habtat and resources.

Concerned by antibiotic reisance apeaing itheenironmen, Australn scientists investigated the potential for antiiotc resstance in *Escherichia coli* (*Ecoli*) resident in th fral pgpoulaton i theKiberley region. The scientists reasonedthat, becuse thee igs have lttlet n ontac with antibiotic, antibiotic-resistance data for their *Ecoli* wold provide an undertanding o the naturaloccurrence of antibi oic resistance ithe wil. he scntist collected samples from thecolon and rectum of rcently culle d feralpigs isoad idivdal colonies of *Ecoli* bacteria from the amples, and then tested each of the *Ecoli* soates aginst tiiotics The results of these experien are shown in Table 9.2.

The study showed the presene oficoplet antbiotic resistance in *E. coli* from the erlpgs. Gven the pig poplaton' solation and he chaacteristics of local farming pracies, te cientsts concluded hat transmission of the resistancerom liveso was nliely. The scientists no ted that freshwater ource normall contain the most diverse range of antbitic eistance,athough the antibiotic sul fadimehoxine was not eteted in soil or groundwater. Other antiiotics suh as cortetracylineasorb strongly to clay, sil andsedmnts. gs norally psh thir snouts into soil and sedimnts in searh of food.

Aim

To investite antibiotic rsistance in bacteria from a remote poultion of feral pigsand reate it to the risks associted wit theapeutic antibiotic use

Questions

1 Suggesthw it s possible that *E coli* from such a remotean isolatd popultion of feral pigs could show reistane to:
   a sufaimetoxine
   b clorteraycline.
2 Asuming that he antibotic are equally effective treatments fo aninection:
   a whch ould you recomnd dministering to graing beef attle in the area and why?
   b what othe advic could you provide a farmer puting cattle on a cour f antibiotics?
3 What risks fortanmission might there be for the dometic attle if they adthe feral pigs congregate around common water sources?
4 Thescientists condutigthe investigation proposed that the data could beuse s a 'baseline' for survillance antiiotic resitace in domestic pigs.
   a Suggest how thurveilance could e conducted.
   b Propose what sort ofdata yo would be seeking.

Table 9.2 Susceptibility to veterinary antimicrobials of 115 *E co* isolates from feral pigs in north-western Australia

Veterinary antibiotic	Number of resistant isolates
Ampicillin	0
Ceftiofur	0
Chlortetracycline	6
Danofloxacin	0
Enrofloxacin	0
Florfenicol	31
Gentamicin	0
Neomycin	0
Oxytetracycline	0
Spectinomycin	0
Sulfadimethoxine	58
Trimethoprim-sulfamethoxazole	0
Tulathromycin	0
Tylosin	0

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Chapter 9 Summary

9 Summary of key concepts

9.1 Mutations are the source of new alleles

KEY CONCEPTS

p 329

- Mutations are canes inDNA. Mutatins occur spontaneouly or are caused by mutagens. The potential effect of a mtation depends on wheter themutation ocurin somatic or germline cells.
- Point mtations can cause changes in a DNAseqnce y subtitution inertion or deletion of a nucleotide.
- Substitutions cn be slen muations, which cause no change in the encoed protein, or they can e missense or nonsense mutatins,whih alter the structure and function of the encoed protein.
- Insrtionsordeletions can cuse frameshift mutations that affect he amino acid sequence downsteam, severey affecting the encoded ptein.

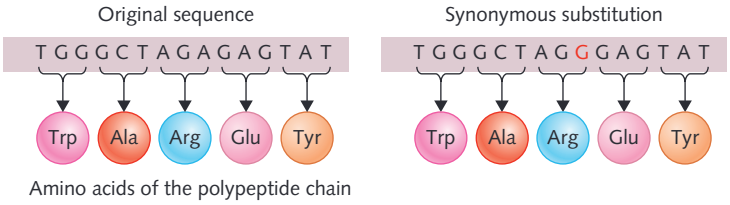

Figure 9.3 A silent mutation

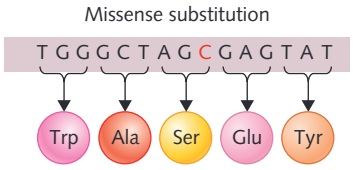

Figure 9.4 A missense mutation in the gene sequence leads to one amino acid being substituted for another in the polypeptide chain.

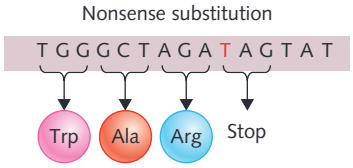

Figure 9.5 A nonsense mutation in the gene sequence results in premature termination of translation.

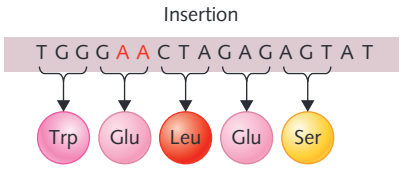

Figure 9.6 An insertion in the gene sequence results in a frameshift mutation. Here, two adenines have been inserted.

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9.2 Chromosomal rearrangements

KEY CONCEPTS

p 333

- Mutations can causechanges in chromosome strucure and chromosome number.
- Deetins, ivrsins, ransloations and dplictions may involve particular segments of a chromosome and may chag te number of aleles in affected areas.
- Mutations ca b silent nonsense, bene ficial r disadvatageous.
- New genes may b acquired bygene duplicationor by horiontal gene transfer.

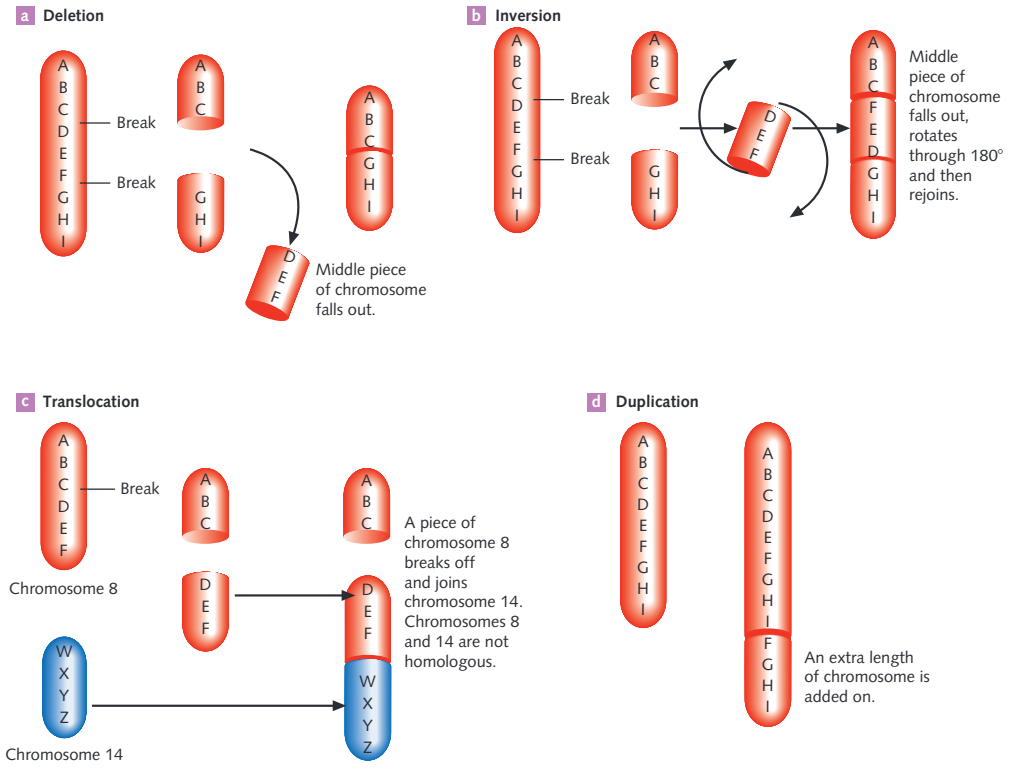


Figure 9.8 Abnormalities caused by chromosomal or 'block' mutations may arise by **a** deletion, **b** inversion, **c** translocation or **d** duplication.

9.3 Challenging allele frequencies in populations

KEY CONCEPTS

p 336

- Geneti drift is thechge in allle frquency in a population due to the random assortment and slection of certaingenes uring ioisand fertilisation.
- The bttleneck effect occurs when an event causes a arge redction in the gne poolo a population, decresing eneic ivrsity in subseuen generations.
- Gene flow reslts from the tranfr of alleles into or ou of a gene pol ecause of the migration of ndvduals betwee poulations.
- The founder effect occurs when a smal number f niviuls, arrying a restricd number of alleles, form a new ppuaton with reducd enetic divers ty compared withth orignal population.

9.4 Natural selection

KEY CONCEPTS

p 343

- Seletion pressures faour the survivalo some membes of a oplation over others; those individuals with a slctive advantage are bet er able to survive and reproduce.
- Naturlselectn is the drivingforce that causes populatons to adap to changing environmental circumstances.
- Poplations with ow enetic dversityar more vulnerable to extinction.
- Exerienta evoution enablesthe effects of slection pressures on populations to be tested and measured.
- There may muliple selection presres actingo a population at the same time.

9.5 Human manipulation of gene pools

KEY CONCEPTS

p 346

- Seletive beeding, or arti ficial slection, occurs hn humans selectivly beed organisms for desired traits.
- In selectiebreeding, t frequency ofalleles encoding for desired traits increases and the frequency of other a eles a the locus decreases, andths educes genetic diversity.
- Human ctivitie have resulted n decreased genetic diver sity in may wil species makng them vulnerable to extiction.

9.6 Natural selection and consequences for disease

KEY CONCEPTS

p 349

- Th glbl ris in ntibioic-resistant bacteria is explained by heprinciples f atural selection. Natrl electionand horizontal gene transfer contribute to the emergne of multidrug-reistant cteria.
- Maagin the rise of ultidrug-resistant bacteria reqires measures o regulat antibiotic use and devlopa ipeline fnw ntibiotics.
- Atigenc driftand atigenic shift account for the emergence of newstrains and sutyp s of viruses.
- Preveting outbreas of nw virus strains or subtypes is b st achivedby vaccination; managing an outbreak requires measures that mt contat within the populaion and regulating access to antviral drugs.

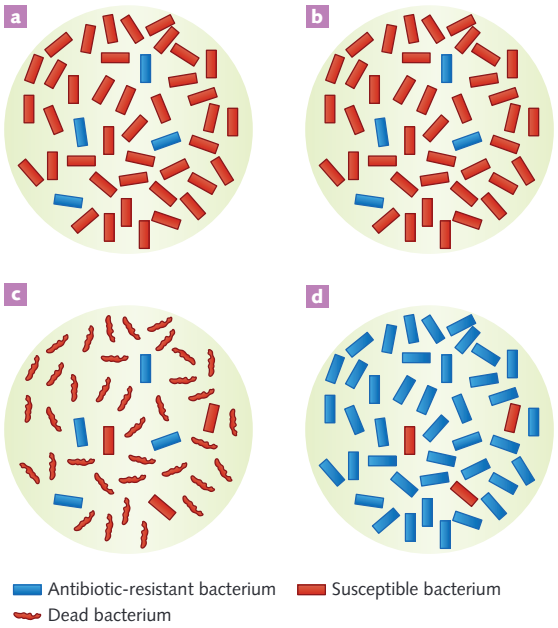


Figure 9.27 a An initial population of bacteria include a few antibiotic-resistant individuals. **b** On exposure to the antibiotic, a new selection pressure is introduced. **c** Many of the susceptible bacteria die off; the survivors tend to be those with antibiotic resistance. **d** Under continuing selection pressure, these bacteria continue to reproduce until, eventually, the population thrives again.

WB
9.7.1 KEY TERMS
PAGE 201

9 Chapter glossary

amino acid sequence the primary structure of a protein; comprises the order of the 20 possible amino acids in the polypeptide, sometimes referred to as 'polypeptide sequence'

antibiotic resistance the capacity for a microbe to withstand the lethal effects of an antibiotic to which it was once susceptible

antibiotic resistance gene a gene that codes for an antibiotic resistant phenotype

antigenic drift a change in the antigen of a virus that would otherwise be recognisable by the adaptive immune system, resulting from the gradual accumulation of mutations in the virus

antigenic shift a sudden change in the antigen of a virus resulting from the rearrangement of genetic material from two or more strains or subtypes of the virus

artificial selection breeding of plants and animals over successive generations to produce traits that are desirable to humans; also known as 'selective breeding'

beneficial mutation a mutation that increases the organism's chances of survival and reproduction

block mutation a mutation involving rearrangements of chromosomal segments

bottleneck effect when a catastrophic event or a period of adverse conditions drastically reduces the size of a population and its genetic diversity

broad spectrum describes an antibiotic (or insecticide) that is effective against a variety of organisms

conserved amino acids of polypeptide sequences or nucleotides of DNA sequences that remain consistent across species

deleterious mutation a mutation that decreases the organism's chances of survival and reproduction

deletion mutation a mutation in which nucleotide pairs have been lost from a segment of DNA

double-strand break a mutation involving breaks in the sugar-phosphate backbones at the same nucleotide pair, resulting in the complete breakage of a chromosome

duplication a mutation that occurs when one or more extra copies are made of a section of chromosome

extinct when all the members of a population or species have died out

fitness the capacity of an individual to survive and produce viable offspring

fixed describes an allele when it is the only variant available for a particular gene in the gene pool of a population

founder effect the type of gene flow that occurs when a few individuals that have become isolated from a larger population do not carry all the alleles that were present in the original population

gene duplication generating an extra copy of a gene within a genome as a result of duplication of a chromosomal segment

gene flow the transfer of alleles that results from emigration and immigration of individuals between populations

gene pool the range of genes and all their alleles present in a population

gene sequence the sequence of nucleotides in a gene

genetic drift the change in the gene pool of a population as a result of chance; usually occurs in small populations

genotype a specific combination of alleles for a particular gene locus belonging to an individual

germline a cell line in eukaryotic organisms from which the gametes are derived

heritable capable of being passed on to the next generation

horizontal gene transfer the process by which genetic material from one organism becomes incorporated into the genome of another organism

insertion mutation a mutation in which nucleotide pairs have been added to a segment of DNA

inversion mutation a mutation resulting in the normal sequence of genes being reversed in a chromosome

missense mutation a mutation that results in one amino acid being replaced by another amino acid in the encoded protein

monoculture the practice of cultivating a single genetically uniform breed of plant or livestock

multidrug resistance when a bacterium becomes resistant to two or more antibiotics

mutagen an agent capable of inducing mutations

mutation when a gene or chromosome has undergone a change relative to the original gene or chromosome; it may also refer to the process of generating such changes

natural selection the process whereby individuals with certain heritable traits survive and reproduce more successfully than other individuals

neutral mutation a mutation that has no effect on the organism's chances of survival and reproduction

nonsense mutation a mutation in which a codon for an amino acid is changed to one that codes for a stop codon, terminating translation

phenotype the actual form taken by a specific feature in a particular individual based on their genotype; can be used in reference to particular traits or characteristics or to the overall form of an individual

point mutation a mutation that affects a single base-pair position within a gene

population a group of individuals of the same species that live in the same area and interbreed, producing fertile offspring

population genetics the study of allele frequencies in populations and how they change over time

selection pressure a factor that favours the survival of some individuals over others within a population

selective breeding *see* artificial selection

silent mutation a mutation in which the DNA codon for one amino acid becomes another DNA codon for the same amino acid; also referred to as a synonymous mutation

somatic describes a body cell that will not pass its genes on to the next generation

species a group of similar organisms capable of breeding and exchanging genes with one another and whose offspring are capable of doing the same; also describes the lowest formal taxonomic rank and forms the second part of an organism's scientific name

subspecies the level of classification below species, referring to races of a species that are geographically isolated from each other

substitution mutation a mutation in which a single nucleotide is swapped for another in the original gene sequence

synonymous mutation *see* silent mutation

translocation a mutation occurring when a section of one chromosome breaks off and reattaches to another chromosome

WB
9.7.2 EXAM PRACTICE 202

9 Chapter review

Remembering

1 Define:
- a gene pool
- b allele frequency
- c genetic drift.

2 Draw diagrams depicting a:
- a bottleneck effect
- b founder effect.

Understanding

3 Classify the following mutations as neutral, deleterious or beneficial to an organism's chances of survival.
- a An insertion mutation in the human hexosaminidase A gene results in improper neural development.
- b A mutation in the beta-lactamase gene of the bacterium *Escherichia coli* generates a new version of the enzyme that detoxifies the antibiotic ampicillin.
- c A nonsense mutation in the human SURF1 gene encodes a protein crucial for formation of a key metabolic enzyme.
- d A silent mutation in the codon for an amino acid occurs at the active site of bovine salivary amylase.
- e Various mutations in a gene for the enzyme alcohol dehydrogenase result in different versions of the functional enzyme.
- f A mutation that extends expression of a human lactase gene enables lactose digestion into adulthood.

4 Draw an annotated diagram of two chromosomes showing that one of them has experienced two double-strand breaks. Draw the possible chromosomal rearrangements that might occur when the fragments of the broken chromosome are re-joined.

5 Figure 9.35 shows segments of chromosomes with genes numbered along their lengths. Identify the mutation that has occurred to produce each of these structural rearrangements from the original segment.

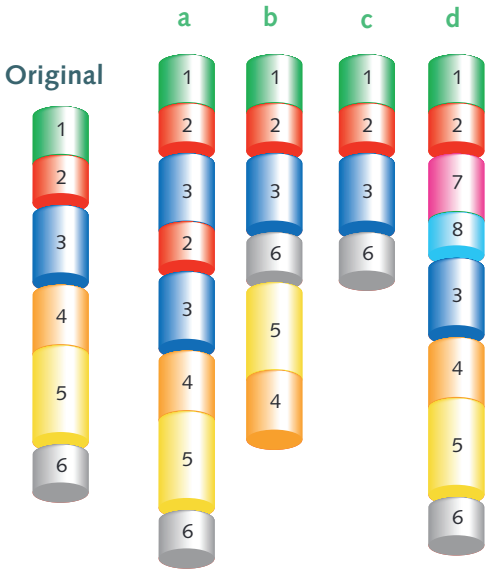


Figure 9.35 Chromosomal mutations

6 Discuss the relationship between substitution, silent, missense and nonsense mutations.

7 Mimicry is a common phenomenon in natural systems. The mimic seeks to take on the appearance of another organism. The organism being mimicked (the model) is harmful, distasteful or unpalatable to predators. Predators learn to avoid the model and therefore the mimic. It is assumed that the origins of mimicry lie in random, spontaneous gene mutations, recombinations and chromosome alterations that result in colour, structure or pattern change.
   a Explain the possible advantages of mimicry.
   b Explain what you would expect the ratio of models to mimics to be in natural systems.
   c Describe how the disappearance of the model might affect the mimic.

Applying

8 Amylase is an enzyme that digests starch. Two forms of amylase are found in humans: one that is secreted in saliva, the other secreted from the pancreas. The amylases are coded by two separate but closely located genes on chromosome 1.
   a Explain how this situation has arisen.
   b In populations with a history of farming starchy grains, most individuals have two or more copies of the salivary amylase gene. Every gene is expressed. How has this situation come about?
   c Draw an annotated diagram of the genes on chromosome 1, depicting your answers to parts a and b.

9 The FTO gene, a gene associated with obesity in humans, is widespread in vertebrate animals (fish, birds, reptiles and mammals). FTO is absent in insects, worms and fungi but is found in a few genera of single-celled algae. What might explain the peculiar distribution of the FTO gene across these organisms?

10 Construct a diagram to summarise the natural selection that occurred among the peppered moths of the UK as described in this chapter.

11 Copy and complete Table 9.3 using information provided in Table 9.4. Note that more than one type of mutation may describe the effect on the protein.

Table 9.3

Genetic mutation	Amino acid	Type of genetic mutation	Effect on protein
GTCCCA ↓ GTCCCT	Valine–Proline ↓ Valine–Proline	Substitution	Silent
TCAATA ↓ TAATA	Serine–Isoleucine ↓		
AGAGGT ↓ AGATGT	Arginine–Glycine ↓		
GCAAGA ↓ GAAAGA	Alanine–Arginine ↓		
CAGTAC ↓ CACGTAC	Glutamine–Tyrosine ↓		

12 A new subtype of influenza virus, H2N8 emerges from pre-existing human H2N1 and avian (bird) H1N8 subtype.
   a Use annotated diagrams to show how this new subtype may have arisen.
   b Explain why the new subtype has the potential to cause a global pandemic.
   c Describe what the WHO does to prepare for such a scenario.

Analysing

13 Using Table 9.4, list all the codons that could result from a silent mutation of GGG. What observation can you make about which of the three nucleotides in the codon is most prone to being mutated?

Table 9.4 Properties, names and DNA codons for each of the 20 amino acids

Characteristics	Name	DNA codons
Small, hydrophobic	Glycine	GGT, GGC, GGA, GGG
	Alanine	GCT, GCC, GCA, GCG
	Valine	GTT, GTC, GTA, GTG
	Leucine	TTA, TTG, CTT, CTC, CTA, CTG
	Isoleucine	ATT, ATC, ATA
Cyclic	Proline	CCT, CCC, CCA, CCG
Bulky, hydrophobic	Phenylalanine	TTT, TTC
	Tyrosine	TAT, TAC
	Tryptophan	TGG
Sulfur-containing, hydrophobic	Methionine (START)	ATG
	Cysteine	TGT, TGC
Hydrophilic	Serine	TCT, TCC, TCA, TCG, AGT, AGC
	Threonine	ACT, ACC, ACA, ACG
	Asparagine	AAT, AAC
	Glutamine	CAA, CAG
Negatively charged, hydrophilic	Aspartic acid	GAT, GAC
	Glutamic acid	GAA, GAG
Positively charged, hydrophilic	Histidine	CAT, CAC
	Lysine	AAA, AAG
	Arginine	CGT, CGC, CGA, CGG, AGA, AGG
	STOP	TAA, TAG, TGA

14 Construct a diagram that illustrates how recessive traits that are deleterious can survive in a population.

15 Explain why processes such as genetic drift and the founder effect are not regarded as examples of natural selection.

16 When a mutation occurs in a large population, it has very little effect on the population as a whole. Explain why mutations are still vital to the process of natural selection despite this small effect.

17 Artificial breeding of horses and cattle is not an example of natural selection but does lead to change in populations. Explain how artificial breeding is relevant to understanding natural selection.

18 The world's stock of Cavendish bananas is sterile and has been derived by cloning from a single original stock. Cavendish bananas in Australia, Africa and Asia are threatened by infection from the fungus *Fusarium oxysporum*. Why is the Cavendish banana so vulnerable to *Fusarium oxysporum* infection and what are the prospects for the population?

19 The red-legged earth mite (*Halotydeus destructor*) is considered a pest of canola and pea crops. In Western Australia, repeated applications of synthetic pyrethroid insecticides over several seasons became ineffective against the mite.

- **a** Explain this observation.
- **b** What might be the long-term consequences for red-legged earth mites in other states if the insecticide-resistant mites breach quarantine borders?

20 Explain how herd immunity reduces the antigenic drift of viruses.

Evaluating

21 Trypsin and chymotrypsin are proteases (enzymes that digest proteins) with very similar structures, but they preferentially split proteins at the site of different amino acids. The enzymes are coded by different genes; however, scientists propose that the two genes arose from a common ancestral gene. Discuss, with annotated diagrams where appropriate, what mutations may have occurred to generate the two different genes from the one original gene.

22 A measles outbreak occurs among an unvaccinated segment of the population. In time, vaccinated members of the population start developing measles. Explain using annotated diagrams how this situation could have emerged. Discuss what strategy health authorities may take to restrict the outbreak.

23 Imagine a situation in which the offspring of dark-skinned parents has inherited a mutated form of a gene that confers light skin pigmentation. Predict whether this mutation would be neutral, beneficial or deleterious if the individual is located in the Arctic Circle as compared with equatorial Africa. Explain your reasoning. Discuss how, if at all, your interpretation of 'neutral', 'beneficial' and 'deleterious' is influenced by the individual's environment.

Creating

24 Imagine you were initiating a captive breeding program for the Tasmanian devil (*Sarcophilus harrisii*), a species threatened by the spread of the transmissible devil facial tumour disease. While most of the population is susceptible, some individual devils show resistance to the disease.

- **a** Explain why a captive breeding program might be valuable even if resistance is showing up naturally in the wild.
- **b** Outline what your objective would be for the gene pool of the captive population.
- **c** Explain how you would seek to achieve your objective.
- **d** Explain which aspects of your scenario are an example of natural selection and which are an example of artificial selection.

Changes in species over time

10

By the end of this chapter you will have covered the following material.

Key knowledge

Changes in species over time

- changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transitional fossils, relative and absolute dating of fossils pp. 373–386
- evidence of speciation as a consequence of isolation and genetic divergence, including Galapagos finches as an example of allopatric speciation and *Howea* palms on Lord Howe Island as an example of sympatric speciation pp. 386–393

Determining the relatedness of species

- evidence of relatedness between species: structural morphology – homologous and vestigial structures; and molecular homology – DNA and amino acid sequences pp. 393–404
- the use and interpretation of phylogenetic trees as evidence for the relatedness between species pp. 404–406

Key science skills

Develop aims and questions, formulate hypotheses and make predictions

- formulate hypotheses to focus investigation pp. 406–409

Generate, collate and record data

- record and summarise both qualitative and quantitative data, including use of a logbook as an authentication of generated or collated data pp. 406–409
- organise and present data in useful and meaningful ways, including schematic diagrams, flow charts, tables, bar charts and line graphs pp. 406–409

Construct evidence-based arguments and draw conclusions

- evaluate data to determine the degree to which the evidence supports or refutes the initial prediction or hypothesis pp. 406–409
- use reasoning to construct scientific arguments, and to draw and justify conclusions consistent with the evidence and relevant to the question under investigation pp. 406–409

Online Chapter Map
Chapter 10 Map

10 Changes in species over time

There is plenty of evidence to tell us that species have not always been as they are today. Species have come and gone, changed and also stayed the same. How do we know? It is written in the rocks!

102 Patterns n evouton p381

Fosss reval vidence for past mass extnctons fo owed by evouton of new groups of organsms Traniionl fosss wth chara cterstcs of both earer and ater speces show how orgaisms are elaed. Dvergent eoltion occurs when new spcies emerge from one poultion underdifferent seecton pressurs. Convergentevlution occurs when unrlated speies evlve smar features under the same seecton pressurs.

101 Studyng fosss p373

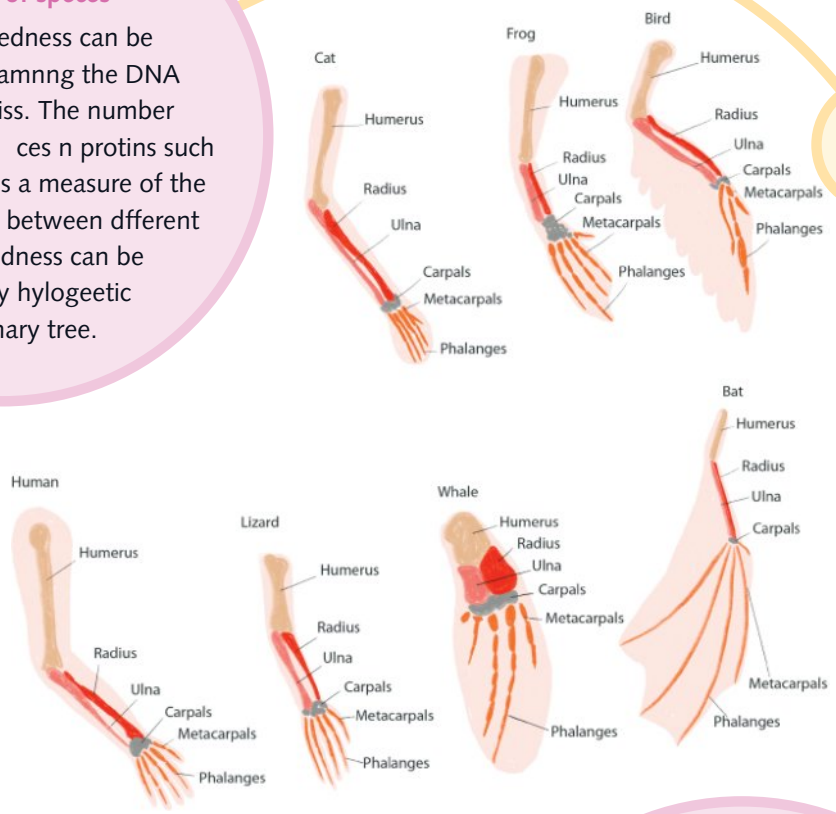
Changes n speces over mons of years can be seen n foss evdence Fosss are eidence of past fe They can be dated by ether comparng the sequence of the rock in whch they are found (rlaive daing) or usng the preict abe natura decay of radoactve e ements n rocks or fosss to determne a numerca age – (absoute datng)

9780170452533

p399

105 Moecuar evdence for reatedness of speces

Evoutonary rlatedness can be nvestgated by examnng the DNA and protins of orgniss. The number of amno acd dfferen ces n protins such as cytochrome c ives a measure of the evoutonary dstance between dfferent speces Ths rlatedness can be represented by hylogeetic or evoutonary tree.



p386

103 Emergence of new speces

f a popuaton becomes dvded then the members of each soated popuaton are unale tointerbreed and exchange aees f each new popuaton s exposed to dfferentselction pressurs, theyll evove ndependenty so that eventuay they cannot nterbreed evenif they are brought together agan – they are new and separate speces

104 Determnng the reatedness of speces

p393

Even though speces may ook competey dfferent from each oter, cose study of ther s tructura morphoogy, embryonc stages and homoogous structures gve cues to ther rlti onshps For exampe the human arm has smartes wth the whale flpper, bat ing an d cat forle, showng that a these speces had a common ancestor.

Evidence for relatedness of species can be found in the fossil record, how species grow and are built and even in their molecular structures. This knowledge can be applied to the human species to trace their evolution over time.

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Online Chapter Mapv
- Chapter 10 map (p. 370)

Onne.e:Ker, m v
- Chapter 10 flashcards (p. 372)

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- Video: how fossils are formed (p. 374)
- Evidence for evolution (p. 393)

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- Fossils (p. 374)
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- Chapter 10: Summary of key concepts (p. 412)

Chape10 flashards

Know your key terms

absolute dating
adaptation
adaptive radiation
allopatric speciation
analogous structure
bioinformatics
biological species concept
clade
cladogram
comparative dating
convergent evolution
correlation
divergent evolution
electron spin resonance
evolutionary distance
fossil
fossil record
fossil succession
homologous
homologous structure
index fossil
isotope
lineage
luminescence
mass extinction
maternally inherited
mineralisation
molecular clock
molecular homology
monophyletic
morphological species concept
mya
niche
node
optically stimulated luminescence
pairwise comparison
phylogenetic tree
phylogeny
phylogram
radioactive decay
radiometric dating
relative dating
reproductively isolated
sequence alignment
speciation
strata
structural morphology
superposition
sympatric speciation
taxonomy
tetrapod
thermoluminescence
trace fossil
transitional fossil
vestigial structure

WB
REMEMBER
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Remember

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 Mutations are the source of new alleles in a gene pool.
2 A point mutation is when a single nucleotide is changed within a DNA sequence. If the mutation occurs within a gene, it could alter the translated protein.
3 A selection pressure is an environmental or ecological factor that promotes the survival of some individuals in a population over others.
4 Natural selection is when selection pressures cause a change in the gene pool of a population.
5 Extinction is when all the members of a population or species have died out.

In 1858, two publications were released simultaneously through the Royal Society in London. These were papers by Charles Darwin and Alfred Russel Wallace. The papers outlined Darwin's and Wallace's ideas on the evolution of species, which they referred to as 'descent with modification'. This term highlights the important idea that all life that exists today has descended from shared ancestors. Darwin and Wallace proposed that this happened by the process of natural selection, which has shaped nearly every feature of living things in the world today. Through natural selection, favourable traits are selected for and inherited and become more common in subsequent generations.

The idea of adaptive evolution through natural selection is one of the most important ideas in Biology. Although Darwin and Wallace did not have a good understanding of the underlying causes of inheritance, they did realise that variable traits must be heritable. Subsequent discoveries of the genetic basis of inheritance, initially through the work of Mendel, fitted perfectly with their theories to produce a coherent theory of evolution referred to as the 'modern synthesis'.

The evidence for evolution has accumulated in Earth's geology, in the form and function of living organisms, and in the structure of their cells and molecules. Moreover, we can use the evidence to hypothesise and construct the relationships between organisms over time. We can reconstruct the actual and hypothetical lines of descent that connect many present-day groups of species through a shared ancestry.

The worldwide collection of fossils as they occur in the surface layers of Earth constitutes the **fossil record**.

10.1 Studying fossils

Fossils are the preserved remains and traces of organisms. They provide evidence of past life. These remains can be hard parts, such as teeth, bones and shells, or impressions in the rock where the organism's tissue decayed. Most fossilised 'hard parts' of animals or plants are found in rocks that have been derived from sediment; that is, sand, silt or clay. Along with animal bones, such as the skeleton of *Ceratoichthys pinnatiformis* (Figure 10.1), fossils can also include trace fossils: footprints, burrows or other evidence for the organism's existence.

To make sense of the fossil record and examine it for evidence of evolution, you need to understand some basic information about the fossils and their geological setting, including how fossils are formed and why some organisms are well represented in the fossil record while others are not. We need to be able to estimate how old the fossils are, which organisms arose first, and which organisms lived together.

Figure 10.1 An immaculately preserved fossil of the extinct fish *Ceratoichthys pnnatforms* : a rare example of a complete fossilised skeleton.

WB
10.1.1 FOSSILISATION PROCESS PAGE 205

Fossilisation

The process of fossilisation requires very specific and rare conditions. The remains of the vast majority of long-extinct animals may never be found, and consequently the fossil record is incomplete and biased towards organisms that fossilise more easily. To become a fossil, organic matter needs to be deposited and covered in sediments in an environment that lacks oxygen. The lack of oxygen reduces the rate of decomposition by bacteria. The overlying sedimentary material also protects the organic matter from scavengers. Plant and animal remains can be preserved if they are covered in waterborne mud, sand or clay, depriving the remains

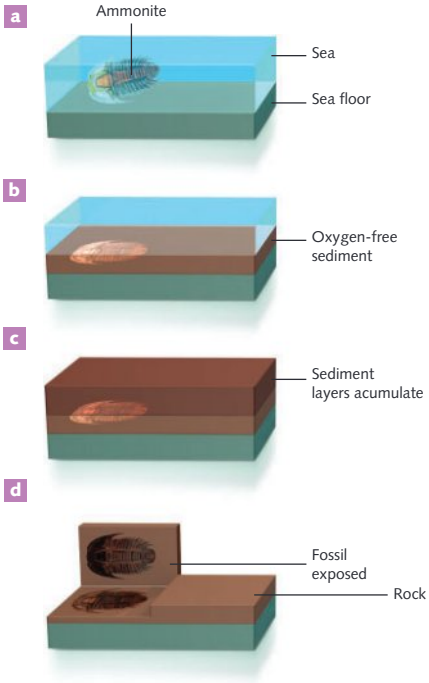


Figure 10.2 Fossil formation. **a** An ammonite dies and sinks to the sea floor. **b** The soft parts of the animal decompose and leave the shell. **c** Left undisturbed, the shell becomes buried under sand and silt. **d** Over millions of years, the shell becomes mineralised and the sedimentary layer hardens into rock.

Weolink
Video: how fossils are formed

Onne Wyrkm heet
Fossils

of oxygen, as can happen in the beds of lakes and rivers or in calcium-rich seabeds (Figure 10.2). In many cases, minerals from the sediments have replaced the natural bone or shell material, making the remains harder and more likely to fossilise. This type of fossilisation is called **mineralisation**. The resulting fossils are generally only the 'hard parts' of organisms, such as bones and teeth that are slow to decay, and rarely more delicate tissue, such as feathers. These layers of mud or silts are consolidated under pressure and over prolonged stretches of time to form sedimentary rock.

Fossils are not ordinarily found in volcanic rocks because molten lava solidifies at about 1000°C, which is hot enough to burn any organic material. However, fossils can be found in sedimentary layers of eroded volcanic ash. Metamorphic rock does not usually bear fossils because the pressure and heat of metamorphism generally (although not always) destroys any remnant of the fossil.

Thin tissue, such as leaves and muscle, is sometimes preserved as films or impressions left in the sedimentary rock. Fossils are also formed when the impression left by a decomposed organism is later filled by soft material such as volcanic ash, or by a mineral solution, resulting in fossils such as those composed of opal. These processes also support formation of trace fossils. **Trace fossils** are not formed directly from the organism itself but from the organism's activites; for example, footprints (Figure 10.3), burrows and even preserved waste products such as 'coprolites' – fossilised faeces – are trace fossils.

There are several other ways fossils can form. They can form through freezing and subsequent dehydration. Plant material may be partly dissolved, and some tissue replaced with dissolved salts (a type of mineralisation) that petrify the material; that is, they turn it to rock. Entire tree trunks have been preserved by petrification in fossilised forests in Arizona and Antarctica.

Figure 10.3 An example of a trace fossil: dinosaur footprints left in ancient mud flats on what is today called Dinosaur Ridge, Colorado, USA

Fossil strata

The fossil record is delineated by layers of successive rocks, or **strata**. Each stratum comprises unique collections of fossils and represents a unique age range. The sequence of strata can be interpreted as depicting the progress of biological evolution on Earth.

To understand the fossil record as a basis for evolution, you need to understand how the strata are formed.

Stratification

Sedimentary rock is composed of weathered material from Earth's surface, such as gravels, silts, sands and muds that have been transported by water and deposited on riverbeds, flood plains and sea floors. Sediment transport and deposition is an ongoing process; it has been occurring continuously on Earth for billions of years and can still be observed today. Over time, these deposited sediments form defined strata that consolidate into sequences of sedimentary rock. Older strata gradually become buried under progressively newer formations. These sequenced layers are the strata, and a section showing successive layers of sedimentary deposition is called stratification (Figure 10.4).

Strata formed at the same time and under similar circumstances are composed of the same sorts of minerals and have the same physical properties. These strata often also contain the same sorts of fossils. Strata become deformed and disrupted over long periods of time by the geological processes that destroy and rebuild Earth's crust. Over millions of years, these geological processes gradually push strata that have formed on the sea floor up above sea level, in many cases forming new mountain ranges. There they are subjected to the weathering effects of wind and rain. After a very long time, ancient fossils may become exposed.

Figure 10.4 Sequential strata of marine sedimentary strata are visible in these weathered sandstone cliffs. The youngest strata are at the top and the oldest are at the bottom.

Only a very small percentage of organisms ultimately leave fossilised remains. Many fossils are destroyed by weathering and erosion. Many more may lie buried or hidden. The fossil record is incomplete; however, it is continually augmented by new discoveries, and curated fossils may be re-examined as new experimental techniques are developed.

Relative dating techniques

WB
10.1.2 RELATIVE DATING TECHNIQUES PAGE 206

Relative dating (also called **comparative dating**) is used to determine the age of a rock, or a fossil contained in the rock, relative to other rocks or fossils found nearby. This approach to dating relies on our understanding of how sedimentary rock is formed.

Strata are deposited in a time sequence, with younger strata progressively formed on top of older strata (Figure 10.5). This is the basis of the principle of **superposition**. Assuming that the movement of Earth's crust has not twisted or inverted the strata, palaeontologists can assign relative ages to fossils according to

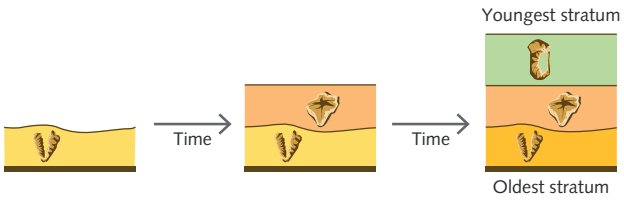


Figure 10.5 Over geological time, younger strata are deposited over older strata. The youngest fossils are found in the uppermost stratum and the oldest are found at the bottom.

the strata in which they are found. The fossils found in the uppermost strata must be younger than those found in the older strata underneath. While this technique cannot give an age in years, it can estimate the ages of the sequence of the strata relative to each other.

Index fossils

Strata that are located in distant sites but have identical mineral and fossil composition can be assumed to be the same age. This is the principle of **correlation**.

Correlating the ages of strata that are far apart is expedited by identifying index fossils. **Index fossils** are fossils that are representative of specific geological ages. Ideally, index fossils appear in only a limited segment of the fossil record so they are tightly linked with a particular geological age. They occur over relatively wide geographical ranges so they can be used to calibrate ages between far-flung sites (Figure 10.6). If rock beds hundreds of kilometres apart contain specimens of the same index fossil, it can be inferred they are of the same geological age.

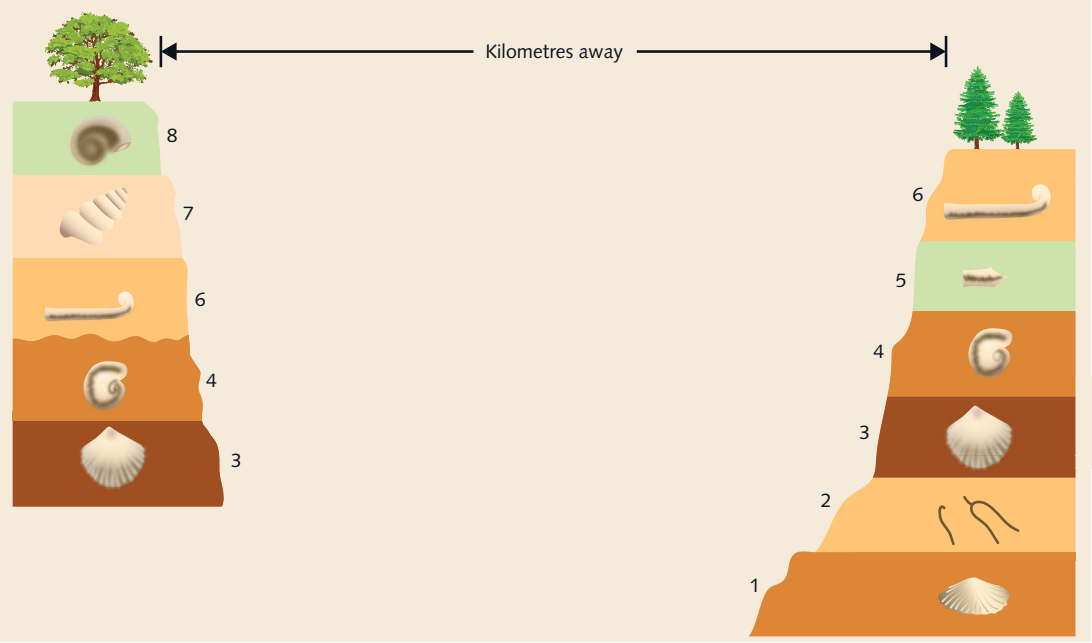


Figure 10.6 **Index fossils are unique to specific strata and enable the ages of strata from distant sites to be correlated.**

Fossil succession

As early as the beginning of the 19th century, when fossils were becoming increasingly studied, it was recognised that each stratum bore a unique collection of fossil animals and plants. By advancing upwards through the sequence of strata (essentially travelling forward in time), one collection of organisms is replaced by another.

Also, many fossil organisms occur in more than one stratum. However, if you proceed down through the sequence (travelling back in time), you reach a stratum beyond which none of that fossil organism is found. In effect, initially the organism does not exist in the fossil record but, after it first appears, it may persist in the fossil record for some time, possibly even to the present.

These observations highlight the principle of **fossil succession**. They demonstrate that the history of life on Earth is described by a number of distinctive time spans, each dominated by a characteristic collection of animals and plants. Progressively, through the course of evolution, one collection abruptly gives way to another.

Absolute dating

WB
10.1.3
ABSOLUTE DATING
PAGE 207

Absolute dating is a technique that assigns a numerical age in years to a fossil or rock. Unlike comparative dating, absolute dating is based on the physical or chemical properties of materials in the rock, rather than the assumption-based sequences that relative dating provides. There are several different absolute dating techniques for absolute dating on vastly different time scales from thousands of years to hundreds of millions of years.

Radiometric dating

The most common method of absolute dating is **radiometric dating**, which is based on the predictable rates of decay of naturally occurring radioactive isotopes present in a rock or fossil. By testing for the proportion of different radioactive isotopes, an age in years can be estimated for the sample.

Many elements occur as **isotopes**: they have the same atomic number (the same number of protons) but a different atomic mass (different numbers of neutrons). For example, carbon has three isotopes: carbon-12 (^{12}C), carbon-13 (^{13}C) and carbon-14 (^{14}C). ^{12}C has six protons and six neutrons in each nucleus, ^{13}C has six protons and seven neutrons, and ^{14}C has six protons and eight neutrons. Some isotopes have an unstable nucleus that splits and emits energy in the form of radioactivity (alpha, beta or gamma rays) at a measurable rate. This process is referred to as **radioactive decay**. The half-life of an isotope is the time taken for half of the radioactive atoms in an initial sample to decay.

^{14}C is a radioactive isotope that forms when cosmic rays strike nitrogen atoms in the upper atmosphere. ^{14}C decays at a known rate to produce nitrogen-14 (^{14}N). Its measurable rate of decay is the basis of carbon dating. When an organism dies, the ^{14}C it contains decays at a steady rate. We can use the half-life of ^{14}C (5730 years) and the ratio of ^{14}C to ^{12}C in the sample to determine the age of the sample – in other words, the time taken for the original ratio to decay to the present ratio (Figure 10.7).

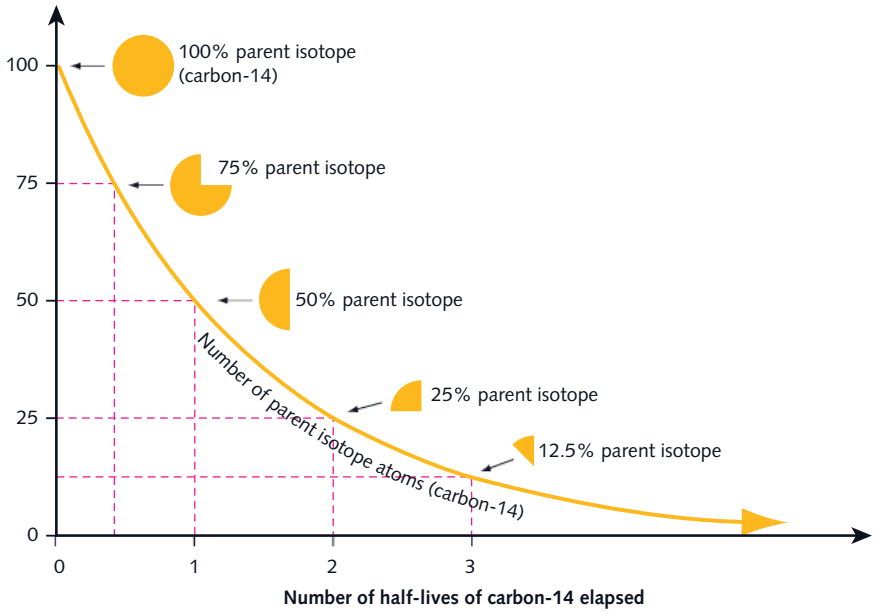


Figure 10.7 A graph of the decay of ^{14}C (half-life 5730 years)

In its simplest form, carbon dating assumes that the proportion of ^{14}C in the atmosphere is constant, but data from tree rings shows that it can change with time. For this reason, the calculated age has to be corrected into calendar years. Ages are expressed with their degree of accuracy (usually as $\pm$ x years).

The older the object, the greater the margin of error. Carbon dating – corrected for atmospheric variation – is thought to be accurate for samples up to about 60 000 years old. After this time it is difficult to measure the level of ^{14}C accurately and so other radioisotopes, such as potassium-40 (^{40}K), which decays into argon, are used instead (Table 10.1).

EXAM TIP
Many radiometric dating methods are suitable for dating sediments on the scale of mya, but ^{14}C is limited to determining ages less than 60 kya.

^{14}C dating is generally used for artefacts such as ash deposits but is not applied directly to fossils for two main reasons.

» In most cases, fossils have been mineralised, meaning that the organic (carbon-containing) tissue has been chemically altered or replaced.
» The process of fossilisation generally takes longer than the maximum age of accuracy for ^{14}C.

Table 10.1 The half-lives and products of decay of some elements used in radiometric dating

Element	Product	Half-life (years)
Carbon-14 (^{14}C)	Nitrogen-14 (^{14}N)	5730
Uranium-235 (^{235}U)	Protactinium-231 (^{231}Pa)	704 million (7.04×10^8)
Uranium-234 (^{234}U)	Thorium-230 (^{230}Th)	246 000
Potassium-40 (^{40}K)	Argon-40 (^{40}Ar)	1.25 billion (1.25×10^9)
Thorium-232 (^{232}Th)	Lead-208 (^{208}Pb)	14 billion (1.4×10^{10})
Rubidium-87 (^{87}Ru)	Strontium-87 (^{87}Sr)	48 billion (4.8×10^{10})

Certain types of rocks are more suited to radiometric dating than others. Igneous and metamorphic rocks are suitable because the radioactive atoms are generated and incorporated into the rock when the rock is first formed. For example, feldspar is a common type of igneous rock that contains potassium but not argon; radioactive decay of ^{40}K to ^{40}Ar (argon-40) in the feldspar thus provides an accurate reflection of the age of the rock. Sedimentary rocks are not suited to radiometric dating because they are formed when older rocks are weathered, and the particles are subsequently deposited in the sedimentary layer. Radioactive decay would have already occurred in the minerals before they formed the sedimentary rock so radiometric dating of the sedimentary layer is misleading. A more reliable estimate for the age of sedimentary rock is to date the igneous or metamorphic layers either side and define an age bracket for the sedimentary rock in the middle.

Luminescence techniques

Thermoluminescence and **optically stimulated luminescence** are absolute dating techniques that measure the properties of electrons in the crystals of minerals (Figure 10.8). The atoms in mineral crystals mostly pack uniformly into a regular lattice structure; however, there are imperfections or holes where atoms are missing from the structure. The energy from natural radioactivity within the mineral lattice and in the surrounding environment chips electrons off atoms in the mineral and they can become trapped as isolated solitary electrons in the holes of the lattice. The rate at which solitary electrons are produced depends on the amount of radioactivity experienced by the mineral. The total amount of solitary electrons trapped in the mineral depends on both the rate at which they are produced and the amount of time since the mineral was last exposed to radiation (i.e. light or heat).

The trapped solitary electrons are subsequently released when the mineral crystal is again exposed to light or heat (Figure 10.8). As the electrons escape, they emit a corresponding amount of energy in the form of light, which can be measured by a specialised instrument. **Luminescence** techniques differ depending on how the solitary electrons are released from the mineral. In optically stimulated luminescence, the electrons escape when the mineral is exposed to coloured light. In thermoluminescence, the electrons escape when the mineral is heated. Either way, the amount of light emitted is a measure of the quantity of solitary electrons trapped in the mineral grain.

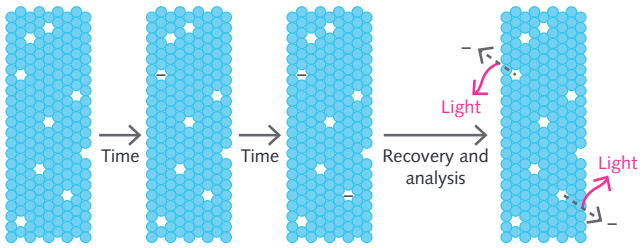


Figure 10.8 **The basis of luminescence dating techniques. A long time after burial (e.g. thousands of years), natural radioactivity in the mineral and the environment drives the production of free electrons (–) that become trapped in imperfections in the mineral crystal. The amount of electrons that are trapped depends on the rate at which they are produced and the total amount of time the mineral crystal remains buried. When the mineral crystal is recovered, it is stored away from heat or light until analysis under controlled laboratory conditions. During analysis, the mineral crystal is exposed once again to heat or coloured light, which stimulates the electrons to escape from the crystal. As they escape, the electrons emit light that is measured to provide an estimate of the total number of trapped electrons in the crystal.**

The minerals thus act as memory devices, storing the record of when radiation (such as light or heat) last struck the mineral crystal. While the mineral grains are being transported by wind or water, they are exposed to sunlight and heat, and their energy signal is set (or reset) to zero. Once buried, the mineral crystal captures energy from surrounding radiation and stores this energy in the form of solitary electrons trapped in the imperfections of the crystal. The solitary electrons accumulate in the mineral crystal for as long as it is buried.

The amount of radioactivity must also be measured within the mineral and in its immediate environment. This makes it possible to calculate the rate at which the solitary electrons are generated. From two parameters, the total solitary electrons in the mineral and the rate at which they are generated, it is possible to calculate the amount of time since the mineral was buried.

Both optically stimulated luminescence and thermoluminescence are useful for dating the last exposure of the minerals to heat or light and the occurrence of mineralisation. Therefore, they can provide an estimate of the time of burial in sedimentary rock. However, they cannot generally provide an estimate for the absolute age of the original mineral that was deposited. The technique is suitable for ages up to 100 000 years and is often used to estimate the age of archaeological artefacts associated with modern humans.

Electron spin resonance

Electron spin resonance also measures the properties of electrons trapped in mineral crystals. In contrast to luminescence techniques, the electrons do not need to be released to be measured.

When a magnetic field is applied to the mineral, the solitary electrons respond as though they are tiny magnets aligning in the magnetic field. A signal is recorded and its strength indicates how many solitary electrons are inside the mineral. The amount of radioactivity is then measured within the mineral and in its immediate environment, which makes it possible to calculate the rate at which the solitary electrons are generated. The total solitary electrons and the rate at which they are generated are used to calculate the amount of time since the mineral was formed.

As with luminescence techniques, it is necessary also to measure how much radioactivity the sediment grains are exposed to, to gauge the rate at which solitary electrons are generated. The measurements for both the total solitary electrons and the rate of their production are used to calculate the time since the minerals were last exposed to light or heat.

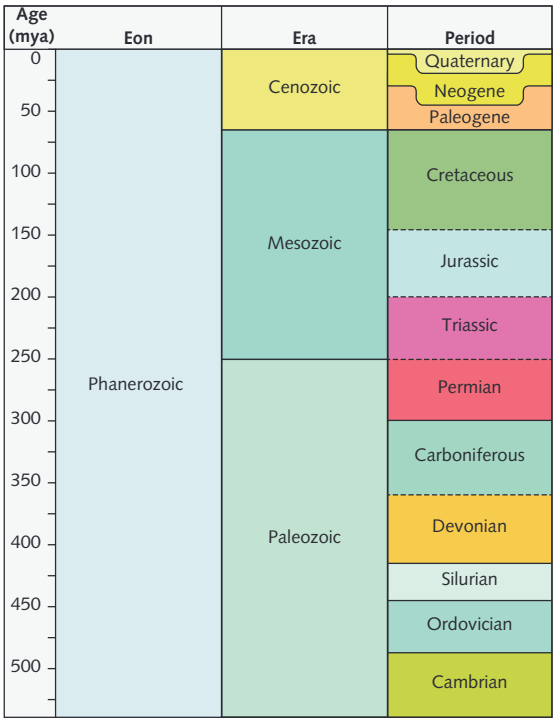


Figure 10.9 The geological time scales constructed from relative and absolute dating methods

The sensitivity of electron spin resonance depends strongly on the nature of the sample and the environment it has experienced but it can be used to measure ages from a few thousand years up to about 2 million years. An advantage of electron spin resonance is that it can be applied directly to minerals produced by organisms, such as teeth and shells.

Geological timescales

Dating techniques credibly demonstrate the colossal time scales it takes for species to diversify. Instead of understanding Earth's history in years, decades or even centuries, we have devised other ways of measuring 'deep time' in segments covering millions, sometimes billions, of years, such as periods, eras, epochs and eons. These measurements are known as geological time and are expressed in millions of years ago (**mya**). Fossils that are large enough to see with the naked eye become apparent from around 600 mya. This fossil record provides another means of refining Earth's geological time scales. A summary of the geological time scales is presented in Figure 10.9.

KEY CONCEPTS

- Foils are preserved remain of organisms or traces of thei exisence. The conditis fr fossilisation occur arelyand this cn ause a bas in the fossil recod.
- The fsil recrds delineated by onsecutive layers of rocksalled strata. Th youngest fossils are found n the uppermost strat and the oldest are found at the bottom.
- Comparative dating can determinehe age of a fossil or foss-bearingrockin relation to he surrounding rock, bt it doesnot giv numerical age.
- Index foils are used o correlate the ages of strata that ocur i deposits far from each other.
- Aboluteda ting gves numerical ages for the time of foss formtion nd inclues rdiomtic dating, umnescence ad elecron spin esonance.

Concept questions 10.1

1. Describe the stagof the fosilisation process.
2. Most of ou knowledge ofte evolutin of sharks is based on theremains o fossilised hark teeth. Suggest why other filised body parts of sharks have not been foun in abundnce.
3. Palaeonoogists havefound trcng the evolution of sea jees ('lly fish') to be ver chlenging. With your knowedge of foils and the process ofossliation, suggest wy this may be the case.
4. Suggest what processmght expain how fossils that ded on the sea floor culd be foud hgh in the Hialayan outains.
5. Recount two moden techniqus fo studying fossils and the kind of information hat can be obtained from each.
6. Lst the methods use to determine te age of fossils andgive the pros and cons of each.
7. What features make anndex fossil?
8. Wich isotoes would be measured to radiodate a fossl that comarative datng suggests is approximately 50 000 yeas old?

HOT Chaenge

9. Radiomtric dting is based on the ncpt of half-life.
   a. What does this mean?
   b. Wht is thhalf-life of ^{4}C?
   c. Wh is carbn datin of novaluen dating fossils that a millions of years old?

10.2 Patterns in evolution

The fossil record provides evidence to trace the evolutionary descent of many broad groups and species of animals and plants over time. Emerging through phases of extinction followed by repopulation, the evolution of organisms is shaped by natural selection over vast amounts of time. The two most striking patterns are divergent evolution and convergent evolution.

Mass extinctions

The collection of fossil animals and plants in a stratum sometimes gives way abruptly to a completely different collection of fossils in the next stratum. Near the top of the older stratum, substantial changes occurred in global environmental conditions that forced the life forms in that stratum to come to an end. The boundary between the two strata marks a **mass extinction** event.

Mass extinctions help to explain major episodes of succession. The interpretation of these boundaries is that large and diverse collections of organisms were rapidly wiped out by sudden and extreme changes in environmental conditions. The organisms' demise left many **niches** (ecological roles) vacant. Descendants of the survivors of the mass extinction evolved relatively rapidly, filled the vacant niches and created novel ones in the emerging new world. The extinction survivors and their evolving descendants ultimately form the collection of organisms that populates the subsequent stratum.

The fossil record thus shows evidence of rapid and diverse evolution of many species from a single (or just a few) initial ancestral species. Each of the descendent species has unique anatomical **adaptations** that allow the organism to exploit specific ecological niches.

WB
10.2.1 TRANSITIONAL FOSSILS PAGE 209

Transitional fossils

The fossil record demonstrates many phases of evolutionary succession. As new species replace older ones, there are lines of continuity between ancestral and descendant forms. Some fossils bear features of both an older ancestral life form and a younger descendant. These intermediate forms are called **transitional fossils**. Transitional fossils provide copious evidence for evolution, documenting changes between groups of organisms over time.

From fish to amphibians

Many transitional fossils between lobed-fin fish and amphibians have been recovered from the period when land-based **tetrapods** (vertebrate animals with four legs) first appear in the fossil record. Fossils, such as *Acanthostega* species, show the early evolutionary progress of tetrapods (Figure 10.10). These animals were about 1 metre long and had a tail and gills typical of fish. However, they also had relatively well-developed limbs with 6–8 digits like tetrapods. Unlike fish, they had a strengthened rib cage and shoulder blades separate from the skull, accommodating a muscular neck that allowed the head to turn relatively freely. They were mainly aquatic but showed the skeletal and morphological adaptations to land that indicate they were the probable ancestors of modern tetrapods.

a

b

Figure 10.10 a A fossil of *Acanthostega* and **b** a digital reconstruction of an *Acanthostega* species from 380–365 million years ago

From dinosaurs to birds

The most well-known mass extinction in the fossil record was the one that ended the age of the dinosaurs. Among the survivors of the extinction were small, ground-dwelling feathered dinosaurs called *Archaeopteryx* (Figure 10.11). About the size of a modern crow, *Archaeopteryx* had the uniform teeth, bony tail and claws typical of dinosaurs. However, it also had the forked wishbone characteristic of birds and the sediment around the animal bears the imprint of feathers. Therefore, *Archaeopteryx* is interpreted as a transitional fossil showing that modern birds descended from dinosaurs. Palaeontologists currently know of five feathered dinosaur species from the fossil record that survived the extinction of dinosaurs. Those ancestors have given rise to more than 10 000 bird species today.

a

b

Figure 10.11 a An *Archaeopteryx* fossil and **b** a reconstructed model of the bird-like dinosaur

WB
10.2.2 DIVERGENT EVOLUTION PAGE 211

Divergent evolution

Divergence is a pattern of evolution where groups of organisms become so different from each other that a new species forms. This is called **speciation**. This is usually the result of the dispersal of a single species to different environments; that is, groups from the same species become isolated from each other. The isolation stops the gene flow between these separated populations and different selection pressures work on each isolated population. Over many generations, members of the population develop adaptations to the different selection pressures, and they eventually become new species. This is described as **divergent evolution**.

For example, koalas (tree-dwelling herbivores), Tasmanian devils (ground-dwelling carnivores) and marsupial moles (dune-burrowing insectivores) have a common ancestor (Figure 10.12). However, they have quite different feeding structures that adapt them to different diets. These animals have evolved by divergent evolution.

Adaptive radiation

Adaptive radiation is a pattern of divergent evolution in which organisms rapidly diversify into many new forms. The fossil record shows that every mass extinction event was followed by adaptive radiation. Adaptive radiation occurs when environmental changes trigger the availability of new resources and environmental niches. A clear example of this can be found in Australia's fossil record, which indicates that

Figure 10.12 a Koalas, **b** Tasmanian devils and **c** marsupial moles evolved from a common ancestor that probably lived during the Eocene epoch. These are examples of divergent evolution.

during the Middle Miocene epoch (approximately 15 mya), dense tropical forests covered central Australia where the Simpson Desert is now.

Forests, lakes and permanent rivers provided a lush habitat for marsupials such as giant koala-like possums, shrewish insectivores and sheep-sized browsers. Flamingos, crocodiles, turtles and dolphins flourished in the waterways. The range of habitats allowed the extensive radiation of animal species that adapted to the available resources and which is an example of adaptive radiation.

Slowly, the tropical centre of Australia began to dry out during the Pliocene epoch (approximately 5 mya). This brought an end to the tropical habitat, which gave way to broad grasslands. Large browsing mammals called diprotodontids (Figure 10.13) and a variety of possums could not survive with the reduction of trees and the subsequent limited food available.

As the tropical forests retreated from central Australia, the animals they once supported were forced to compete for diminishing resources and became vulnerable to extinction. Remnants of these forests and their inhabitants are now confined to Papua New Guinea and pockets of northern Queensland. The grasslands that replaced the forests provided new habitats that allowed for adaptive radiation of other Australian mammals: the kangaroos and wallabies. A summary of the adaptive radiation of marsupials is shown in Figure 10.14.

Figure 10.13 The giant *Dprotodon optatum* was a type of megafauna that browsed on leaves.

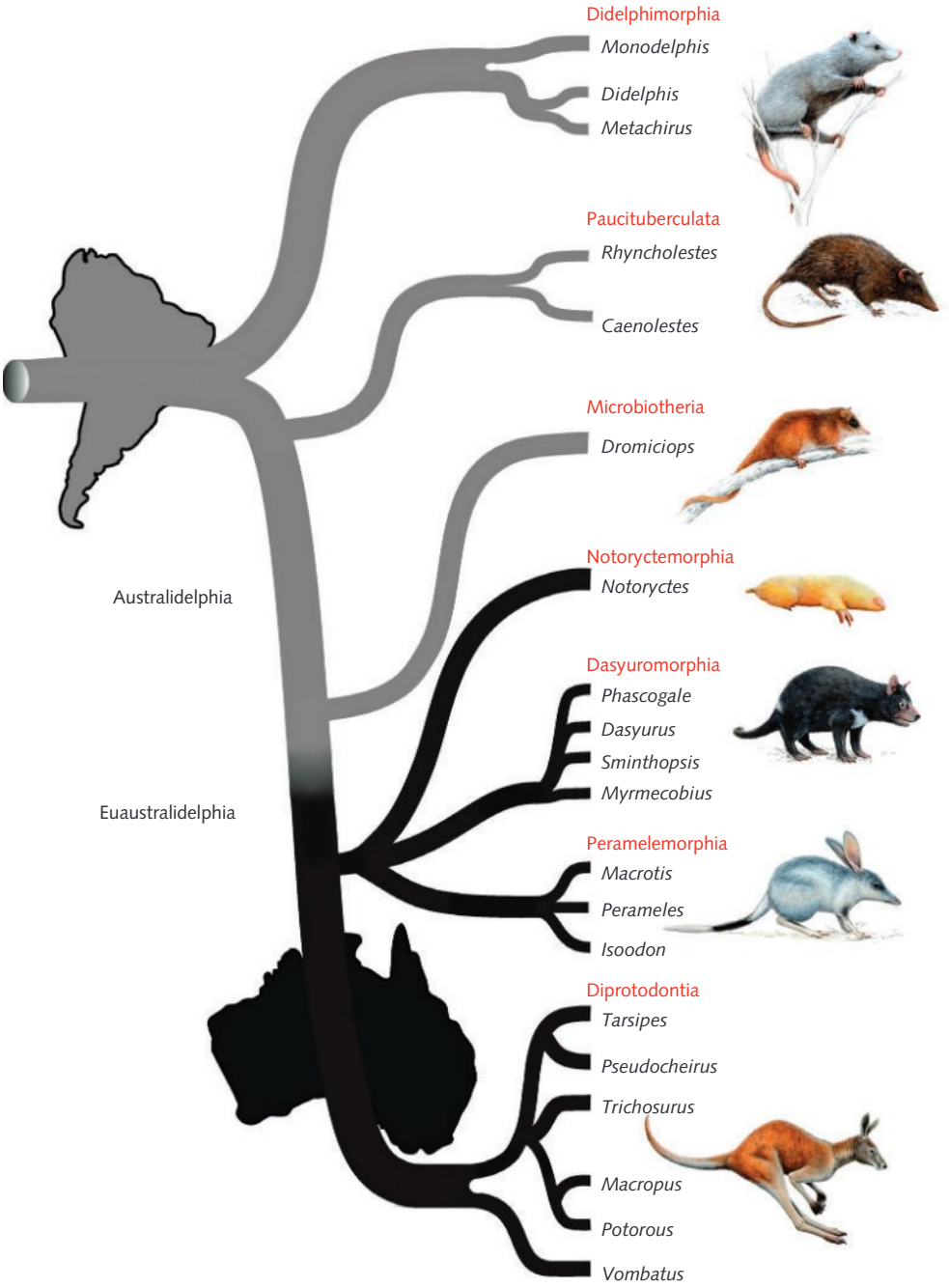


Figure 10.14 Adaptive radiation of marsupials began in South America, which was joined to Antarctica and Australia in the supercontinent Gondwana. The Australian continent detached and began drifting north about 45 mya. Most surviving marsupials are now restricted to the Australian continent.

WB
10.2.3 CONVERGENT EVOLUTION PAGE 212

Convergent evolution

Convergent evolution occurs when unrelated organisms evolve similar adaptations in response to similar selection pressures. Ant-eating mammals are an example of convergent evolution. Many animals eat ants and termites and have developed similar structures, but they are not closely related.

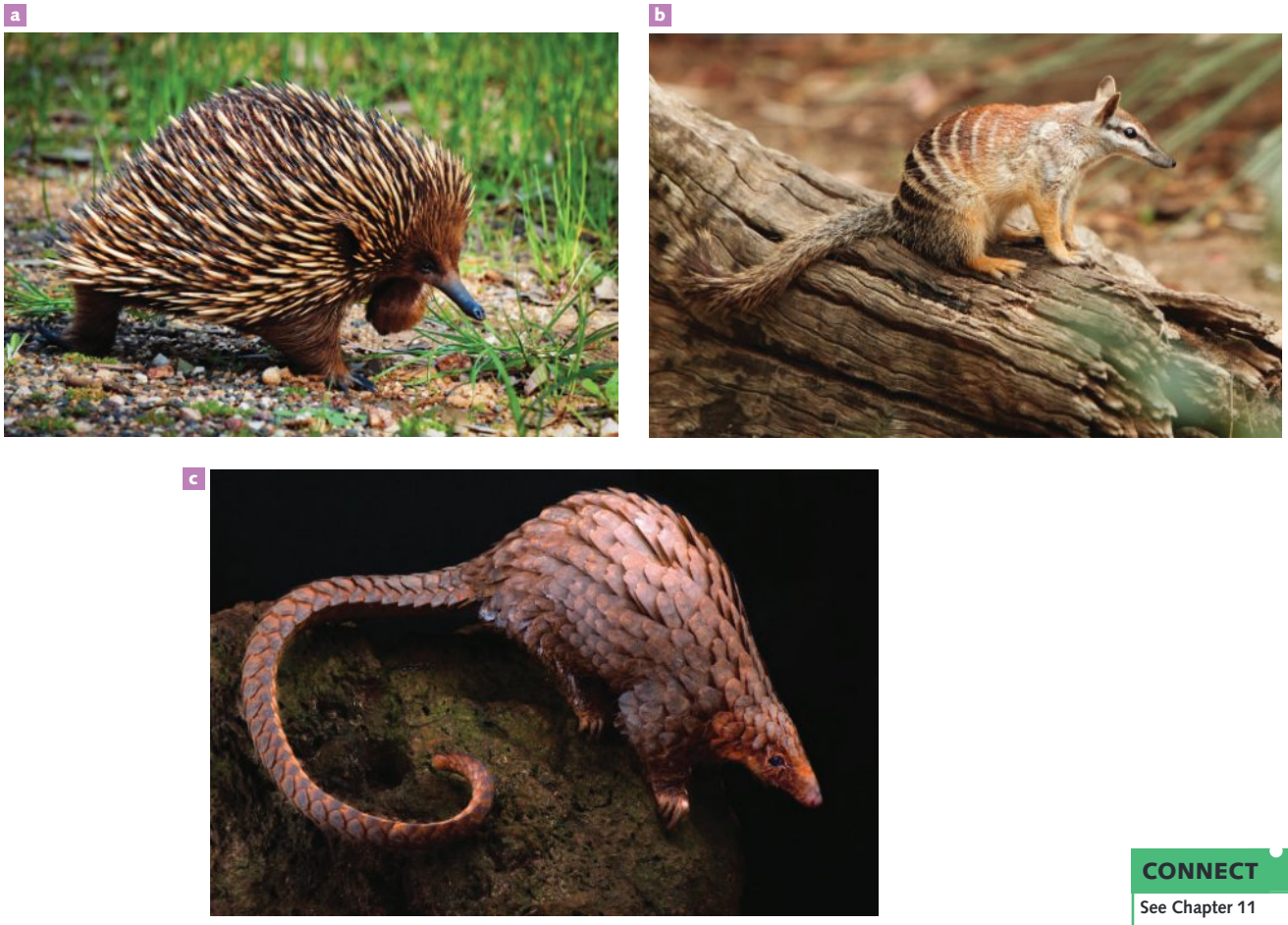


Figure 10.15 Ant-eating mammals, including **a** echidnas (monotremes), **b** numbats (marsupials) and **c** pangolins (placental mammals) show convergent evolution with ant-eating structures.

CONNECT

See Chapter 11 for more on monotremes, marsupials and placental mammals.

Modern ant-eating mammals include echidnas, which are monotremes; numbats, which are marsupials; and aardvarks and pangolins, which are placental mammals (Figure 10.15). All of these species have an elongated snout for smelling and digging, a long, extendible tongue that can extract ants from crevices, and powerful claws that can dig up ant and termite nests.

The different species of ant-eating mammals do not share a common ancestor. They have developed ant-eating habits independently and coincidentally under the same selection pressure to survive by sourcing ants for food.

KEY CONCEPTS

- The foil record demonstratesthat mass extinction events have occured many times in the past. After each mass etntio, acollectionof new species replaces theextinct ones.
- Tranitona fossis prvide evidecefor evolutionary reaioships between grous of organisms and document chnge i organismsover time.
- ivergentevolution occrs whendifferent selection pressures apply o differentppulations of a ancestral speces.The diffeen populations accumulate many changes, becomingnew pecieswih characteristics tha differ from those of he ncestral species. When tis occur on a lrgescle it i called adaptive radain.
- Converget volution occrs when unrelated organisms (or rgaisms with a very distant common ancesto) elve similar strctures or adaptations to performaimila fnction in response to the same slection presures.

Concept questions 10.2

1 How do strat provide evidence formas extinction events?
2 Explain what rnsiinal fossl is and how it shows evdence of the descen of a later grou of organisms from anarlier group o oganisms.
3 Give anexampleo a raitiona fossil, the ancestral and descendantgoupit 'transitions' between, and the features of thfossil that serve as evidence for the transiion.
4 Draw an annoated diagrm contrasting the patterns of dvergent and converget voution. Include ancestral and descendent pecies with arrows connecting the ancesralspecies to the descenent species.
5 From your nowledge ofhe fosl record, give an example o a ituation that eads t aaptive radiation of speces.

HOT Chaenge

6 a What type of eolutionmight es in the following groupings?
  i Dophin and whale
  ii Shark an whale
  iii Dg, fox nd wolf
  v Humingbird an humingbird moth
  b Ho is adaptie radiationofa species different fromdivergen eolution?
  c s the example of Dawin's Glapagos tortoises an example o adativeradiation or divergent evolution?

Figure 10.16 a The famous Galapagos tortoises are similar to **b** the much smaller Chaco tortoise (*Cheonods chenss*), found in South America.

10.3 Emergence of new species

The Galapagos Islands lie about 1000 km west of Ecuador (South America) in the Pacific Ocean. Charles Darwin drew inspiration for his proposal for the origin of species from observations and collections he made at the islands in 1835. During his famous voyage on the HMS *Beagle*, he realised that these islands were geologically quite young. They were teeming with life but the animals and plants on the islands were of recent origin. Many of these appeared to be related to similar species on the South American mainland but were also clearly different from them. One of the most famous groups of animals on the Galapagos Islands are the 15 or so species of giant tortoise, whose closest living relative, the Chaco tortoise, is found in mainland Argentina (Figure 10.16). Darwin wondered how the tortoises had got to the islands, and how there could be so many different species. He hypothesised that the tortoises on the Islands originally came from the mainland population but had changed over time to become better suited to the environment of the Galapagos in the process of speciation. How this occurred was a key aspect of Darwin's theory.

The species concept

Species can be defined in a variety of ways. In 1940, Ernst Mayer proposed that species are groups of actual, or potentially, interbreeding natural populations that are reproductively isolated from other such populations. This is called the **biological species concept**. According to this definition, individuals from different species are unable to produce viable offspring under natural conditions. The biological species concept is the most widely used in evolutionary biology. It relates directly to the concept of a species as a genetically isolated group, which can only breed within itself. In this sense, a species is represented by a totally isolated gene pool.

Sometimes, the only evidence that a species existed is its fossil. The **morphological species concept** identifies different species based on their physical characteristics but is limited to what can be observed in the fossil record. For example, kangaroos are well represented in the fossil record. Twenty-five million

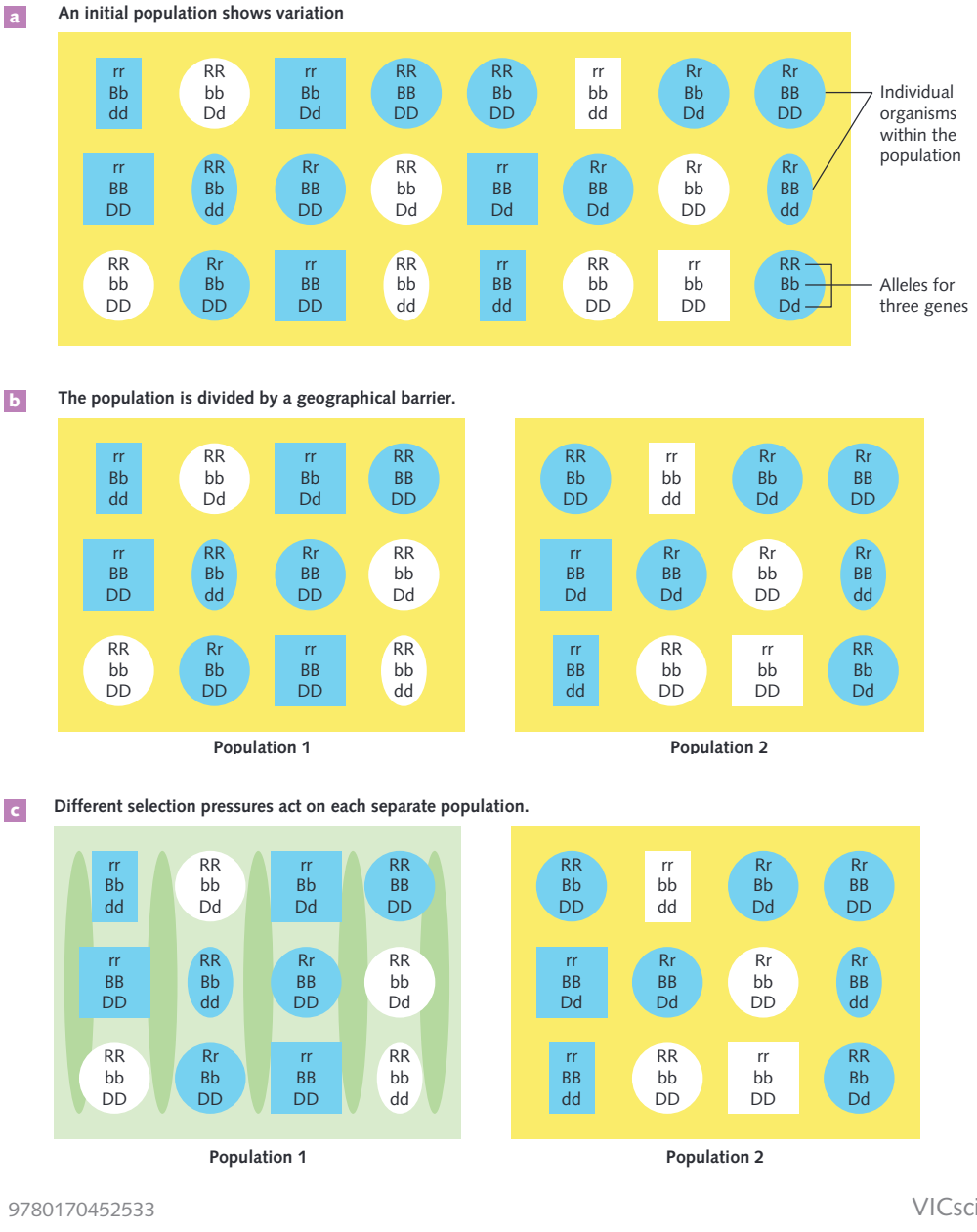
years ago the ancestors of modern kangaroos lived in rainforests and fed on fruit. Kangaroo species of today are connected to these distant ancestors through an unbroken line of descent.

Allopatric speciation

Allopatric (from the Greek *allo* meaning other, and *patric* meaning home) speciation is regarded as the most common mechanism by which new species emerge. **Allopatric speciation** occurs when members of an initial population become geographically separated and each isolated population develops into a new species. The mechanism builds on the principles of natural selection introduced in the previous chapter. The key stages of allopatric speciation are summarised in Figure 10.17 and are as follows:

1. The process commences with a population of individuals that contribute variation to the initial gene pool (Figure 10.17a).
2. The population becomes divided by a geographical barrier such as a river or a canyon. The geographical barrier prevents gene flow between the gene pools of the two isolated populations (Figure 10.17b).
3. Different selection pressures act on each isolated population, favouring different variants in each gene pool (Figure 10.17c).

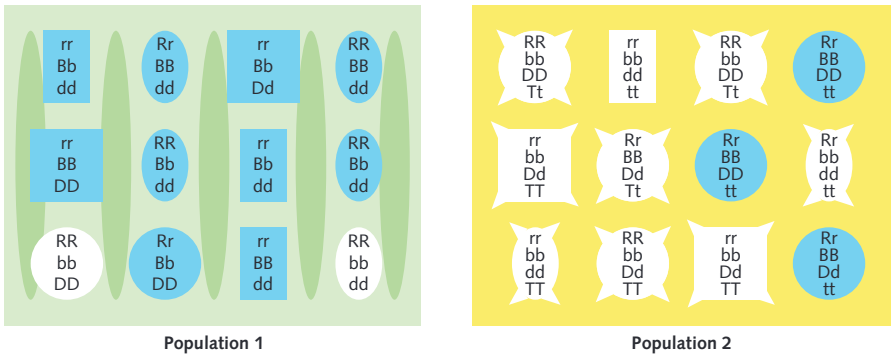
EXAM TIP
A common misconception is that evolution occurs because it benefits the organisms, as though it is intentional. More accurately, evolution occurs as a result of natural selection acting on the variation in a population.



4 Over time, point and block mutations accumulate independently in the gene pool of each isolated population. This implies that new genes, as well as new alleles, are appearing. The gene pool of each isolated population acquires unique mutations that are favoured by local selection pressures, some of which become fixed (Figure 10.17d and e). Note that once an allele is fixed, it no longer contributes variation to the population.

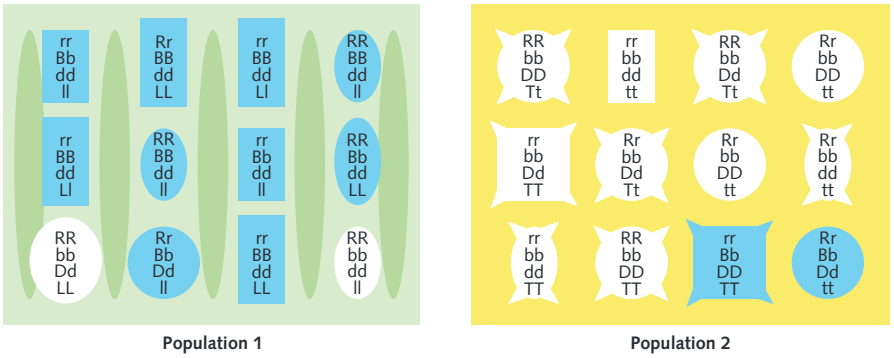
5 Eventually, after enough time has elapsed, the two isolated populations are distinctly different. Members of one population are genetically incompatible with those of the other so they are no longer capable of interbreeding, even if they encounter each other. Each population is now a distinct new species (Figure 10.17f).

d **New mutations accumulate independently in each separate population. A new gene for thorns has appeared in Population 2**



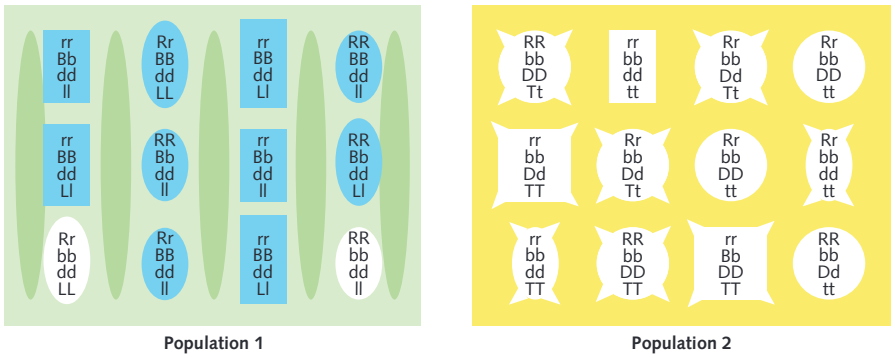
Population 1 Population 2

e **A new gene for size has appeared in Population 1.**



Population 1 Population 2

f **After sufficient time, each population has become a distinct species. The two species have different genes for different characteristics and are genetically incompatible with one another: members of Species 1 cannot interbreed with members of Species 2 and vice versa. Note that allele d has become fixed in Species 1, and allele b has become fixed in Species 2.**



Population 1 Population 2

CONNECT

See Chapter 9 for more on gene duplication and mutation.

Figure 10.17 a–f The stages of allopatric speciation. Alleles: R = round, r = square; B = blue, b = white; D = broad, d = narrow; T = thorns, t = no thorns; L = long, l = short.

Allopatric speciation and Galapagos finches

WB
10.3.1
ALLOPATRIC SPECIATION AND GALAPAGOS FINCHES
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The Galapagos finches are 14 songbird species native to the Galapagos and Cocos islands. Specimens were first collected by Charles Darwin, who was particularly fascinated by the birds' beaks. The beaks differ in size and shape depending on how the birds obtain their food (Figure 10.18). In 1845, reflecting on the finches' morphologies, Darwin wrote that it was as though 'one species had been taken and modified for different ends'.

Morphological and genetic evidence suggests the Galapagos finches are a closely related group of bird species. They are more closely related to each other than to any other bird species. The finches are found on the Galapagos and Cocos islands but nowhere else in the world. A couple of the finch species are located only on one or two islands but most species co-inhabit a number of different islands. Field observation over multiple generations shows the fundamental ingredients for speciation occur in the finch populations (Figure 10.19). Each population exhibits variations in beak characteristics, as well as other traits, and the traits are heritable. The genetic evidence suggests they share an evolutionary history extending to approximately 3 million years ago.

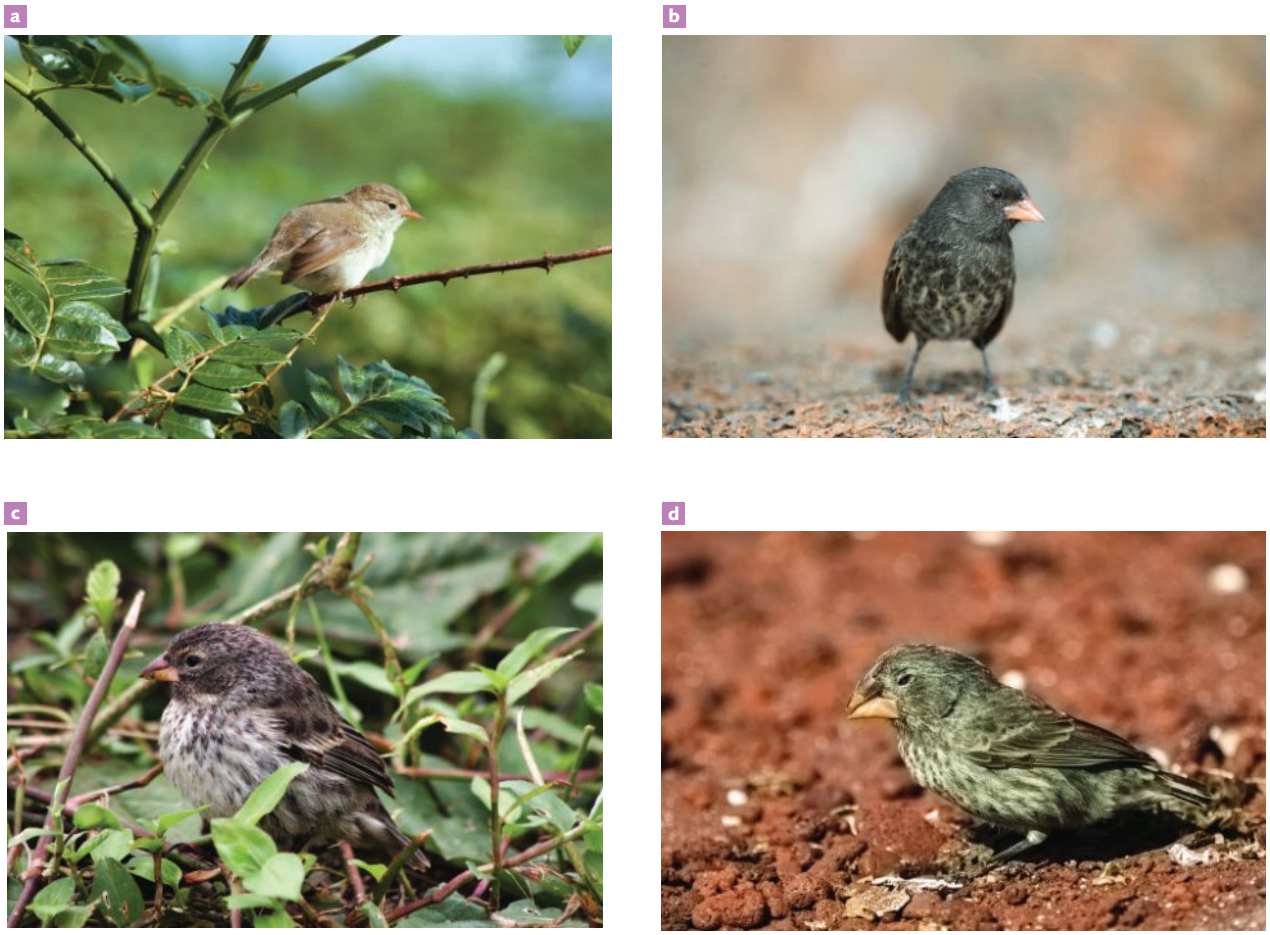

Figure 10.18 Galapagos finch species show variations in beak shape depending on the type of food they eat. **a** The green warbler finch (*Certhdea ovacea*) feeds mainly on small insects and spiders. **b** The sharp-beaked ground finch (*Geospiza df fics*) has a varied diet but feeds primarily on small seeds. **c** The vegetarian finch (*Patyspza crassrostrs*) feeds mainly on plant leaves, flowers and fruit. **d** The large ground finch (*Geospza magnrostrs*) has a varied diet that includes large woody seeds, cactus fruits and large insects.

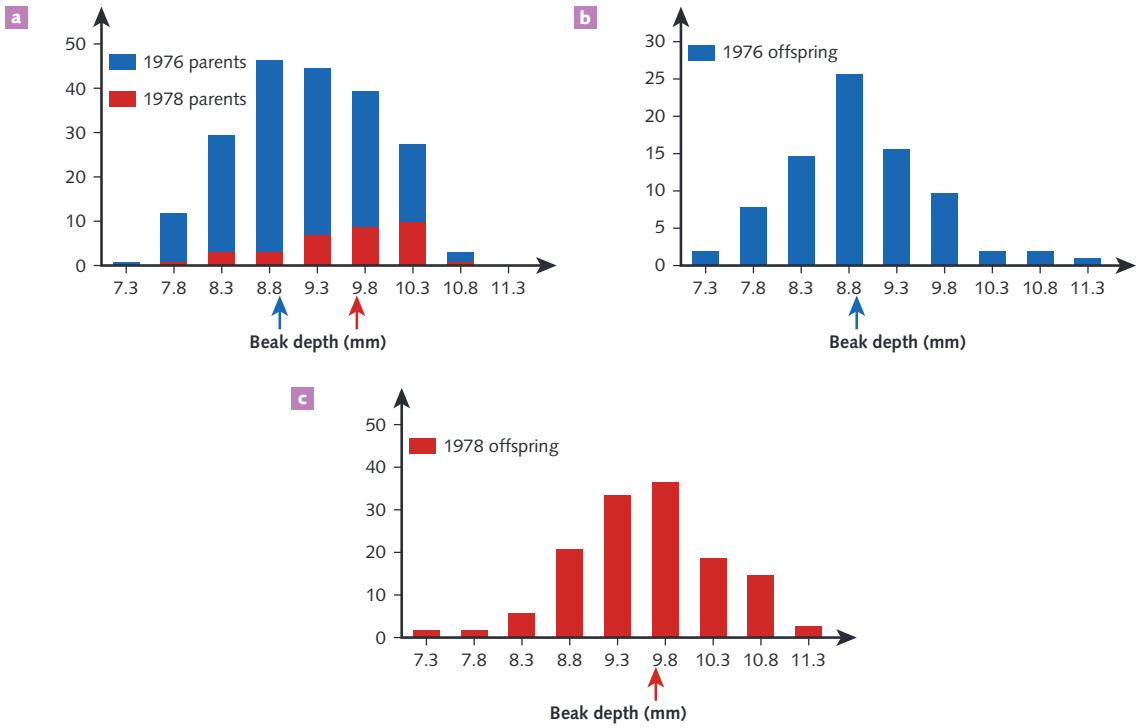

Figure 10.19 Evolutionary change in beak depth in the population of the medium ground finch (*Geospza forts*) on the Galapagos island of Daphne Major. **a** The distribution of beak depths in the breeding population in 1976 (blue) and 1978 (red). The 1978 breeding population comprises the survivors of a drought that affected the island in 1977. Large ground seeds were the main food source available during the drought. The difference between the means, indicated by the arrows, is a measure of the strength of natural selection. **b** The distributions of beak depths of fully grown offspring hatched from the 1976 parents. **c** The distributions of beak depths of fully grown offspring hatched from the 1978 parents. Evolutionary change between generations is measured by the difference in mean between the 1976 offspring before the effect of the selection pressure and the 1978 offspring afterwards.

The Galapagos Islands are 18 main islands and countless small islands and rocky outcrops spread over an area of 45 000 km², about 1000 km west of Ecuador (Figure 10.20). They are a geologically recent volcanic island chain (archipelago) that emerged between approximately 4.2 and 0.7 mya, although the submerged portions of the islands are millions of years older. Cocos Island is a separate volcanic island formed approximately 2 mya about 840 km north of the Galapagos Islands. The global and local climate changed continuously throughout the islands' formation up to the present. These include cycles between glacial and interglacial periods every 100 000 years. Glacial periods were associated with lower sea levels so the area and distance between islands changed throughout the archipelago's history. Ongoing volcanism combined with climatic variations probably resulted in dynamic variations in the vegetation and food types available across the archipelago.

Figure 10.20 The location and arrangement of the Galapagos Islands

Evolution of Galapagos finches

The most straightforward interpretation of these observations is that the Galapagos finches evolved by allopatric speciation. The scenario implies that the ancestral finches arrived from the South American mainland possibly approximately 3 mya at a time when the Galapagos archipelago consisted of five or six islands. The founder population probably entered an environment that was warmer and wetter than at present. The islands were already colonised by plants and insects and were probably covered in rainforest. The founding finches were likely to be generalist feeders of insects, flower nectar and pollen.

Conditions did not remain static. Continuing volcanism, changing climate, and differing patterns of colonisation by plants and insects gradually created new islands with new environments. In time, members of the original finch population dispersed to settle new island habitats. Competition among individuals for the available food presumably exerted selection pressures on resettled finch populations. One of the habitats occupied by resettled finches would have been relatively dry and dominated by bushland. Individuals with sharp beaks were better able to manipulate and crush the small nutritious seeds that fell to the ground. These finches had the best chance of surviving and reproducing, and they passed their alleles for sharp beaks on to their progeny. The finches with the least suitably shaped beaks for feeding on such seeds were more prone to die without leaving offspring. Over many generations, individuals in the population predominantly had sharp beaks. Together with the effects of additional new mutations, alleles that had become fixed by genetic drift and isolation from gene flow, this finch population diverged to become a species distinct from other populations.

Similar selection pressures probably occurred in other island habitats (Figure 10.21). One finch population may have occupied a dry habitat dominated by *Opuntia* cactus. This environment favoured individuals with long slender beaks better able to probe cactus flowers for pollen and nectar. Another population may have occupied a moist upland forest dominated by trees in which individuals with strong large beaks may have had an advantage. These finches were capable of tearing bark from the trees to expose insects upon which they could feed.

Figure 10.21 Examples of different environments in the Galapagos Islands: **a** bushland on Isabella Island; **b** dry landscape dominated by *Opunta* cactus on Santa Cruz Island; **c** lush forest on the highlands of Santa Cruz Island.

This model of allopatric speciation assumes that geographically separated finch populations adapted under natural selection to local conditions and gradually evolved in isolation into separate species. Allopatric speciation helps us to understand the evolution of Galapagos finches but it is sometimes inadequate to explain other cases of speciation.

Sympatric speciation and *Howea* palms

Lord Howe Island is a remote island in the Tasman Sea located 580 km from the east coast of Australia, which is the island's nearest land mass (Figure 10.22a). It was formed by volcanic activity between 6.4 and 6.9 million years ago. Steadily eroded by oceanic forces, it is now only a minute fraction of its original size and it is likely to become submerged in the next few hundred thousand years.

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10.3.2 SYMPATRIC SPECIATION AND *HOWEA* PALMS PAGE 217

Figure 10.22 a The location of Lord Howe Island. **b** Curly palm (*Howea bemoreana*) and **c** Kentia palm (*Howea forstriana*).

There are approximately 240 plant species on Lord Howe Island, almost half of which are endemic to the island. Among them are four palm species, including two closely related endemic species, *Howea belmoreana* (the curly palm) and *Howea forsteriana* (the kentia palm) (Figure 10.22b and c). The kentia palm was first brought to Europe and propagated more than 150 years ago and it has become one of the world's most popular commercial palm species. Genetic studies suggest the two *Howea* species diverged from their common ancestor about 1 mya, implying the speciation occurred on Lord Howe Island. However, both palms are wind pollinators often found growing near one another, and the island is so small (about 12 km^2) that allopatric speciation on the island is practically impossible.

The two *Howea* palms have evolved through a process of **sympatric speciation** (from the Greek *sym* meaning same, and *patric* meaning home). This occurs when two species evolve from an ancestral population while still inhabiting the same geographical area. Mechanisms other than geographical isolation must be at work for sympatric speciation to occur. Some members of the population must become **reproductively isolated** from all the others so that sexual reproduction can no longer occur freely in the population. Each individual is able to reproduce with only a limited number of other individuals in the population. The two reproductively isolated populations thus form separate gene pools that independently accumulate unique mutations over many generations, eventually becoming separate species.

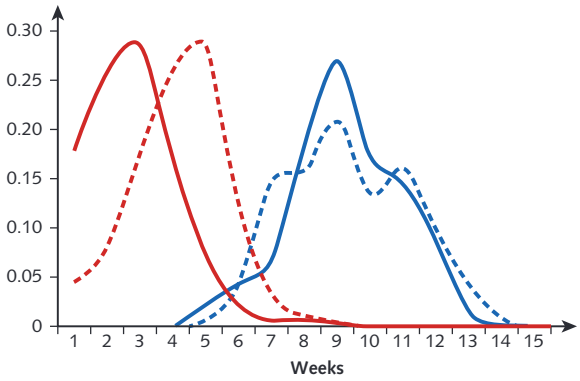

Figure 10.23 The differing flowering times of the two *Howea* species. The blue lines represent 198 curly palms (*Howea bemoreana*) and the red lines represent 177 kentia palms (*Howea forsterana*) showing female (dashed) and male (solid) flowering phases.

Reproductive isolation between the two palm species came about because of a difference in the timing of their flowering (Figure 10.23). Kentia and curly palms have both female and male flowers but the kentia palms flower about 6 weeks before the curly palms. Kentia palms are also androgenic, which means the male flowers open before the female flowers (Figure 10.23). Flowering timing ensures that kentia palms reproduce mainly with other kentia palms, and curly palms reproduce mainly with other curly palms. Temporal reproductive isolation rather than geographical separation was the mechanism that resulted in two *Howea* species.

Evolution of the *Howea* species

It is reasonable to ask what caused the difference in flower timing within the original population of *Howea* palms. Most of the curly palms grow on the fertile, relatively acidic volcanic soils located throughout the island.

By contrast, the kentia palms tend to grow on the more alkaline and nutrient-poor calcareous soils on the lowlands of the island. Geological evidence suggests the calcareous deposits began accumulating about 1 mya, probably with the emergence of the island's coral reefs. As the coral weathered, the chalky calcium carbonate washed up and accumulated in pockets throughout the island's lowlands. The timing of these geological events coincides with the estimated speciation of the *Howea* palms about 1 mya based on genetic evidence.

Putting together the geological, physiological and genetic evidence suggests the following evolutionary sequence. The ancestor of the *Howea* palms arrived on Lord Howe Island probably about 5 mya. These *Howea* palms grew on volcanic soils throughout the island. Around 1 mya, coral reefs developed, leading to the accumulation of calcareous deposits on the island. *Howea* palms growing on those deposits purely by chance were physiologically stressed. As a stress response, these palms flowered earlier than usual. Under the circumstances, the earlier flowering *Howea* palms were able to reproduce with each other but not with the *Howea* palms growing on the volcanic soils. After the flowering season was over for the *Howea* palms growing on calcareous soils, flowering commenced for the *Howea* palms growing on volcanic soils. These palms could reproduce with one another but were too late to reproduce with the palms growing on the calcareous soils. This pattern repeated over many years and led to eventual speciation of the two *Howea* palms.

It has also been observed that curly palms are less tolerant of calcareous soils, while kentia palms grown on nutrient-rich volcanic soils flower at about the same time as curly palms. Some first and second generation hybrids of the two *Howea* palm species have been identified but they are rare and it remains uncertain whether they are fertile beyond the second or third generation.

KEY CONCEPTS

- llopatricspeciation is the rocess by which new species diverge from members f an ancestral species that have become grahiadly for long periods.
- Theevolution of Galapags and Cocos Island finches s an exampl ofalloaric speciation.
- The eolution of *Howea* palmsis an exmple of sympatri seiation in whch new species emerge from an eistig popaionwile inhabiting the same geographicl area.

Concept questions 10.3

1 Define speition'.
2 Constructa tab summarisingthe fundamental stages oflloatrc pciation.
3 Describ the difference bwen the biological and the morhlgial species concepts.
4 What slection pressures acted on Galapagos and Coco sland finches and what effect did they have on the morhology of the finches?
5 What is meant by rerodutive isoation' and how did tis ocur in the peciation of *Howea* palms?

HOT Chaenge

6 Forty per cent of e world's pecies of fruit fly are found on theislands of thHawaian rchipelago.
   a Propose why th Hawaiin archipelago might proide a uitabl habitat forso many different species o fruit fles
   b Explain how daptv radiation may have been nvolved in th evolutn ofHawaiian fruit fles
   c Describe three ways hat ancestral fruit fly genes may have been transportedfrom one island to anoter.

10.4 Determining the relatedness of species

The fossil record demonstrates how older ancestral organisms have evolved into later descendant forms. This implies many species today are related by lines of descent, rather like a branching tree, as first proposed by Darwin. The fossil record for many modern organisms is scant, or even absent, and evaluating the evolutionary relationships between them requires other lines of evidence; for example, anatomical, physiological and molecular evidence that can be observed directly from the modern organisms.

Weolink
Evidence for evolution

Onne Wyrkm heet
Evidence for evolution

Classifying relatedness

Biologists have traditionally used a variety of features to categorise organisms. The goal is to group organisms according to the degree to which they share similarities. This is the basis of **taxonomy**, the system of scientific conventions for naming and classifying organisms. Organisms are classified in a hierarchy from phylum (the broadest), class, order and family through to genus and species. The term 'division' in plant taxonomy is equivalent to phylum in animal taxonomy. The scientific name of an organism is derived from its genus and species name.

In modern biology, taxonomy serves as a tool to describe hypotheses about the **phylogeny**, or evolutionary relatedness of organisms. If two species are classified in the same genus (grey wolf and coyote) we are hypothesising that they are more closely related to each other than either is to a third species (puma) that is classified in a different genus. By classifying organisms in taxonomic schemes, we are recognising that organisms that are more closely related have more features in common. Some examples are shown in Table 10.2.

Table 10.2 Taxonomic classification of five predators

Common name	Scientific name	Phylum	Class	Order	Family	Genus	Species
Great white shark	*Carcharodon carcharias*	Chordata	Chondrichthyes	Lamniformes	Lamnidae	*Carcharodon*	*carcharias*
Killer whale	*Orcinus orca*	Chordata	Mammalia	Cetacea	Delphinidae	*Orcinus*	*orca*
Puma	*Puma concolor*	Chordata	Mammalia	Carnivora	Felidae	*Puma*	*concolor*
Coyote	*Canis latrans*	Chordata	Mammalia	Carnivora	Canidae	*Canis*	*latrans*
Grey wolf	*Canis lupis*	Chordata	Mammalia	Carnivora	Canidae	*Canis*	*lupis*

As you navigate through the taxonomic ranks from phylum (or division) to species, you are proceeding from the ancestors towards more closely related descendants. Essentially, the taxonomy provides a guide to the progress of evolution over time. The higher ranks, such as phylum or class, represent very ancient divergences, whereas the lower ones, such as genus and species, represent relatively recent divergences between organisms.

Structural morphology

Some species can appear very different, with very few obvious similarities to each other, while others appear so similar that their shared common ancestor must have existed relatively recently. Closer examination of the physical structure and form of species, that is their **structural morphology**, at both the embryonic and adult stages reveals further evidence for evolution.

Embryology

Structural morphology is used to establish evolutionary relationships on the basis of structural similarities and differences, including the comparative study of embryos. For example, all members of the phylum Chordata (or 'vertebrates') have, at some stage of their development, a dorsal notochord (a cartilaginous rod running along the back), pharyngeal slits (which turn into gill slits in fish), a dorsal nerve chord and a tail that extends past the anus. Sea squirts are the most unlikely members of this phylum; the adults look more like marine invertebrates than the more closely related vertebrates (Figure 10.24a). However, sea squirt larvae have all the chordate characteristics, including a notochord (Figure 10.24b). Vertebrates have lost the notochord and it is replaced with vertebrae.

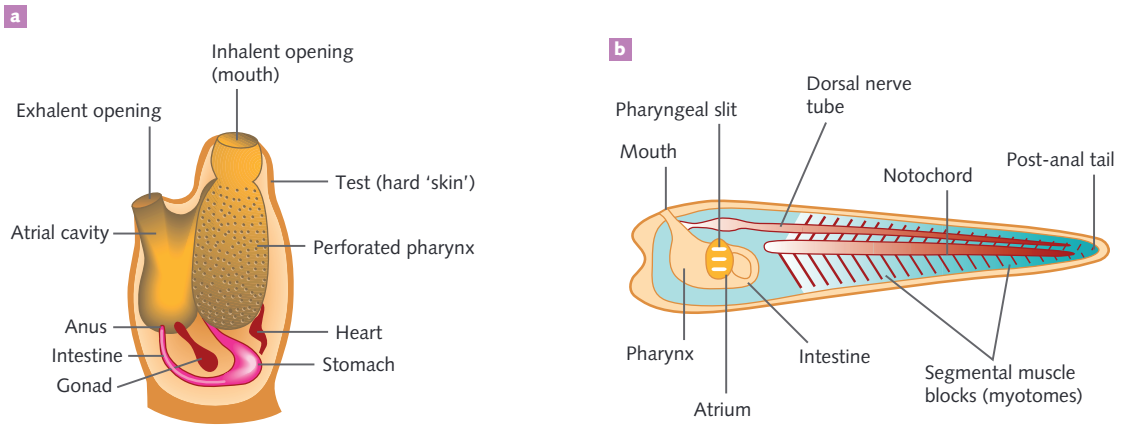

Figure 10.24 a Adult sea squirts show few characteristics of chordates. **b** The free-swimming larvae of sea squirts show the characteristic features of chordates, revealing sea squirts' evolutionary affinity with chordates.

The similarities between embryos of fish, humans and many other organisms suggest a shared ancestor from which all these species have eolved (Figure 10.25). No theory other than evolution can adequately explain why the same structures occurin all chordate embros, whose adult forms are so diverse.

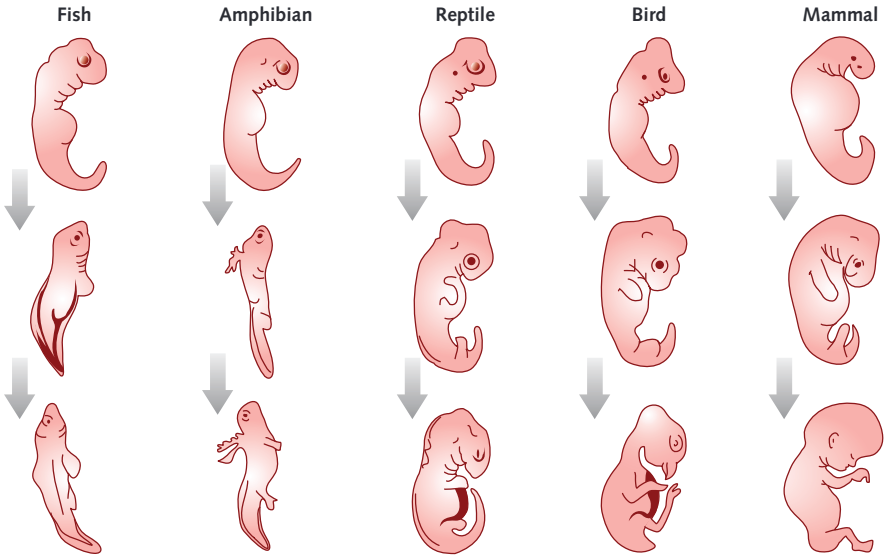

Figure 10.25 Similarities between chordate embryos suggest a common ancestor.

Homologous structures

Homologous structures are common anatomical structures shared by different organisms that stem from their descent from a common evolutionary ancestor. When an adaptive radiation occurs, organisms retain the same basic structures because they have the same genetic history. For example, all lizards have scaly skin; this is a defining characteristic of their classification. However, the scales can differ in colour, hardness and shape depending on the habitat that they occupy, and may function in defence, temperature maintenance or camouflage. The different types of scales are examples of homologous structures.

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Some homologous structures have evolved very different functions. The wing of a bird, the wing of a bat, the leg of a crocodile, the flipper of a whale and the arm of a human all have different functions and appear superficially different. However, they all have the same basic skeletal structure: the pentadactyl limb, a hand or foot with five fingers or toes (Figure 10.26). In each species the limb has become modified to suit the organism's way of life, demonstrated by the different bone lengths and coverings of the limbs. For example, as birds evolved and their forelimb became adapted for flight, the bones of the hand reduced to three fingers. The fact that the bone structures of all these organisms have the same pentadactyl pattern implies they all descended from the same evolutionary ancestor, which also had the pentadactyl limb.

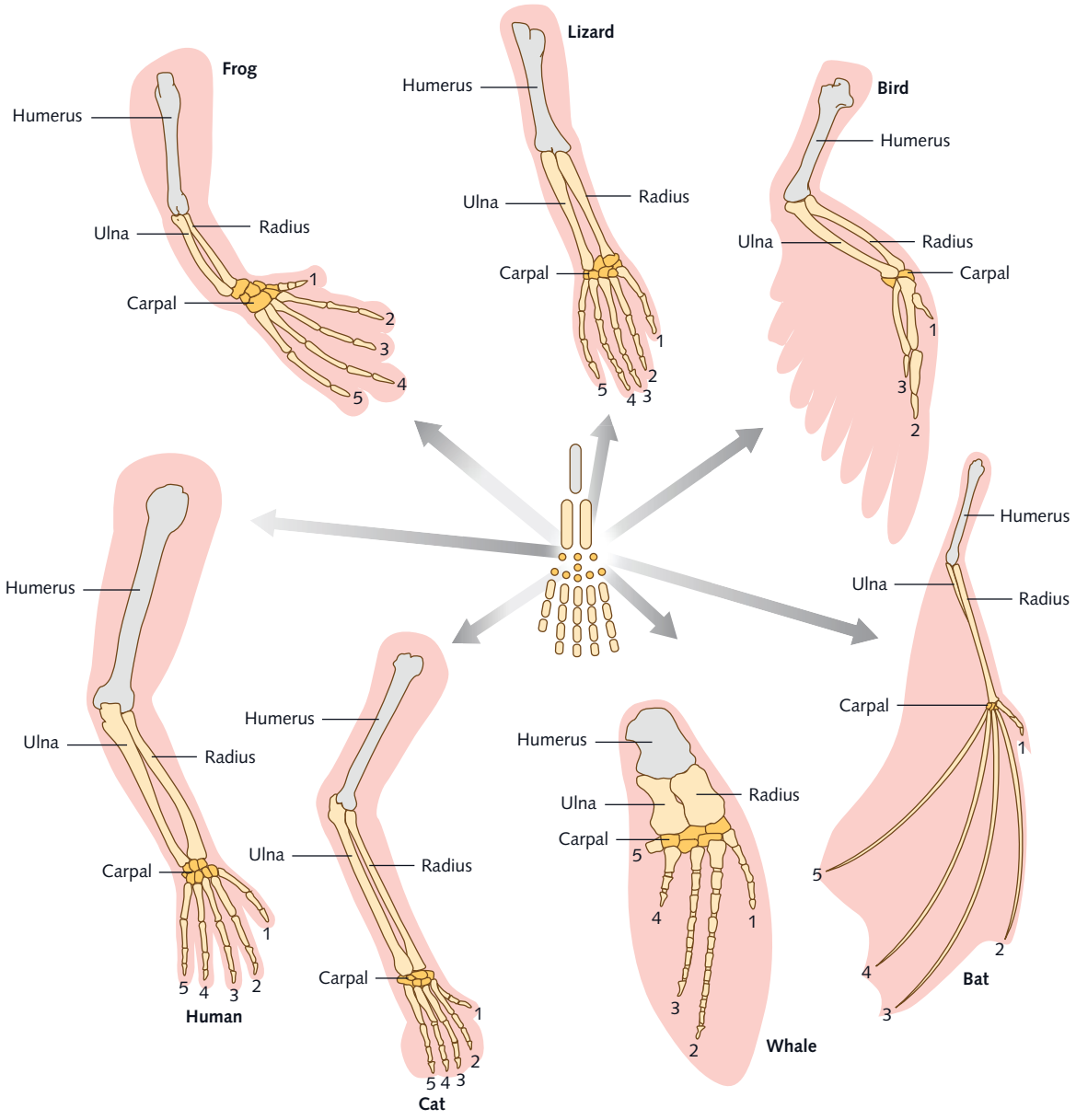

Figure 10.26 The principle of homologous structures can be illustrated by the adaptive radiation of the forelimb of a selection of vertebrates, which all show the basic pentadactyl pattern modified for different uses.

The leaves of land and aquatic plants all have the same basic components, but the structure shows enormous variety in size, shape, colour and function. Some leaves function as coloured petals, some as support structures in buds and others as defensive spines or fleshy water stores (Figure 10.27).

Figure 10.27 Homologous structures derived from leaves. a The spines of a cactus and b the coloured bracts that enclose the flowers of *Hecona* are derived from the same basic leaf structure but now have different forms. In this case, they are homologous structures but serve different functions. In other examples, homologous structures can share functions, but different environments can influence how these functions are necessarily performed.

Homologous structures can be used to infer phylogenetic (i.e. evolutionary) relationships because only organisms with a common ancestor can have the structures with the same fundamental arrangement.

Vestigial homologous structures

In some cases, homologous structures stemming from a common descent can eventually cease to provide a functional use for an organism; the structure may not necessarily impede a particular adaptation of an organism, but at the same time the structure no longer serves a 'useful' purpose. These structures are called **vestigial structures**. Vestigial structures can take a variety of forms, including skeletal structures on vertebrates, soft tissue such as organs, or even features at the cellular and molecular levels.

Wherever vestigial structures may be found, they are usually either rudimentary or atrophied. Vestigial structures are quite common and are yet another line of evidence that points to shared ancestry. Among humans, features that are thought to be vestigial include the coccyx (tailbone), the muscles that allow some people to wiggle their ears, and the palmar grasp reflex that causes babies to grip tightly onto something placed in the palm of their hand. Ostriches still have small wings even though they cannot fly and some cave animals have remnant eyes and optic nerves even though there is no light in a cave and they cannot see.

Analogous structures

Analogous structures are features of organisms that have the same function but not the same basic structure. The eyes of octopuses and vertebrates are remarkably similar, even down to fine points of detail,

and an observer could conclude that they are homologous structures (Figure 10.28). However, there is one important difference. In the vertebrate eye, the nerve fibres lie in front of the sensory cells of the retina, whereas in the octopus eye they lie behind them. Because of this, the vertebrate eye has a blind spot where the optic nerve emerges from it, whereas the octopus eye has no blind spot. The reason for this difference lies in the ways the two eyes developed, which indicates that they are the products of two distinct lines of convergent evolution. The same selection pressures probably resulted in the evolution of similar eyes in such unrelated organisms.

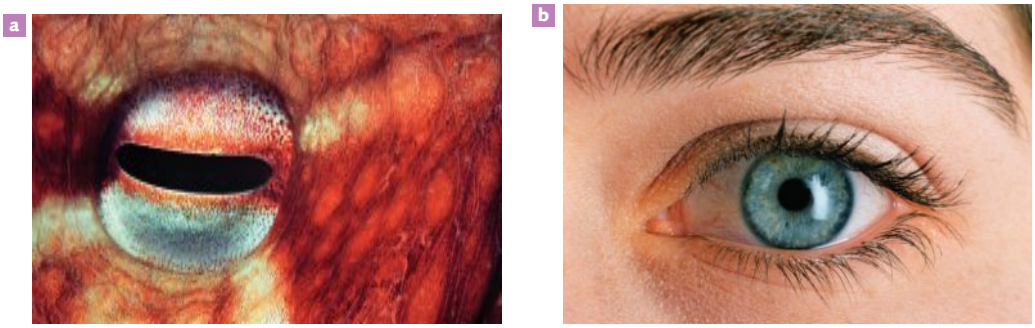

Figure 10.28 a Octopus eyes and b human eyes are the solution to the same problem with similar adaptations.

KEY CONCEPTS

» Taxonmy is naming system that categorises organisms based on hypothes s about evolutionary reaionhis.
» Compring the development and anatomy of organisms can rovde evidence tat organisms evlved from a common ncestor.
» Hoologous strutures evolved from the same ancestral form bu have different foms or functions due to dfferent selction pressures.
» Aalogous strucures evolved uner he same selection pressures fromdifferent ancestral forms so they have a common fuction but show some undamental differences.

Concept questions 10.4

1 Wich two species remore closely related among *Solenopsis fugax* *Carebara castania* and *Solenopsis invicta*? How can you deuce this romtheir scienti fic names alone?
2 Why does ebryolgyprovide evidence for a shared common ancesto of all chrdate organisms?
3 Name twohomologous structues and two analogous strucures.
4 n a group of species that arose from a common ancestor trough dierent volution, would you expect to se homologous structures or analogous structures?
5 The pentaaty limb is a key tructural piece of evdence used whe cassifin organsms into taxa.
   a Wich goupings f organisms demonstrate a pentadayl limb?
   b Wh is the penactl limb eviene for evolution?
   c s the pentadcyl limb n analogous structure or a homologous structure?
   d How do pentaatyllimb differnces relate to dfferent funtion?

HOT Chaenge

6 Fsh are thought to be the ancestors of terrestrial aials. The penactyl imb exists in terrestrial aials but nt in modern fish Why do yo thnk this might be?

10.5 Molecular evidence for relatedness of species

Almost as soon as the amino acid sequences of proteins were being determined in the 1960s, it became clear that proteins could be used to infer evolutionary relatedness. For any given protein, the numbers of amino acids that differ between a pair of species provided a guide for the **evolutionary distance** between the two species. For example, the mitochondrial protein cytochrome c is a vital protein in the electron transport chain of cellular respiration. The cytochrome c sequences have been determined for the species shown in Figure 10.29. Compared with the human, the number of differences increases in order from the rhesus monkey (another primate), to the rabbit (another mammal), the rattlesnake (a reptile), the bullfrog (an amphibian), the fruit fly (an insect) and wheat (a plant). Interpreted this way, the number of amino acid differences in the cytochrome c sequence reflects the evolutionary distance between humans and each of the other organisms. Humans are most closely related to rhesus monkeys and least related to wheat.

CONNECT
The Electron Transport Chain of cellular respiration was discussed on page 144.

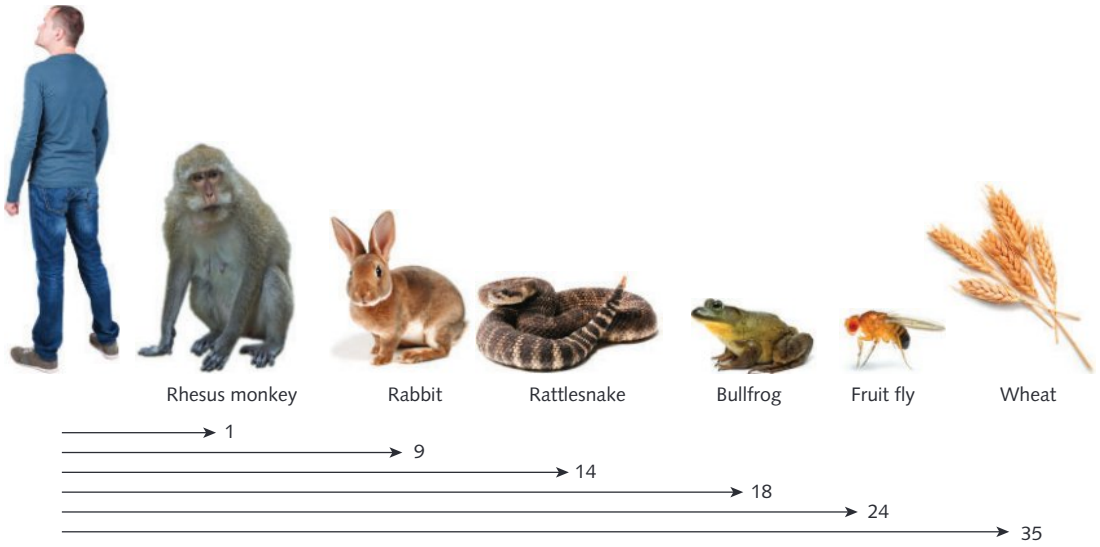

Figure 10.29 The number of amino acid differences between the cytochrome c of humans and that of other organisms.

The concept of molecular homology

In the study of structural morphology, 'homology' refers to the similarity in patterns of anatomical structures between different organisms. The more similar the structural pattern, the closer the evolutionary relationship between the organisms. Molecular biologists have applied the concept of homology to molecules. The term **molecular homology** refers to the similarity of patterns in the nucleotide sequences of DNA or the amino acid sequences of polypeptides from different organisms as evidence for a common evolutionary origin. Genes or polypeptides from different species that exhibit molecular homology are described as **homologous**.

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10.5.1 THE CONCEPT OF MOLECULAR HOMOLOGY PAGE 221

CONNECT
See Chapter 2 for more on the transcription and translation of genes into polypeptides.

DNA and proteins suit studies of molecular homology

There are two key reasons why DNA and proteins are suited to the study of evolution.

The first reason relates to their structure. DNA and proteins are unbranched polymers. They are very long, linear molecules composed of a limited number of possible building blocks. DNA is made up of four possible nucleotides in different orders. Proteins consist of polypeptides, which are made up of the

20 amino acids in different orders. This makes it straightforward to align and compare the sequences of DNA or proteins from different organisms. Similarities in sequences can be identified readily, and differences, corresponding to changes over time, can be easily calculated.

The second reason relates to technological developments in the field of molecular biology, which reflect advances in both the efficiency of chemical analysis of the molecules and the speed and power of computer processing. One outcome of this is automated, high-throughput sequencing to generate large amounts of DNA sequence data. Complementing this is the enhanced capacity to manage and explore the large amounts of data produced. A relatively new branch of science called **bioinformatics** has emerged from the application of computer science to storing, retrieving and analysing large volumes of biological data. Bioinformatics is an interdisciplinary field combining mathematics, computer science, engineering, chemistry and biology. There are many different aspects to the field of bioinformatics, one of which is that it can be used to determine how closely related different species are.

A model to explain molecular homology

Figure 10.30 illustrates how speciation results in sequence variation between species. Originally (5 mya) there was a single interbreeding population of skinks. Assume there is a gene with a nucleotide sequence that is characteristic for the population. In reality, there may be very few individual variations (alleles) in this gene sequence among members of the population, but it is broadly representative of the whole population. After some geological time, the original population has separated to become two reproductively isolated populations (Figure 10.30). The separation may be because a geographical barrier has formed to divide the original population, or because of physiological or behavioural differences that segregate members of the original population.

The example illustrates how a single ancestral species diverges, or splits, to eventually become two descendant species. Two **lineages**, or two populations that represent separate lines of descent, emerge from the point in time when the split occurs. Over time, the descendant lineages evolve different morphological, physiological and behavioural features and become recognisably distinct from one another as separate species. It is possible to infer speciation events that have occurred in the past by comparing DNA sequences of different species that are alive today.

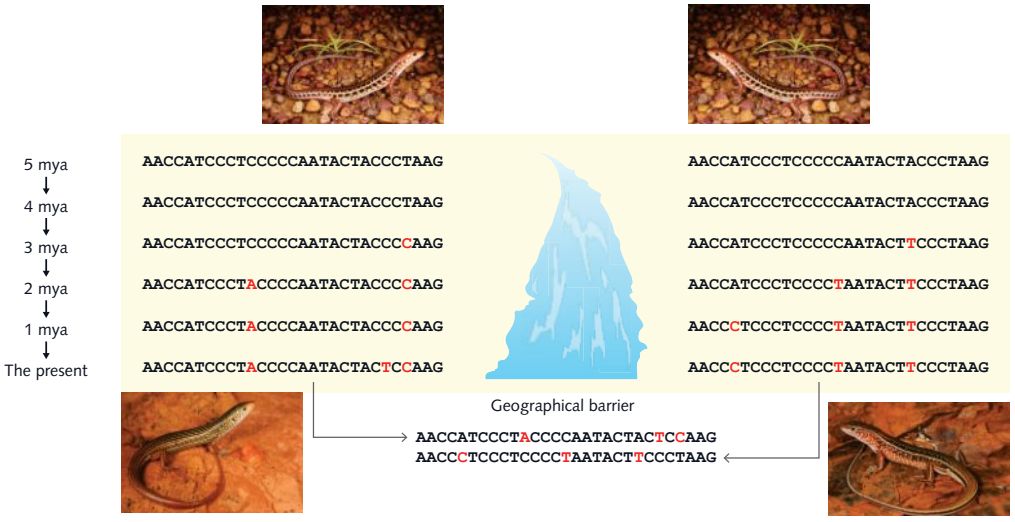


Figure 10.30 **A representative DNA sequence in a population of skinks from 5 mya to the present. After the original population becomes divided by a geographical barrier (such as a body of water), mutations accumulate independently in the DNA sequences of each isolated population over time. Today, the two independent populations are separate species. The representative DNA sequences of the two species are aligned to show their homology (similarities in the sequence). The differences between the two sequences provide a measure of the evolutionary distance between the species.**

Sequence alignments as a tool for investigating molecular homology

The DNA sequences in the present-day skink species illustrated in Figure 10.30 are homologous. They are related to each other by descent from a common ancestral DNA sequence. If we make a **sequence alignment** of those sequences today we can identify the original pattern in the gene by the similarities (homology) in the nucleotide sequences (Figure 10.30). Comparing this alignment with the mechanism depicted in Figure 10.30 highlights three key observations.

1. The nucleotides that are identical in both sequences are presumably ancestral. That is, these nucleotides were in the gene of their common ancestor and both descendant species inherited those nucleotides. These nucleotides are conserved nucleotides. They were retained during the course of evolution in the sequences of the descendant species.
2. The number of differences between the gene sequences of the two species – the independent mutations in each sequence – provide a measure of how related the two species are. This is the measure of evolutionary distance. Generally, for a given gene, the greater the differences in the sequence, the less related the two species are.
3. The degree of difference between the sequences indicates the time of divergence from the last common ancestor. Generally, for a given gene, the more differences there are between the two sequences, the more time has elapsed since the species last shared a common ancestor.

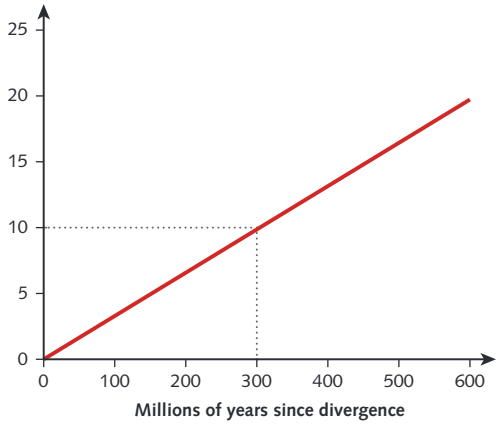


Figure 10.31 The rate of the molecular clock for cytochrome c. The dotted lines show how the number of amino acid differences in the sequence from two organisms (in this case, 10 per 100 residues) can be used to estimate the time since they diverged from a common ancestor (approximately 300 mya).

The last point explains the concept behind the **molecular clock**. The rate of the molecular clock describes the number of point mutations that occur in a specified polypeptide or gene over a defined period of time (Figure 10.31). In principle, the 'ticking' of the molecular clock is used to estimate the time since two species diverged from their last common ancestor. The rate of the molecular clock varies for different genes and proteins and must be calibrated against the fossil record.

Mitochondrial DNA

The mitochondrial genome is contained entirely on a double-stranded, circular chromosome (Figure 10.32) and possesses a suite of genes for many of the proteins involved in cellular respiration. So it can express these genes inside the organelle, the mitochondrial genome also has genes for ribosomal RNA (rRNA) and transfer RNA (tRNA) molecules. Polypeptide synthesis is achieved by ribosomes within the mitochondrion.

Mitochondrial DNA (mtDNA) is often used in evolutionary studies because it is found in essentially all eukaryotic organisms, it is abundant, it has sufficiently variable DNA sequences, and its inheritance is easily traced. The mtDNA is useful for studying the evolutionary relationships of recently extinct organisms up to 100 000 years old.

Eukaryotic cells have many identical copies of the mitochondrial chromosome. This is because there are many copies of the chromosome inside each mitochondrion, as well as many mitochondria inside each cell (Figure 10.32). A given eukaryotic cell will contain only a diploid set (two copies) of each nuclear chromosome but thousands of copies of the mitochondrial chromosome. Furthermore,

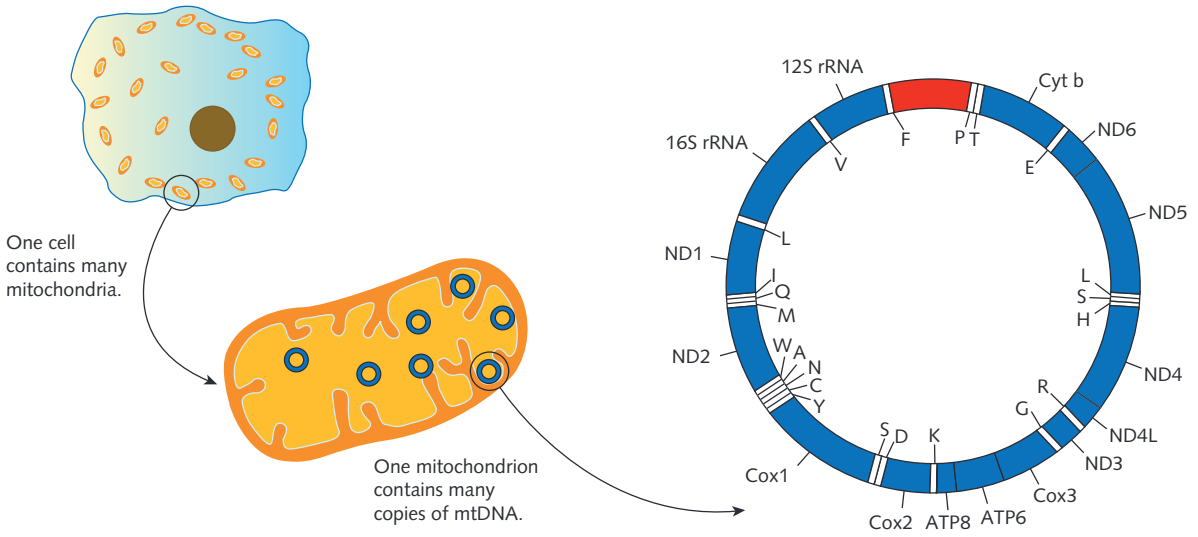


Figure 10.32 **Eukaryotic cells contain many copies of the mitochondra genome Genes for mitochondrial proteins and rRNA are labelled on the outside of the chromosome; genes for mitochondrial tRNA are labelled with single letters on the inside.**

the mtDNA is a very compact genome. For example, in humans the mitochondrial chromosome comprises about 16 600 base pairs coding for just 37 genes (Figure 10.32). This contrasts with the nuclear genome, which comprises about 3.1 billion base pairs encoding between 21 000 and 25 000 genes. The very high number of relatively small mtDNA molecules makes it comparatively easy to extract and manipulate for sequencing. It also means that, compared with nuclear DNA, there is a better chance of recovering an intact mitochondrial genome than a nuclear genome from fossil specimens.

Nuclear DNA is inherited equally from both parents, but mitochondrial chromosomes are inherited independently of nuclear chromosomes. mtDNA is **maternally inherited**. Offspring inherit their mtDNA only from their mother because essentially all the cytoplasm in the fertilised zygote is derived from the egg and not the sperm. This ensures the zygote contains an almost uniform population of mitochondria derived from the mother. Furthermore, mtDNA does not undergo independent assortment or crossing over in the way nuclear chromosomes do during meiosis. Consequently, while the ancestral history for a mutation in nuclear DNA is quickly obscured or lost after just a few generations of recombination and random fertilisation, the ancestry of mtDNA variation can be simply and continuously traced through the female line of inheritance, from mother to offspring, or vice versa (Figure 10.33). This aspect of mtDNA favours its use for identifying individuals long deceased, as well as for exploring patterns of ancestry and migration within and between populations.

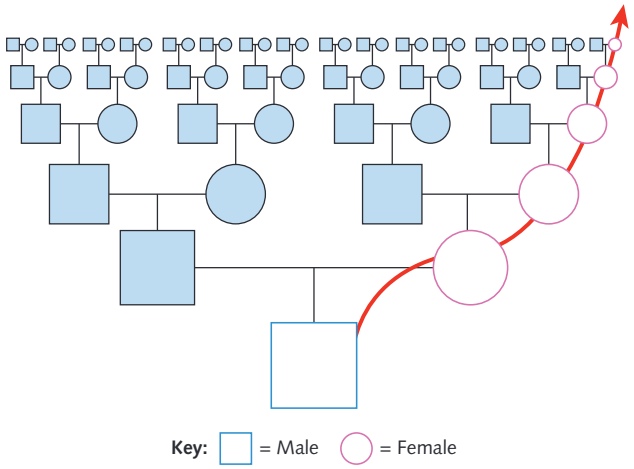


Figure 10.33 **Inheritance of mitochondrial DNA can be traced simply through the female line of descent.**

Reconstructing evolution from gene sequences

Figure 10.34 illustrates how DNA sequences of organisms alive today can be used to reconstruct evolution. The Asian elephant, African savannah elephant and African forest elephant live in different locations and are different in size and morphology. In Figure 10.34c, a 100-nucleotide segment of the ND4 gene in the mtDNA from each of the three elephant species is aligned by the conserved nucleotides – the nucleotides that are identical in each gene. While most of the nucleotides are conserved, some nucleotides differ between each sequence. These differences have arisen by mutation in one of the sequences.

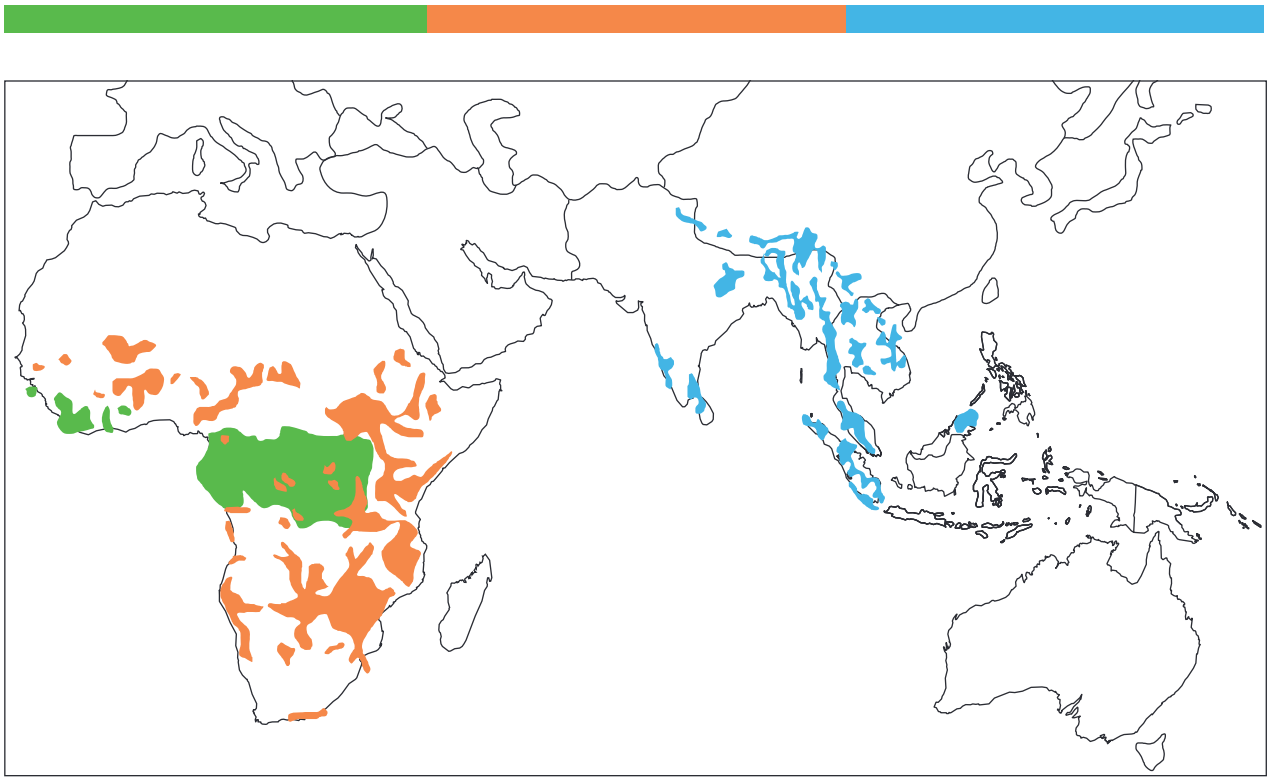
a

African forest elephant

Afrcan savannah eephant

Asian elephant

b



c

```
Forest     TATTATAATAATAGCCTGATTTATTATCCACTTAAATTCCTGAGAGTTTCAACAAATCTTCTTAACTAACCCCAAGAACACTACACTCCCACTACTAGGT
Savannah   TATTATAATAATAGCCTGATTTACTATCCATTTAAATTCTTGAGAGTTTCAACAAATCTTCTTAACTAACCCCAAGAACACTACACTCCCACTACTAGGT
Asian      CATTATAATAATAGCCTGATTTATTATCCATTTAAACTCCTGAGAATTTCAACAAATCTTTTTAACTAACCCCAAGAACACTACACTCCCACTACTAGGC
           ********************** ****** ***** ** ***** ************** ************************************
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Figure 10.34 a Three elephant species and **b** their corresponding global ranges. **c** A sequence alignment of 100 nucleotides from the mitochondrial ND4 gene of each elephant species. Asterisks indicate conserved nucleotides in the sequences of the three species.

It is possible to determine how similar the sequences are. For example, a **pairwise comparison** of the sequences of the African forest elephant and African savannah elephant shows that 97 of the 100 nucleotides are identical (Table 10.3). The mtDNA sequences of the African forest and African savannah elephants are 97% conserved.

Table 10.3 Pairwise comparisons of mtDNA sequences between three elephant species

Pairwise comparison	Number of nucleotides in the DNA segment	Number of conserved nucleotides	Number of nucleotide differences	% sequence conservation
African forest elephant with African savannah elephant	100	97	3	97%
Asian elephant with African forest elephant	100	94	6	94%
Asian elephant with African savannah elephant	100	93	7	93%

Pairwise comparison of the DNA sequences of the Asian elephant with either the African forest or the African savannah elephant gives lower levels of sequence conservation: 94% and 93%, respectively (Table 10.3). Therefore, the mtDNA sequences from the two African species share more nucleotides with each other than either does with the Asian elephant. This suggests the two African species shared a more recent common ancestor than either did with the Asian elephant. In evolutionary terms, the data indicates that the two African species are more closely related. This reasoning is based on the concept of evolutionary distance and is described as the 'distance method' for inferring evolutionary relationships.

The example of the three elephant species can be re-framed in a slightly different way. Rather than asking how conserved the mtDNA sequences are, it is possible to ask how different the mtDNA sequences of each species pair are. Counting the differences between each pair of sequences provides a measure of the evolutionary distance between each species. The more nucleotide differences, the greater the evolutionary distance. The data shows that the Asian elephant is the most distantly related of the three species.

10.5.2 ASSEMBLING PHYLOGENETIC TREES PAGE 222

Assembling phylogenetic trees

Phylogeny is the study of lines of descent from ancestral organisms and the relationships among descendant organisms. A convenient way to visually represent phylogeny is with a **phylogenetic tree**.

The evolutionary relationships inferred from the data in Table 10.3 can be represented in a phylogenetic tree (Figure 10.35). The tips of the phylogenetic tree are labelled with the organisms under study. Each **node**, or branch point, represents the last common ancestor of the organisms whose lineages emerge from it. The root is the ancestral lineage leading to all the descendants in the tree. The first node encountered nearest the root is the common ancestor of all the organisms in the tree. In these phylogenetic trees, the two African elephant species are the most closely related because they share the most recent common ancestor.

The phylogenetic tree also implies the passage of time. The tips represent the present. To trace back to the root is to journey back in time. The most ancient lineages branch near the base of the tree and the most recently derived ones branch closer to the tips. In the trees shown in Figure 10.35, the lineages leading to the Asian elephant and to the African elephants diverged first. The lineage leading to the two African species diverged more recently. Figure 10.35 depicts two types of phylogenetic trees: cladograms and phylograms.

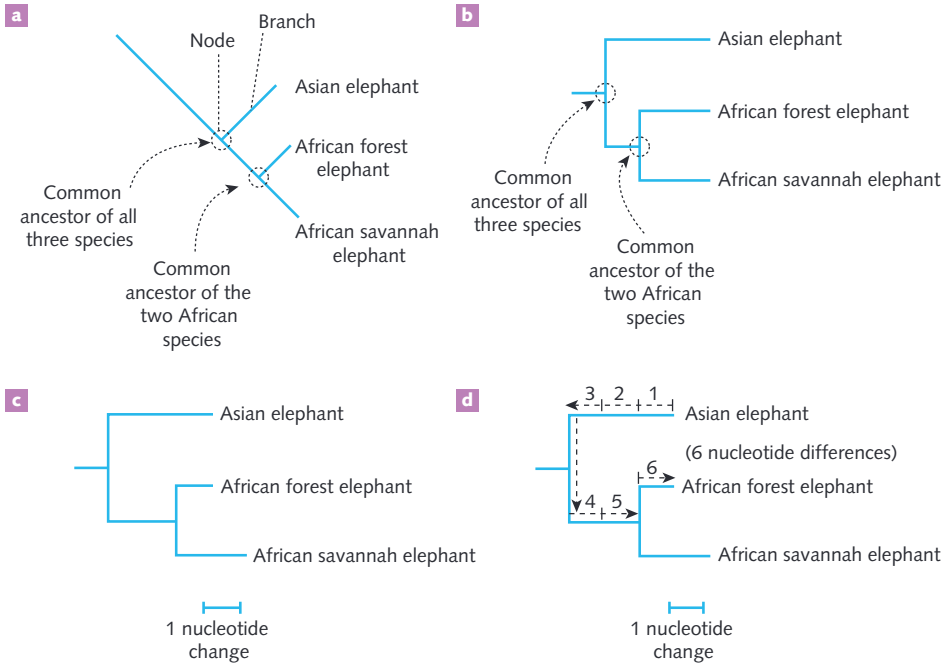

Figure 10.35 Phylogenetic trees depicting the evolutionary relationships of three elephant species. **a, b** Two representations of the same cladogram. The nodes are circled and each indicates a common ancestor to the lineages branching from it. **c** A phylogram with horizontal branch lengths representing the number of nucleotide changes occurring during evolution of the lineage. **d** A demonstration of how the scale of the phylogram is used to measure the evolutionary distance between the Asian and African forest elephants (six nucleotides)

Cladograms

A **cladogram** (Figure 10.35a and b) represents a hypothesis for the evolutionary history leading to the descendant species. It is characterised by **clades**, where each clade is a branch of the cladogram that comprises an ancestor and all its descendants. In each of Figures 10.35a and 10.35b, two clades can be identified. One clade comprises all three elephant species and their common ancestor. The other comprises the two African elephant species and their common ancestor.

The goal is to define each taxonomic group as a clade within the cladogram. A taxonomic group is described as **monophyletic** if all the species in that taxonomic group are descended from the same common ancestor. Therefore, a clade is the representation of a monophyletic group. If the taxonomic group is missing one or more descendants, or if it includes descendants that belong to a different clade, the taxonomic group is not monophyletic and is considered invalid (Figure 10.36).

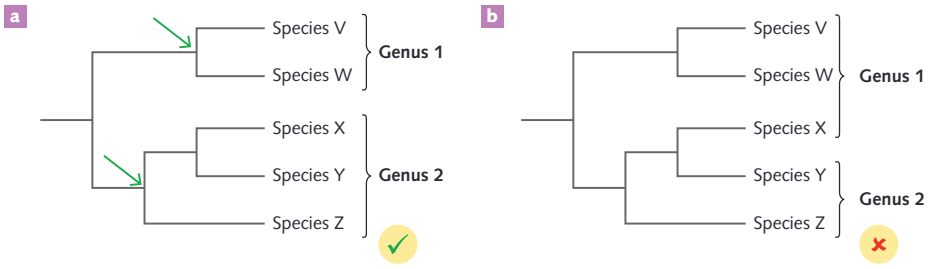

Figure 10.36 Describing monophyletic taxonomic groups. **a** Genus 1 (with species V and W) and Genus 2 (with species X, Y and Z) are both monophyletic groups. The species contained within each genus share a common ancestor (green arrows). **b** In this case, neither Genus 1 (with species V, W and X) nor Genus 2 (with species Y and Z) is monophyletic. Genus 1 contains an additional species (X) which does not share a common ancestor with species V and W. Genus 2 is missing a species (X) that shares a common ancestor with species Y and Z.

Phylograms

A **phylogram** is a scaled, or quantified, version of the phylogenetic tree. The branch lengths are proportional to the amount of inferred evolutionary change or the number of nucleotide changes that have occurred during the evolution of the lineage. The scale bar represents a single nucleotide change (Figure 10.35c and d). The nodes – the points of divergence from a common ancestor – are counted as zero length. For example, the path traced along the horizontal branches from the Asian elephant to the African forest elephant is six times longer than the scale of one nucleotide. Therefore, the difference between the sequences of the two species is six nucleotides.

Representing phylogeny using taxonomy

We can now apply taxonomy to formalise our hypothesis for the evolution of the three elephant species. All three species are classified within the same monophyletic family (Elephantidae). Reflecting the evidence from molecular homology, the Asian elephant is classified in one genus (*Elephas*), and the two African species are classified in another (*Loxodonta*). The placement of the two African species in the same genus formally recognises them as more closely related to each other than either is to the Asian elephant. The genus *Loxodonta* is therefore also monophyletic. The taxonomy of the three species is represented in Figure 10.37.

We still describe the phylogeny as a hypothesis because it is based on an interpretation of the available evidence. Classification schemes may be modified as new evidence emerges or as existing evidence is re-examined and re-interpreted.

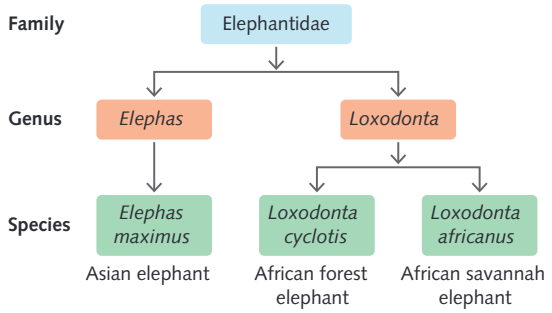

Figure 10.37 The taxonomy of elephant species

INVESTIGATION 10.1

Evidence fit for a mammoth task

The argetlivng landaimals in the wor d today are he elphants, members of the order Probosidea. The roboscideans were far more numerousand dverseinte geological past than they are today. There are more than 100 species in the foss record over some million year s but nealall are owextinct. The ore famou extict proboscideans include mammoths (genus *Mammuthus*) and mastodons (genus *Mammut*)

The wolly mammoth (*Mammuthus primigenius*) and the merican mastodon (*Mummut americanum*) (Fgure 138) occupied thecold arctc and sub-aric regions of he world. A few preserved specimens have been excavated from permafrost and thei ancient DNA has been extrace from teir ur. Althog still relatively degraded, scientists have managed to sequenc e the etire mithondrial genome (approximately 16 500 bas pairs) othe woolly mamoth and he American mastodon.

a

b

Figure 10.38 Two extinct members of the order Proboscidea: **a** the woolly mammoth and **b** the American mastodon

The DNA sequences of organismscan be analysed and used to buld phylogeetic res. This apprach has provided nsghts intoevolutioay elatiohps beween living specie s. Seqencig ancient DNA eansthat phylogenetic trees can be usedto expoe evoltonary relati onshps betwn living nd exict species.

Am

To usemoeculr homlogy to invesigat the volutionary rlationhips between the woolly mammoth, the American mastodon and mers of lving proboscideans

Observatons

Mammoths apear inhe fossil re cord 4lion years ago and were wdespread throughout Erope, Asia andNrth America. They persisted untl about 4000 yars ago, disapearing as the climate warmed after th la t ice age. There were about 11 species, including the wolly mammoth (*Mammuthus primigenius*) (Fgure 038).

The foils of mastodons (genus *Mammut*) date bac at lt 40 million yeas, with icoveies in Afric, Euro and North America. Like woolly mammoths, mastodons were cered by thick, shaggy fur but mastodons were sller and tockie antheir skulls were larger and flattr. The most recent secies i the American mastodon (*Mummut americanum*) (Fgure10.38) which die ou approximately 10000 years ago.

Fosss suggest modrn elephants riginat in Africa 4 million years ago and dispersed fom here. Af ican savannah elephants (*Loxodonta africana*) nhait the sub-Saaran reions of Africa as far south as South Afica. Thy are larer han Asian elephants (*Elephas maximus*), hich occur fromInia in the west t Myanmar in the eas. The ranges of elephants are rapidlysrinking and fragmentig u to habiat loss. Elephant species are threatned, withth Asia elephant lised as endangered.

n the Proboscidea, h distinctive tusks volve from the 'adult' incisors, rather than rm cnines, as in oter tusked mammals such as walruses and wd bors. Teethare ne diagnosticfeature used to distinguish between the many differtlivingand extinct members of the order.

Mastodons (from the Greek mstoont meaning nipple tooth) are named for her mlars, which have 6– 8 cone-shaped cusps tha resemble npples (Figue 1.39b). African savannah elephants also haveraised diamond-shaed extesion ontheir molars. By analogy with modern Afrcan elephants, mastodons are be eved to have been browsers that ae eaves and twigs.

n contrat, mammoths hadteethwiththi, parallel ridges (Fgure 10.39a) that resemble thse of modern Asian elephans.It is inferred tt mammohs, lie Asian elephants, wee grazer that preferentially ate grass.

Figure 10.39 Molar teeth of **a** the woolly mammoth and **b** the American mastodon

Predctons

1. Suggest tw pysical(orphological) faturs hat characterise animals of the order Proboscidea.
2. Wich one of the four roboscideans mght have evol ved erliest?What evidence supports your answer?
3. Wich of the four roboscidens are extinct today?
4. Divide the four prooscdeans into tw mos similar pairs (pair 1 andpair 2) based on ther ur covering.
5. Divide the four prooscdeans into tw mos similar pairs (pair 1 andpair 2) based on their eeh morphology.
6. Cosider th evidence and propose your ow hypothesis for probocidea eolution, whch gave rse to the woolly mammoth, te American mastodon and the African and Aia elephnts. Coy the evolutionar tree in Figure 10.40 nto you logbook and complete it y by adding the names of the four speies.

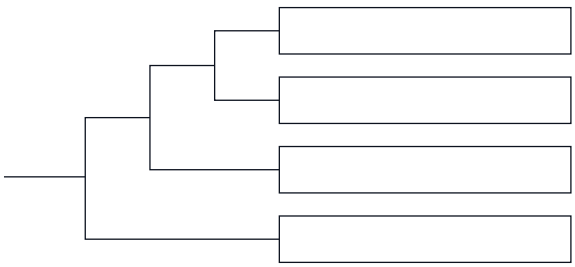

Figure 10.40 Hypothesis for the evolution of four species of the order Proboscidea

Anayss and resuts

Tabl 1.4 list he two liing andtwo extinct proboscidea n speciesused in this study. Fgure 10.41 shows a sequence aignment of a segment f th itochondrial ND5 gene from the four prbosciean species.

Table 10.4 Probiscidean specimens used in this study

Common name	Scientific name	Sample age
Woolly mammoth	*Mammuthus primigenius*	~17 000 years
American mastodon	*Mammut americanum*	~90 000 years
African savannah elephant	*Loxodonta africana*	Present day
Asian elephant	*Elephas maximus*	Present day

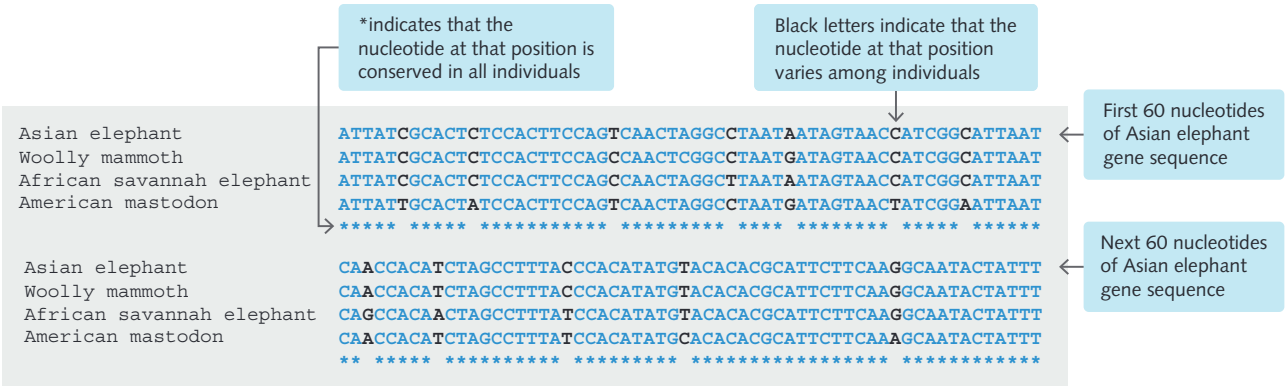

Figure 10.41 Alignment of 120 nucleotides of the *ND5* gene from four probiscidean species. The alignment is chunked into two blocks of 60 nucleotides. Conserved nucleotides are shown in blue and variable nucleotides are shown in black.

1 CopyTale 10.5 into your logbk nd complete i, using the sequnce alignment in Figure 10.41.

Table 10.5 Summary of pairwise comparisons between each of the four proboscidean species

Pairwise comparison	Number of nucleotide differences	% sequence conservation
Asian elephant with woolly mammoth		
Asian elephant with African savannah elephant		
Asian elephant with American mastodon		
Woolly mammoth with African savannah elephant		
Woolly mammoth with American mastodon		
African savannah elephant with American mastodon		

2 Explain how yu woud determine the two most close y reated species from he atin Table 10.5.

3 Copy the claogram inFiure 10.42 ino your logbook.
   a denify the two mos closely related species from the data n Tabl 10.5 andwrite their nams in the appropriate boxes of the cladogram.
   b denify the netmot closely related species and wr te ts nae in the ppropriat box f the cladogram.
   c denifythe least rlated seies and write it s nam in the apropriate ox ofthe cladogram.

Dscusson

Use your cmpletd cladogram to anserthe following questins.

1 Wich one of the for speciesiverged earliest? Explain how yo interpreted this from th cladogram.
2 Wich seies ismostclosely reated to the Asian elephant?
3 Wold a taxnomifaily Elphntidae, containing only the Africa and Asin elephants,be a monophyletic group? Exlan.
4 Does yur cladogram generated from the *ND5* gene sequences(Figur 10.42) support or reject the hypothesis you proposed in Fiure 10.40? Explain.

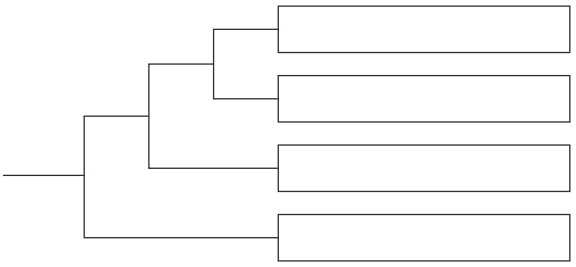

Figure 10.42 A cadogram generated from a 120-nucleotide segment of the mitochondrial *ND5* gene from four species of the order Proboscidea

5 Wich character – fu covering o teeth mrphooy – rovides evolutionary inferences that agree best with the *ND5* gene? Explain with reference to trcural homology.
6 s mlecula homoogy more,less o equally useful toth character you identi fied in Questio 5 fo inferring evltionaryreltionships aong proboscide ans Give reasos to justify your response.

Concuson

Wite a rief cnclusion tating the outcome f your analysis and summising you evaluaton of h value of molecular homlogyfor iferring ev oluionar elatonships.

KEY CONCEPTS

» Molecular hmolo i the similarity of patterns in the ncleotide sequences of NA r the amino acid sequences o polypeptids from difeent organisms. t s explined y diverget evolution from a common ancestor.
» The eolutionry distance between two species is estimated by the numer of amin acids differing in a homlogouspolpptide, or the numerof nucleotides dffeing in a hmologos gene, between the two speces. volutonry relationshps between species can be constructed base on evlutioary distances; the morelike the squences te mre closely related are the oransms.
» Eolutionar reationshipsamong species can be represented b phyogenetic trees that depict the patterns of descent from common ancestors.

Concept questions 10.5

1 Give two reasons why DN and prteins are suited to stuies o mlecula homology.
2 What thee things doesa sequence alignment of homlogous genes eveal?
3 Describe the features of mtDN tat make it suitable for evoutionar tudies.
4 Explain how sequence coservation can be used to estimate hw closely ordisantly elated species are.
5 Use thalignment prsnted i Figure 10.30 to estimate how conservedhe reptile sequences are.
6 What does a node reresentin a phylogenetic tree?
7 Descrie a cladogramanda hylogram. Distinguish betwee them.

HOT Chaenge

8 Exmine teanimals pesetedi Table 10.2.
  a denify oe pairta is likelyto have homologous structures an one pairhat is likely to have analogous structures.
  b How does the taxonomy support your interpretation?
  c Construct a cladogram that represents the reaioships between heorganism in the table.

BRANCHING OUT

Speciation and conservation: eastern barred bandicoot

Poplatins with reuced diversity hav an inraed risk o extinctin, so conervation efforts usually focus on maintaining gentic dvrsity. When large-scle xtinctions occur, not all speci es ar lst, and some seem tobe at more risk than others. Rapid extinction evets tend t lead t the los of larger organismsat the top of od chains rather than smaller ones. Large oulations can more relien han small populations probably because tepopulation as a more diverse gene poo. This it holds a greate resere of different alleles to draw on as the pressures from natural selection change.

The eastern bared bandicoot (*Perameles gunnii*) blongs to the arsuial faiyPramlidae. It is small (body about 300 m, ail 20 mm), grey-brow i colour,with four pale stripes or'brs' on its hidquarers(Figure 10.43). It has thre claws on the frot fet, whic t se for digging,while theack feet are long, nd similar to those of a kangaroo.

Figure 10.43 The eastern barred bandicoot (*Peramees gunn*)

Poultions of the easter barred bandicoot wre once common ove a wd area of south-western Victoria. Numbers reduced drmtically in the 1900s and now there are fewer tan 0 eastern rred bandicoots isolated to a small area around Hmlto. Ths resulted fro a cange n enronmnal cnditions e.. cearing of woodlands, growing exotic pasture grases grazing by destic stoc, introduction of rabbitsand xes), whchsrely reduced its available habitat in Victori a. However, the eastern bareandicoot is still widespread throughout most of Tania.

Consevation plans for the eastern barredbandicootdeend heaviy on how populations are classi fied. A subspecie i a lvl of classi ficationbelowspecies, referring to races of a species tat are fairly permanently geograhcally isolated fromeach oter and may in fuure divergeto become two different species. Because of the rltively healthy badioo populations in Tasmania, the bandicoot is regarded a vulnerable bt not endangered. f the Vctorin popuaton were identi fied asa differentspecies, o sbspcis, thenit could be recognised ndependetly for consrvatio purposes.

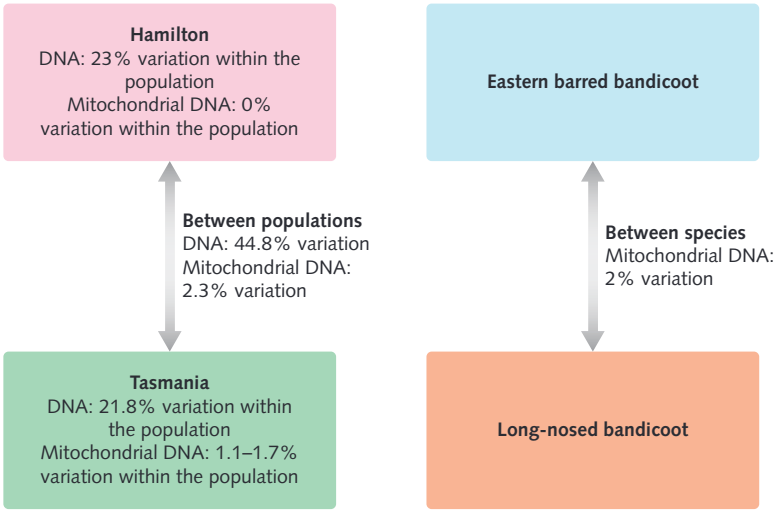

Figure 10.44 DNA variability in different populations of eastern barred bandicoot. A 2% variation is the average difference between subspecies and closely related species of mammals.

A number of studies have been conducted on the Vctorianand Tasmanian poplatios in an attempt to protect the Vicorian opulatin. The bandicoots were trappe,small blood samples were taken and th animals ere rleased immediately nto the samearea. The blood was snap-frozen nd later a DNA fingerrint was taken by anlysin genmic variable nucleotide tandem repeats. The average percentage dfferencein vriable ucleotide tandem repeat ithin the opulations around Hailton was abut 23%, and for those n Tasmnia it ws21.8%. The average percentae difference betwen the Hamilton and Tamanian opulatios was 44.8% (Fgure 10.44).

Further esting was done using mitochondrial DNA (tDNA) restriction fragment lengthpolyohim analysis. This revealed a %nucleotide ariation within theVitora populatios and a 1.1–1.7% varation for te Tamanin populations. Te percentage vari aion betwee the Victoran and asmanian populations was 23%. ariatin of 2% is he average differnce between subspecies of mammals.

Ther is no doubt that the wo populati ons have diverged to some extent due to geograpca soation. It is mportant to know whether theto populations are eparate ubspecies becaus this affects how the conservation of these tw populations of eastern arred bdicoot is anaged. Bioloists curtly use a variety of species concepts, all of whch are based on the theo y of evolution.

The ilogialspecies concept de fines a secies as a reprodutive omunity o popultions that occupies a speci fic nche n natue.Theidenti fication of species often uses dat frm etic analysis. 'DNA fingerpriing' ispredominantly used to deterine which groups are related – that is, share genepool – and which are not. A species de fined according to this concept would b the smalles group of organisms th at share a common ancestor not shared byany other organism.

The Ausralian Gvernment, through th Deptment of the Environment, lists two subspecies of *P. gunni*. The foloin is an excerpt fm he listing for the eastrn barred bandicoot.

> Sieti fic name: *Perameles gunnii* Vicorian ubspecies
> Common name: Eastern Brred andicoot (Mainland)
>
> The genetic iversity, as mesred by the variable number f tandmrepeat markers and mitochondrial DNA retriction ragment lngth polymorphisms, amon g specimens fm Hmilton, Victoria, was greater than that fund in widesred populatio ns of the Tsmaniansubspecies (*Perameles gunnii gunnii*). The justification for onsdering e mainland fom to be disti nctis basd in part onmrphological comparisons of sand andminland orms, and that mDNA data indicated separation 270000–620000 years ago.

Questions

1 Whatspecies de fintion could be used o jut fy classif ig the two popuibns as separate subspecies?
2 Does thercognition of two separate subspeies appear toe well accepted by the Australian Government at this stage?
3 What does te DNA evidence suggest about how the popu aions became separated? To wht extent does this examp illustrate the nceptof alloatric speciation?
4 Wuld th small genec ariability in th eastenbarred bandicot popltions affect their survival? Explain.
5 Elain why te identi fication of the o possble subspecisof bandico t is impor ant for their conservation.

Online . e: CynTeptm
Chapter 10: Summary of key concepts

10 Summary of key concepts

10.1 Studying fossils

KEY CONCEPTS

p373

» Foils are preserved remains f organisms or traces of theirexistnce. Th onditions fosilisation occur rarly andthis can cause a bias in thfossil ecord.
» The foil recrd idelineated b cnsecutive layers of rocksalledstrata. The yo ungest fsils are fond in the uppermost strataand the oldest ar found at the bottom.
» Compaatie datin can determine he age of a fossil or fossl-bering rok in relation tothe srrounding rock, but t does no give nmerical age.
» Index foils are used t correlate the ages of st rata thatoccu in deposits far rom each other.
» Abolutedating gves numerical ages for the time offossil ormain and nclues radiometric daing luminescenc and eectronspin resonance.

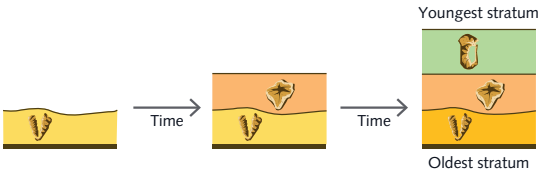

Figure 10.5 Over geological time, younger strata are deposited over older strata. The youngest fossils are found in the uppermost stratum and the oldest are found at the bottom.

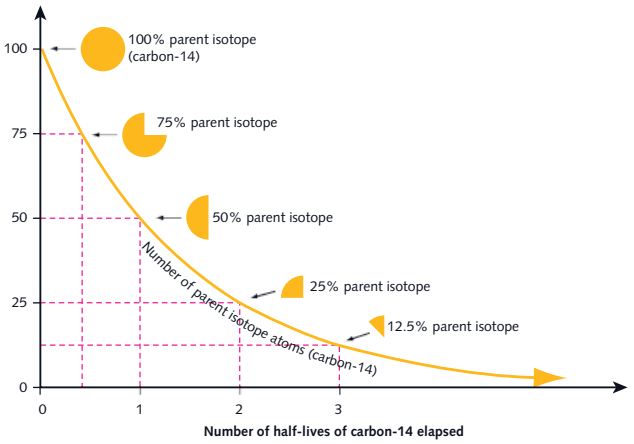

Figure 10.7 A graph of the decay of ^{14}C (half-life 5730 years)

10.2 Patterns in evolution

KEY CONCEPTS

p381

» The fosil record demonstrate that mass extinction evnts have occurred many times in the past. After each mass extinction a ollection of ne speciesreplaces the extinct ones.
» Tranitonalfossil proide evidence for evltionaryreltionships betw een groups of organisms and document change in oganismsoer time.
» ivergentevolution occrs whendifferent selection pres sures apply o differentppulations of n ancestral speces.The diffeen populations accumulae many changes, becming ne spcies wih charcteristics that differ from those of th anetral pecies.When thi occrs on lare cale it is called adaptive radiation.
» Converget volution occurs when nrelated organims (or oganisms with a very distant common ancestor) evlvesimilar structues or adaptatis to perform a similar functio in response to the same selection pressures.

10.3 Emergence of new species

KEY CONCEPTS

p386

- llopatricspeciation is the process by whch new secies diverge from members of an ancstal species that have become geograpially isolated or long periods.
- The eolution f Galapagos and Cocos sand finche is an eampe of allopatric spciaion.
- The eolution of *Howea* palmsis an example of sympariespeciation in which new species emerge foman existing poplationhie inabiting the same geographicl area.

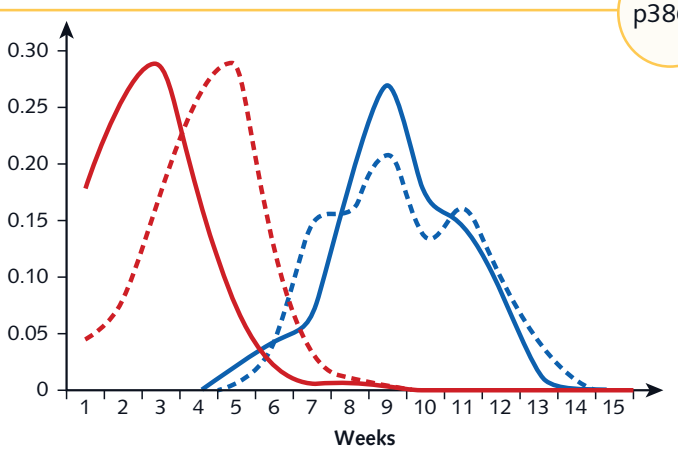

Figure 10.23 The differing flowering times of the two *Howea* species. The blue lines represent 198 curly palms (*Howea belmoreana*) and the red lines represent 177 kentia palms (*Howea forstriana*) showing female (dashed) and male (solid) flowering phases.

10.4 Determining the relatedness of species

KEY CONCEPTS

p393

- Taxonmy is naming system that categrisesorganisms based on hypothesesaout evolutionary reaionhis.
- Comparing he development and anatomy of organisms can rovde evidence that organisms deeloped from a common ancestor.
- Hoologous strutures evolved from the same anestral form bt have developed dfferent forms or functions dueto having dfferent selction presures.
- Aalogous strucures evolved under the same slection pressurs from different ancestral forms so they have a common function but show some fndamental differences.

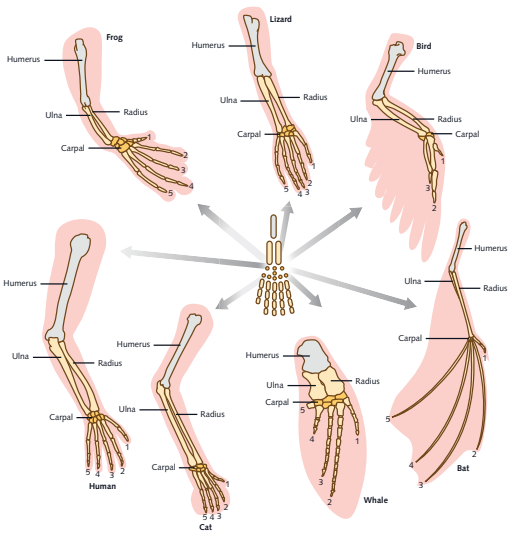

Figure 10.26 The principle of homologous structures can be illustrated by the adaptive radiation of the forelimb of a selection of vertebrates, which all show the basic pentadactyl pattern modified for different uses.

10.5 Molecular evidence for relatedness of species

KEY CONCEPTS

p399

- Molecular hmolo i the similarity fpatterns in the nucleotide sequences of DNA or the amino acid sequences o polypeptids from difeet organisms. It s explainedby divegnt evolution from a common ancestor.
- The eolutionry distance between two species is es timated by the numbe of amin acds iffering in a homlogouspolpptide, or the numerof nucleotide s dffeing in a hmologou gene, between the two speces. volutonry relationshps between species canb consructd based on evolutionary distances; the morelike the squences te mre closely relaed are the organisms.
- Eolutionar reationship among species ca be epresented y phylogenetic trees that depict the patterns of descent from common acestors.

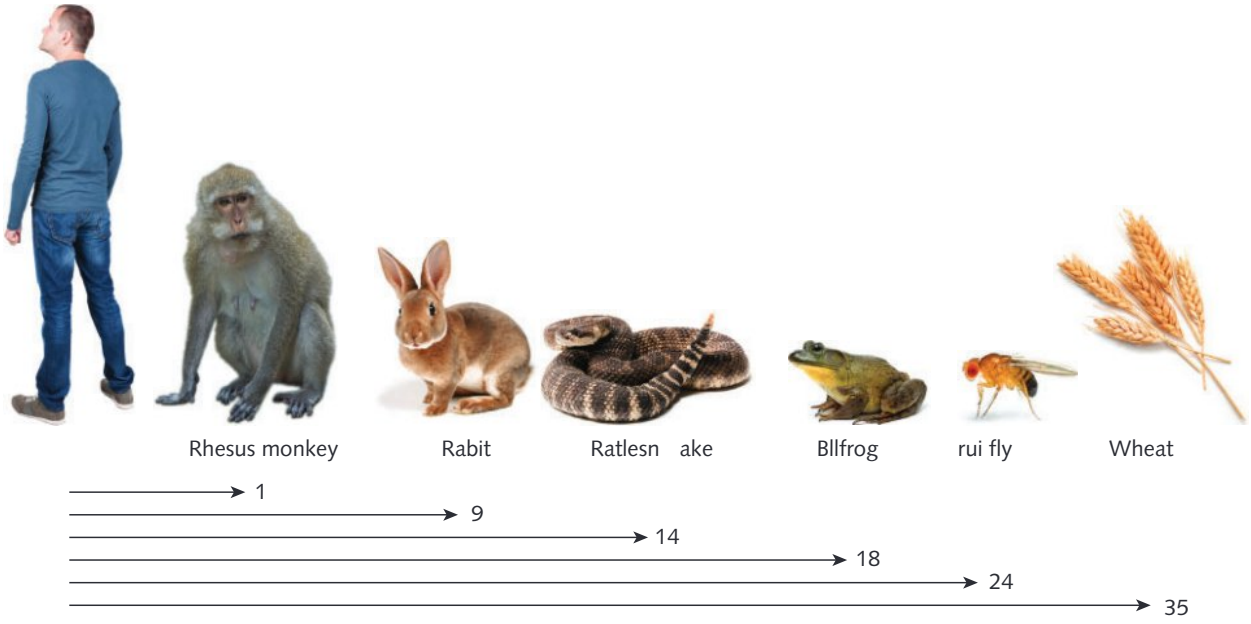

Figure 10.29 The number of amno acd dfferences between the c ytochrome c of humans and that of other organisms.

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WB
10.6.1 KEY TERMS PAGE 224

10 Chapter glossary

absolute dating the process of determining the age in years of rocks and the fossils they contain on the basis of the physical or chemical properties of materials in the rock

adaptation an anatomical, behavioural and physiological characteristic that allows an organism to exploit a specific ecological role

adaptive radiation when a single species diversifies relatively rapidly into many new species because of the availability of many different ecological niches

allopatric speciation speciation that occurs when members of an ancestral population become geographically separated and each isolated population evolves into a new species

analogous structure an anatomical or morphological feature in different organisms that has the same function but not the same basic underlying structure

bioinformatics the application of computer science to the digital storage, retrieval and analysis of large volumes of biological data

biological species concept the concept that species are groups of natural populations that could potentially interbreed but are reproductively isolated from other populations

clade a branch of a cladogram that represents a common ancestor and all of its descendants

cladogram a phylogenetic tree that depicts a hypothesis about the evolution of a group of organisms from a common ancestor

comparative dating *see* **relative dating**

convergent evolution when organisms that are not closely related independently evolve similar traits as a result of having to adapt to similar environments or ecological niches

correlation the inference that rock layers located in distant sites must be of the same age if they have identical mineral and fossil composition

divergent evolution when members of a population develop adaptations to different selection pressures over many successive generations and eventually become new species

electron spin resonance a method for determining the age of a rock or fossil based on the properties of electrons trapped inside the crystals of minerals

evolutionary distance the number of substitutions that have occurred in the amino acid sequences of homologous polypeptides or nucleotide sequences of homologous genes since two organisms diverged from a common ancestor

fossil the preserved remains or traces of an organism

fossil record the worldwide collection of fossils as they occur in the surface layers of Earth

fossil succession when fossils appear in a consistent order in the fossil record from older rock layers to younger overlying rock layers; the same order is found worldwide

homologous refers to genes or polypeptides that have similar sequences and indicate a shared evolutionary ancestry

homologous structure an anatomical feature in different organisms that has the same basic underlying structure but different functions

index fossil a fossil that is representative of a specific geological time

isotope one of two or more atoms of the same element with the same atomic number and number of protons, but different numbers of neutrons and therefore different relative atomic masses

lineage in evolution, a population that represents a separate line of descent from a common ancestor to modern species

luminescence in absolute dating, a method for determining the age of a mineral crystal based on measuring the emission of light by electrons as they are stimulated to escape from the crystal

mass extinction the extinction of many species over a relatively short (geological) period

maternally inherited describes a genotype that is transmitted entirely from the female parent to the offspring

mineralisation the process by which minerals from sediments have replaced the biological matter in a deceased organism, making it prone to become a fossil

molecular clock the number of substitutions that have accumulated in the amino acid sequence of a polypeptide or the nucleotide sequence of a gene in a given lineage; the rate of the molecular clock is used to estimate the time since two species diverged

molecular homology the similarity of patterns in the nucleotide sequences of DNA or amino acid sequences of polypeptides as evidence for a common evolutionary origin

monophyletic describes a taxonomic group of species that have all descended from the same common ancestor

morphological species concept usually applied to fossils, defines a species by measurable anatomical criteria and characteristics

mya millions of years ago

niche an organism's ecological role; the way the organism lives and functions in its environment

node a junction point in a phylogenetic tree that represents the common ancestor of the lineages that diverge from it

optically stimulated luminescence a luminescence technique that stimulates electrons to escape a mineral crystal when the crystal is exposed to coloured light

pairwise comparison in evolutionary studies, a comparison between two polypeptide sequences, two DNA sequences or two genomes to determine how similar they are

phylogenetic tree a branching diagram showing the evolutionary relationships between species; groups joined together in the tree are believed to have descended from a common ancestor

phylogeny the evolutionary relationships that exist between species, often expressed as a tree-like diagram or represented by taxonomic classification

phylogram a type of phylogenetic tree with branch lengths scaled to represent the number of nucleotide or amino acid changes that have occurred during the evolution of each lineage

radioactive decay a process by which the nucleus of an unstable isotope splits and emits energy in the form of radioactivity

radiometric dating a method for determining the age of a rock or fossil based on the predictable rates of decay of naturally occurring radioactive isotopes present

relative dating the process of determining the age of rocks and the fossils they contain relative to each other, allowing an estimation of 'oldest to youngest' without assigning an actual age in years

reproductively isolated when sexual reproduction can no longer occur freely among any adult members of the population

sequence alignment a display in which homologous polypeptide or DNA sequences are positioned against each other to identify patterns of conserved sequence

speciation the evolution of one or more new species from an ancestral species

strata (singular: stratum) successive layers of sedimentary rocks; each layer represents a unique age range and contains a unique collection of fossils

structural morphology the study of the physical structure and form of organisms

superposition the principle that strata are deposited in a time sequence, with the oldest at the bottom and the youngest at the top

sympatric speciation when two species evolve from an ancestral population while still inhabiting the same geographical area

taxonomy a system of scientific conventions for naming and classifying organisms

tetrapod a 'four-footed' vertebrate animal; includes amphibians, reptiles and mammals

thermoluminescence a luminescence technique that stimulates electrons to escape a mineral crystal when the crystal is heated

trace fossil a fossil produced by an organism's activities, such as fossil footprints or burrows

transitional fossil a fossil that bears features of both an older ancestral life form and a younger descendant

vestigial structure a structure found in organisms that has lost most, if not all, of its original function in the course of evolution; in ancestral organisms, the structures served a purpose, but in their descendants, the structures become atrophied or rudimentary

WB
10.6.2 EXAM PRACTICE PAGE 226

10 Chapter review

Remembering

1 Define:
   a fossil succession
   b transitional fossil
   c vestigial structures
   d homologous structures
   e evolutionary distance.
2 The fossil record is a vital stream of evidence for evolution, but it is patchy and incomplete. Recall why it is incomplete.
3 Describe what makes an ideal index fossil and how it is applied to the principle of correlation.
4 Explain the difference between a phylogram and a cladogram.

Understanding

5 Draw an annotated diagram of an *Archaeopteryx* fossil. Explain why *Archaeopteryx* is an excellent example of a transitional fossil between dinosaurs and modern birds and label the relevant features on your diagram.
6 The phrase 'survival of the fittest' has been used to describe Darwin's concept of natural selection. Outline the ways in which this term could be misleading.
7 In North America, species of fruit fly of the genus *Rhagoletis* are confined to different species of apple trees and hawthorn bushes.
   a Describe how this could lead to speciation.
   b Would this be allopatric or sympatric speciation? Explain.
8 Both birds and bats have wings, while mice and crocodiles do not. Explain if this means that birds and bats are more closely related to one another than to mice and crocodiles.
9 Defend or refute the statement, 'There is nothing more to be learnt from fossils that have already been examined'. Use evidence to support your position.
10 What would it imply if the sequences of homologous genes from two organisms were 100% conserved?
11 You are related to your first cousins because you share two recent 'common ancestors' (your grandparents). The theory of evolution states that all organisms on Earth today have also arisen from a single common ancestor. How are these two usages of this term similar and how are they different?

Applying

12 Provide an example of how an understanding of changing gene pools is important to understanding evolutionary change.
13 Embryological studies show bird embryos develop a fourth finger and a fifth toe that vanish as the foetus develops. This vestigial developmental structure is evidence for common descent.
   a Explain what this evidence explicitly says about the characteristics of the ancestors of birds.
   b Explain whether you would expect a complete fossil skeleton of a common ancestor showing this characteristic to have been found.

14 Stone tools have been found with campfire charcoal. Explain how the technique of carbon dating could be used to determine the time at which the tools were made.

15 Explain the difference between the techniques of electron spin resonance and optically stimulated luminescence.

16 A fossilised fish skeleton is found in sandstone, 1 m below the surface, at location X. A very similar skeleton is found at location Y, 2 m below the surface and 1 km away from location X. Another similar skeleton is found at location Z, 3 m below the surface and 3 km away from location X. Describe what can be inferred about the:

- **a** way in which the rocks were formed
- **b** age of the fossil at location Y.

17 Examine the cladogram of the Galapagos finches in Figure 10.45.

- **a** Which species is most closely related to *Geospiza fortis?*
- **b** Which species is most closely related to *Camarhynchus parvulus*?
- **c** What evidence from the cladogram supports the ground finches being a monophyletic group?
- **d** How is a monophyletic group of ground finches recognised in their taxonomy?

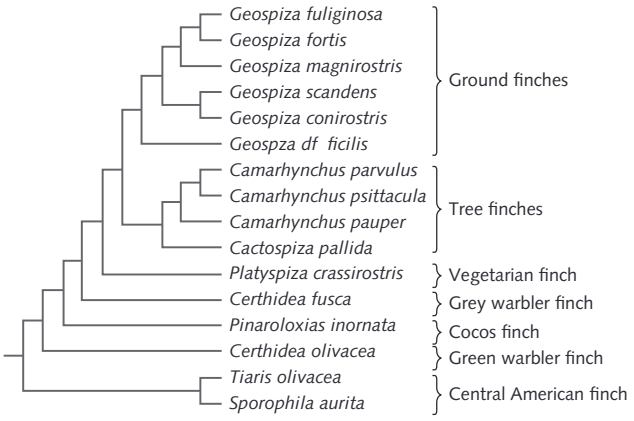


Figure 10.45 A cladogram representing the Galapagos finches

18 The cladogram in Figure 10.46 represents evolutionary relationships among groups of land plants.

- **a** Which group diverged the earliest?
- **b** Which two groups are most closely related?
- **c** A biologist proposed recognising a taxonomic group of 'non-flowering plants' consisting of mosses, lycophytes, ferns and conifers. What is your recommendation for that proposal? Justify your recommendation.

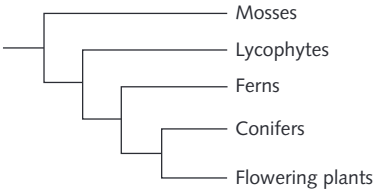


Figure 10.46 A cladogram representing evolution of land plants

Analysing

19 Over the last 30 years many new pre-human fossils have been found, but scientists often find it difficult to agree whether they should be identified as new species or not. Account for this limitation in terms of our current understanding of the species concept.

20 Identify a limitation of luminescence in dating sedimentary rock.

21 There is a variety of types of tortoise on the Galapagos Islands. One species has a domed shell and a short neck and is found on islands with high moisture content. Another species has a long neck and a shell that flares up at the front so that the tortoise can lift its long neck up. The long-necked tortoise is found on the more arid islands. The main food of the tortoises is the prickly pear cactus. On the islands with no tortoises, the prickly pear has a low spreading form with soft spines. On the islands with the long-necked tortoise, the prickly pear has a tall form with hard spines.

- **a** Explain how the tortoises could have first reached the Galapagos Islands.

b Assess and explain if it is likely that the ancestor tortoises would be identical to the modern tortoises.
c Explain why prickly pear would grow in different plant forms on different islands.
d Define and explain what type of evolution is illustrated by the association of the long-necked tortoise and the tall prickly pear.

22 The last known Tasmanian tiger (thylacine) died in the Hobart Zoo in 1936 and the species is now recognised as extinct. Hypothesising a close evolutionary relationship between the Tasmanian devil and the Tasmanian tiger, scientists sought to explore the phylogeny of Australian carnivorous marsupials using DNA. They sequenced the mitochondrial genome from a pelt of the Tasmanian tiger and from tissues of living marsupials. They compared the mitochondrial genomes and constructed the phylogram shown in Figure 10.47.

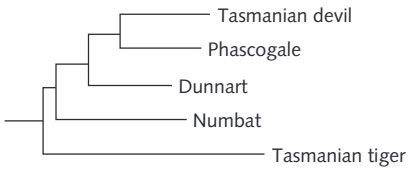


Figure 10.47 A phylogram of Australian marsupial carnivores based on mitochondrial genome sequences.

a Suggest why the scientists chose to sequence the mitochondrial genome rather than the nuclear genome.
b What do the branch lengths represent in the phylogram?
c What has happened during evolution to result in the different branch lengths?
d Which is the most closely related species to the Tasmanian devil?
e Was the scientists' hypothesis supported or rejected?
f Decide whether the following taxonomic families are monophyletic or not and give reasons to justify your decision.
i Family Dasyuridae, which includes the Tasmanian devil, phascogale and dunnart
ii Family Thylacinidae, which includes the Tasmanian tiger

Evaluating

23 The hoatzin (*Opisthocomus hoazin*) is a remarkable bird from South America. It has only one known fossil ancestor, identified from a 10-million-year-old skull fragment found in Colombia. The age of the fossil demonstrates that hoatzins were endemic to South America; the fossil pre-dates the land bridge between North and South America by 8 million years. Genetic analysis of the living hoatzin has shown it to be unique, perhaps because of its extensive history of geographic isolation, and it has its own suborder. Chicks of the hoatzin show a characteristic seen in no other living bird: a pair of claws on their wings, a characteristic similar to those seen on the bird-like dinosaur *Archaeopteryx*, which had three wing claws. From this description, identify lines of evidence for evolution from the disciplines of:
a palaeontology, via the fossil record
b morphology
c genetics.

24 You are invited to construct a phylogenetic tree to represent the relationships between *Eucalyptus* species. You have the resources to copy and sequence the genes listed in Table 10.6.

Consider the merits of using any one of these genes for investigating evolutionary relationships.
a Discuss which features of a gene you would consider important in deciding whether or not to use it for phylogenetic studies.
b Which gene would you choose to explore the phylogenetic relationships of species within the genus *Eucalyptus*? Give reasons to support your choice.
c Which would you choose to explore the phylogenetic relationships between genus *Eucalyptus* and other representatives of kingdom Plantae? Give reasons to support your choice.
d In parts **b** and **c**, would you choose to represent the evolutionary relationships as a cladogram or phylogram? Justify your answer.

Table 10.6 Genes found in *Eucayptus* and some of their characteristics

Gene	Genomic location	Approximate size (bp)	Notes
his4	Nucleus	500	Codes for a subunit of the proteins around which DNA is wound to form chromatin inside the nucleus
gapdh	Nucleus	2900	Codes for an enzyme involved in glycolysis
rbcL	Chloroplast	1400	Codes for a subunit of Rubisco, an enzyme involved in the light-independent reactions of photosynthesis
ATP8	Mitochondrion	450	Codes for a subunit of mitochondrial ATP synthase
CO1	Mitochondrion	700	Codes for a protein involved in the mitochondrial electron transport chain
ITS1	Nucleus	200	Transcribed but untranslated region of DNA situated between two rRNA genes; may occur in thousands of copies
hmgb1	Nucleus	4000	Non-functional and does not code for protein

25 Defend or refute the statement: 'Gene phylogeny is the same as species phylogeny'. Outline the arguments in support of your position.

Creating

26 Consider the gene sequences shown in Table 10.7.

a Propose a series of operations for using the sequences to infer the evolutionary relationships between the four organisms. Depict your proposal as a flow chart.

b Construct a cladogram from the sequences and outline your rationale for the arrangement of the branches.

Table 10.7 A 100-nucleotide segment of the *CO1* gene from four bird species

Species name	Common name	Locality	DNA sequence
Eclectus roratus	Eclectus parrot	Northern Australia, New Guinea	CTTCGGCGCATGAGCTGGCATAATC GGTACCGCCCTAAGCCTACTTATCCG CGCAGAACTAGGCCAACCTGGAAC CCTACTAGGAGACGACCAAATCTAC
Ciconia boyciana	Oriental stork	China, Russia	CTTCGGCGCATGAGCTGGCATAGTTG GAACCGCCCTTAGCCTTCTTATTCG CGCAGAACTTGGTCAACCAGGAAC CCTCCTAGGAGACGACCAAATCTAC
Agapornis roseicollis	Rosy-faced lovebird	South-western Africa	CTTCGGCGCATGAGCTGGCATGATTG GTACATCCCTAAGCCTCCTCATCCGCG CAGAACTAGGCCAGCCAGGAACCCT GCTAGGAGACGACCAAATCTAC
Rhynchopsitta terrisi	Maroon-fronted parrot	Mexico	CTTCGGCGCATGAGCAGGCATGGTCG GTACCGCCCTAAGCTTGCTTATTCGTGCA GAGCTCGGTCAACCAGGGACCCTCCTAG GAGACGACCAGATCTAC

Human change over time

11

By the end of this chapter you will have covered the following material.

Key knowledge

Human change over time

- the shared characteristics that define mammals, primates, hominoids and hominins pp. 425–433
- evidence for major trends in hominin evolution from the genus *Australopithecus* to the genus *Homo*: changes in brain size and limb structure pp. 433–442
- the human fossil record as an example of a classification scheme that is open to differing interpretations that are contested, refined or replaced when challenged by new evidence, including evidence for interbreeding between *Homo sapiens* and *Homo neanderthalensis* and evidence of new putative *Homo* species pp. 442–454
- ways of using fossil and DNA evidence (mtDNA and whole genomes) to explain the migration of modern human populations around the world, including the migration of Aboriginal and Torres Strait Islander populations and their connection to Country and Place pp. 455–464

Key science skills

Plan and conduct investigations

- design and conduct investigations; select and use methods appropriate to the investigation, including consideration of sampling technique and size, equipment and procedures, taking into account potential sources of error and uncertainty; determine the type and amount of qualitative and/or quantitative data to be generated or collated pp. 452–453
- work independently and collaboratively as appropriate and within identified research constraints, adapting or extending processes as required and recording such modifications pp. 452–453

Comply with safety and ethical guidelines

- demonstrate safe laboratory practices when planning and conducting investigations by using risk assessments that are informed by safety data sheets (SDS), and accounting for risks pp. 452–453
- apply relevant occupational health and safety guidelines while undertaking practical investigations pp. 452–453
- demonstrate ethical conduct when undertaking and reporting investigations pp. 452–453

Generate, collate and record data

- systematically generate and record primary data, and collate secondary data, appropriate to the investigation, including use of databases and reputable online data sources pp. 452–453

Analyse and evaluate data and investigation methods

- identify outliers, and contradictory or provisional data pp. 452–453

Analyse, evaluate and communicate scientific ideas

- use appropriate biological terminology, representations and conventions, including standard abbreviations, graphing conventions and units of measurement pp. 452–453
- discuss relevant biological information, ideas, concepts, theories and models and the connections between them pp. 452–453

Online Chapter Map
Chapter 11 Map

11 Human change over time

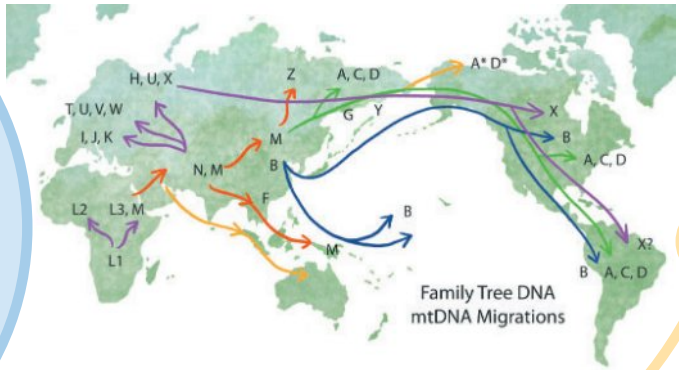
Humans have evolved under the same constraints as all other species. Human evolution can be traced over millions of years using fossil and molecular evidence.

113 Meet the ancestors p442

The foss recorddeicting human evouton s patchy and contnuay beng re fined or chaenged by new foss dsc overes and the appcaton of new technoogy. The evdence suggests bpedasm evoved before nlargement of the rin, eventuay eadng to sophstcated cutur.

114 Modern humans and Neanderthas p451

Fosss archaelogy and mtochondra and nucear DNA se quences support the Out of Afrca hypothess They ndcate modern humans patterns of mgraton across the wrd iclding the peopng of Austraa DNA evdence shows that modern humans nterbred wth Neanderthas and other ancent humans as they mgrated nto Europe and across Asa



Humans share characteristics with other mammals, primates and hominoids. We are set apart from other hominoids by having certain characteristics that enabled us to take advantage of the natural world and use it for our own advantage. Humans can trace their ancestry to Africa, which they left to populate the rest of the world.

Online Key Terms
Chapter 11 flashcards

Know your key terms

arboreal
australopithecine
bipedalism
brachiation
brain case
brow ridges
carrying angle
cerebral cortex
cognitive capacity
cranial capacity
cranium
cultural evolution
Denisovan
dentition
exocrine gland
foramen magnum
gracile
hallux
haplogroup
haplotype
hominin
hominoid
kya
language
mammal
mammary gland
mandible
midden
Out of Africa hypothesis
oviparous
palaeoanthropology
placental
postcranial
precision grip
prefrontal cortex
prehensile
primate
prognathism
quadrupedalism
recent single origin
robust
sagittal crest
sagittal keel
sebaceous gland
sexual dimorphism
stereoscopic
superfamily
suspensory locomotion
sweat gland
tribe
viviparous

Remember

WB
REMEMBER PAGE 229

This chapter will build on the following concepts that you will have already met. Take the time to refresh these concepts before you start this chapter.

1 Allopatric speciation occurs when members of an ancestral population become geographically separated and each isolated population evolves into a new species.
2 Sympatric speciation occurs when two species evolve from an ancestral population while still inhabiting the same geographical area.
3 The fossil record is not a complete record of past living things, as it is biased towards living things that fossilise more easily than others.
4 Radioisotope dating takes advantage of the decay of naturally occuring radioactive materials and is used to absolutely date fossils, artefacts or the sediments they come from.
5 All mammals possess hair at some stage in their development and feed their young on milk produced in mammary glands.
6 A phylogenetic tree is a branching diagram showing the evolutionary relationships between species. Groups joined together in the tree are believed to have descended from a common ancestor.
7 A phylogram represents the number of nucleotide or amino acid changes that have occurred during the evolution of each lineage.
8 A cladogram depicts a hypothesis about the evolution of a group of organisms from a common ancestor.

To access resources below, visit www.nelsonnet.com.au

Online Chapter Map:
- Chapter 11 map (p. 422)

Online Key Terms:
- Chapter 11 flashcards (p. 424)

Weblinks:
- Four billion years of evolution (p. 442)
- A brief history of dogs (p. 463)

Online Worksheets:
- Myths and misconceptions about evolution (p. 442)
- A brief history of dogs (p. 463)

Online Key Concepts:
- Chapter 11: Summary of key concepts (p. 466)

We, *Homo sapiens*, are the dominant vertebrate animal on Earth, and humanity's activities have a profound impact on other species. However, biologists cannot assume humans are special, separate from nature on Earth, because of some divine plan. Rather, *Homo sapiens* is a species that has evolved under the same constraints and biological principles that apply to all species. In this chapter, we will explore the various lines of evidence that argue for the evolutionary history of *Homo sapiens* and the features that have emerged to make the species unique.

Our understanding of human evolution is incomplete, sometimes contentious, and is continually being reshaped as new evidence arises. The evidence may come from new discoveries that are made by using existing investigative methods or by developing new methods. Consequently, the study of human evolution serves as an illustrative model for how biologists infer evolutionary relationships between organisms and assess how closely related one group of organisms is to another. This chapter therefore discusses the current knowledge of human evolution and some of the gaps in our understanding.

Throughout this chapter, we will look at how evolution has enabled *Homo sapiens* to rise to such prominence (Figure 11.1).

Figure 11.1 What has occurred during evolution to enable *Homo sapens* to rise to prominence?

11.1 Taxonomy of modern humans

WB
11.1.1 TAXONOMY PAGE 230

Like all other animals, humans can be classified within taxonomic schemes. As a tool of systematics, the taxonomy reflects a hypothesis for the evolutionary relationships between humans and other organisms. Taxonomy organises our current understanding about the evolutionary descent of modern humans and which animals are our most closely related living species.

CONNECT
Taxonomy is discussed in more deta n Chapter 10.

Humans are mammals

Within formal taxonomic schemes, modern humans are members of the phylum Chordata and the class Mammalia. There are approximately 5000 species of **mammals**. They are a diverse group (Figure 11.2) that includes many of the largest terrestrial and marine carnivores, the largest terrestrial herbivores, burrowing and flying species and many of Australia's most iconic animals.

Features that characterise mammals

Many mammals are covered in hair or fur. However, in some mammals the hair or fur is not immediately recognisable. For example, on the echidna, the hairs are fused into quills. Some aquatic mammals, such as dolphins, have fine whiskers at birth that are quickly lost. Humans have a diminished covering of hair.

Other shared features of mammals are shown in Table 11.1. Mammals have several unique types of **exocrine glands**, which secrete fluids to the outer surface of the body. Most notably are the **mammary glands** from which the class name, Mammalia, is derived. Newborn offspring are nourished by nutrient-rich milk secreted by the mother's mammary glands.

Humans have other types of exocrine glands that function as the adaptations of an endothermic (warm-blooded), fur-covered animal. For example, we have **sebaceous glands**, which secrete oils that lubricate and protect patches of the skin and hair. We also have **sweat glands** that help regulate body temperature.

EXAM TIP
Do not get secretion and excretion mixed up. Secretion is when a required substance has been produced in a cell or a gland and is removed from the cell or gland to perform a function. Excretion is the removal of cellular waste from cells or the body.

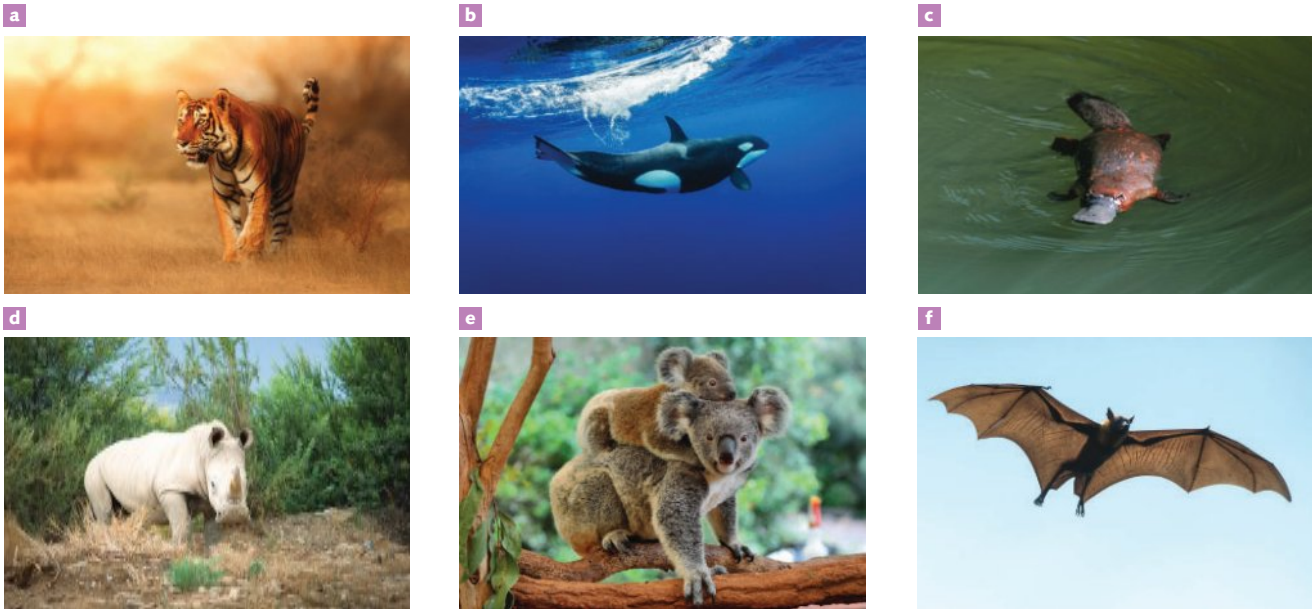


Figure 11.2 The diversity of mammals: **a** a tiger (*Panthera tgrs*); **b** a killer whale (*Orcnus orca*); **c** a platypus (*Ornithorhynchus anatnus*); **d** a white rhinoceros (*Ceratotherum smum*); **e** a koala (*Phascoarctos cnereus*); and **f** a bat (*order Chroptera*)

Table 11.1 Conspicuous anatomical features that distinguish mammals from other classes in the phylum Chordata

Feature	Mammals	Other chordates
Body covering	Fur or hair	Scales (fish, reptiles), permeable skin (amphibians), feathers (birds)
Temperature regulation	Endothermic	Ectothermic (fish, amphibians and reptiles) or endothermic (birds)
Unique exocrine glands: mammary, sebaceous, sweat and scent glands	Present	Absent
Number of chambers in the heart	Four	Two (bony fishes), three (cartilaginous fish, amphibians and reptiles) or four (birds and crocodiles)
Diaphragm	Present	Absent
Number of bones in the middle ear	Three: malleus, incus and stapes	One: stapes
Structure of lower jaw	Single bone	Multiple bones (fish, amphibians, reptiles) or absent (birds)
Teeth	Specialised into incisors, canines and molars	Uniform (fish, amphibians, reptiles) or absent (birds)

Our heads, like those of other mammals, have conspicuous external ears, a middle ear consisting of three bones, a fused lower jawbone, and teeth that are differentiated by size, shape and position into incisors, canines and molars. Like other mammals, humans have a four-chambered heart with a unidirectional cardiovascular system that circulates oxygen-rich blood independently of the oxygen-poor blood. Humans also have a diaphragm that separates the abdominal and thoracic cavities and acts to inflate and deflate the lungs during rhythmic breathing.

Placental mammals

Most mammals are **viviparous** (bear live young). Other mammals are **oviparous** (lay eggs).

The oviparous subclass Prototheria, or monotremes, constitutes just a few modern egg-laying species, the platypus and the echidnas. Monotremes produce leathery eggs, like those of reptiles. Juvenile

monotremes are nourished by the mother's mammary glands, with the milk secreted from a patch of skin, rather than from a discrete teat, enclosed inside a skin fold. Anatomical and DNA evidence suggests that monotremes are the earliest divergence of the mammals.

The viviparous subclass Theria consists of two 'infraclasses'. In the infraclass Metatheria, or marsupials, the embryo is nourished by a yolk-based placenta. The nutrients are soon consumed and the embryo is born at a relatively early stage of development. The embryo moves from the mother's uterus to the pouch, where it attaches to a nipple and suckles for the rest of its development.

Most mammals are members of the infraclass Eutheria, or **placental** mammals. In this group, the placenta is a relatively more complex organ that connects the blood supply of the developing embryo or foetus with that of the mother. The placenta exchanges nutrients and wastes, and provides some immunological protection. By connecting to the mother's blood supply, gestation can proceed for much longer in the uterus and placental mammals are normally born at a substantially more advanced stage than marsupials.

Humans are placental mammals. Humans clearly share an anatomical, physiological and reproductive heritage with thousands of other eutherian mammals that have descended from an ancient, highly successful and highly divergent lineage extending back more than 225 mya.

KEY CONCEPTS

- Mamals share a rane of uniqu anatomical features, ncluing a body cvering of har r fur, a single jaw wth speialised teeth threbons in the middle ear, specalised eocrie glands, a diaphragm and a four-chamberedheart.
- Mamals e diided into mnotres, marsupials andplacntalmmmals.
- Humans ae placent mammals.

Concept questions 11.1a

1 Describe three type of eocrine glands unique to mammas.
2 Describe e secialiationsin mammalian teeth.
3 Ditnguish between oviarousand viviparous. Wich mamalian suclass is ovpaous and which is viviparous?

HOT Chaenge

4 Mamals have a four-chambered eart. Mammals have exocin glands. Mammals have three bones n themidleear.Referringt Table 11.1, postulate whether or not thre is a volutionary advantage in these characteristics over oher chordates.

Humans are primates

Humans have features in common with certain animals. Humans have obvious similarities with apes, monkeys and an even broader group, the order Primata, or **primates**. These similarities inspired Charles Darwin to study primates in the later part of his career. His conclusions about human evolution were publicly ridiculed at the time but are supported today by the evidence collected by modern science.

The primates include lemurs, lorises, tarsiers, New World monkeys, Old World monkeys and apes, as well as modern humans (Figure 11.3). They all have features that unite them as a group.

Features that characterise primates

Many features distinguish primates from other mammals. Primates have hands and feet that bear five digits that include an innermost, opposable digit (Figure 11.4). These are the thumb of the hand and the **hallux** (enlarged toe) of the foot. The hands and feet curl – they are **prehensile**. The tips of the digits have sensitive touch receptors. In contrast to non-primates, the digits typically have flattened nails instead of claws. These adaptations enable primates to grasp, climb and manipulate objects.

Figure 11.3 Representative primates: **a** a crowned lemur; **b** a pygmy loris; **c** a Siau Island tarsier; **d** a Colombian night monkey (a New World monkey); **e** a mandrill (an Old World monkey); and **f** a western gorilla (an ape).

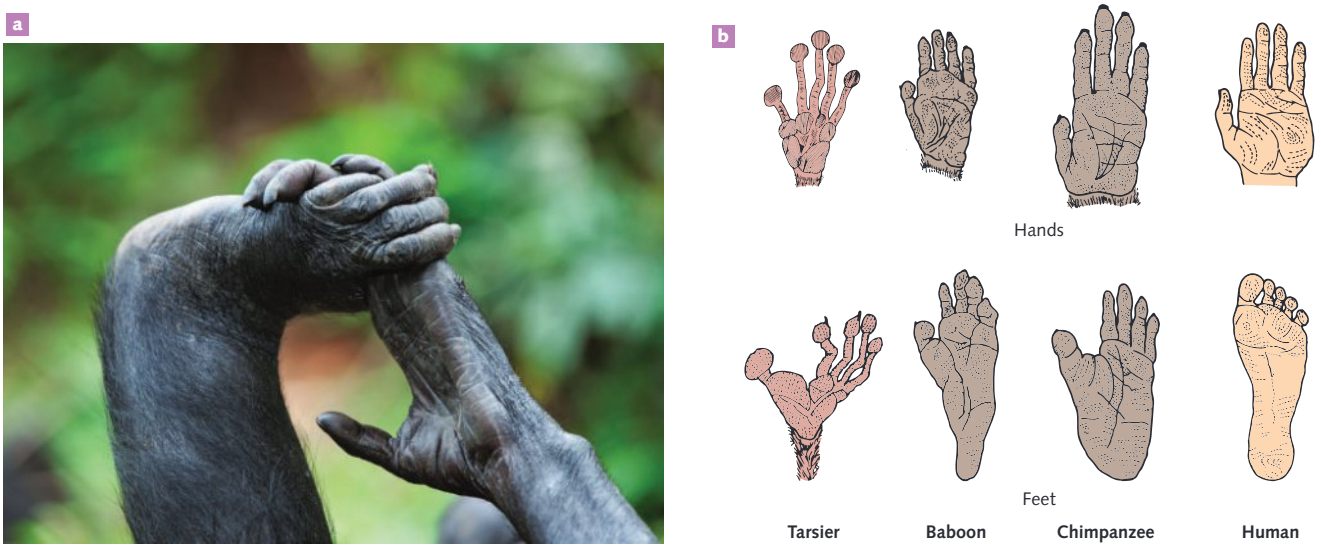


Figure 11.4 a A chimpanzee has prehensile hands and feet. **b** Hands and feet of four primates. Note that only the human lacks an opposable hallux (big toe).

Primates depend heavily on their sense of vision, for which they have distinctive features. They have forward-facing eyes, which enable **stereoscopic** (3D) vision. This is probably an adaptation for an **arboreal** lifestyle – most primates live in trees. Animals that leap from tree to tree must be able to judge distances. Most primates also have retinas that contain many cone cells (colour photoreceptors), enabling them to see in colour.

Primates tend to have small snouts because they are less reliant on a sense of smell than many other mammals. To accommodate the reduced snout, primates have fewer teeth than other mammals. However, the precise number, structure and arrangement of teeth varies.

Compared with other mammals, primates have a relatively large **cranium** for their body weight. This is due to the expansion of the **brain case**. The outer region of the brain, the **cerebral cortex**, has expanded the most during primate evolution. The cerebral cortex is responsible for the higher functions of the brain, such as processing visual and tactile sensory information, as well as memory and reasoning. It has been proposed that the enlarged cerebral cortex is an adaptation to life in the treetops, which depends heavily on processing visual and tactile input and coordinating it with responses from the body's muscles.

Primates have flexible spines and have considerable rotation about the hips and shoulders, enabling them to move in various ways. Their modes of locomotion include climbing, **brachiation** (swinging through the air between branches), **suspensory locomotion** (using all fours to amble while hanging from a branch), **quadrupedalism** (moving on all four limbs) and **bipedalism** (moving on the hind limbs) (Figure 11.5). Primates tend to shift their body weight onto their hind limbs, whereas other terrestrial mammals shift their body weight onto the forelimbs (e.g. dogs and horses).

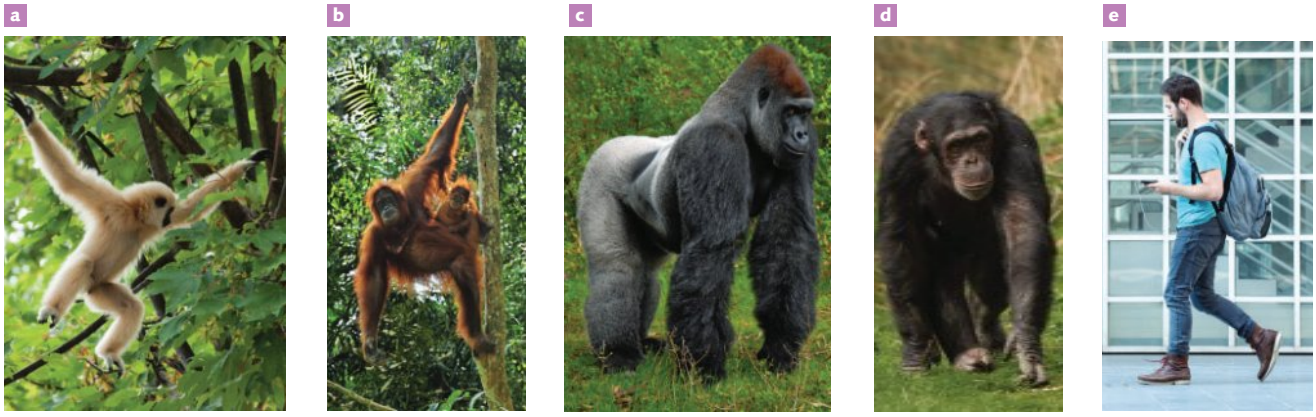


Figure 11.5 Representative hominoids displaying their chief mode of locomotion. a A Lar gibbon brachiating; b a Bornean orangutan moving by suspensory locomotion; c a western gorilla knuckle-walking (quadrupedal locomotion); d a chimpanzee knuckle-walking; e a human walking upright (bipedal locomotion).

Primates also have intangible characteristics that are not necessarily useful for classification. For example, many primates are social animals, often living in extended family and social groups. Most give birth to a single offspring at a time, and the juveniles need relatively intense care for an extended period.

It is within the context of these features that modern humans evolved.

Classifying primates

The Primata is divided into two suborders: the Strepsirrhini or 'wet-nosed primates' and the Haplorrhini or 'dry-nosed primates' (Figure 11.6). The Strepsirrhini comprises lemurs and lorises. The Haplorrhini includes all the other primates. This division has not always been clear-cut and the position of the tarsiers is still being debated. Traditionally, tarsiers were considered more closely related to lemurs and lorises based on presumably shared morphological features. The modern classification reflects DNA sequence data. DNA sequence data tends to suggest that tarsiers represent the earliest split within the Haplorrhini.

Humans are classified within the suborder Haplorrhini, along with monkeys, apes and tarsiers, which are regarded as a distant relation within the suborder.

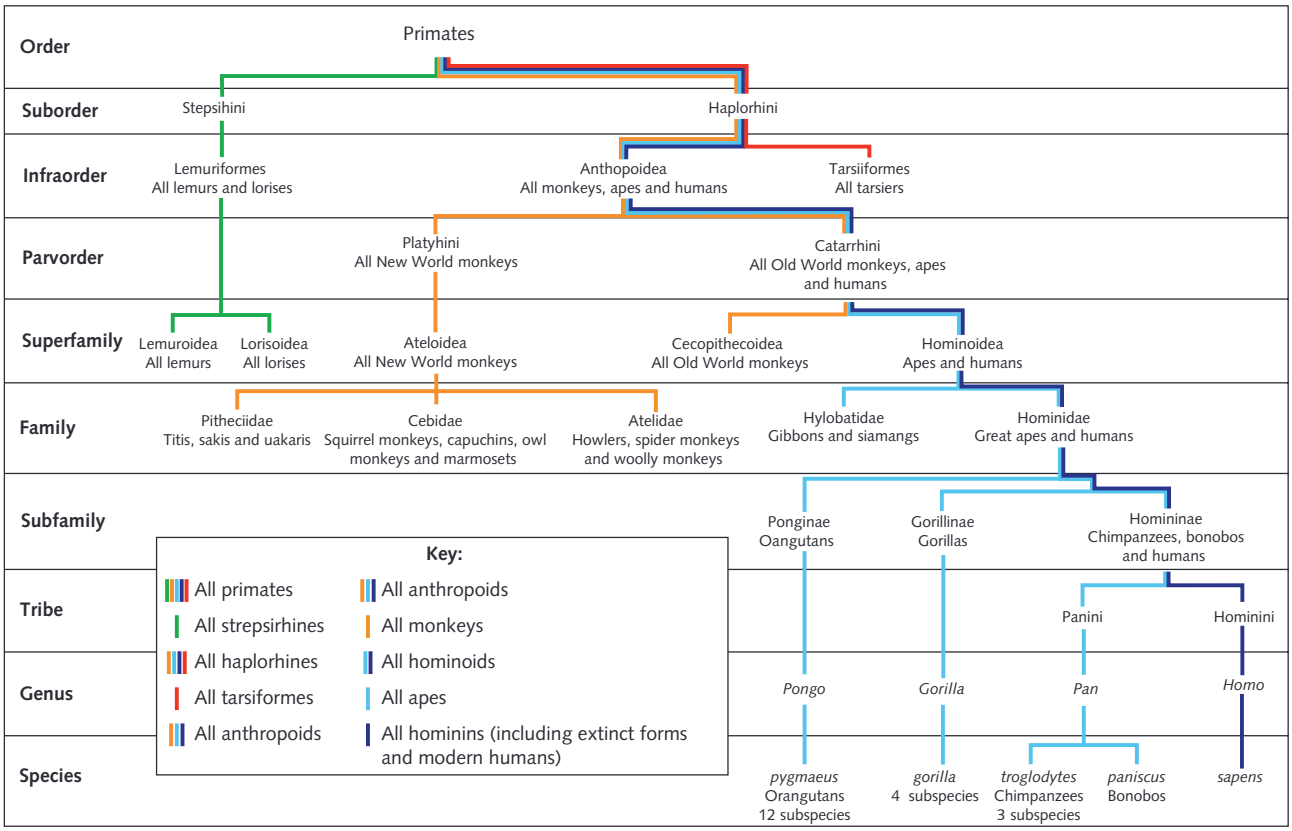


Figure 11.6 Taxonomy for the primates superimposed on a cladogram showing the position of modern humans. Additional levels of classification are introduced (e.g. superfamily, tribe) to better represent the evolutionary relationships within the order.

Classifying monkeys

Monkeys are divided into Old World monkeys and New World monkeys. These common names refer to the continents the monkeys inhabit. Historically, the 'Old World' refers to Europe, Africa and Asia, whereas the 'New World' refers to the Americas. The geographical isolation imposed by continental separation shaped the evolution of the monkeys on each continent and they now have discernibly different anatomy, physiology and behaviour.

The fundamental feature that distinguishes monkeys is their nostrils. New World monkeys have compact noses with nostrils that angle sideways ('platyrrhine'). Old World monkeys, as well as apes, have expanded noses with downward-facing nostrils ('catarrhine', Figure 11.3e). New World monkeys are thus classified in the taxonomic group of Platyrrhini, and Old World monkeys are classified in the Catarrhini (Figure 11.6).

Additional differences relate to the number and pattern of premolar teeth, the form of the bony ring in the ear drum and the type of tail.

On the basis of anatomy, morphology, physiology, biogeography and DNA sequence data, the Old World monkeys are considered most closely related to the apes. Therefore, apes are classified with Old World monkeys in the Catarrhini (Figure 11.6).

KEY CONCEPTS

- Modern humansare classi fied in the orde Primata (primate) within the subordratarrhini.
- Key charatristcs o rimates include opposable dits, steeoscopiccolouvision, ail on the digits, and a eltivly enlaed brain.

Concept questions 11.1b

1 Define prehee'.
2 What are our different moes of prmate locomotion?
3 How are the key caacterstic of primates relevant to organisms tatlive high in the treetops?
4 What is the fudamenal natomical difference between Old World monkey and New World monkeys?

HOT Chaenge

5 Whatevolutionary avantage might stereoscopic vson confer on pimates?

Humans are hominoids

Humans are **hominoids**, which are classified within the **superfamily** Hominoidea (Figure 11.6). The superfamily is a taxonomic rank immediately above the traditional rank of family. The hominoids include the gibbons, orangutans, gorillas, chimpanzees, bonobos and humans. Hominoids are fundamentally apes. They are distinguished from monkeys by lacking a tail.

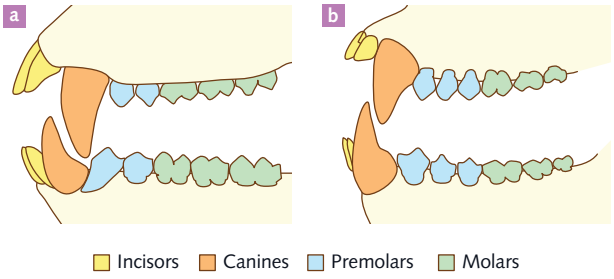


Figure 11.7 The dentition of a hominoids and Old World monkeys and b New World monkeys

Hominoid dentition

The **dentition** (teeth arrangement) of hominoids has similar features to that of Old World monkeys but differs from those of other primates. For example, adult hominoids and Old World monkeys have eight premolars, whereas New World monkeys have 12 (Figure 11.7). The molars of hominoids and Old World monkeys have five cusps on their molars with a Y-shaped upper valley. By contrast, New World monkeys have four cusps with a cross-shaped valley (Figure 11.8). These observations support a closer evolutionary relationship between Old World monkeys and hominoids.

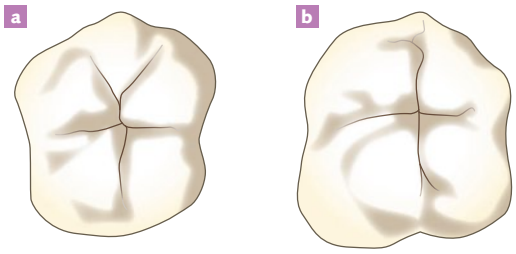


Figure 11.8 a Molars of hominoids and Old World monkeys have five cusps. b Molars of New World monkeys have four cusps.

Hominoid posture

The rib cage in hominoids is flattened compared to that in monkeys, which gives hominoids a broader chest. In addition, for their body length, hominoids have a comparatively shorter spine between the rib cage and the pelvis. It is inferred that these features enable hominoids to sit comfortably with an upright posture.

The relative length of the forelimbs and hind limbs varies among primates depending on the animal's lifestyle and mode of locomotion. Most hominoids, except humans, have longer forelimbs (arms) than hind limbs (legs). Most hominoids have flexible shoulder and elbow joints that allow a range of locomotion styles (Figure 11.5, p. 429). The shoulder blades of hominoids sit further back than in other primates. This allows hominoids to move their arms freely around the shoulders. To maintain their balance when moving, hominoids display an 'orthograde' form of locomotion. The limbs on the same side of the body move in opposition to each other so that the forelimb swings back as the hind limb swings forward.

By contrast, monkeys have relatively narrow rib cages and comparatively longer spines for their body length. The forelimbs and hind limbs of most monkeys are approximately equal in length, adapting them for quadrupedalism on tree limbs or on the ground. Monkeys are highly dependent on their tails to control their balance. Consequently, when monkeys 'walk' on their hind legs, their forelimbs tend to hang loosely. Some monkeys, such as New World monkeys of the family Cebidae, have a prehensile tail that they use for gripping, swinging and climbing.

Humans are great apes

Hominoids are divided into the lesser apes and the great apes (Figure 11.6). The lesser apes (family Hylobatidae) comprise the gibbons, and great apes (family Hominidae) comprise the orangutans, gorillas, chimpanzees, bonobos and humans.

The division between the lesser apes and the great apes reduces to a key difference in the anatomy of the wrist bones. The wrist of lesser apes (gibbons) has a ball-and-socket joint, which permits the wrist to swivel and facilitates highly agile brachiation. Gibbons move by swinging briskly between tree branches (Figure 11.5a), and they can freely reverse their direction by pivoting on one wrist while in motion.

By contrast, great apes, including humans, have a gliding wrist joint. The joint moves up and down with limited twisting movement. Great apes do not practise brachiation in the same sense as gibbons do. Great apes more commonly use suspensory climbing and knuckle walking. They can also stand and walk on their hind legs to a limited extent. Humans are exceptional among the great apes by being exclusively bipedal.

EXAM TIP
Remember the key features that identify hominoids (absence of a tail) and hominins (bipedalism).

Humans are hominins

A **hominin** is any organism classified in the taxonomic tribe Hominini (Figure 11.6). The **tribe** occupies a taxonomic position between family and genus. The defining feature of hominins is that they are principally bipedal and walk on their hind limbs (Table 11.2). Humans are uniquely classified as hominins for this reason; however, they also have many features that distinguish them from other apes (Section 11.2, p. 433).

Table 11.2 Summary of defining features for taxonomic groups within which *Homo sapens* is classified

Group	Defining features
Primates (Primata)	Hands and feet with five digits that include an innermost, opposable digit Forward-facing eyes enabling stereoscopic colour vision Enlarged cranium relative to body weight Flexible spine with considerable rotation about the hips and shoulders
Hominoids (apes – Hominoidea)	Distinguished from monkeys by lacking a tail Dentition includes eight premolar teeth Molars with five cusps and grooves in a Y-shaped pattern Broad, flattened rib cage Arms generally longer than legs (except for humans)
Great apes (Hominidae)	Gliding wrist joint with limited twisting movement
Hominins (Hominini)	Bipedal mode of locomotion

Delineating the human lineage

Within the Hominini, modern humans are classified in the genus *Homo* as the species *sapiens* (Figure 11.6). *Homo sapiens* is Latin for 'wise man'.

The scientific name was introduced by scientist Carl Linnaeus in 1758 and highlights a bias of reasoning that confounded taxonomy for centuries. Humans seemed so different from other great apes that they were placed in a separate taxonomic family. During the second half of the 20th century, the taxonomy was progressively amended. Humans were grouped together with the other great apes in a single family. By the mid-1980s, orangutans were regarded as the earliest divergence of the great ape lineage, based on morphology and molecular evidence. However, there was much debate about which two were more closely related among gorillas, chimpanzees and humans. As a compromise, for a time the three were regarded as equally related.

In the late 1980s, a clearer picture of the phylogeny of humans emerged with the first analysis of genetic data. This data showed that the gorilla lineage split first and that the chimpanzee and human lineages diverged later (Figures 11.6 and 11.9). This implies that humans and chimpanzees are the most closely related great apes. This phylogeny is now strongly supported by molecular homology of many gene sequences, as well as whole-genome sequences. Estimates for how long ago the human, chimpanzee and gorilla lineages diverged have been drawn from molecular clock estimates. These are derived from the predicted rate of mutation in a gene sequence. Molecular clock estimates tend to be controversial because they vary with the molecular data, statistical methods and calibration systems used. However, recent molecular clock estimates based on whole-genome analyses (Figure 11.9) indicate that the human and chimpanzee lineages diverged about 7.5 mya. The gorilla lineage is estimated to have diverged about 10 mya. These estimates may be refined in the future.

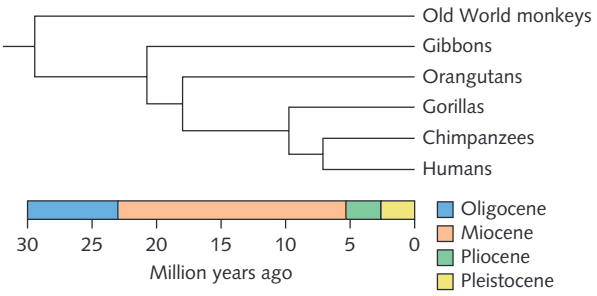


Figure 11.9 **A phylogenetic tree depicting molecular clock estimates for divergences between major hominoid lineages**

CONNECT
Refer to Chapter 10 for a discussion about molecular clock estimates.

KEY CONCEPTS

- In contrast to other pimae, hminod lack tails, have eight pemolars rater than 2, and molars with five cusps rather thn four.
- Hminins ar unquely bpedal.
- Cimpanzees re theliving species that are most cloely related t humans.

Concept questions 11.1c

1 Explain he difference betwen a hominoid and a homii.
2 How may livi homnin species are there?
3 Ouline te evidence that sugests hominoids are moreclosely relatd toOld World monkeys than to New World mnkeys.
4 Ouline te evidence that sggests chimpanzees and humans are the mst clsely relate great apes.

HOT Chaenge

5 A palaeoanthopologist excavate rimate mandible. He observe that it hd six premolar teeth and the mlars each had four usps.
   a Has he iscovered a hom noid? Provideevidence to support your answer?
   b f he excavated the res the skeleton, what other anatmicalfeature might he find thatwould ndcae tis, r is ot, hminoid?

11.2 Adaptations that define humans

Humans have the same basic characteristics as apes, which places them in the hominoid superfamily. Humans also have features that distinguish them from other hominoids, such as the anatomical features that allow humans to stand upright and walk with a fully striding gait. These fundamentally define humans as hominins. Humans are also relatively hairless, have a greatly expanded brain, and have modified teeth and jaws. Furthermore, humans behave very differently from other apes. They communicate with speech, display advanced intellectual abilities, apply sophisticated technology, and practise elaborate symbolism.

Adaptations for bipedalism

Most hominoids are fundamentally quadrupedal – they use all four limbs for locomotion. Humans' closest living relatives, the gorillas and chimpanzees, mainly knuckle-walk (Figure 11.5c and d).

WB
11.2.1 ADAPTATIONS FOR BIPEDALISM PAGE 233

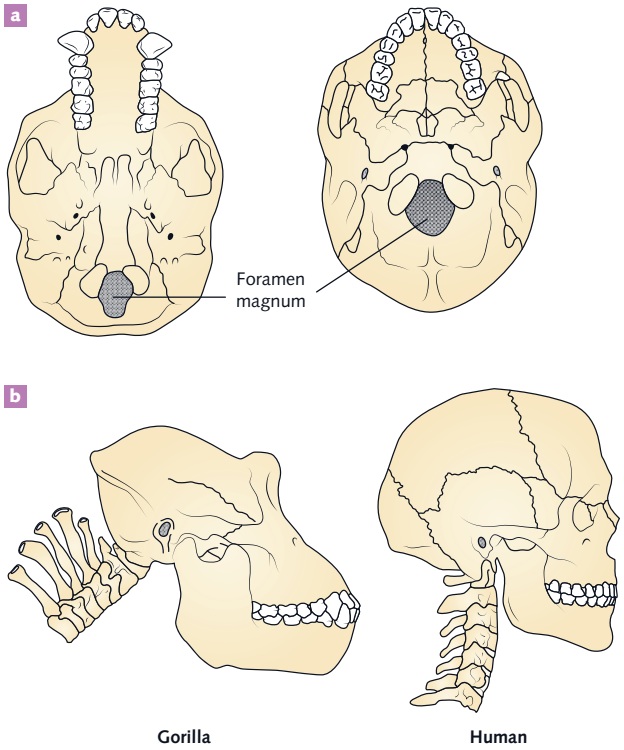


Figure 11.10 a The position of the foramen magnum (shaded) in the gorilla and the human. **b** The corresponding position of the spine and skull in each.

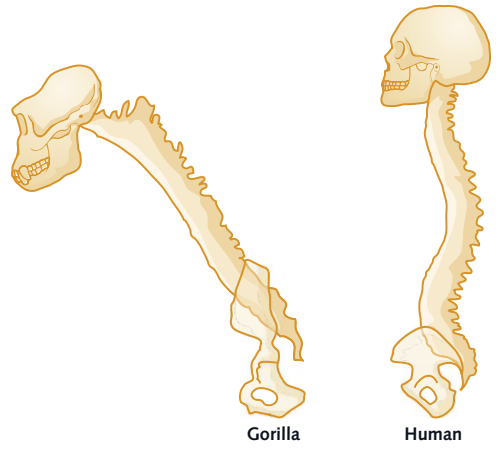


Figure 11.11 A gorilla's spine curves forward, whereas a human's spine is an S shape.

Humans are distinctly different because they stand upright and walk on their hind limbs. They are bipedal (Figure 11.5e). The shift to bipedalism has been accommodated by changes throughout the skeleton. No other animal is as adept as humans at full striding bipedal locomotion. From head to toes, the human body is configured for bipedalism.

Position of foramen magnum

In vertebrate animals, the spinal cord feeds through a hole in the skull called the **foramen magnum** to connect with the brain. The position of the foramen magnum in the base of the skull varies according to how the animal moves. In quadrupedal animals, such as gorillas and chimpanzees, the foramen magnum is towards the back of the cranium (Figure 11.10). This is because the spine is almost horizontal where the spinal cord enters the skull. In the fully bipedal human, the foramen magnum is positioned more centrally at the base of the skull (Figure 11.10). This permits the head to face forward comfortably while resting almost vertically over the spinal cord when upright.

Curvature of spinal column

The posture of modern humans contrasts with that of other apes. In apes such as gorillas and chimpanzees, the spine curves forward (Figure 11.11). Their body weight is evenly distributed by support from the forelimbs during quadrupedal locomotion (Figure 11.5c and d). However, the curve of the human spine follows an S-bend to support the weight vertically (Figure 11.11). The vertebrae in the lumbar (lower back) region of humans are wedge-shaped and pack together with the thin edges pointing backwards. This causes the lower spinal column to adopt a convex (forward) curve.

Pelvis

The lower spinal column connects with the pelvis. In most apes, the pelvis is relatively long and narrow (Figure 11.12a). However, in humans the pelvis is comparatively shallow and bowl-shaped (Figure 11.12b). This shape creates a basin that sustains the weight of the abdomen and provides support for the upper body. The broader hip bones also provide expanded attachment sites for the buttock muscles. The relatively enlarged buttock muscles of humans extend the leg and help steady the pelvis and upper body during walking.

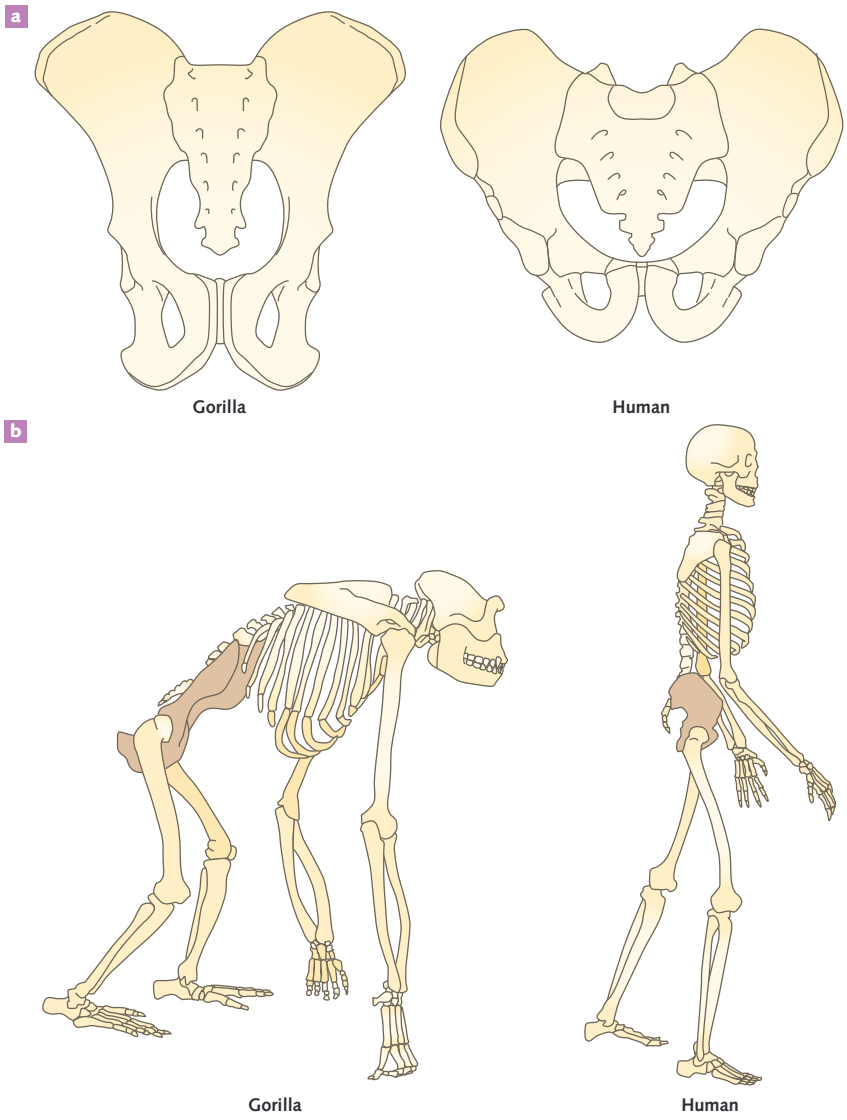


Figure 11.12 a A comparison of the pelvises of the gorilla and human. **b** An illustration of the position and orientation of the pelvis during locomotion in gorillas and humans.

Carrying angle

In humans, the flaring of the pelvis aligns the hip joints directly beneath the head and torso. Therefore, the weight of the upper body is transferred via the pelvis to the legs. However, the broad pelvis spreads the top of the femurs (thigh bones) away from the midline of the body. This is potentially destabilising. The body would have to sway from side to side during walking so one leg could support the body's weight. For this reason, the femurs are angled in towards the knees.

This **carrying angle** relative to the vertical is evident when viewed front-on (Figure 11.13). The angle ensures one knee and foot are directly beneath the body while the other knee and foot are lifted during walking. It allows the body to rotate about the lower leg and foot, and one foot to be set ahead of the other when striding. Orthograde movement of the arms compensates for body rotation. The knees are strengthened to support the weight at the lower end of the femur. This arrangement allows humans to extend the leg fully during walking. The longer legs of humans relative to other great apes increases the length of the stride overall.

WB
11.2.2
EVOLUTION OF TWO-LEGGED WALKING
PAGE 233

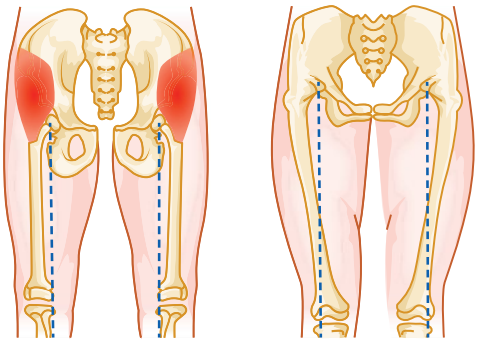


Figure 11.13 A comparison of the pelvis and femur of chimpanzees and humans, showing how the femur of humans is angled inwards towards the knee. The dotted line shows the direction weight is transmitted.

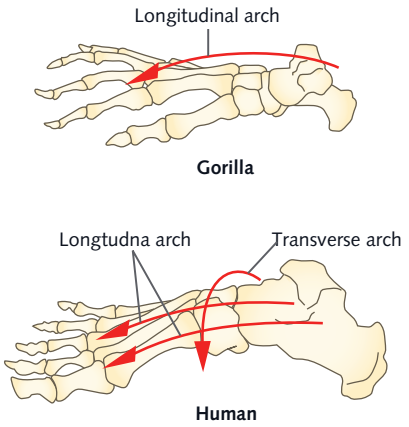


Figure 11.14 A comparison of the arches of a gorilla foot and a human foot

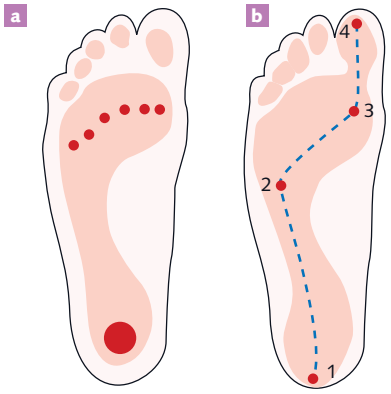


Figure 11.15 How body weight is distributed across the human foot when a standing and b walking. When walking, the body weight shifts in sequence from position 1 (heel strike) to position 4 (the big toe thrusts off).

Feet

Unlike other apes, humans use their feet mainly for propulsion rather than for grasping or climbing. As a result, the human foot has lost its prehensile capacity and the hallux aligns alongside the other toes. In addition, the human foot has a comparatively wide heel (Figure 11.14) that serves as a shock absorber upon heel strike. Apes' feet have a single longitudinal arch that runs from the back to the front of the foot. By contrast, human feet have two arches, one longitudinal and one transverse, which cross over the foot (Figure 11.14). When a human is standing erect, the foot acts as a pedestal to support the body's weight (Figure 11.15). When the human foot presses to the ground during walking, weight is transferred progressively forward and across the foot via the arches (Figure 11.15). With the weight transmitted to the toes, the big toe pushes off to launch the next step.

KEY CONCEPTS

» Many features of the uma kleton, incuding the skull, sp ne, vis, femurs and feet, have a range of daptations for bipdalism.

Concept questions 11.2a

1 Describe the foramen magnm,its function, and what ts pstionindicates abou hminoids locomotion.
2 How does the shape of hehuman spine differ from that of other aes? Why is this the case?
3 What advatage s there in the shape of the human pelvis?
4 Define caryiane'.Epain why it is different in humans and otherapes.
5 What features does the human foot hve that adapt it to wking rather hn climbing?

HOT Chaenge

6 Exmine te ollowig two ideas.
- n the 1980s, Rodman ad McHeny, of University of Clifoia, Los ngeles, USA, suggested that homiids eolve to walkupright in response to cimate chnge.
- n 200, researchrs studying chimpanzees deterined that they required 75% more energy whle walking than two-lgged humans.

Whattrait of modern humans dothese two ideas support?

Human hands

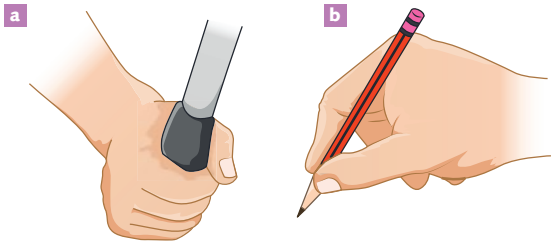


Figure 11.16 A comparison of a the power grip and b the precision grip

Bipedalism means human forelimbs do not have to carry the body's weight. Instead, hands have become adapted for manipulation. Compared with other primates, human hands are short and broad, with relatively short, straight fingers and a long, strong thumb. These adaptations allow the human thumb a substantial amount of freedom and the ability to extend to each of the fingertips. The degree of contact between the thumb and forefinger allows humans to grip and manoeuvre small or delicate objects, such as a needle for sewing or a pencil for writing (Figure 11.16). It is the basis of the **precision grip**, which enables humans to grasp and manipulate objects with exquisite dexterity.

Expansion of cranium

Humans behave very differently from other apes. Humans are very inventive and technologically sophisticated. Humans have an unparalleled aptitude for manipulating and interpreting symbols. They are capable of complex **language**. They indulge in personal ornamentation, art and music. They establish extensive communities and observe societal conventions.

Compared with other apes, humans have an advanced **cognitive capacity**. Cognitive capacity describes an organism's innate intelligence, ability to learn, plan, evaluate, make decisions, and apply new knowledge and skills. Human cognitive capacity is largely due to their relatively big brain (Figure 11.17). Brain volumes of most apes vary between about 350 and 500 cm^3. The volume of human brains varies between 900 and 2200 cm^3 but the average is about 1350 cm^3. Most of the enlargement is associated with expansion of the cerebral cortex, the outermost region of the brain. The surface area of the primate cerebral cortex is further increased by extensive folding, called 'convolutions'. Convolutions are not random. Rather, they form specific patterns in the cerebral cortex of different species. Relative to brain size, the human cerebral cortex is estimated to have about 40% greater surface area than the chimpanzee cerebral cortex.

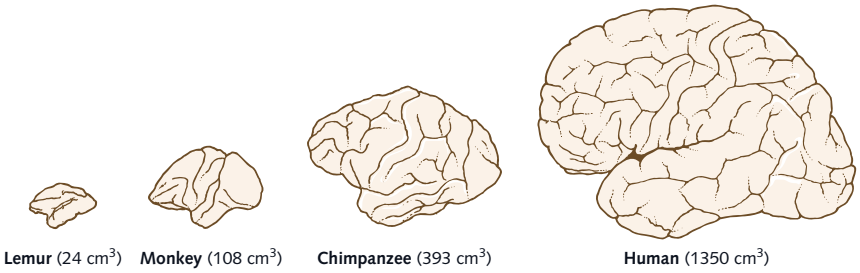


Figure 11.17 The brain sizes of various primates (drawn to scale)

There are also differences in how the brain operates. The cerebral cortex is associated with the executive functions of the brain, including reasoning, planning and judgement. The **prefrontal cortex**, which covers the front part of the brain, is the portion of cerebral cortex that has undergone the greatest expansion. The human prefrontal cortex is about six times larger than that of other apes. The prefrontal cortex governs a variety of functions, including abstract thinking, analysis of conflicting outcomes, and planning and strategising. It is also associated with complex social behaviours, such as impulse control and ethical choices.

The human cerebral cortex consists of more than 50 distinct regions based on cellular staining patterns. The difference between the human and chimpanzee genomes seems small, approximately 1% of total gene sequences. However, it is likely that changes to the genome have affected genes that affect brain development, anatomy and function.

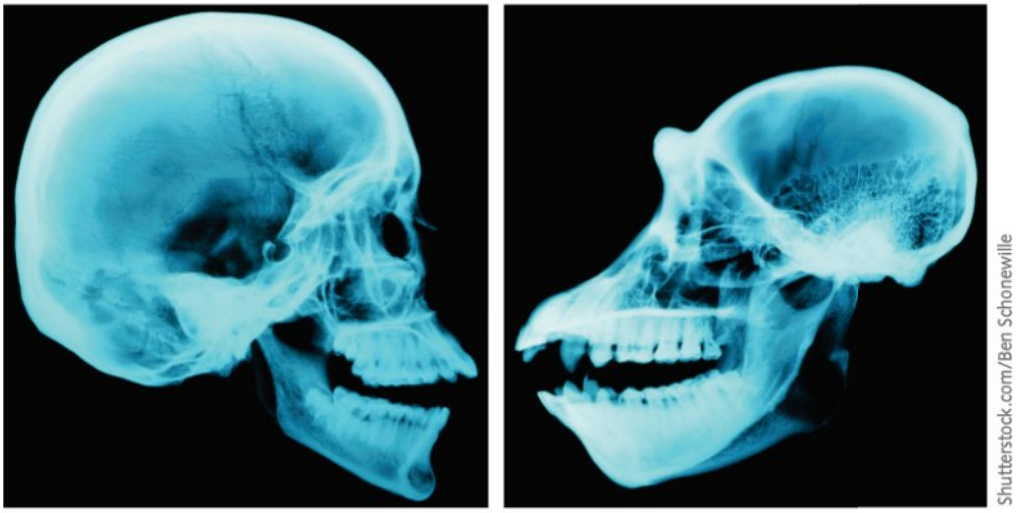


Figure 11.18 Profile images of human and chimpanzee skulls showing differences in cranial capacity, brow ridges, jaws and teeth.

Modification of skull

To adapt to the extraordinary change in brain size, the human cranium has substantially changed during evolution (Figure 11.18).

An enlarged brain case accommodates the brain. **Cranial capacity** is a measure of the volume of the brain case. The shape of the cranium in humans is also altered. In the skulls of most apes, the forehead is sloped back. The **brow ridges** (the bony ridges above the eye sockets) are typically prominent. However, in humans the front of the cranium is higher and more rounded, and the brow ridges are significantly reduced. These changes accommodate the enlarged prefrontal cortex. The effect is the distinctively raised forehead of humans.

Chimpanzees and other apes have protruding jaws (Figure 11.18), a condition known as **prognathism**. In humans, the jaw does not protrude so far. Corresponding to the difference is the shape of the lower jaw, or **mandible**. The chimpanzee mandible has an extended rectangular shape, whereas the human has a shallower, parabolic mandible (Figure 11.19). Most apes have prominent, interlocking canine teeth, whereas human canines are reduced so that they appear similar to the incisors (Figure 11.19). It is proposed that the difference in canines relates to differences in behaviour rather than diet. The canine teeth are most conspicuous in male apes. Male apes exhibit their canines in competitive displays so as to avoid violent aggression.

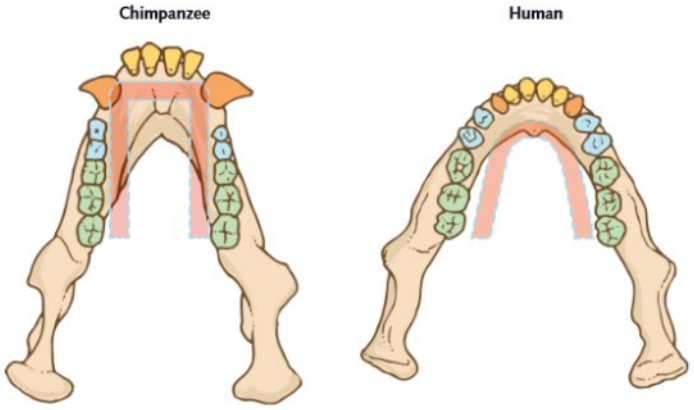


Figure 11.19 The mandible of a chimpanzee and a human. The pink shapes highlight the rectangular shape of the chimpanzee mandible and the parabolic shape of the human mandible.

Overall, the changes to the cranium and jaws have flattened the human face. The chin and prominent nose are distinctly human characteristics.

Table 11.3 summarises the anatomical features that distinguish humans from other hominoids.

Table 11.3 Summary of anatomical features that distinguish *Homo sapens* from other hominoids (apes)

Feature	*Homo sapiens*	Other hominoids
Body covering	Relatively hairless	Relatively hairy
Mode of locomotion	Bipedal	Quadrupedal
Position of foramen magnum	Closer to the centre of the base of the skull	Closer to the rear of the skull
Spinal curvature	S-bend with convex curve near the base of the spine	C-shape forward curvature
Pelvis	Shallow and bowl-shaped	Long and narrow
Carrying angle of femur	Relatively high	Relatively low
Feet arches	One longitudinal and one transverse	One longitudinal only
Hallux	Not prehensile	Prehensile
Hand	Thumb long compared to fingers for precision grip	Thumb short compared to fingers
Cranial capacity	Relatively large	Relatively small
Brain	Expanded prefrontal cortex, many more convolutions	Smaller prefrontal cortex, fewer convolutions
Brow ridges	Subtle or absent	Prominent
Prognathism (jaw protrusion)	Subtle	Substantial
Mandible shape	Parabolic	Rectangular
Canines	Reduced	Enlarged

KEY CONCEPTS

» Humans have recsion grip hat nables manual dexterty.

» The anatomy oth bain, ncluding the enlarged prefrontal corte and increasdconvolutions, enhances the cogitive apacity of humans.

Concept questions 11.2b

1 Describe how the anatomy of the human hand differs from that of oher aps, and how it adapts the human hand for a precisiongip.
2 Define cognitiv apacity'.
3 Describe three ways he uman brain evolved to becom different from those ofother apes.
4 Describe how the shape of theodern human skull dffers from that of ther apes, and how these changes accommodate the huan brain.

HOT Chaenge

5 Why are modern humn canine reduced in size when compared wt similar structuesin other hominoids?

Communication, technology and culture

WB
11.2.3
HUMAN INGENUITY
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The most significant characteristics that result from human evolution are bipedalism and the expansion and development of the brain. As a consequence of bipedalism, the hands are also freed for fine manipulation. The result is an organism that combines an enhanced capacity to imagine and plan with the manual acuity to modify items and reshape its environment. These are the basic biological ingredients for developing tools and utilising technology.

Humans are social creatures, like many other primates. However, humans have a unique ability to communicate abstract ideas in detail. This has ensured that knowledge and ideas are transmitted freely between individuals. It is also the foundation for extensive cooperation, which accelerates the pace and scale of innovation. In this section, you will explore some of the behavioural adaptations that distinguish humans from other primates.

Figure 11.20 These common chimpanzees (*Pan trogodytes*) are using sticks to 'fish' for termites.

Language: a mechanism for innovation

Humans are not the only animals to use tools. For example, chimpanzees use a kind of 'toolkit' for capturing termites (Figure 11.20). They use one stick with brush-like leaves to clear the entrance leading into the termite mound. They use a second stick deliberately stripped of leaves for 'fishing' the termites by inserting it into the hole. When the stick is withdrawn, it is covered in termites, which the chimpanzees lick off. Juvenile chimpanzees learn these techniques by watching and imitating the adults.

Humans also learn by imitating others. However, humans have a unique capacity to communicate far more complex and abstract ideas. Humans have the astounding ability to vocalise thousands of sounds, to attach meaning to each, and to reorganise them in expressive new sequences. Humans have the anatomy and neural wiring to coordinate the lungs, mouth, throat and nasal organs to make talking possible. Language provides a functional framework for human speech. As the utterings of infants demonstrate, humans are genetically predisposed to learn vocabulary and to order words according to strict grammatical rules. The language learned by any individual human is influenced by their environment. So if Chinese parents in China adopt a native-born German baby, the infant learns Chinese as his or her principal language.

Humans can write. They record symbols to represent spoken language. Writing allows humans to formalise, store and reference abstract ideas. The ability to manipulate and interpret symbols also allows humans to express meaning in fields such as music or mathematics (Figure 11.21). Spoken language is immediate. It transmits knowledge directly among people at a particular time. Written language preserves ideas for transmission to wider audiences, between generations, and over extended periods of time (Figure 11.21). For example, Charles Darwin's 19th century publications can still inspire biologists more than a century after his death. The result is a collective knowledge that accumulates over time. New ideas and technology can be developed by people today that are based on those of people who lived in another time and place.

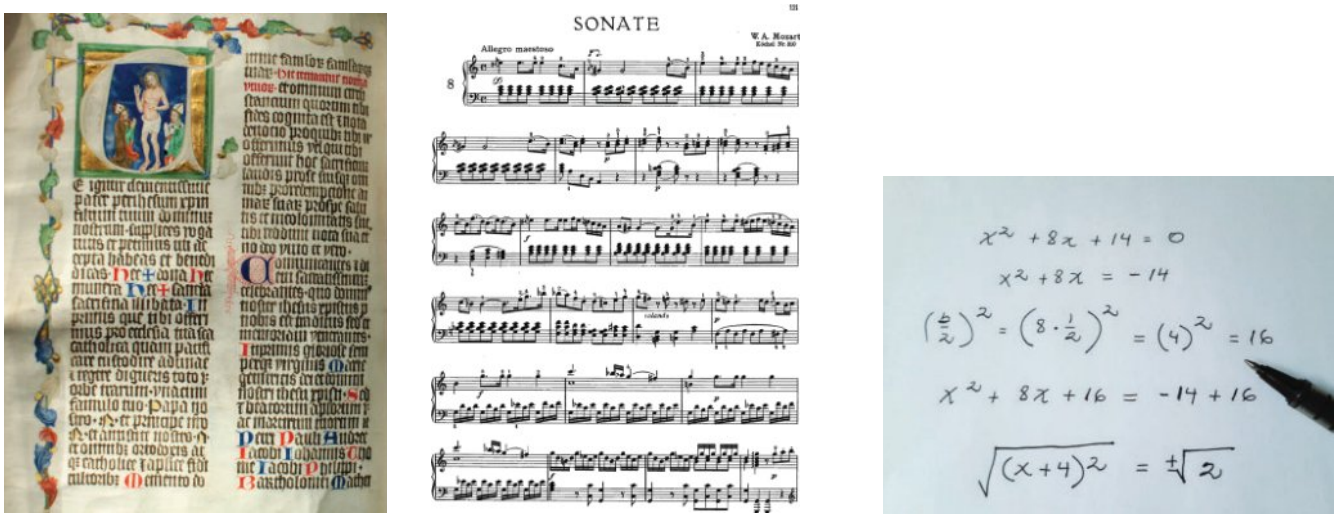


Figure 11.21 Examples of written communication

Other animals, including chimpanzees, can recognise and communicate using sounds or abstract symbols. A famous case is that of Washoe, a common chimpanzee, who learnt to communicate with up to 200 symbols of American Sign Language. Washoe could string up to three signs into short expressions, and she set about teaching her adopted infant chimp the language. Chimpanzees in the wild also produce scores of vocalised sounds to communicate with one another.

The difference between humans and other animals is a matter of scale and sophistication. Vocabulary size varies from one person to the next, but it has been estimated that, by age 4, most humans know about 4000 words. For adults, it is suggested to be between 15 000 and 25 000 words. A standard English dictionary contains more than 170 000 definitions. The size and versatility of human language means we have an exceptional ability to convey abstract ideas. It enables us to describe models for concepts that are beyond our personal experience. The structure of the atom, principles in electromagnetic theory, supermassive black holes and evolution on geological time scales are all examples.

Cultural evolution

Humans' aptitude for communication has enabled knowledge to spread rapidly between individuals, throughout populations and between different populations. It also enabled individuals to organise themselves and work cooperatively in ever larger groups. Our physical, cognitive and communicative characteristics underpin the evolution of human culture. **Cultural evolution** describes the way human beliefs, social practices, skills and technology change over time.

Cultural evolution contrasts with biological evolution in the speed and means by which it is transmitted. The significant differences between cultural and biological evolution are summarised in Table 11.4. Biological characteristics are exclusively transmitted from parent to offspring. It normally takes many generations to observe biological changes in a population. However, culture can be transmitted rapidly between unrelated individuals of the same generation or of different generations. Cultural characteristics can be communicated informally (e.g. spoken word), formally (e.g. education) and even over long distances without the individuals concerned ever meeting each other (e.g. via books or the Internet). Consequently, cultural evolution occurs rapidly within the lifetime of an individual.

Table 11.4 Summary of differences between features of biological and cultural evolution

Feature	Biological	Cultural
Data coding	Genetic	In written, spoken or symbolic language
Transmission of traits	Inherited from parents. No choice in traits acquired	Communicated from unrelated individuals. Taught and learnt. Choice in traits acquired
Generation	From one generation to the next	Within or between generations
Speed of dispersal	Slow. Many generations required to spread trait in population	Fast. Spreads rapidly in population by immediate learning
Intent	None. Unplanned, resulting from random processes	Deliberate, result of conscious action

KEY CONCEPTS

- Written andspoken laguage enables humans to convey abstract oncepts.
- Cultra evolutio isdistingushdfrom biological evltion by th speed, the mens and the choice exerisd in ts diersal.

Concept questions 11.2c

1 Define cultra eoution'.
2 List five way cultua evolutio contrasts with booial evlution.
3 Explain whetherhe followin g representcultral or booial evlution.
   a nventon and subsequent mnatursaton of mobe phones
   b Changes n harstyes n a popuaton
   c ncreaing uptake of the vegan det n a population
   d ncreaing rsistance to maara over many generations
4 How doe wrtten langage contibte to cultural evolution?

HOT Chaenge

5 Gundtjmara country is found around Lake Condah n south-westVictoia. I 1841, the Chief Protector of Abrigines, George Agustus Robinson found an extenive area of cannelspurosely uil by the local ndgenous peole. H surmised that the channels were used for c tchng eels. Shor y after this, other settlers deterined that this area was only a swamp that needed to be drained and usedfo heep grazing. In the 1970s, arhaeologic l surves revealed the actual compleity of the costructions and the ef ficiency of the chaels' peainsThis implied that the chanels were an advanced fish-trapping system that suppied thepeople wih a yar long supply of food. n 201, the area wa recognised a a UNESCO World Heritge site be litdexclsivelyfo its Aboriginal cltra values as one of the finest exmple of ancient aquaclture and hyrauli enineering. Three hundred stone houseshave also bee fund at the site.
s tis an eample o cutral evolution? Justify your response.

11.3 Meet the ancestors

When examining fossils, scientists are often trying to deduce the morphology, lifestyle and behaviour of an extinct organism from a few fragments. To do this, they compare fossils with the skeletal structures of living organisms. Knowing the living organism's appearance and lifestyle allows scientists to infer some things about the appearance and behaviour of the extinct organism.

Hominin classification includes species of fossil great apes that have similarities with modern humans. Therefore, as a taxonomic group, hominins are modern humans and their extinct bipedal ancestors. The hominin fossil record (Figure 11.22) demonstrates that human evolution was not a simple, linear

Video
Four billion years of evolution

Online Worksheet
Myths and misconceptions about evolution

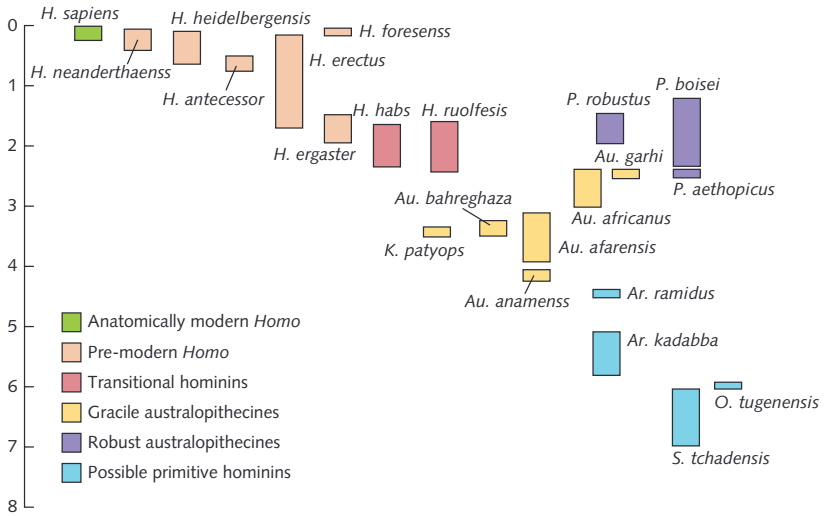


Figure 11.22 Species recognised in the hominin fossil record under the genera *Homo* (H.), *Ardpthecus* (Ar.), *Austraopthecus* (Au.), *Kenyanthropus* (K.), *Orrorn* (O.), *Paranthropus* (P.) and *Saheanthropus* (S.). The rectangles represent an estimate of the periods in the geological record during which the species are presumed to have lived, based on fossil evidence.

progression from one species to the next. Instead, many human species coexisted at one time. Some may have been competitors; others may have occupied different niches. Some species persisted for a long time; others less so.

Palaeoanthropologists draw lines that connect these species into an evolutionary tree. What that tree looks like is inevitably contentious because interpretations vary among scientists. New fossil discoveries occasionally challenge existing hypotheses. However, it is evident that the hominin evolutionary tree has many branches, and all but one of those branches terminates in extinction. Modern humans are the last living legacy of this rich evolutionary history.

In this section we review specific hominins and what they reveal about the course of human evolution. To establish the trends, we will consider fossil hominins mostly in the chronological order of their appearance in the geological record. The oldest of these were recovered in Africa but many more recent ones have been found across Europe and Asia (Figure 11.23). This observation reveals an African origin for the hominins, followed by subsequent migration.

Australopithecines

The fossil record for hominins older than about 4.2 million years is limited. There are fossils of possible hominins that date to about 7 mya (Figure 11.22) but their identity and significance as hominins are frequently debated. The earliest universally accepted hominin fossils, those from which modern humans evolved, are the **australopithecines**. These are a varied group of small, bipedal apes that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya. They were evolving during a time of climatic change accompanied by a shift from forests to wooded grasslands. During that period, the australopithecines flourished and diversified into a number of species. The species are distinguished by their morphology as slender **gracile** forms or stocky **robust** forms. There are five generally recognised gracile species, which are classified in the genus *Australopithecus*. The robust forms are placed in the genus *Paranthropus*, which contains three accepted species (Figure 11.22). Some australopithecines, such as *Australopithecus afarensis*, are widely regarded as direct ancestors of modern humans. A number of others are considered evolutionary dead-ends.

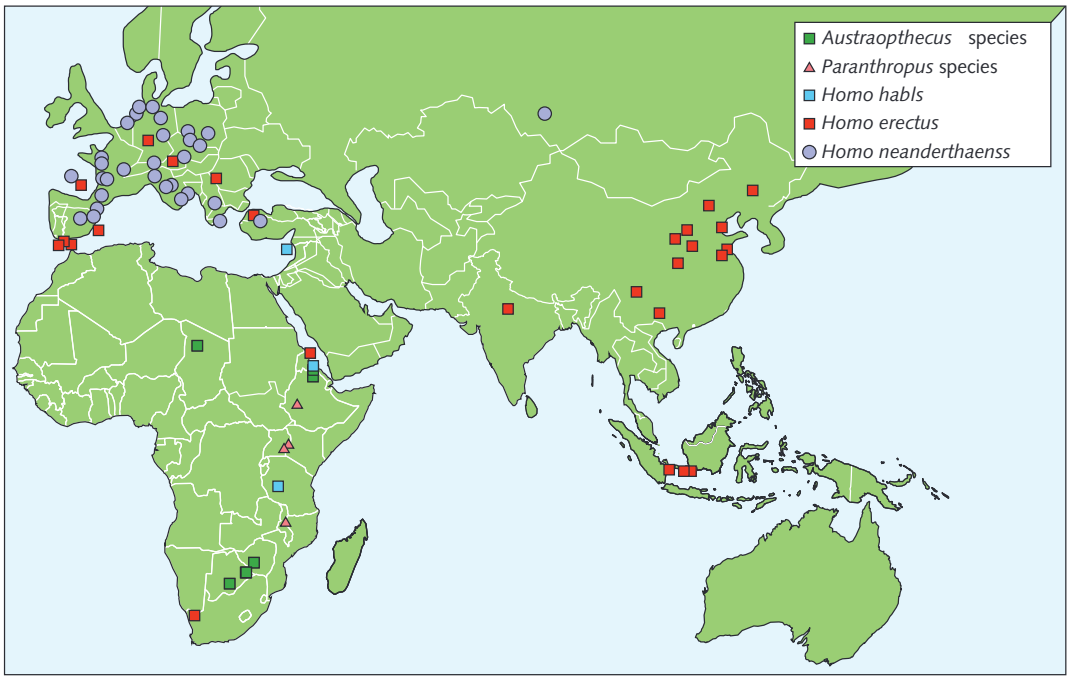


Figure 11.23 Location of major hominin fossil finds

Archetype for genus *Australopithecus: Au. afarensis*

Australopithecus afarensis was discovered by chance in 1974 when paleoanthropologists were surveying potential excavation sites in the Afar region in Ethiopia. The specimen eventually unearthed and reconstructed to 40% completion was nicknamed 'Lucy' (Figure 11.24). Now known from hundreds of fossils collected in eastern Africa, *Au. afarensis* is considered a key australopithecine. The species persisted 3.8–2.9 mya and is believed to be a direct ancestor of modern humans. Like other australopithecines, the species displayed **sexual dimorphism**, with adult males significantly bigger than females. Males bore a **sagittal crest** at the top of the skull. Male gorillas also have a sagittal crest, which provides expanded surface area for the uppermost attachment of their powerful jaw muscles. By implication, the male *Au. afarensis* had a strong bite.

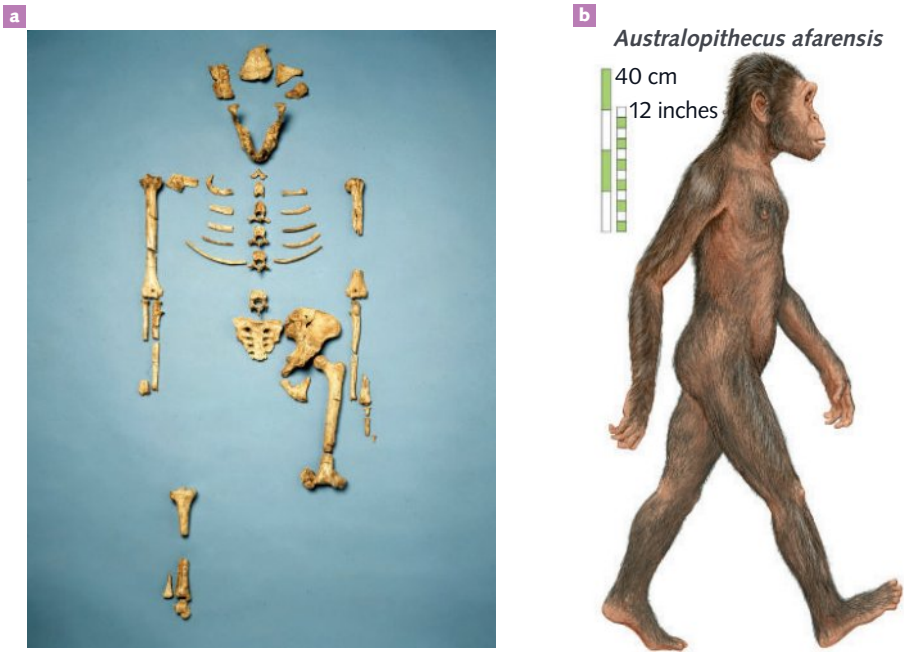


Figure 11.24 ***Austraopthecus afarenss*** **: a the specimen nicknamed 'Lucy'; b an artist's impression of the organism.**

As a model for the genus, *Au. afarensis* clearly showed bipedal features (Figure 11.25). It had a relatively wide and shallow pelvis, femurs angled in towards the knees, strengthened weight-bearing knees, arched feet, wide heels, and a hallux aligned with the other toes. The interpretation was substantiated by a discovery of a trace fossil in 1978 near Laetoli in Tanzania. This was a set of fossilised footprint impressions in a volcanic ash bed laid down some 3.6 mya (Figure 11.26). Attributed to *Au. afarensis*, they show the tracks left by two adults walking one in front of the other and a juvenile walking beside them. These provided direct evidence that *Au. afarensis* was capable of bipedal locomotion. Yet *Au. afarensis* also had relatively long forearms, long curling fingers and toes, and shoulder blades akin to those of other great apes rather than modern humans. These adaptations indicate *Au. afarensis* was a proficient tree climber. The collections of features suggests this species lived in mixed habitats that included forest and grassland, and it both climbed and walked. Microanalysis of fossil teeth indicates its diet consisted mainly of leaves and fruits.

For its body size, *Au. afarensis* had a relatively small cranial capacity (about 430 cm^3). The cranium of its successor, *Au. africanus*, was only marginally larger (about 480 cm^3). In fact, relative to their estimated body masses, the brains of australopithecines were comparable in size to those of modern chimpanzees.

The australopithecines demonstrate that bipedalism preceded expansion of the cranium during hominin evolution.

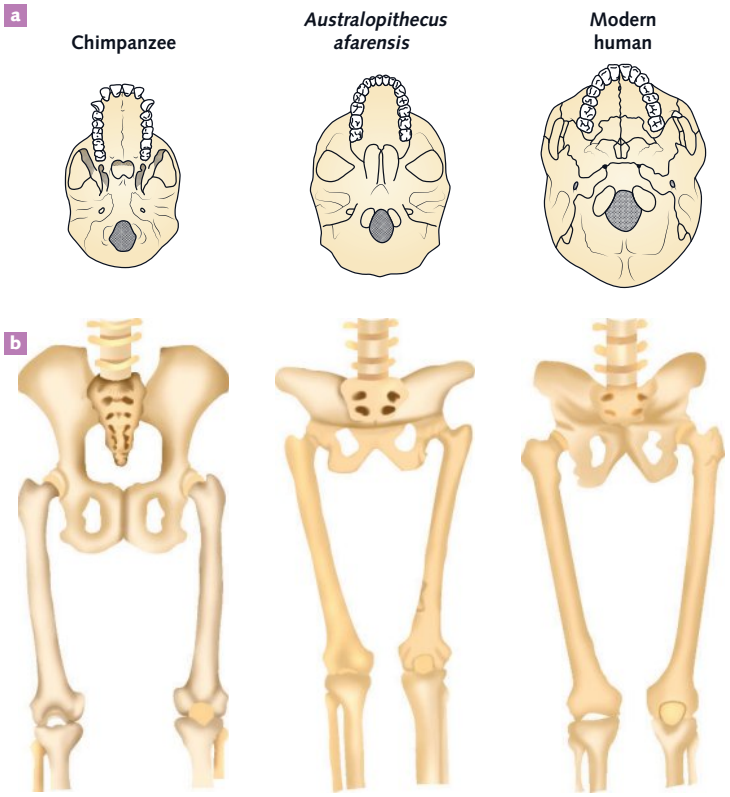


Figure 11.25 *Austraopthecus afarenss* showed bipedal features. **a** The position of the foramen magnum was intermediate between that of the chimpanzee and modern human. **b** It had a bowl-shaped pelvis and carrying angle more like those of the modern human.

Figure 11.26 The Laetoli footprints in Tanzania provide evidence that australopithecines were bipedal.

EXAM TIP
For written responses, make comparative statements when describing trends in human evolution. For example, write 'the foramen magnum is more centrally located than in other hominoids' rather than 'position of the foramen magnum'.

Genus *Paranthropus*

The name *Paranthropus* is derived from two Greek words that mean 'beside human'. The 'robust' australopithecines are classified in *Paranthropus*. 'Robust' in this case refers not only to their heavier build but also to their extremely large jaws, premolars and molar teeth (Figure 11.27). One species, *P boisei*, discovered in Ethiopia in 1959, was nicknamed 'nutcracker man' because it was assumed their strong jaws and large molars were used for crushing and grinding hard, fibrous foods, such as nuts. More recent microwear evidence of fossil teeth suggests the diet of *P boisei* was much more varied, and nuts were not a staple food item. Despite the striking fossil evidence for *Paranthropus* skulls, there is limited evidence for the remainder of the skeleton. The little that has been recovered for *P boisei* shows the pelvis and hip joint to be similar to members of genus *Australopithecus*. This suggests it was bipedal but does not prove that walking was its main mode of locomotion.

Paranthropus species persist in the fossil record from around 2.5 to 1 mya. From their age and anatomical features, scientists consider the accepted *Paranthropus* species to be descendants of *Australopithecus* or an undiscovered *Paranthropus* species. Whatever the origin, scientists agree that *Paranthropus* is an extinct side branch to the direct line from which modern humans evolved.

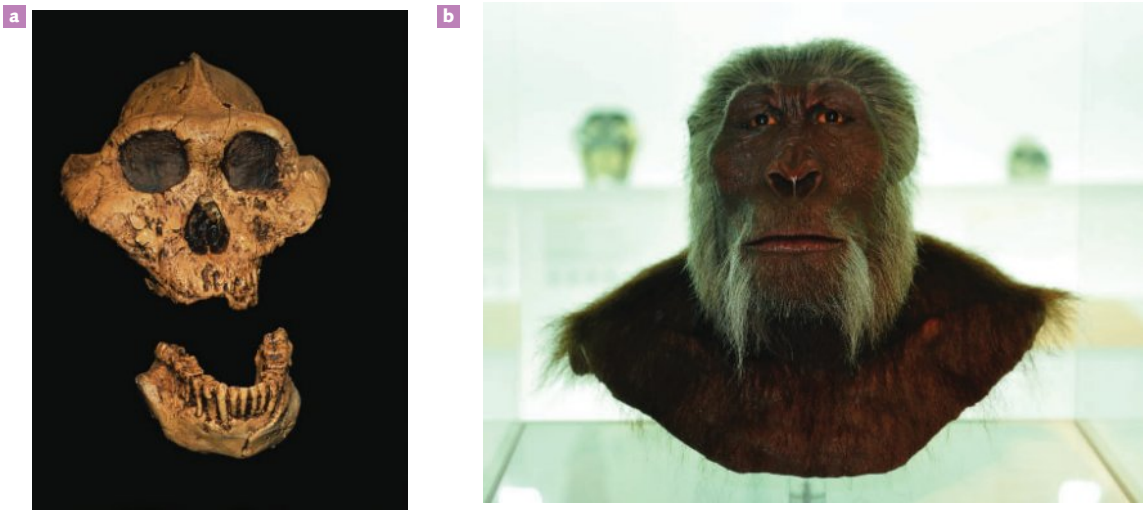


Figure 11.27 ***Paranthropus bose*** **: a a fossil skull showing the prominent mandible and the sagittal crest along the midline of the cranium; b an artist's reconstruction.**

Figure 11.28 Samples of Oldowan tools associated with australopithecines

Oldowan technology

Australopithecines apparently occupied home bases from which they ventured to forage for food. There is no evidence for fire use among australopithecines. Stone tools, such as choppers, scrapers and chisels, have been recovered in areas where australopithecine fossils are found. These relatively simple tools are known as Oldowan technology, named for the site in Tanzania where they were first discovered. Most implements are about the size of a tennis ball or smaller (Figure 11.28). Oldowan tools were sculpted to shape by striking the tool stone with a 'hammerstone' to chip off flakes. Although relatively simple, Oldowan tools demonstrate that their makers were fashioning materials towards an imagined

end product. They were also using precision grip to carry out the work. Oldowan tools represent the first stage of technological evolution in hominins, presumably enabling australopithecines to exploit their environment more effectively.

Oldowan tools date from around 2.5 to 1.2 mya. They are found along the east coast of Africa and throughout the Old World. These observations suggest Oldowan technology was migrating with early hominins. They also show that cultural evolution was underway. The technology was transmitted across massive geographical areas over many generations.

KEY CONCEPTS

- Hminin vluion is representd by a 'bushy' evltionary ree. Interp retations about th fossil record are subject t ongoing debate and re finement as newdiscoveries are made.
- Austrloithecie were raively mall, bipedal apes.
- Austrloitheine species are grouped into the genera *Australopithecus* (graile) and *Paranthropus* (robust).

Concept questions 11.3a

1. What des i mean ifa speies issexually dimorphic?
2. What was th evolutionary fate of genus *Paranthropus*?
3. *Australopithecus afarensis* shows a range of features that sugests it was oth aroreal and bipedal. Defend tis stateent ithevidence.
4. What does the cratsmansip nd distribution of Oldowan stoetols indicateabout hminin evolution?

HOT Chaenge

5. What evidece is there that *Paranthropus* s an evltionay side branch and not a direct ancestor of modern humans?

Evolution of genus *Homo*

The evolution of genus *Homo* is associated not only with refinements to bipedalism but also with expansion of the cranium (Figure 11.29). There are 11 species classified in genus *Homo*, including *H. sapiens*. The composition of genus *Homo* is continually being revised. For example, a new species, *H. luzonensis*, was added in 2019 after the discovery of ancient skeletal remains in the Philippines. In addition, the validity of a few revisions is often questioned. Some argue that one or other of the existing species should be split into more species. Three species highlight what is understood and what is debatable about the course of hominin evolution.

A transitional fossil: *Homo habilis*

Homo habilis is described mainly from fragments of skull (Figure 11.30a), hand and arm bones discovered in Tanzania in 1960. Dating to 1.8 mya, *H habilis* is one of the earliest fossil hominins verified to show an increased cranial capacity (Figure 11.29), although its total volume was less than half that of modern humans. It was also the earliest hominin to be found unequivocally associated with Oldowan stone tools, providing direct evidence for the use of technology. These features were used to justify its placement in genus *Homo* and it was accordingly named *H. habilis*, or 'handy man'. However, some scientists contest this placement because selected fossils assigned as *H habilis* have arm and leg dimensions resembling those of australopithecines.

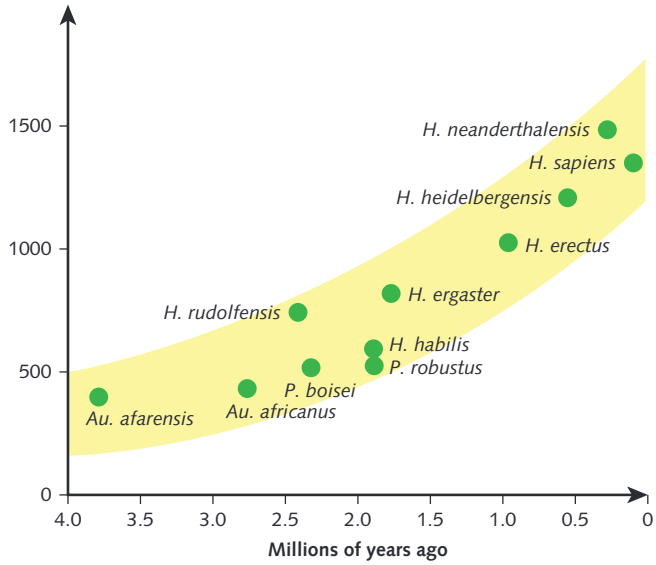


Figure 11.29 The gradual increase in cranial capacity of hominins over time

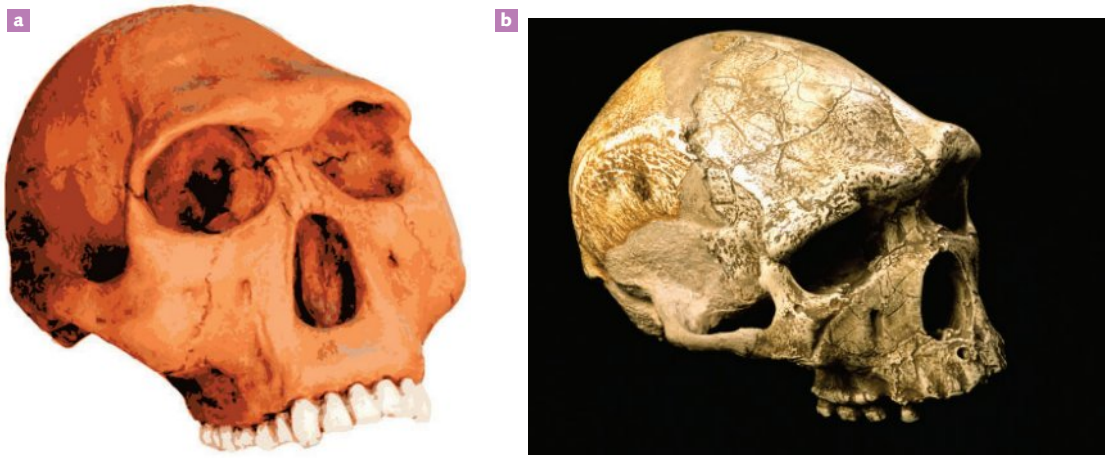


Figure 11.30 Detail of skulls from **a** *Homo habs* and **b** *Homo erectus*. Note the sagittal keel (thickened midline) on the *Homo erectus* skull.

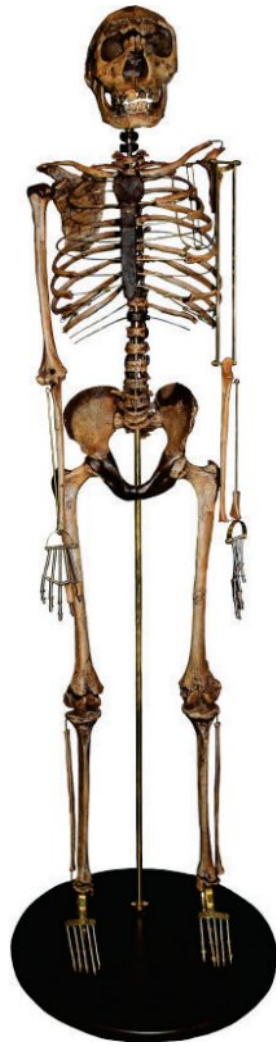


Figure 11.31 A relatively complete skeleton of *Homo erectus*

H habilis is currently interpreted as a transitional fossil that shows features of both the australopithecines and the genus *Homo*. *H habilis* used stone tools, which indicates a significant advance in the cognitive abilities of hominins.

Homo erectus

H. erectus (Figure 11.30b) is the first *Homo* species to resemble modern humans. *H. erectus* is the earliest hominin that combines modern human dentition, fully upright posture, obligatory long-range bipedalism, and at least a middle-sized brain.

The **postcranial** (all the skeleton except the skull) anatomy (Figure 11.31) indicates that *H. erectus* was a dedicated biped capable of walking long distances and running, if necessary. Its fossils are found throughout Africa, Europe and predominantly in Indonesia and China. This indicates *H. erectus* is the earliest known hominin to migrate out of Africa, dispersing across the Old World by about 1.5 mya. In China, repeated cooling and drying through multiple glacial periods encouraged the establishment of grasslands, attracting large grazing animals that *H. erectus* might have hunted.

The cranial capacity of *H erectus* was greater than that of the australopithecines and *H. habilis* (Figure 11.29), suggesting further evolution of cognitive abilities. Skulls had thickened midlines referred to as a **sagittal keel**. Hands were no longer used for climbing but had become more refined for manipulating objects. Stone tools have been found with *H erectus* fossils in western Asia, Europe and Africa. Their smaller teeth indicate that their diet had changed in some way compared with that of the australopithecines. *Homo* species were possibly eating different foods or preparing the same foods differently; for example, by cooking them before eating them. Deer, antelope, boar and fish bones found at various sites indicate some of the prey items of *H. erectus*. Burnt stone and animal bones, charcoal and ash deposits dating to about 0.5 mya suggest *H erectus* used fire (Figure 11.32), but it is difficult to prove that they could control fire.

Acheulean technology

The stone tools found with *H. erectus* fossils are characterised mainly by tear-drop or pear-shaped hand axes. The tools are known as Acheulean technology, after the site in France (St Acheul) where they were first discovered. The axes are 12–20 cm long and are crafted by chipping on both faces of the stone (Figure 11.33). These tools appear in the fossil record from around 1.6 mya to 200 000 years ago (200 kya) (**kya** = thousands of years ago).

Figure 11.32 An artist's impression of a *Homo erectus* campsite. Males in the foreground use fire to fashion spears, while a female and a male in the background skin an animal.

Figure 11.33 Acheulean hand axes associated with *Homo erectus*

Homo floresiensis

WB
11.3.1 FROM *AUSTRALOPITHECINES* TO *HOMO SAPIENS* PAGE 239

Occasionally, a new fossil discovery challenges existing assumptions about hominin evolution. For example, *Homo floresiensis* is widely regarded as the most surprising fossil hominin find in decades. *H. floresiensis* was discovered in 2003 by a joint Australian–Indonesian team. Skeletal remains unearthed in Liang Bua cave on the island of Flores in Indonesia (Figure 11.34a) were dated to 100–60 kya. The most important specimen, dubbed 'the Hobbit', is the unfossilised skeletal remains of an adult female (Figure 11.34b,c). At just 1.1 metre tall, the Hobbit was a diminutive hominin. Associated with ample stone tool artefacts, *H. floresiensis* evidently hunted and processed island animals. Charred bones demonstrate that *H. floresiensis* used fire for cooking.

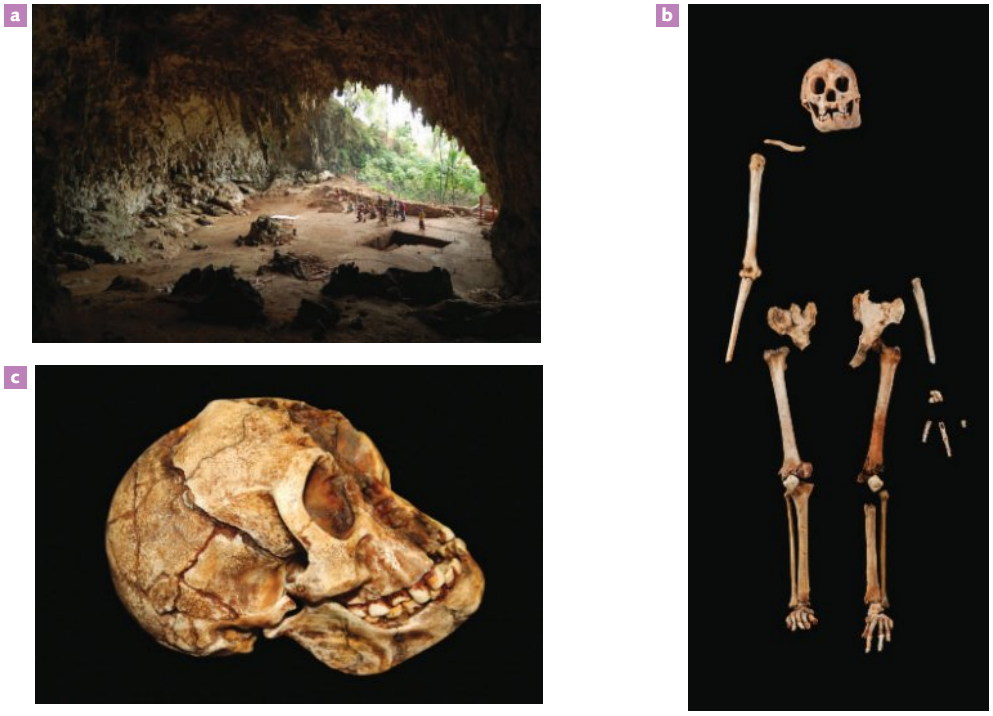


Figure 11.34 a Liang Bua cave on the island of Flores, the excavation site where *Homo floresenss* was unearthed. **b** The skeleton and **c** detail of the skull of the *H. floresenss* specimen dubbed 'the Hobbit'.

It is not known how *H. floresiensis* arrived on Flores. Even with the sea level changes occurring in the last million years, Flores was never connected to mainland Asia and is separated by tens of kilometres of sea. Chance colonisation by drift rafting is a possibility. Archaeological evidence indicates *H. floresiensis* occupied Liang Bua cave from at least 190 kya until 50 kya. Stone tools older than 800 kya have also been found on Flores and may belong to *H. floresiensis* or an earlier hominin. The disappearance of *H. floresiensis* broadly correlates with the timing of modern humans' arrival on Flores. However, there is no direct evidence that the two species interacted.

The discovery of *H. floresiensis* upset the field of **palaeoanthropology** (the study of fossil hominins) for a couple of reasons. First, the remains were originally dated to 38–18 kya, suggesting that *H. floresiensis* coexisted with modern humans until relatively recently. That suggestion was rejected in 2016 after the dating evidence for the remains and surrounding deposits was re-examined. The remains are now considered to be older, 100–60 kya.

Second, and more compelling, the origin of *H. floresiensis* is mysterious. One possibility is that *H. floresiensis* evolved from *H. erectus* that had migrated into Asia. This interpretation recognises similarities in the shapes of their skulls, particularly the brow ridges and sagittal keel. If true, *H. floresiensis* must have evolved to become smaller after its ancestors settled on Flores. Examples of 'insular dwarfism' have occurred on other islands around the world. Dwarfism presumably evolves in colonising species that experience long-term isolation with a restricted food supply and limited predators. The smaller cranium of *H. floresiensis* (about 400 cm^3) (Figure 11.34c) may be such an evolutionary adaptation to reduce the brain's energy demand. This proposal is supported by fossils of other extinct miniature species on Flores, such as those of *Stegodon*, an unusual form of pygmy elephant.

An alternative proposal for the origin of *H. floresiensis* is drawn from the primitive features of its body. *H. floresiensis* had relatively long arms and short legs with long feet, which are more like those of australopithecines. This interpretation is supported by australopithecine features of the wrist, hip and collar bones. It may be that *H. floresiensis* descended from an australopithecine or a *H. habilis*-like ancestor and always was of a comparable size to them. If this were the case, *H. floresiensis* could have initiated the earliest independent migration of hominins out of Africa. Whatever its origin, *H. floresiensis* ultimately represents an extinct side branch to the direct line of human evolution.

KEY CONCEPTS

» Eolution of the genus *Homo* s associated with expanion of thecrnium,s well as ehancements in bip da eolution.

» New foildiscoveries (e.g. *Homo floresiensis*) chllene xistin assumptin about hominin evlton.

Concept questions 11.3b

1 What evidence demonstratethat the cognitive alties ohominins had advanced ith the evolution of *Homo habilis*?
2 What do the ptcrania aatomy and global dstriution offosis indicateabt bipedalism in *Homo erectus*?
3 What des i dentition suggest about the *Homo erectus* det? What other evidence may support or refute that assertion?
4 Ouline two aspects of *Homo floresiensis* anatomy that argue for cntradiory origins.

HOT Chaenge

5 What evidece available tosupport the idea that the *Homo* speciesate different foods from the *Australopithecines*?

11.4 Modern humans and Neanderthals

The first formal record of fossil hominins to be excavated was in 1856 in north-west Germany. The fossils were initially mistaken for the remains of a bear. They would famously come to be known by the name of the valley in which they were exhumed: Neanderthal. The discovery launched the field of palaeoanthropology and ignited public imagination. An icon of pop culture, the Neanderthal was rendered as a 'dull-witted prehistoric brute' (to paraphrase the early 20th century palaeontologist Marcellin Boule). No other ancient hominin has received as much attention or fascination.

There are many questions about the relationship between Neanderthals and modern humans. Why did the Neanderthal disappear while modern humans survived? Did Neanderthals and modern humans coexist peacefully or were they competitors, or adversaries? Did they interbreed? Are modern humans descended from Neanderthals? To answer these questions we will draw on a range of evidence, from over a century and a half of palaeontology to the most recent developments in biotechnology.

Fossil evidence for *Homo neanderthalensis*

The thousands of Neanderthal fossils that have been recovered reveal a hardy, resourceful people, an image that is at odds with the pop culture stereotype.

The distinctive facial appearance of Neanderthals arises from the enlarged brow ridge, sloping forehead and expanded nose. They also had a larger average cranial capacity than modern humans, approximately 1485 cm^3. The enlarged portion of the Neanderthal cranium is at the rear, associated with the visual cortex of the brain. Together with relatively larger eyes, these features suggest Neanderthals were equipped especially for vision in low light, which may have been an advantage in northern European latitudes. Neanderthals were stockier than modern humans, and had a flared rib cage, accommodating an expanded abdomen, and shorter limbs (Figure 11.35). Well-formed muscle attachments indicate strong muscular bodies and a strenuous lifestyle. Neanderthals had to survive episodes of glaciation, so some scientists interpret these features as adaptations for conserving heat in a cold climate. However, others disagree. For example, it was accepted for a long time that the broader nose was associated with a larger

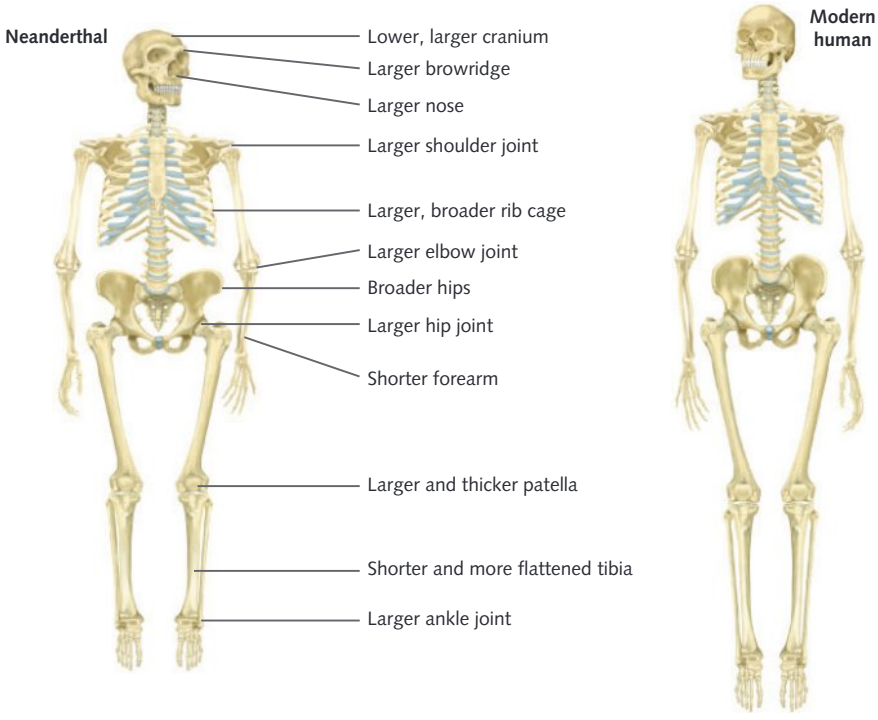


Figure 11.35 A skeletal reconstruction of *Homo neanderthaenss* and modern *Homo sapiens*

sinus cavity, enabling a greater volume of air to be warmed during inhalation. This view was contradicted by 2D X-ray analysis of skulls that revealed Neanderthal sinus cavities are similar in size to those of modern humans.

An alternative hypothesis is that the unique Neanderthal morphology arose by genetic drift in a relatively small, sparsely distributed population.

INVESTIGATION 11.1

Hominoid skull analysis

Am

To analye vaius hoinoid kulls nd explr various anatomical adaation tht have emerged in hominids over their evolutio n

Time reuirement

45 inutes

Materas

» Models o skuls from:
  – *Pan troglodytes* (chimpanzee) (modern)
  – *Gorilla* (golla) (modern)
  – *Homo sapiens* (human) (modern)
  – *Homo neanderthalensis* (Neanderhal human) (120 000–30 000 years ago)
  – *Homo erectus* (uright human) (2.0 mon years ago)
  – *Australopithecus boisei* (2.3–1million years ago)
  – *Australopithecus afarensis* ('Luy' (4 million years ago)
» Tape measurin millimetres)

What are the rsks n dong th s nvestgaton?	How can you mana ge these rsks to stay safe?
Skus may have sharp edgesHanle	with care and do not run your fingers over skull teeth

Method

Examnng the bran case

For eachull:

1 Exmine the rontal bone (forehead) of ech of the skul s and detemine whether they appear mor vertical or flatter. Ensure that th skull eys are orientd frward while doing this.
2 Exmine above the eye socket and determin wether suprorbitl (brow ridge) is present. If so, see if the brow ride is cntinuous rdivied in hemiddle.
3 Measure te width of the brain case t the dest poit.akeall measurements in millimetres.
4 Look for eidence of sgitta crestruning lengthwise along themline of the top ofe sull. Identiy whether it is prominen, present or absent.
5 Measure te distance between the front teth and the front ridge of the foramen magnum.
6 Examinebehind the ear f the skulland determine if the astoid process is fairly flat or ntieably poruding.
7 Copy esults tble 1 nto yur logbook. Add extra rows as reqired. Record the results of yurobservations.

Examnng the faca structure

For eachull:

1 Poiion thskull s ha it i faing you. Examin the nasal bones. Identify whether they are flat or protuding.
2 Measure th maximum breath (width) f th nasal opening.
3 Measure th maxium height ofhe asal opening.
4 Starting at the outside fthe back mlars, meure the widthof the maxilla (the upper jaw).
5 Thebizygomaticbreadt is the width of the face from the widest part of one cheek bone to the widest part of the other cheek bne. Measur thisdistance.
6 Copy esults tble 2 nto yur logbook. Add extra rows as reqired. Record the results of yurobservations.

Examnng the dentton (teeth)

For eachull:

1. Exmine thedental arcade (the shape made by the rows ofteeth in the upper jaw). Observe the teeth towards the back an idetify whether the teeth on each side of tejawar arallel or diverging.
2. Repoiion thekull so that ou are viewing it from the sde. Eamin the inciorsand identify whether they are veticalor angled forward.
3. Measure the width of the incisors on the left side of the jwand then eaure the incisors on the right side of the ja. Add te widtofall incisors together to get the ombne width.
4. Exmine thmaxilla (upper ja and mandible (lower jaw) togethe Idntify whether the canine teeth project above or blow the hewing surfaces of th other teeth.
5. Se if you anidentfy anine diastema (the ga between the canie and the adjacent teeth).
6. Measure from the backof the last molar to the front of the first preolar n the left sideof t jw. This will give you a measurement of th chewing surface o the teeth.
7. Copy esults tble 3 nto yur logbook. Add extra rows as reqired. Record the results of yurobservations.

Resuts

Resuts tabe 1 Examnng the bran case

Specmen	Forehead	Browidge present/absent	Browrdge contnuous/dvded	Bran case	Saitta crest	Foramen magnum	Mastod

Resuts tabe 2 Examnng the faca structure

Specmen	Nasl bones	Nsal oenin width asal	opnig hight	axillawidth	Bizygomatic breadth

Resuts tabe 3 Examnng the de ntton (teeth)

Specmen	Dentl arcadlcisor	s	Incisors width	Canine	Dastema	Cheing surface

Draw a graph of one caaceistic (e.g. resence of bo rde) fom each table. Write 'Specimen' on the *x*-axis and arrage in order from great apes to mdern humans.

Dscusson

1. The canine teeth haedrastialy reduced in size from gret apes to odrn hmans. Explain why this might be.
2. Explain why the face has become progressively flatter over the volutino hominids.
3. Describe howthe position of the framen magnum relatesto bodyposture and locomotion.
4. Certain areas ofthe brain case enlarged before others in our evlton. Dscribe how the areas enlarged throughout our evlton.
5. Whattritsdifferentiate modern apes and modern humans?
6. Usng your measurements andthe facial feature yo observed as evidence. Are modern humans or modern apes moreclosely relate to exnct hominids?
7. maine you found the remains f a skul that only containe d the manie. s this enou gh evdence to deterin i it blonged to a modern human, early hominnor an ape? Explain your answer.

Concuson

Wite a statement on th trends instructural changes from great apes to modern humans.

Figure 11.36 Mousterian tools associated with Neanderthals

Mousterian technology

There is ample archaeological evidence to show that Neanderthals used relatively advanced stone tools. This Mousterian technology (named from a site in France) dates from about 300 to about 30 kya. Mousterian tools are mostly found throughout Europe but evidence for the technology also occurs in the Middle East and northern Africa. The technology appears to have evolved from Acheulean industry and is characterised by sharp, pointed blades (Figure 11.36) crafted by chipping flint, a type of dark quartz.

Near the end of their history, Neanderthal sites are found with flint-based serrated blades normally associated with modern humans, suggesting Neanderthals were copying or trading the technology.

Neanderthal lifestyle

The Neanderthal diet was mixed, and depended on what was locally available. Chemical analysis of fossil teeth residues and faecal deposits provides direct evidence that Neanderthals consumed starchy tubers, nuts, fruits, grasses and meat. Bone remains indicate Neanderthals effectively hunted and butchered game, particularly reindeer, but also bigger prey such as bison and mammoths. Asymmetric anatomy and frequent broken bones suggest Neanderthals hunted by thrusting spears at large game at close range. Archaeological evidence shows that Neanderthals built hearths and controlled wood-fuelled fires for cooking and for warmth. Neanderthals consistently took refuge in caves and rock shelters, a practice that contributes to the caveman stereotype. Indeed, the rich fossil record for Neanderthals exists because a number of deceased Neanderthals were buried in caves.

There is evidence that Neanderthals buried their dead and occasionally marked their graves. Although disputed, there is no definite evidence that Neanderthal burials were associated with rituals, nor is there any rock art firmly attributed to them. For these reasons, the prevailing if controversial view is that Neanderthals were pragmatic and even altruistic but they displayed little of the symbolic expression that defines the art and ceremonies of *Homo sapiens*.

WB
11.4.1
EVOLUTION OF MODERN HUMANS
PAGE 241

Evolution of modern humans

The fossil, cultural and molecular evidence suggests that modern humans evolved initially in Africa and then migrated across the world, displacing earlier hominins as they advanced. This model is referred to as the **recent single origin** hypothesis, or more informally as the **Out of Africa** hypothesis. This hypothesis accommodates the possibility that *H sapiens* interbred with localised populations of ancient humans. However, the migration of modern humans was overwhelming. Evidence suggests that older hominin populations were assimilated into a dominant modern human population, if they were not wiped out altogether.

Fossil evidence

Anatomically modern humans first appear in the fossil record in Africa. Fossil skull fragments from Morocco dated to about 300 kya have a blend of modern and older hominin features and may represent an early version of modern humans. The earliest unequivocally modern human fossils currently known come from Ethiopia and are dated to about 195 kya. The fossil evidence suggests modern humans evolved first in central Africa. As climatic conditions changed, drought in central Africa pressured modern humans to migrate to the east coast. A coastal existence may have contributed to cognitive evolution by supplementing the diet with seafood rich in omega-3 fatty acids. Modern humans then migrated northwards and southwards along coastal routes.

Palaeoanthropologists do not yet agree on the precise timing, the routes or even the number of migration waves of modern humans out of Africa. The current hypothesis is that there were at least two major migration waves north. One of these occurred about 100 kya but ended after reaching the Middle East. A later wave occurred 70–50 kya and pushed into coastal routes along Europe and Asia. The second migration wave ultimately spread the human population worldwide and brought the ancestors of modern Aborigines to Australia.

Skull fragments of an anatomically modern human dated to about 50 kya have been recovered in Niah Cave, Malaysia. This fossil hints at the route modern humans were likely to have taken to enter Australia. Fossil evidence confirms that anatomically modern humans inhabited south-east Australia by 40 kya. The evidence comes from dating of skeletal remains from two burial sites at Lake Mungo on the lands of the Paakantji, Ngyiampaa and Mutthi Mutthi people of southern New South Wales. These remains of a woman and a man are informally referred to as 'Mungo Lady' and 'Mungo Man'.

Modern human fossils in Europe are dated from about 40 kya and are traditionally referred to as Cro-Magnon Man, after the French cave where the first specimens were discovered. The earliest evidence of modern humans currently known on the Asian mainland is dated to about 30 kya. The anatomical proportions of the earliest modern human skeletons in Europe and mainland Asia resemble those of modern Africans, supporting the Out of Africa hypothesis.

Evidence of art and culture

Hominin evolution is associated with the evolution of tools. Evolution of modern humans is also associated with resources being used for artistic, as well as functional, purposes. Ancient art represents the most enduring record of symbolic expression during human evolution. The first convincing signs of art are associated with anatomically modern humans about 120 kya in scattered sites in South Africa. Blombos Cave in South Africa provides evidence of continuous human occupation for more than 100 000 years. It is a significant site for tracking the development of art and culture. Consistent use of particular artistic styles, or 'industries', appears there by about 90 kya. The evidence includes artefacts such as perforated seashell 'beads', engraved ostrich eggs, and patterned engravings in stone tools, bone and ochre (Figure 11.37), as well as evidence for symbolic burial practices. The appearance of industries is significant because it shows that human groups were organising according to shared beliefs, values and behavioural practices. That is, they demonstrate the establishment of culture. Furthermore, some resources at the excavation site, such as abalone shells filled with liquefied ochre, originated from distantly separated locations. This implies the cave inhabitants had the capacity to plan. They were identifying resources, relocating and storing them at their 'workshop', and combining them for later use.

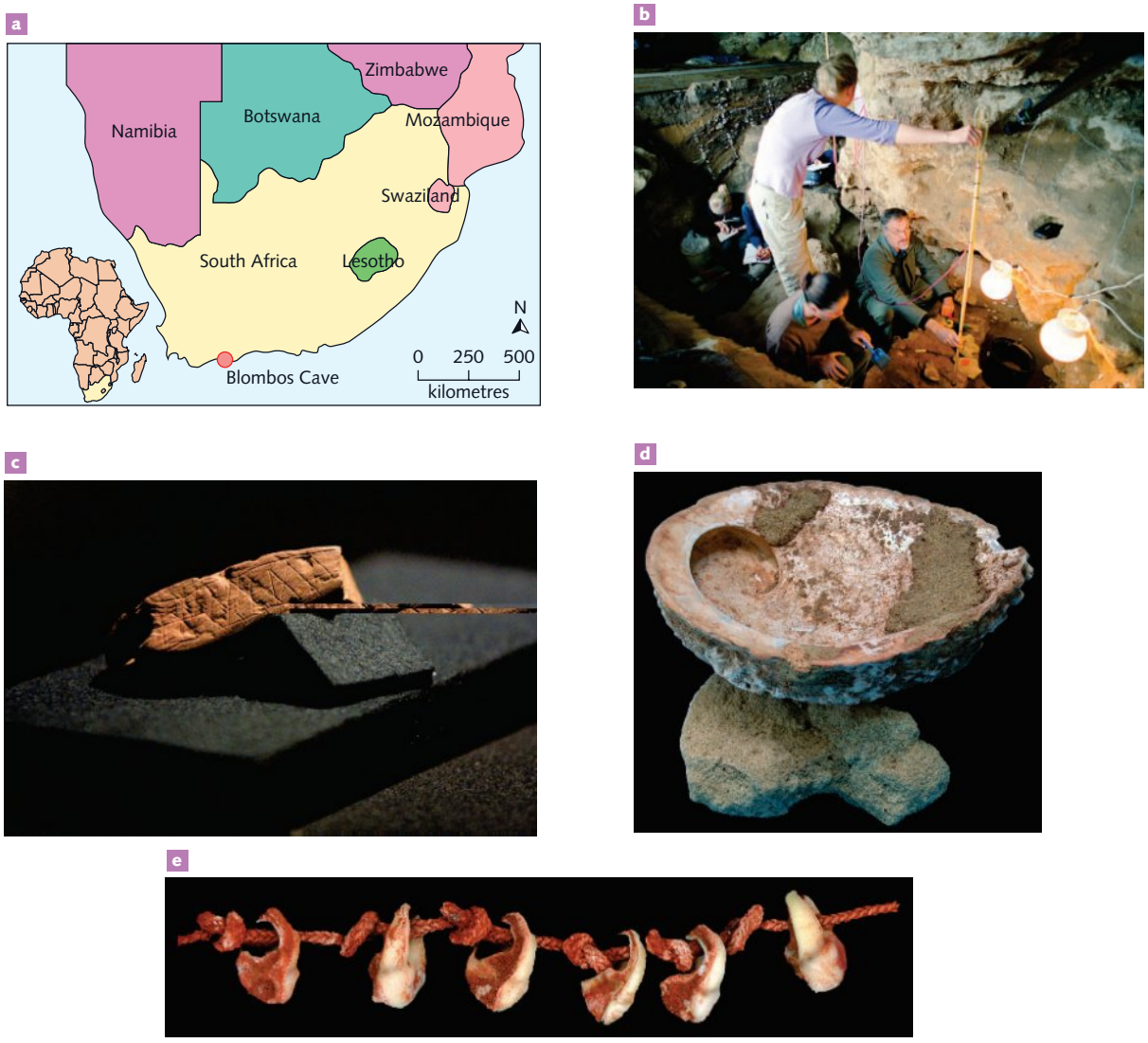


Figure 11.37 a The location of Blombos Cave in South Africa. **b** The interior of Blombos Cave. Artefacts found at the cave include **c** a stone block with patterned carvings and **d** an abalone shell used to prepare ochre. **e** A reconstruction of Blombos Cave beadwork using modern shells.

The quantity, sophistication and geographical distribution of human art expanded abruptly about 40 kya. This is largely represented by cave art in Europe and Australia, including paintings, engravings and carvings (Figure 11.38). The oldest confirmed rock art is from about 45 kya in southern Sulawesi, Indonesia. In Australia, the oldest reliably dated cave art is a charcoal drawing from 28 kya excavated at Nawarla Gabarnmang rock shelter located in Jawoyn country in Arnhem Land. Australia's Aboriginal cave art is considered to be the longest unbroken record of ancient art in the world.

Mungo Lady and Mungo Man, dated to about 40 kya, are the oldest known examples of human cremation and burial in the world. Although the Mungo site skeletons proved to be one of Australia's most significant archaeological discoveries, their removal for scientific study in the 1960s and 1970s caused considerable distress to the local Indigenous communities whose ancestors they represented. The local Indigenous people believe that the spirits of the deceased are restless until their remains are laid to rest on Country. The matter was settled when Mungo Lady and Mungo Man were returned to the Paakantji, Ngyiampaa and Mutthi Mutthi people in 2017.

The removal of ancient artefacts for study and the angst their removal causes Indigenous owners is a cultural and political issue worldwide. The tension underscores a signicant lesson about cultural evolution. As modern humans cooperated in larger groups, artwork, ceremony, language and affinity with territory served to bond members and define group identities.

Evidence from mitochondrial DNA

From the late 1980s, mitochondrial DNA (mtDNA) was used to explore evolutionary relatedness among modern humans. mtDNA was chosen because its pattern of maternal inheritance provided a relatively uninterrupted lineage of descent from ancestral populations. Global human populations were grouped according to the specific and unique mutations in their mtDNA (their **haplotype**). Members of a group that share a haplotype must be descendants of a common ancestor. These are described as **haplogroups**. Molecular homology was used to produce phylogenetic trees from the mtDNA haplogroups.

a

b

c

Figure 11.38 Examples of ancient rock art featuring local fauna from **a** Altamira Cave, northern Spain; **b** Gabarnmang rock shelter, Jawoyn country, northern Australia; and **c** Maros karsts, southern Sulawesi, Indonesia.

It was discovered that, among modern humans, most of the variation in mtDNA sequences occurs in African populations (L haplogroups, Figure 11.39). mtDNA of Europeans, Asians and the Indigenous peoples of Australia, the Americas and Pacific islands represent just a subset of total human mtDNA diversity (M and N haplogroups, Figure 11.39). This provides further evidence for the Out of Africa hypothesis. Molecular clock estimates suggested diverse populations of modern humans evolved more than 200 kya in Africa, with the haplogroups that migrated out of Africa diverging

CONNECT

For more detail on using mitochondrial DNA for evolutionary studies, see Chapter 10.

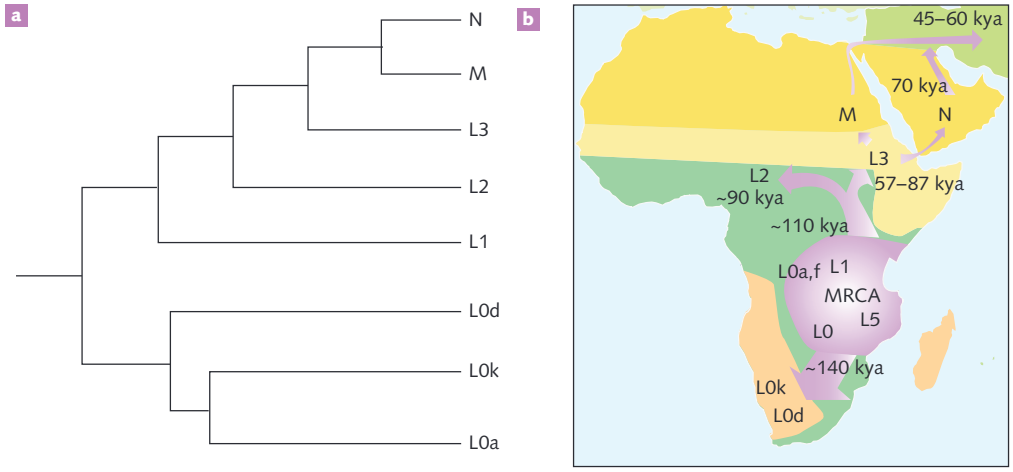


Figure 11.39 a A phylogenetic tree generated from mtDNA sequences of global human populations. The labels at the tips of the tree represent the haplogroups into which human mtDNA mutations can be classified. **b** The location of each haplogroup and migration patterns inferred from them taking place thousands of years ago (kya). All haplogroup originate in Africa and the Middle East. Only the M and N haplogroup are found in Indigenous populations throughout the rest of the world. MRCA = most recent common ancestor.

70–50 kya. The two surviving mtDNA groups (M and N) that colonised the other continents are most closely related to the African L3 group located north-east of Africa and nearest to the Middle East.

Mitochondrial DNA sequences exhibit affinities between northern Indigenous Australian and Papua New Guinean populations, suggesting joint colonisation of the two land masses by related peoples.

Evidence from nuclear DNA

Historically, sequencing nuclear genomes has been far more challenging than sequencing mitochondrial genomes. However, technical advances since the mid-2000s have accelerated the number and rate of whole nuclear genomes sequenced. Nuclear genomic sequences collected from representatives of human populations throughout the world indicate modern humans outside Africa share a common ancestry, consistent with a single major wave of migration dispersing from Africa. Models based on molecular estimates of mutation rates in these sequences suggest that a population bottleneck occurred in the non-African populations about 72 kya, converging with the likely time of migration out of Africa.

The models also indicate a likely early split between populations of modern humans. The common ancestors of Australian Aborigines and Papua New Guineans diverged from the ancestors of all other non-Africans about 58 kya. Europeans and east Asians subsequently diverged about 42 kya. This evidence suggests at least two waves of migration across Asia, with the earlier wave establishing the population of modern Indigenous Australian populations, who arrived perhaps about 50 kya. Nuclear DNA sequences indicate native Americans trace back to a common ancestry about 12.6 kya, representing the last major continent-wide migrations by modern humans.

CONNECT
Go to Chapter 9 to review bottlenecks.

Australian settlement

WB
11.4.2 AUSTRALIAN SETTLEMENT PAGE 243

Migration across the Australian continent was facilitated by lower sea levels that connected New Guinea, the Australian mainland and Tasmania into a single land mass called Sahul until about 8 kya. Sahul was separated from a consolidated southeastern Asian land mass called Sunda. This implies that an approximately 90 km sea crossing was required to settle Sahul.

Dating archaeological artefacts associated with processing food, pigments and stone tools in Madjedbebe rock shelter in Mirarr country 300 km east of Darwin confirms human occupation in northern Australia from at least 65 kya. This early date for human arrival in Australia indicates that models for human migration out of Africa and across the globe still need refinement.

Geographically widespread data from archaeological artefacts across Australia confirms extensive human habitation by about 45 kya (Figure 11.40). The evidence includes stone and bone implements, charcoal and burnt bones dated at many Australian sites, including Lake Mungo (about 48 kya), Devil's Lair, Wardandi country in southwestern Western Australia (about 48 kya), Gledswood rock shelter, Waanyi country in northwestern Queensland (about 42 kya) and Warreen Cave on the lands of the Tommeginne in Tasmania (about 40 kya).

Variations in autosomal sequences of Indigenous Australians indicate a genetic divergence between northeastern and southwestern populations with genetic intermediates shared by populations in the interior of the continent. The data informs models for gene flow and shows that the arid interior of the continent acted as a barrier to migration. Migration occurred preferentially in both directions around Australia's coastlines before dispersing inland (Figure 11.40). The combination of nuclear genome data and dating data for human fossils and archaeological artefacts suggests the migration and settlement of Australia was relatively rapid and completed within a few thousand years. Archaeological artefacts indicate ongoing or recurrent human occupation at many Australian sites after they were settled.

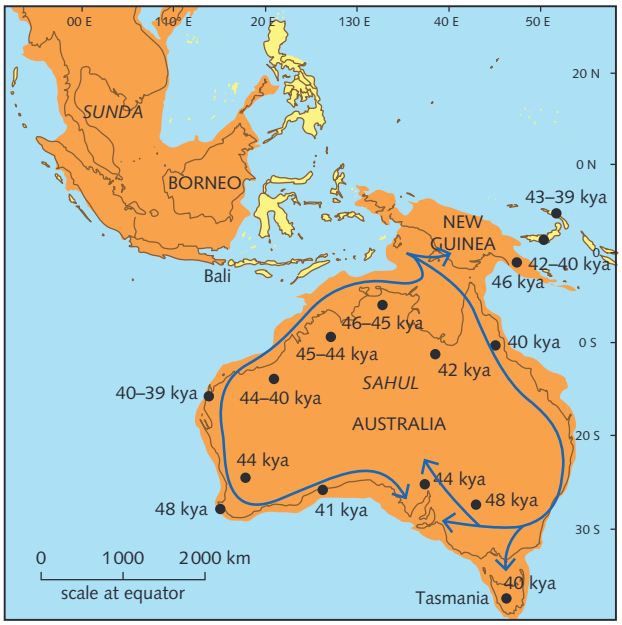


Figure 11.40 **Migration routes (blue arrows) taken by Indigenous Australians populating the ancient continent of Sahul inferred from nuclear genome sequences, archaeology, and diversification of language. The dots represent archaeological sites with dates for the oldest artefacts currently known at each site.**

Today, Indigenous Australians are represented by hundreds of Indigenous nations and language groups and the clans within them. The principal roots for approximately three-quarters of the Indigenous languages are the Pama (northeastern Australian) and Nyungan (southwestern Australian) languages. The divergence and spread of these languages correspond well with hypothesised migration routes based on nuclear genome sequences (Figure 11.40). However, the timing of the language divergences remains a matter of debate.

Sea levels began rising about 8 kya, eventually cutting off Australia from New Guinea and forming more than 270 islands and 1300 coral reefs in the shallow seas of the Torres Strait (mostly <20 metres deep). Ongoing human occupation in the Torres Strait islands is indicated by archaeological evidence consisting of charcoal and burnt dugong and turtle bones from about 7 kya. The native islanders' connection with the sea is highlighted by archaeological evidence for marine food consumption (Figure 11.41). For example, ancient **middens** from at least 4 kya are rich in discarded bones of small sharks, rays and other fish, indicating subsistence mainly on near-shore fish. Genetic, archaeological and linguistic evidence indicates the local islander populations remained in ongoing contact with the populations of northern Australia and Papua New Guinea.

Figure 11.41 **Artefacts recovered from a fish bone midden in Tigershark Rockshelter on the granite islet of Pulu. Almost 60% of the bones are derived from small sharks and rays (about 1 metre) in this and other middens in western Torres Strait.**

KEY CONCEPTS

- Neanderthals were aiet hoiinswith distinctive anaomicalfeaturs, who coeistd for a time with modern humns.
- Neanderthals demonstrted culurl and technological evltion b lttle ymboli expressin or art.
- Evidence from sils, archaeoogy and mitochondrial and nclear DNA sequences support the recent single origin, or Out ofAfrica, hypothesis.
- Genetic and arhaelogicl evdence provides evidence for patterns f igration of modern humans across the world ncludin ustralia.

Concept questions 11.4a

1 Describethree differences bteen the skeletal anatomy of Nanderthals ad modern humans.
2 Describ fssil evidence that supports the Out of Africa hothesis.
3 What are haplogroups and how do they support the Out of fricahypothesis of modern humans?

HOT Chaenge

4 Among modern umans, the mtDNA f Europeans, Aians andte Indigenus people f Australia, the Amricas and Paci fic sands repres ent just a subset of total human mtNAdiersity, through M and N halogoups. Hapogoup N is derived from the ancestral L3 acroaplogroup, which represents the migrtin discsse in the 'ut of Africa' theory. Halogrop N is the ancestral haplogroup to almost alclades toaydistibuted in Europe and Oceania, as wll as manyfoun in Asia and theAmericas. Only the M and Nhaplogroups r fund in Indigenous poplations throughout the resof the world.

How do these findngs suggest that oden Aboriginal peples arethe direct desendants of migrants who eft Africa up to 75000 years ago?

WB
11.4.3 RELATIONSHIP BETWEEN MODERN HUMANS AND NEANDERTHALS PAGE 246

Relationship between modern humans and Neanderthals

It is unclear why the second wave of modern human migration was more successful than the first. It may be that modern humans in Africa developed better survival skills in the intervening period. Or perhaps they were cooperating in larger or better organised groups. Whatever the causes, it is certain that, during the second migration wave, anatomically modern humans coexisted with Neanderthals across Europe and Asia. What was the outcome of this encounter?

A split in the tree

Ongoing speculation about interbreeding between Neanderthals and modern humans was fuelled in part by discoveries of fossils presumed to be anatomical hybrids. At the beginning of the 21st century, modern molecular methods enabled ancient DNA to be isolated and sequenced. This development offered an innovative approach to exploring evolutionary relatedness. Among the first to be studied was the mitochondrial DNA of sufficiently preserved Neanderthal fossils from Asia to western Europe spanning from 70 to 30 kya. These were compared with mtDNA sequences of modern human populations. The data showed that the degree of variation within

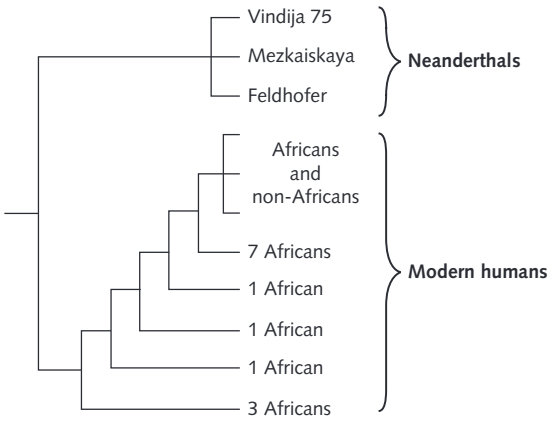


Figure 11.42 A cladogram for mtDNA sequences derived from ancient DNA of fossil Neanderthal bones and DNA from modern humans. The tree shows that the Neanderthal and modern humans samples diverged into separate branches.

the mtDNA of both Neanderthals and modern humans was relatively narrow. The mtDNA sequences of modern humans and Neanderthals were very different and had no overlap (Figure 11.42). These early studies offered no proof of interbreeding between Neanderthals and modern humans. Rather, they suggested that Neanderthals and modern humans diverged as two isolated populations.

Branches cross again

In 2010, scientists published a draft nuclear genome sequence from the ancient DNA of Neanderthal bones. This extraordinary technical feat overcame challenges posed by the size of the genome (more than 3 billion nucleotides) and the age of the samples (about 40 kya). The Neanderthal DNA was severely degraded and heavily contaminated by bacterial DNA. The genomes of the scientists working on the project were also sequenced and compared to ensure their DNA had not contaminated the Neanderthal samples.

The nuclear genome of the Neanderthals was compared with nuclear genomes of various modern humans. The comparison revealed that 1–4% of the genomes of modern Europeans and Asians are uniquely identical to those of the Neanderthal. However, these sequences are not shared between genomes of the Neanderthal and sub-Saharan African populations. The simplest interpretation is that 1–4% of the nuclear DNA of modern humans living outside Africa was derived from Neanderthals. This constitutes evidence for a limited amount of interbreeding between Neanderthals and modern humans. Estimates suggest the Neanderthal alleles entered the modern human population 40–80 kya. It is proposed that modern humans encountered and interbred with Neanderthals as they migrated out of Africa and through the Middle East. This is the reason the signature for Neanderthal DNA today is found mainly in descendants of Europeans and Asians but not those of African populations.

As no living human has been found to contain mtDNA of Neanderthals, it is likely that individuals of those lineages have not survived. In 2016, it was reported that chromosome 21 was sequenced from two European Neanderthal specimens dating to about 30 kya. The sequences provided evidence for earlier interbreeding between Neanderthals and modern humans. This was estimated to have occurred about 100 kya, most likely around the Middle East. The evidence indicates multiple interbreeding events between Neanderthals and modern humans.

Another branch, another crossing

In 2010, scientists announced the discovery of bone fragments from a previously unknown ancient hominin in Denisova Cave in Siberia. The bone fragments and the few associated artefacts were dated to about 40 kya. The anatomy of the **Denisovan** hominin remains a mystery but good quality DNA was recovered and sequenced from a single finger bone. The mitochondrial DNA of the Denisovan indicated it was more closely related to the Neanderthals but was sufficiently different to be a distinct branch in the hominin evolutionary tree. Phylogenetic trees show that the split between the Neanderthals and Denisovans occurred after their common lineage diverged from modern humans (Figure 11.43). Molecular clock estimates date the divergence between the Neanderthal/Denisovan lineage and the modern human lineage at about 800 kya. The Neanderthals and Denisovans subsequently diverged about 640 kya.

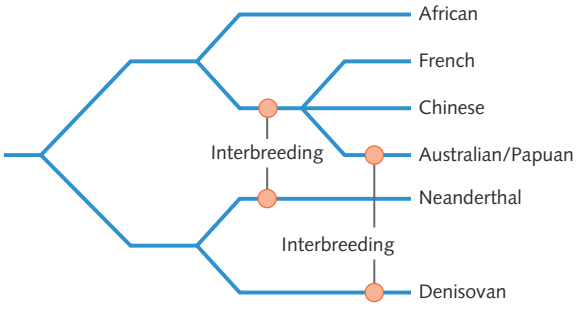


Figure 11.43 Patterns of divergence and subsequent interbreeding during human evolution. The diagram is drawn from evidence revealed by mitochondrial and nuclear DNA sequences.

A fossil jawbone recovered from the Tibetan plateau of northern China was dated at 180 kya. The DNA in the jawbone had degraded but the fossil was later identified as Denisovan based on analysis of teeth proteins. The discovery suggests the Denisovans were an enduring and widespread population, occurring at high and low altitudes in central Asia.

Analysis of nuclear genomes revealed that modern Melanesians share 3–5% of their DNA sequences uniquely with the Denisovans. The Melanesians include Indigenous Australians, native Papuans of New

Guinea and the native populations of several western Pacific islands. No other modern humans carry this signature of the Denisovan nuclear DNA. The evidence suggests Denisovans interbred with the ancestors of Indigenous Australian and Papuan populations as they were migrating through southern and southeastern Asia. It is estimated this interbreeding occurred between 50 kya and 44 kya. However, most modern Chinese and southeastern Asian populations lack Denisovan DNA. This supports the view that there were many waves of modern human migration through eastern and southeastern Asia, only some of which resulted in interbreeding with Denisovans. Figure 11.43 summarises the major divergence and interbreeding patterns between modern humans, Neanderthals and Denisovans.

At a hypothetical boundary between the Neanderthal populations of the west and the Denisovan populations of the east, Denisova Cave continues to be an important focus for the study of human evolution. In 2018, the mitochondrial and nuclear genome sequence of another finger bone found at the cave was reported to belong to a 13-year-old female who was estimated to have lived about 90 kya. Most significantly, her DNA showed she was a first-generation hybrid of a Neanderthal mother and a Denisovan father. The discovery proved that the ancient hominins interbred. The exchange and selection of the fittest genes among hominin populations was surely a key factor contributing to the survival of hominins. Our understanding of gene flow between prehistoric hominins and modern humans will continue to be reappraised whenever the genomes of ancient hominin remains are sequenced.

CONNECT

Artificial selection is discussed on pages 346–348.

Domestication and industrialisation

In the last 10 000 years, human innovation has been transformed by the practice of domesticating species by artificial selection. The development of agriculture depended upon the availability of domesticable species. The Fertile Crescent in the Middle East was the home range for a variety of suitable species, including wild wheats, barley, peas, cows, sheep, pigs and goats (Figure 11.44). This coincidence of

a

b

Figure 11.44 a The ocaton of the Fe rte Crescent (green) **b** artwork from Ancent Egypt depicting a cow being milked

biogeography is principally why the earliest conventional farmers and herders were located in that region. The plants and animals they domesticated are still some of the most valuable species to humans today.

Domestication also depended on advantageous genes and alleles in humans, as well as in wild plant and animal species. Just as humans selected for favourable traits in other species, the novel sources of nutrition applied selection pressures on modern humans. For example, modern dairying populations have higher allelic frequencies for the gene for lactase persistence. People who produce lactase in adulthood can digest the lactose sugar in milk derived from cattle or goats. Individuals in populations with a long history of agriculture tend to have more copies of the salivary amylase gene. This adapts people to a starch-rich diet of cultivated grains. However, the traits favoured by humans tend to make domesticated species less capable of surviving if re-introduced into the wild. Therefore, domesticated species rely on humans for their dispersal and survival. In essence, humans and their domesticated species have co-evolved and become interdependent.

Indigenous Australians developed holistic agricultural practices to manage the land for a sustainable food supply. Notably among them was Indigenous Australians' relationship with fire. 'Fire-stick farming' was used to stabilise the landscape and control the local food supply of plants and animals. Another example was the unique form of aquaculture practiced by the Gunditjmara people of south-western Victoria. They dug and maintained an extensive system of water channels connecting natural ponds to manage the local eel stocks. Basalt blocks installed in the channels trapped eels at different stages of growth. The transition to an agricultural lifestyle enabled humans to settle and eventually specialise and develop advanced technology.

Domesticated crops selectively bred for yield are grown at higher densities than their counterparts in the wild. The enhanced supply of kilojoules is a key driver of increased human population sizes. The rate of human population growth has accelerated with the pace of technological innovation and industrialisation (Figure 11.45). The tools of modern humans enhance the speed, scale and efficiency of energy production (e.g. power stations), transportation (e.g. motor vehicles and aeroplanes), long-range communication (e.g. television, telephones and Internet), cognitive processing (e.g. computers and software), and the ability to perceive the imperceptible (e.g. microscopes, telescopes and subatomic particle accelerators). Humans have colonised every continent and the human population will soon exceed 8 billion. The scale of the human population now affects every other plant and animal species. The impacts of human activities include depletion of wild species through overharvesting, habitat destruction, widespread pollution, the spread of invasive species and accelerated climate change.

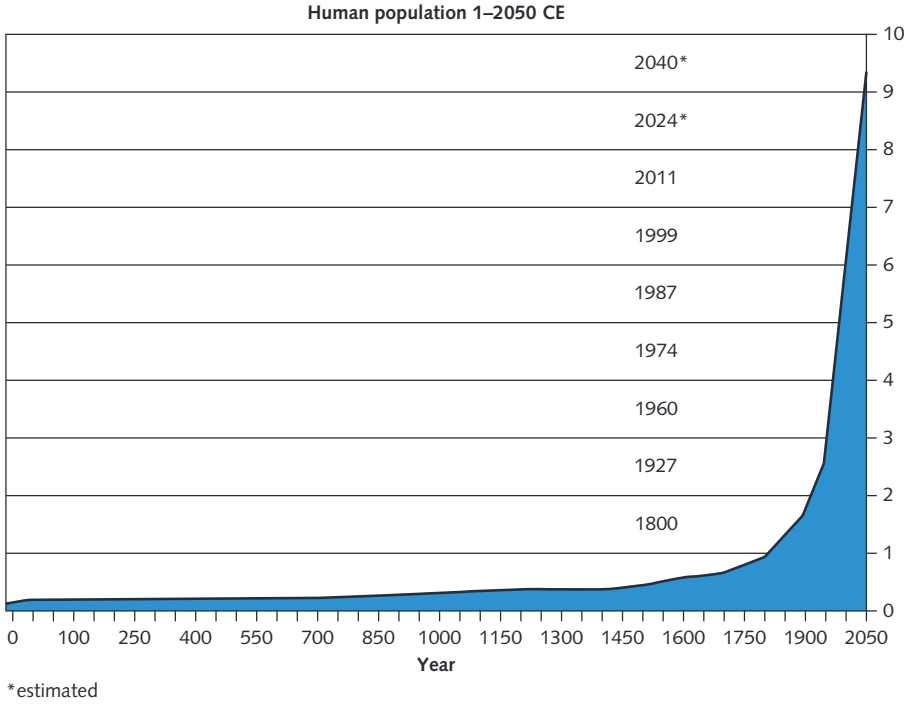


Figure 11.45 Growth in the human population during the past 2000 years

Weblink
A brief history of dogs

Online Worksheet
A brief history of dogs

Homo sapiens is an extraordinary outcome of evolution: a conscious, cooperative ape with an unprecedented capability to evaluate, plan and manipulate its environment. It is one species that can, to a considerable extent, shape its own future and that of countless other species. In the course of your lifetime, years from now, how will that future unfold?

KEY CONCEPTS

- Evidence fro mitocndrial DNA suggests Neandethals and modern humans diverged up to 800kya.
- Coplemntin fossi evidence,mitochondrial and nuclear genome dta indicates that modern humans evolved firs in Afica, ad the migrated via the Midle East to the resof the world.
- Evidence fromnuclear DNA sequences suggests slect groups of moden hmans latr interbred with Neandethals and another roup oancient hominins clled the Deisovans.
- About 10kya, te shift from hunter–gatherer to agrcultural lifestyles ccelerated cultural and techooica eolution.

Concept questions 11.4b

1 Explain ow mitoondrial DNA shows that the Neandethal and the moern humn lineages diverged about 800kya.

2 Wich modern humans hae the signature of Neandethal DNA in heir nuclea genomes today, and hich modern humans do not? How do these observations fit wth the Out of Afria hypothesis?

3 Who are th Densovans, and hw do they help to explain pattern of migratin and colonisation by modern humans?

4 Describe two aspects of the shift to agriculture that made the exansion of the h uman poplation psile.

HOT Chaenge

5 Receny, Ausralan Aborigis have been linked geneticlly to a group of people knw as Dravidian ndans from the suconinent. How might this have happened?

BRANCHING OUT

Is 'Ardi' a hominin?

There are very w fossilsof possib e homiins dating to before bout 4.2 mya andtei identi as hominis is frequently debatd. The mst inormaive fossil from that time is a early haf-coplete skeleton of *Ardipithecus ramidus* (icknamArdi). It was dsoere in Ethiia in 1994, and the delicate wok of excavating and reconstructing Ardi took 15 yars.

Sietists debate whether A di is a direct ancestor of modern humas, anealy side branch o ominin evolution, or a representative of a separate group from th huans and the chimps.

Aim

To exmine the charcteristics of *Ardipithecus ramidus* and ealute tsclassi fication asahominin

Observations

Exmine the data for *Ardipithecus ramidus* outined inTale 1.5 ad Figurs 1.46 and 11.47. Use your logbook to record your observations for the lowing features.

- Crial capacity
- Reative size ofthe brow ridges
- Reative size of the canines
- Amount of pronathism
- Lengthf libsrelative to the rest of the body
- Length of the thmb relative to other digits of the hand
- Shape ofthe spine
- Shape ofthe pelvis
- Caryig angle
- Length o halux relative to other toes of the foot

Table 11.5 Characterstcs of *Ardpthecus ramdus*

Characteristic	Value
Age	About 4.4 million years
Sex	Female
Height	1.2 m
Weight	50 kg
Crana capacty	350 cm

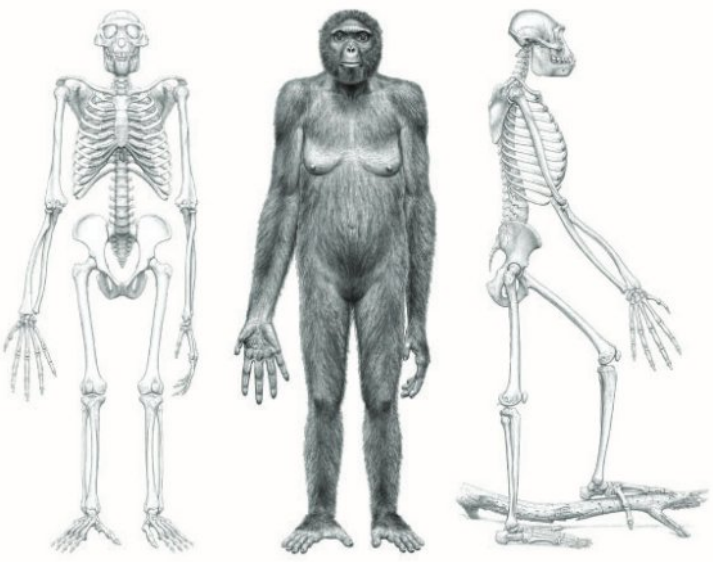


Figure 11.46 Sketches of the front view (left) and profile view (right) of the skeleton of *Ardpthecus ramdus* , together with an artist's reconstruction (centre)

Figure 11.47 A 4.4 million-year-old reconstructed fossil skull of *Ardpthecus ramdus* , found in the Aramis site, Middle Awash Valley, Afar depression, in north-east Ethiopia. The fossil is housed in the National Museum of Ethiopia.

Discussion

1 Whatattribue(s) wuldyou conider important in de terning whether ominoid s a hinin? Explain what features yo might expectto see n the skelto ofthe hominoid if i posessed those attributes.
2 What can you deducebout Ardi's ode o locomotion? Would o say she wa quadrupedal, bipedal or both? Did she cimb trees?What evidence do yo u have to support your inerpretations?
3 Do thecanial features of Ardi (ro ridgs, canines onthsm)sow moe similarities with hominins or with other hoinoids?
4 Wht iscranal capacitysupposed to ndcate about homioid? Wa oes Ardi's cranial capacity indicate?
5 Use th evidence andyour intrpretation to argue the casefo includn or excluding *Ardipithecus ramidus* n the homnns.Is there aything mor you would need to know abo ut the fssil tht ould help you me your decision?

Conclusion

Wite a rief cnclusion tatingyour dcision to include or excludeArdi aa hominin nd summarie your reasons.

Online key concepts
Chapter 11: Summary of key concepts

11 Summary of key concepts

11.1 Taxonomy of modern humans

KEY CONCEPTS

p425

- Mamals share a range of unqu anatomical featurs icuding a boy coveringof hair or fur, a snle ja with pcialied teeth, three bones n themidle er,secialiedexcrine glands, a daphragm and a four-chambered heart.
- Mamals e dvided into mnotremes, marspials and pacental mammals.
- Humans ae placent mammals.
- Modern humans are classified in the order Pimata (priates) within e suborder Catarrhini.
- Key chaacteritic f primtes include opposable dits, stereoscpic oour sion, nails on the dits, and rlaivelyelarged brain.
- In contrast to oter primae, hminoids lack tis, have eight premolars rtherthan 12, and molars with five cusps rathe four.
- Hminins ar unquly ipedal.
- Cimpanzees re theliving specistht are most closely related to humans.

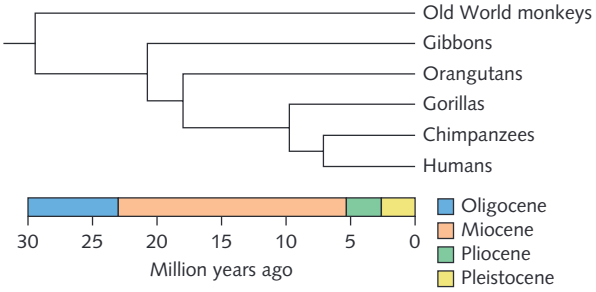


Figure 11.9 A phylogenetic tree depicting molecular clock estimates for divergences between major hominoid lineages

11.2 Adaptations that define humans

KEY CONCEPTS

p433

- Many features of the uma kleton, including the sk u, sie, lvis, femurs and feet, have a range of adapttions fr bpdalism.
- Humans have recsion grip hat nables anual dexterity.
- The anatomy oth bain, ncluding the enlarged prefrontal cortexand increaed cnvolutions, enhances the cogiive cpacity f humans.
- Written andspoken laguage enables humans to convey abstract concepts.
- Cultualevoltin s dstinguihd from biological evo ution by the peed, the meas andth choice exercised in ts ispesal.

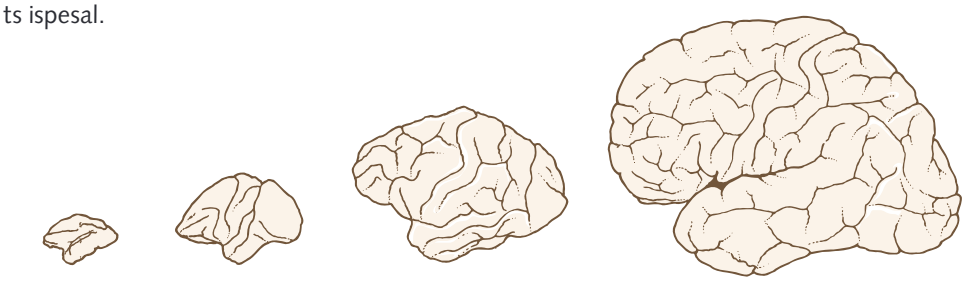


Figure 11.17 The brain sizes of various primates (drawn to scale)

9780170452533

11.3 Meet the ancestors

KEY CONCEPTS

p442

- Hminin vluion is represented by 'bshy' volutionary tr. Interpretations about the foss record are subject to onging debate and re finement as ne discveries are made.
- Austrloithecie were raively mall, bipedal apes.
- Austrloitheine specis are grouped into the genera *Australopithecus* (graile) and *Paranthropus* (robust).
- Eolution of the genus *Homo* s associated wit expansion of the crnum, a well as enhancemnts in bipedal evlton.
- New foi discvees (e.g. *Homo floresiensis*) chllenge exsing asumptions about hminn evolution.

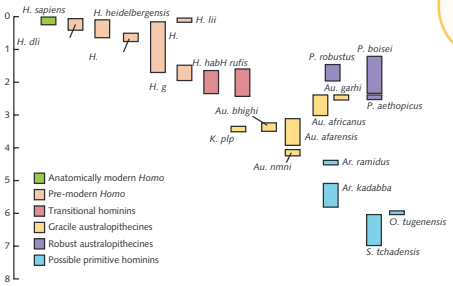


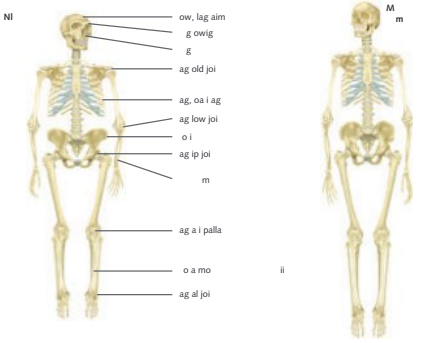
Figure 11.22 Species recognised in the hominin fossil record under the genera *Homo* (H.), *Ardpthecus* (Ar.), *Austraopthecus* (Au.), *Kenyanthropus* (K.), *Orrorn* (O.), *Paranthropus* (P.) and *Saheanthropus* (S.). The rectangles represent an estimate of the periods in the geological record during which the species are presumed to have lived, based on fossil evidence.

11.4 Modern humans and Neanderthals

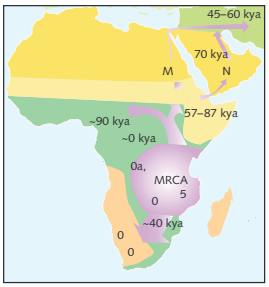
KEY CONCEPTS

p451

- Neandrthal wereancenthmnins wit distictiv anatomicl features, who coexisted for a time with modern humns.
- Neanderthals demontrated cutral and technologica evltion b ltle sy mblic exprssion or rt.
- Evidence fromosils, achaeology d mitochondrial and nclear DNA sequences support the recent single origin, or Out ofAfrica, hypothesis.
- Gentic and arheolgicalevidnce provides evidece for patterns of migration of modern humans across the world ncludinAstralia.
- Evidence fom mitoondrial DNA sugests Neanderhals and moder humans diverged up to 800 kya.
- Copleentin fosil eidnce, mitoch onrial ad nuclear genme data indicates tat modern humans evolved firs in Afica then igrae via the Middle Eastt the rest of the world.
- Evidence fro nuclear DNA sequence suggests sel ect groups of modern uman later inerbred with Neandethals and another roup oancent homiins called the Denisovans
- About 1 kya, the shift from huntergatherer to agricultur allifetyle accelerte cultura nd tecnlogical evolution.



Fgure 1135 A skeeta reco nstructon of *Homo neanderthaenss* and modern *Homo sapens*



Fgure 1139 b The ocaton of each hapogroup and mgraton patterns nferred from them takng pace thousands of years ago (kya) A hapogroup orgnate n Afrca and the Mdde East Ony the M and N hapogroup are found n ndgenous popuatons throughout the rest of the word MRCA = most recent common ancestor.

Fgure 1140 Mgraton routes (bue arrows) taken by ndgenous Austraans popuatng the ancent contnent of Sahu nferred from nucear genome sequences archaeoogy, and dvers ficaton of anguage The dots represent archaeoogca stes wth dates for the odest artefacts currenty known at each ste

WB
11.5.1
KEY TERMS
PAGE 247

11 Chapter glossary

arboreal related to, or living in, trees

australopithecine a term for bipedal apes of the fossil genera *Australopithecus* and *Paranthropus* that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya

bipedalism a type of locomotion in which an organism walks on two hind limbs

brachiation a type of locomotion in which an organism swings between the limbs of trees

brain case the part of the cranium that encloses the brain

brow ridge a bony ridge above the eye sockets

carrying angle the angle at which the femur is tilted in towards the knee

cerebral cortex the outermost layer of the brain

cognitive capacity an organism's innate intelligence, ability to learn, plan, evaluate, make decisions and apply new knowledge and skills

cranial capacity the volume of the brain case

cranium the skull, excluding the mandible

cultural evolution the way beliefs, social practices, skills and technology change over time

Denisovan a distinct, but undescribed, ancient hominin known primarily from bone fragments found in Denisova Cave in Siberia

dentition arrangement of the teeth

exocrine gland a gland that secretes a substance through a duct to the outer surface of the body

foramen magnum the hole in the base of the skull through which the spinal cord passes

gracile of slender build

hallux the big toe, or innermost toe of the foot

haplogroup a group of organisms that have the same genetic mutations in a single chromosome and are descendants of a common ancestor through either the maternal (mtDNA) or the paternal (Y chromosome) line of inheritance

haplotype the unique combination of genetic mutations in the DNA sequences of a single chromosome (e.g. mtDNA or Y chromosome) that are shared by different organisms and indicate common ancestry

hominin a member of tribe Hominini; modern humans and their extinct bipedal ancestors

hominoid a member of the superfamily Hominoidea; an ape, or tail-less primate

kya thousands of years ago

language the system of spoken or written communication comprising distinctive words and the rules by which the words are organised and expressed

mammal a warm-blooded vertebrate animal that has hair or fur, and the females secrete milk to nourish their offspring

mammary gland a gland in female mammals that produces milk

mandible the lower jawbone of the skull

midden a pile of discarded waste left by humans in the past

Out of Africa hypothesis *see* recent single origin

oviparous an animal that lays eggs that are expelled from the body and from which juveniles hatch

palaeoanthropology the field of study concerned with fossil hominins

placental describes an animal in which the foetus develops inside the mother while attached to an organ that provides nutrients and oxygen and removes wastes

postcranial all of the skeleton, except the skull

precision grip a grip defined by the tips of the thumb and fingers pressing together to finely manipulate an object

prefrontal cortex the portion of cerebral cortex that covers the front part of the brain

prehensile capable of curling and grasping

primate a member of the order Primata; includes lemurs, lorises, tarsiers, monkeys, apes and modern humans

prognathism a condition in which the jaws protrude from the plane of the face

quadrupedalism a type of locomotion in which an organism walks on four limbs

recent single origin a hypothesis that modern humans evolved in Africa and subsequently migrated out and colonised the other continents; also known as Out of Africa hypothesis

robust of sturdy build

sagittal crest a prominent raised bony ridge along the midline of the skull

sagittal keel a thickening of bone along the midline of the skull

sebaceous gland a gland in the skin that secretes oils that lubricate and protect patches of skin and hair

sexual dimorphism where males and females of a species have different morphologies, often in shape or size

stereoscopic describes vision that has a sense of depth

superfamily a taxonomic rank immediately superior to the traditional rank of family; a superfamily may contain multiple taxonomic families

suspensory locomotion a type of locomotion in which an organism hangs or moves beneath the limbs of trees

sweat gland a gland in the skin that secretes sweat

tribe a taxonomic rank inserted between family and genus

viviparous an animal that gives birth to live young

WB
11.5.2
EXAM PRACTICE
PAGE 249

11 Chapter review

Remembering

1 Sketch a diagram of a modern human and label the features that show the human is a mammal.
2 Describe how the fingers and toes of modern humans differ from the digits of other great apes. What advantages do these adaptations confer to modern humans?
3 Outline the ways that *Homo floresiensis* contrasts with other ancient *Homo* species.

Understanding

4 Summarise features of the postcranial anatomy of modern humans that adapt them for bipedal locomotion.
5 Describe at least five changes that have occurred to the hominin skull during evolution and indicate likely reasons for why they came about.
6 Describe the similarities and differences between the ways a monotreme, marsupial and placental mammal nourish their unborn and newborn young.
7 Sketch a cladogram that traces the evolutionary relationships among hominoids. On the cladogram, include the genera *Pongo*, *Gorilla*, *Pan*, *Australopithecus*, *Paranthropus* and *Homo*. Justify your positioning of each of the genera in the cladogram. Explain whether or not the hominins in your cladogram are a monophyletic group.
8 Explain how three anatomical and/or behavioural features of *Homo sapiens* have contributed to cultural evolution.
9 Is cranial capacity enough to explain the cognitive capacity of modern humans? Explain your point of view and provide evidence, wherever available, to support it.

Applying

10 Discuss the advantages conferred on primates by at least three adaptations that enabled them to live in trees.
11 Draw an annotated timeline from 2.5 mya to 100 kya showing:
   a the appearance of stone technologies and other cultural artefacts
   b which hominins the technologies were associated with
   c what these artefacts say about hominin cultural and technological evolution.
12 Draw a map outlining the major migration pathways of modern humans out of Africa and their timing based on nuclear genome sequences.
13 Describe key observations from mitochondrial and nuclear DNA of modern and ancient hominins that provide evidence for patterns of migration and interbreeding.

Analysing

14 Alleles for lactase persistence occur in many human populations around the world. The alleles are different, for example, between North African, European and Indian populations, but the phenotype is the same: lactase production persists beyond infancy and adults can digest lactose in milk. Studies of ancient DNA show the alleles were present at low proportions in early dairying populations 5–10 kya. Explain what has happened to result in higher frequencies of lactase persistence alleles in modern human populations. Your explanation should discuss:
   a why the alleles differ in different populations
   b which events represent natural selection
   c which events represent artificial selection
   d which events represent cultural evolution.
15 Social cooperation is observed in insect species such as termites and honey bees. Would you argue that this social cooperation is similar to or different from that observed in humans? Outline the evidence in support of your argument.

16 Distinctive pottery of Lapita culture is dated to 3.5 kya on islands off northern New Guinea, 2.9 kya in eastern mainland New Guinea and 2.5 kya in the Torres Strait islands. Pottery is not a feature of Torres Strait islander culture.
   a What might explain how the pottery arrived in the Torres Strait islands?
   b What evidence would you seek to support or disprove the hypothesis that Lapita people were absorbed into the Torres Strait islander population?

Evaluating

17 Consider the representative species of genus *Homo* discussed in this chapter. How satisfied are you with their designation as a distinct species? Give reasons to support your response.
18 Humans exercise great control over their environment and often adapt the environment to their needs. So have humans ceased to evolve? Provide evidence to justify your response.

Creating

19 Write an account of the changes in diet during hominin evolution from australopithecines to modern humans. Support your account with evidence from a range of sources, including the fossil record, comparative anatomy, biogeography and archaeological artefacts.
20 Sketch and annotate a drawing of what you predict the common ancestor of the chimpanzee and modern human might have looked like. Summarise the features you expect to see in the ancestor and list dot points to explain your reasoning.

Unit 4 Area of Study 2 review

Multiple choice

Question 1 ©VCAA 2012 EXAM 2 Q25 ADAPTED EASY

Cultural evolution

A includes physical responses to life events and the behaviour of individuals in a population.
B occurs faster than biological evolution.
C describes change in a population over many generations through the inheritance of traits.
D is defined as simply change over time.

Question 2 ©VCAA 2017 Q30 ADAPTED EASY

Farming practices adopted by Mongol farmers involved separating horses with docile temperaments and ease of riding from the native stock to interbreed within themselves for future horse herds. This is an example of

A genetic drift.
B natural selection.
C allopatric speciation.
D selective breeding.

Question 3 ©VCAA 2010 EXAM 2 Q18 ADAPTED MEDUM

Index fossils are useful because they

A represent the oldest rocks in a series of strata.
B show the absolute age of any sedimentary rock stratum.
C are easily recognisable and have a wide geographic distribution.
D date the age of rocks from the Triassic period only.

Question 4 ©VCAA 2006 EXAM 2 Q16 ADAPTED HARD

A type of genetic drift is

A speciation.
B founder effect.
C gene flow.
D selection pressures.

Question 5 ©VCAA 2019 Q28 ADAPTED MEDUM

The image shows a marine ammonite discovered by scientists. This type of fossil is best described as

A preserved remains.
B a petrified fossil.
C a cast.
D a trace fossil.

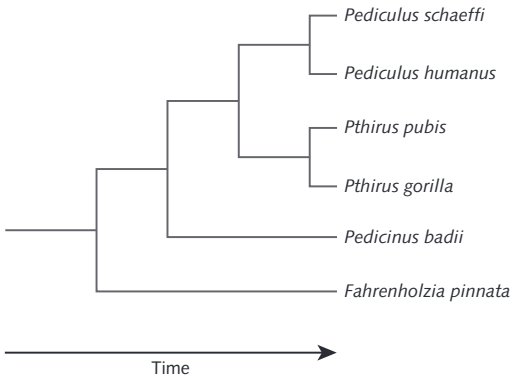
Question 6 ©VCAA 2012 EXAM 2 Q23 ADAPTED EASY

When scientists discover a new species of any organism, their claims need to be supported by evidence that shows that the new species

A looks similar to other species.
B cannot produce fertile offspring with members of known species.
C exhibits different behaviours to other known species.
D has DNA sequences that are similar to known species.

Question 7 ©VCAA 2016 Q29 ADAPTED MEDUM

The following diagram is a phylogenetic tree for six different species of lice. The tree has been constructed from molecular and morphological data.



This information suggests that

A *Pediculus humanus* is the ancestor of *Pediculus schaeffi*.
B *Pediculus humanus* is more closely related to *Pedicinus badii* than it is to *Pthirus gorillae*.
C *Pedicinus schaeffi* shares a more recent common ancestor with *Pthirus gorillae* than it does with *Pediculus humanus*.
D *Pedicinus badii* shares a more recent common ancestor with *Pthirus pubis* than it does with with *Fahrenholzia pinnata*.

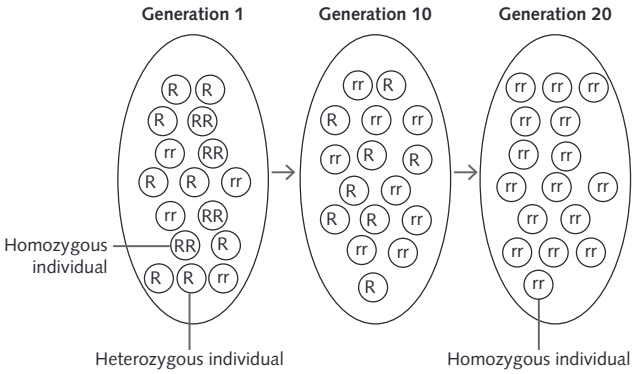
Question 8 ©VCAA 2016 Q30 ADAPTED EASY

Height is an example of a human trait that displays continuous variation. A trait that displays continuous variation

A may be influenced by many genes and the environment.
B is controlled by a single gene with very few alleles.
C is human ABO blood groupings.
D would have equal numbers of individuals in the population carrying each of the different genotypes.

Question 9 ©VCAA 2019 Q26 ADAPTED HARD

Consider the following diagram, which shows the gene pool of a population over 20 generations.



It would be correct to conclude that, over the 20 generations

A new advantageous alleles for this gene were introduced via individuals joining the population.
B individuals with the genotype rr had a selective disadvantage in this population.
C the frequency of each allele is equal in Generation 10 but not in other generations.
D genetic diversity is decreasing in this population.

Question 10 ©VCAA 2013 Q31 MEDUM

The thylacine (*Thylacinus cynocephalus*) was a large, dog-like marsupial whose last known specimen died in 1935. A mummified carcass of a thylacine, found in a cave on the Nullarbor Plain, was dated at about 5000 years old. Dating a mummified carcass this old is most successfully done by

A carbon dating of the mummified remains.
B uranium–thorium dating of the mummified remains.
C relative dating of the layers of rocks within the cave.
D comparing the teeth in the mummified carcass to the teeth of other fossilised dogs.

Question 11 ©VCAA 2019 Q33 ADAPTED MEDUM

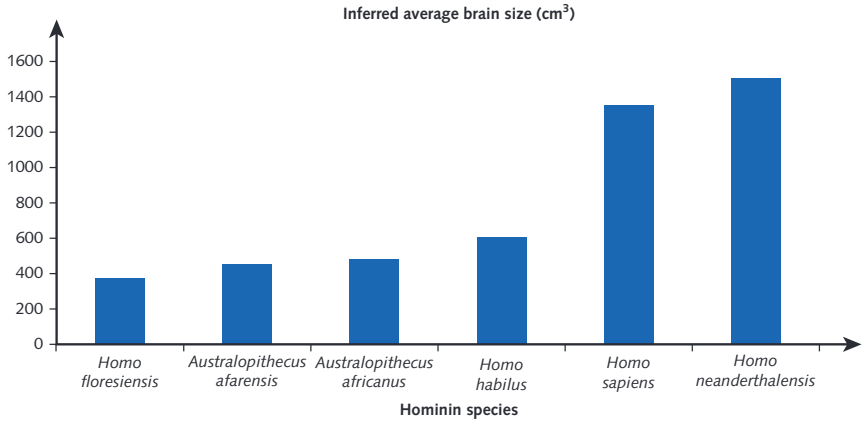
Significant trends in hominin evolution occurred in the transition from the *Australopithecus* species to the *Homo* species. Which of the following shows the group of characteristics that best reflects these trends?

A Increasing size of brow ridges, decreasing tooth size, increasing size of zygomatic arch, increasingly bowl-shaped pelvis
B Increasing cranial capacity, decreasing size of canines, increasingly bowl-shaped pelvis, increasing arch of feet
C Decreasing cranial capacity, increasing jaw size, increasing arch of feet, decreasing tooth size
D Decreasing tooth size, decreasing arch of feet, decreasing size of brow ridges, more-opposable big toe

Question 12 ©VCAA 2019 Q32 HARD

The inferred average brain size of a number of hominin species is represented in the following bar graph.
Using the information in the graph and your knowledge of human evolution, which one of the following species could be the transitional form between *Australopithecus* and *Homo* species?

A *H. habilis*
B *H. sapiens*
C *H. floresiensis*
D *A. afarensis*



Source: Australian Museum, https://australianmuseum.net.au

Question 13 ©VCAA 2007 EXAM 2 Q7 ADAPTED EASY

Comparison of sequences in mitochondrial DNA is often used to establish the degree of relatedness between organisms, and thus to suggest evolutionary relationships, particularly in complex, higher-level organisms.
Mitochondrial DNA is used because it

A is only inherited through the female line.
B has a higher rate of crossing over than nuclear DNA.
C has more genes than nuclear DNA.
D contains different nitrogen bases from those found in nuclear DNA.

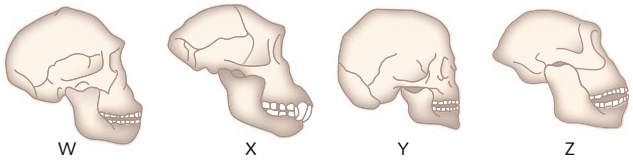
Question 14 ©VCAA 2017 Q35 ADAPTED MEDUM

The genome of modern-day African *Homo sapiens* does not contain Neanderthal DNA. Modern non-African *H. sapiens* contain a small percentage of Neanderthal DNA due to interbreeding between *H sapiens* and Neanderthals. This interbreeding is thought to have happened between 65 000 and 47 000 years ago. A recent study has found that the genomes of 100 000-year-old Neanderthal remains contain *H. sapiens* DNA. From this new discovery, it would be reasonable to conclude that

A modern Africans are descended from Neanderthals.
B between 65 000 and 47 00 years ago, the ancestors of modern Africans migrated from Europe to Africa.
C *H. sapiens* migrated out of Africa before 100 000 years ago.
D approximately 100 000 years ago, *H. sapiens* bred with Neanderthals in Africa before the Neanderthals migrated out of Africa to the rest of the world.

Question 15 ©VCAA 2006 EXAM 2 Q 22 HARD

Consider the following diagrams of skulls.



The skull most likely to be that of a Neanderthal is:

A W.
B X.
C Y.
D Z.

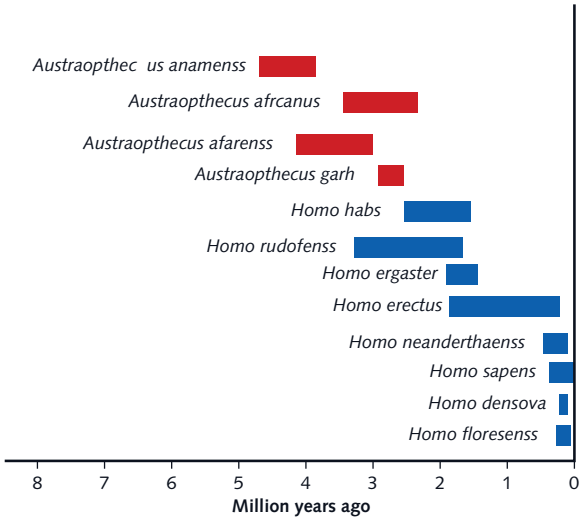
Short answer

Question 1 ©VCAA 2017 SECTON B Q7 ADAPTED

In 2013, about 1500 fossil bones of a hominin species were found in a cave in South Africa. From these bones, scientists have managed to construct an almost complete skeleton. The fossil bones have some features in common with those of the genus *Australopithecus*; however, they have enough similarities to the genus *Homo* that scientists have classified the fossil skeleton as belonging to a new species, *Homo naledi*.

a What two features would the fossil skeleton of *H. naledi* have shown to cause the scientists to classify it into a different genus from *Australopithecus*? 2 marks

Determining the age of these *H naledi* fossils has been both difficult and controversial. A group of scientists claims that the age of the fossils is more than 2 million years and suggests that *H naledi* might be a 'link' between *Australopithecus* and *Homo*. A second group of scientists has calculated the age of the *Hnaledi* fossils to be only about 900 000 years and claims that *Hnaledi* cannot be the 'link' between *Australopithecus* and *Homo*. The following diagram indicates the time periods for different *Australopithecus* and *Homo* species.



b Draw a horizontal arrow on the diagram to show where *H naledi* would have occurred if the first group of scientists was correct.

1 mark

Question 2 ©VCAA 2009 EXAM 2 SECTON B Q4 ADAPTED

A slab of rock contained a fossil of an ancient fish form.

a What type of rock is this most likely to be?

1 mark

b List the sequence of events that led to the formation of this fossil.

2 marks

c Scientists discovered more fossils in layers below this fish fossil. They declared that these fossils were older than the fish fossil. Were they right or wrong? Explain your choice.

2 marks

Question 3 ©VCAA 2003 EXAM 2 SECTON B Q6 ADAPTED

The following table shows the number of nucleotide differences between a region of mitochondrial DNA in humans, chimpanzees and a Neanderthal.

a Based on the data in the table, list the other organisms in order of relatedness to Human 1.

2 marks

b The Neanderthal DNA was extracted from a fossil approximately 25 000 years old.

i What other type of information obtained from the fossil could be used to assist in determining the evolutionary relationship of Neanderthals with humans and chimpanzees?

ii One scientist suggested using potassium–argon dating to determine the absolute age of the fossil. The other scientists disagreed. Explain why they disagreed and what they would have suggested instead.

iii Would dating this fossil using relative dating techniques provide a more accurate estimate of age than using absolute dating techniques? Explain your answer.

1 + 2 + 2 = 5 marks

	Human 2	Chimpanzee 1	Chimpanzee 2	Neanderthal
Human 1	15	77	76	20
Human 2		79	80	27
Chimpanzee 1			23	72
Chimpanzee 2				71
Neanderthal				

Glossary

A

α-helix a type of secondary protein structure in which the polypeptide chain folds into a tight coil

absolute dating the process of determining the age in years of rocks and the fossils they contain on the basis of the physical or chemical properties of materials in the rock

accurate without any mistakes

acetyl CoA a molecule used to convey carbon atoms to the Krebs cycle

activation energy the energy required to initiate a reaction

active immunity when, after vaccination, memory cells are created to provide immunity against further exposure to antigens

active site the place on the surface of an enzyme molecule where substrate molecules attach

adaptation an anatomical, behavioural and physiological characteristic that allows an organism to exploit a specific ecological role

adaptive immune response an immune response directed against a specific antigen; it retains memory of that antigen so that, on subsequent exposure to the same antigen, it responds with a secondary response

adaptive radiation when a single species diversifies relatively rapidly into many new species because of the availability of many different ecological niches

adenosine diphosphate (ADP) a low-energy compound made of adenine and ribose with two phosphate groups attached; it is converted to ATP for energy storage when it gains a phosphate group

adenosine triphosphate (ATP) a high-energy compound made of adenine and ribose with a chain of three phosphate groups attached; it releases energy for cellular reactions when its last phosphate group is removed and it is converted to ADP

aerobe an organism that requires oxygen to survive and reproduce

aerobic cellular respiration a metabolic reaction that requires oxygen to produce energy for the cell

agarose gel a gel matrix used for electrophoresis

agglutination when antigens or pathogens become stuck together because of antibody binding

alcoholic fermentation a form of anaerobic respiration (no oxygen present); glucose is converted to ethanol, a type of alcohol

allele a different version of the same gene (at the same locus) determined by small differences in the DNA sequence of the gene

allergen an antigen that is normally innocuous but can sometimes cause an over-reaction from the immune system known as an allergy

allergy an immune response characterised by IgE production to an innocuous substance

allopatric speciation speciation that occurs when members of an ancestral population become geographically separated and each isolated population evolves into a new species

allosteric site a binding site on a protein (usually an enzyme) that is not part of the active site; binding of a specific molecule at this site results in a change in activity of the protein

alternative splicing a process in which one or more exons are removed with the introns to produce mRNA molecules of different length and sequence

amino acid a nitrogen-containing compound that is the monomer from which proteins are built

amino acid sequence the primary structure of a protein; comprises the order of the 20 possible amino acids in the polypeptide, sometimes referred to as 'polypeptide sequence'

anabolic reaction a reaction in which atoms and small molecules are joined together to make larger molecules

anaerobe an organism that does not require oxygen to survive and reproduce

anaerobic cellular respiration cellular respiration in the absence of oxygen

analogous structure an anatomical or morphological feature in different organisms that has the same function but not the same basic underlying structure

anaphylactic shock a severe allergic reaction that causes widespread swelling, including of the face and neck, which can lead to difficulty breathing and a life-threatening reaction

annealing a process used in the polymerase chain reaction to join separate strands of DNA together as a result of hydrogen bonds pairing; occurs when the temperature is lowered

antibiotic a naturally or synthetically produced compound that is toxic to bacteria

antibiotic resistance the capacity for a microbe to withstand the lethal effects of an antibiotic to which it was once susceptible

antibiotic resistance gene a gene that codes for an antibiotic resistant phenotype

antibiotic selection growing bacteria in the presence of an antibiotic so only cells containing a gene for antibiotic resistance (encoded on a recombinant plasmid) can grow

antibody a Y-shaped protein that binds to foreign substances that invade the body; also called immunoglobulin

anticodon the three nucleotides in tRNA that bind to the complementary codon in mRNA according to base-pairing rules, resulting in the addition of a specific amino acid to the polypeptide chain

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

antigen-presenting cell (APC) a cell that displays peptides derived from processed antigens on major histocompatibility complex class II molecules for presentation to T_H cell; can be B cells, macrophages and dendritic cells

antigenic drift a change in the antigen of a virus that would otherwise be recognisable by the adaptive immune system, resulting from the gradual accumulation of mutations in the virus

antigenic shift a sudden change in the antigen of a virus resulting from the rearrangement of genetic material from two or more strains or subtypes of the virus

antiparallel parallel but orientated in the opposite direction

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cells by various enzymes, including caspases

arboreal related to, or living in, trees

artificial selection breeding of plants and animals over successive generations to produce traits that are desirable to humans; also known as 'selective breeding'

ATP synthase an enzyme that provides energy for the cell through synthesis of ATP

australopithecine a term for bipedal apes of the fossil genera *Australopithecus* and *Paranthropus* that inhabited eastern and southern Africa between 1.4 and at least 4.2 mya

authentication confirming that the submitted assessment has been completed by the student

autoantibody an antibody produced by a person's immune system that is directed against the person's own proteins

autoimmune disease a disease caused when a person's immune system mistakes self-cells and tissues as non-self and initiates an immune response against them

autotroph an organism that makes its own food from inorganic substances, using light (through photosynthesis) or chemical energy (through chemosynthesis); includes green plants, algae and certain bacteria

B

β-pleated sheet a type of secondary protein structure in which segments of the polypeptide chain bond side by side into a flattened assembly

B cell a class of lymphocyte that, once activated, produces antibodies; also called a B lymphocyte

B cell receptor a surface-bound antibody that serves as a receptor so that B cells can detect antigens

B plasma cell a cell that originates in the bone marrow and produces large quantities of antibodies

bacteria unicellular prokaryotes that can be pathogenic and therefore carry disease

bacterial capsule a polysaccharide layer surrounding some bacteria that makes them resistant to phagocytosis and thus more virulent

bacteriophage a virus that can infect bacteria and replicate

base pair two complementary nitrogen bases linked by hydrogen bonding

beneficence an ethical concept that involves taking positive action that maximises the benefit or 'good', and minimises the risks and potential harms

beneficial mutation a mutation that increases the organism's chances of survival and reproduction

bias an error that occurs when an investigation is not randomised, particularly if the investigator is affected by their expectations of the outcome

biochemical pathway a series of chemical reactions, each controlled by an enzyme, that brings about the step-by-step conversion of an initial substrate molecule to a final product

bioethics the study of ethical issues emerging from advancements in biology

biofuel a fuel that has used biomass as its original source

bioinformatics the application of computer science to the digital storage, retrieval and analysis of large volumes of biological data

biological functionality the function of a protein

biological species concept the concept that species are groups of natural populations that could potentially interbreed but are reproductively isolated from other populations

biomass the total dry weight of organic material

biotechnology the use of living organisms and biological systems and processes for human benefit

bipedalism a type of locomotion in which an organism walks on two hind limbs

block mutation a mutation involving rearrangements of chromosomal segments

blunt end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at the same position on both strands

bone marrow soft tissue found inside some bones that contains stem cells that produce cells of the immune system

bottleneck effect when a catastrophic event or period of adverse conditions drastically reduces the size of a population and its genetic diversity

brachiation a type of locomotion in which an organism swings between the limbs of trees

brain case the part of the cranium that encloses the brain

broad spectrum describes an antibiotic (or insecticide) that is effective against a variety of organisms

brow ridge a bony ridge above the eye sockets

C

C_3 plant a plant that directly uses CO_2 as an input for photosynthesis

Calvin–Benson cycle a biochemical pathway in which sugar molecules are produced using carbon dioxide

CAM (crassulacean acid metabolism) plant a plant that shuts its stomata during the day and fixes carbon during the night when its stomata are open; an adaptation to hot dry environments

cancer uncontrolled abnormal division of cells that are not kept in check by the immune system and invade other areas of the body

carbon fixation the conversion of atmospheric carbon dioxide into carbohydrates in the stroma of chloroplasts in eukaryotic cells

carrier a person who does not show symptoms of a disease but can transmit the infection to others

carrying angle the angle at which the femur is tilted in towards the knee

Cas9 protein an endonuclease that cuts double-stranded DNA at a target location in the genome

catabolic reaction a reaction in which larger molecules are broken down into smaller molecules

catalyse to speed up a biochemical reaction by using an enzyme

catalyst a substance that increases the rate of a reaction without itself undergoing any permanent chemical change

cell-mediated immunity an immune response initiated by cells, which does not involve antibodies

cellular metabolism the sum of metabolic reactions in a cell

cellular pathogen a disease-causing pathogen that is made up of one or more living cells such as bacteria or fungi

cellular respiration a process occurring in all living cells where large molecules are broken down to release energy

cerebral cortex the outermost layer of the brain

chemoautotroph an organism that makes its own food from inorganic substances, using chemicals as the primary energy source

chemokine a type of cytokine that induces chemotaxis

chemotaxis the movement of an organism or a cell along a chemical concentration gradient either towards or away from a chemokine

chlorophyll the green pigment in plant chloroplasts; it absorbs light energy, making it available for photosynthesis

chloroplast a membrane-bound organelle containing chlorophyll and found in the cytoplasm of plants and algae; its main function is photosynthesis and storage of carbohydrates

chromosome a thread-like structure made of nucleic acids and proteins that encode genetic information

cilia slender hair-like structures projecting from a cell surface that beat against fluid

cisterna a flattened membrane disc that makes up the Golgi apparatus and endoplasmic reticulum

clade a branch of a cladogram that represents a common ancestor and all of its descendants

cladogram a phylogenetic tree that depicts a hypothesis about the evolution of a group of organisms from a common ancestor

clonal selection the process in which lymphocytes that have bound to an antigen divide rapidly and become more numerous than other clones

codon a group of three nucleotides in mRNA that specifies an amino acid

coenzyme a small molecule that assists enzyme activity by carrying groups of atoms to or from the reaction

cofactor a molecule that assists enzyme activity by helping the enzyme to fold properly or to facilitate the reaction

cognitive capacity an organism's innate intelligence, ability to learn, plan, evaluate, make decisions and apply new knowledge and skills

companion plant a plant that is grown with another plant because one species improves the growth of the other

comparative dating *see* relative dating

competitive inhibitor a substance that competes with a substrate for an enzyme's active site and thereby reduces the enzyme's activity

complement a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

complementary base pairing the linking together of complementary nitrogen bases by hydrogen bonding; A pairs with T and C pairs with G

condensation polymerisation a reaction in which monomers are linked together into a polymer with the release of a small molecule, such as water, as a by-product

conformation the proper or functional shape of a protein

conserved amino acids of polypeptide sequences or nucleotides of DNA sequences that remain consistent across species

control group a group in an investigation that receives no treatment (independent variable) so a baseline value can be established

controlled variable the variable that is kept constant during an investigation in order to determine the relationship between the independent and dependent variables

convergent evolution when organisms that are not closely related independently evolve similar traits as a result of having to adapt to similar environments or ecological niches

correlation the inference that rock layers located in distant sites must be of the same age if they have identical mineral and fossil composition

cranial capacity the volume of the brain case

cranium the skull, excluding the mandible

CRISPR-Cas9 a bacterial immune defence mechanism in which short RNAs target complementary sequences in viral genomes to guide Cas9 proteins to destroy an invading virus

cristae the folding of the inner mitochondrial membrane into the matrix, thus increasing the total surface area of the inner membrane

crRNA (CRISPR RNA) RNA transcribed from the CRISPR locus; guides Cas9 proteins to their complementary sequence in the invading viral DNA genome, targeting it for destruction by Cas9

cultural evolution the way beliefs, social practices, skills and technology change over time

cytochrome a family of membrane-bound proteins that carry out electron transport; located in the mitochondrial inner membrane and in chloroplast thylakoid membrane

cytokine a signalling molecule that coordinates inflammation and immune responses and that leukocytes use to communicate with one another; includes interleukins and interferons

cytotoxic T cell (T_C cell) a class of lymphocyte that destroys virally infected or cancerous cells by secreting proteins that result in the extrinsic pathway of apoptosis; also called a cytotoxic T lymphocyte

D

defensin a type of small antimicrobial peptide secreted by nearly all plants and animals

degenerate a property of the genetic code in which most amino acids are encoded by two or more codons

degranulation a cellular process in which the granules of neutrophils, mast cells, basophils or eosinophils are emptied into extracellular surroundings

deleterious mutation a mutation that decreases the organism's chances of survival and reproduction

deletion mutation a mutation in which nucleotide pairs have been lost from a segment of DNA

denature to permanently change the molecular structure of a protein or DNA

dendritic cell a phagocyte with membranous extensions that engulf pathogens process them and present them to other cells of the immune system

Denisovan a distinct, but undescribed, ancient hominin known primarily from bone fragments found in Denisova Cave in Siberia

dentition arrangement of the teeth

deoxyribonucleic acid (DNA) the information molecule that is the basis of an organism's genetic material

dependent variable the variable that is measured and whose value depends on the independent variable, i.e. it responds to the independent variable

desensitisation a treatment to make a person more tolerant to a substance to which they are allergic

disease any condition that interferes with how an organism, or any part of it, functions

divergent evolution when members of a population develop adaptations to the different selection pressures over many successive generations and eventually become new species

DNA ligase an enzyme that catalyses the formation of a phosphodiester bond between two pieces of DNA

DNA polymerase the enzyme that catalyses the bonding of nucleotides to form new strands of DNA

DNA profiling comparison of individuals based on patterns of non-coding base sequences in the genome

DNA sequencing the process of establishing the nucleotide sequence of a piece of DNA

double-strand break a mutation involving breaks in the sugar-phosphate backbones at the same nucleotide pair, resulting in the complete breakage of a chromosome

duplication a mutation that occurs when one or more extra copies are made of a section of chromosome

E

electron spin resonance a method for determining the age of a rock or fossil based on the properties of electrons trapped inside the crystals of minerals

electron transport chain the process involving the stepwise transport of electrons to a final electron acceptor, such as oxygen (in aerobic cellular respiration); ultimately, it creates an electrochemical gradient across membranes to drive the addition of phosphate of ADP to yield ATP

endemic restricted or native to a certain locality

endergonic reaction a chemical reaction that requires the input of energy for it to proceed

endoplasmic reticulum an organelle made up of a network of membranous tubules involved in protein synthesis and folding for secretion

endothermic reaction a reaction that absorbs energy from its surroundings

enzyme a specific protein catalyst that increases the rate of a biochemical reaction within the cell by lowering the amount of energy required for the reaction to proceed

eosinophil a leukocyte that secretes powerful enzymes capable of rupturing multicellular organisms

epidemic the rapid spread of a disease across a number of countries

epitope a small part of a larger molecule that binds to a receptor site such as B cell receptors and T cell receptors

ethics a system of moral principles that considers what is good and bad for society

evolutionary distance the number of substitutions that have occurred in the amino acid sequences of homologous polypeptides or nucleotide sequences of homologous genes since two organisms diverged from a common ancestor

exergonic reaction a spontaneous reaction that releases energy

exocrine gland a gland that secretes a substance through a duct to the outer surface of the body

exon a segment of DNA or RNA containing information that codes for a polypeptide or part of a polypeptide

exothermic reaction a chemical reaction that releases energy, usually in the form of heat or light

extinct when all the members of a population or species have died out

extraneous variable a variable, other than the independent variable, that can influence the dependent variable

F

$FADH_2$ the loaded form of flavin adenine dinucleotide, a coenzyme that acts in both cellular respiration and photosynthesis

feedback inhibition a control mechanism used by cells in which an enzyme's activity is stopped or reduced by the product

first line of defence physical and chemical barriers that keep pathogens from entering the body of a living thing

fitness the capacity of an individual to survive and produce viable offspring

fixed describes an allele when it is the only variant available for a particular gene in the gene pool of a population

flagellum a helical filament that rotates to give bacteria locomotion

foramen magnum the hole in the base of the skull through which the spinal cord passes

fossil the preserved remains or traces of an organism

fossil record the worldwide collection of fossils as they occur in the surface layers of Earth

fossil succession when fossils appear in a consistent order in the fossil record from older rock layers to younger overlying rock layers; the same order is found worldwide

founder effect the type of gene flow that occurs when a few individuals that have become isolated from a larger population do not carry all the alleles that were present in the original population

frameshift mutation a mutation in DNA caused by the addition or deletion of a nucleotide or nucleotides resulting in a change in the amino acid sequence and protein being made

functional proteomics the study of how proteins work together in different cells or tissues, or under different circumstances

fungus a heterotropic organism made up of one or many cells; has cell walls but is not a plant

G

gel electrophoresis a technique that separates DNA fragments according to their size and charge

gene a segment of DNA in a chromosome that codes for a polypeptide; comprises the promoter, exons and introns

gene cloning the process of using plasmids and bacteria to make numerous identical copies of a gene

gene duplication generating an extra copy of a gene within a genome as a result of duplication of a chromosomal segment

gene expression the process by which the information in a gene is turned into a polypeptide

gene flow the transfer of alleles that results from emigration and immigration of individuals between populations

gene pool the range of genes and all their alleles present in a population

gene regulation the process by which gene expression is switched on or off

gene sequence the sequence of nucleotides in a gene

genetic code the complete set of mRNA codons and the corresponding amino acids they specify

genetic drift the change in the gene pool of a population as a result of chance; usually occurs in small populations

genetic engineering manipulation of genetic material, including altering DNA in an organism to suppress or enhance a gene's activity, or combining genetic material from different species

genetically modified organism (GMO) an organism whose genome has been genetically engineered

genome the complete sequence of DNA in a single (haploid) set of an organism's chromosomes, including nuclear, mitochondrial and chloroplast DNA

genotype a specific combination of alleles for a particular gene locus belonging to an individual

germline a cell line in eukaryotic organisms from which the gametes are derived

glycogen an energy-storage polysaccharide in animals that is made of many connected glucose molecules

glycolysis an energy-yielding process occurring in the cytosol of cells in which glucose is partially broken down to pyruvate in enzyme reactions that do not require oxygen; this first stage of cellular respiration produces two ATP molecules

Golgi apparatus a collection of membranes that package and store substances into vesicles in preparation for their release from the cell

gracile of slender build

gradient the slope of a graph

grana the stack of thylakoid membranes in a chloroplast that contain chlorophyll

granulocyte a white blood cell that has granules in the cytoplasm

guide RNA RNA that guides the Cas9 protein to the target sequence in a genome for gene editing

H

hallux the big toe, or innermost toe of the foot

haplogroup a group of organisms that have the same genetic mutations in a single chromosome and are descendants of a common ancestor through either the maternal (mtDNA) or the paternal (Y chromosome) line of inheritance

haplotype the unique combination of genetic mutations in the DNA sequences of a single chromosome (e.g. mtDNA or Y chromosome) that are shared by different organisms and indicate common ancestry

helper T cell (T_H cell) a lymphocyte that assists cytotoxic T cells, B cells and macrophages by secreting cytokines and providing contact-dependent signalling; also called a helper T lymphocyte

herd immunity when unvaccinated individuals are protected against a disease because a large number of people between 60–95% depending on the disease have been vaccinated, thereby making it unlikely that unvaccinated people will come into contact with anyone suffering from the disease

heritable capable of being passed on to the next generation

heterotroph an organism that cannot make its own organic compounds from simple inorganic material; it depends on other organisms for nutrients and energy requirements

histamine a chemical released by mast cells and basophils that increases blood flow and the permeability of capillaries

histone a protein that binds and packages DNA in eukaryotic chromosomes

hominin a member of tribe Hominini; modern humans and their extinct bipedal ancestors

hominoid a member of the superfamily Hominoidea; an ape, or tail-less primate

homologous refers to genes or polypeptides that have similar sequences and indicate a shared evolutionary ancestry

homologous structure an anatomical feature in different organisms that has the same basic underlying structure but different functions

horizontal gene transfer the process by which genetic material from one organism becomes incorporated into the genome of another organism

host the organism in which a parasite lives

humoral immune response an adaptive immune response mediated by antibodies

hybridoma a cell involved in the production of large amounts of monoclonal antibodies

hydrogen bond a weak chemical bond between a hydrogen atom on one molecule and a more electronegative element, usually an oxygen or nitrogen atom, on another molecule

hydrophilic describes substances such as polar molecules and ionic compounds that dissolve readily in water

hydrophobic describes substances such as non-polar molecules that are insoluble in water

hypothesis a tentative prediction, or explanation of an observation, based on an existing model or theory

I

immune having resistance to infection by a specific pathogen

immune system a complex network of cells, tissues and organs in the body that detect differences between self-molecules and foreign (non-self) organisms, and mounts an immune response that results in the formation of memory lymphocytes

immune tolerance tolerance of the presence of an antigen by the immune system so it does not mount an immune response to the antigen

immunisation the process of making a person immune to a disease through vaccination

immunoglobulin (Ig) a Y-shaped protein produced by plasma cells that binds to a specific antigen also called antibody

immunotherapy boosting the ability of a person's own immune system to fight cancer

independent variable the variable changed or manipulated by the scientist and assumed to have an effect on the dependent variable

index fossil a fossil that is representative of a specific geological time

induced-fit model a model of enzyme action that explains that the shape of an enzyme's active site undergoes specific changes, induced by the substrate, to achieve a high degree of specificity with the substrate

inducer a signalling molecule that switches on expression of a gene

infectious disease a disorder caused by bacteria, viruses, fungi and other organisms, that can often be transmitted to other members of a population

infectivity the ability of a pathogen to spread from one host to another host

inflammation an innate response to infection or damage that causes pain, swelling, heat and redness

innate immune response a response to a pathogen that is not specific to the antigen, only that it has been identified as being non-self; the response does not generate antibodies or memory lymphocytes

insertion mutation a mutation in which nucleotide pairs have been added to a segment of DNA

integrity an ethical concept that means being honest about one's actions; in science it means fully reporting data (even if it doesn't fit your hypothesis) and acknowledging all sources of information

interferon a type of cytokine produced by the cells of the immune system in response to challenges by foreign agents such as viruses, bacteria, parasites and tumour cells

interleukin a subset of cytokines that assists with the coordination of cells involved in the immune response

interstitial fluid a fluid that lies between cells; also known as tissue fluid or extracellular fluid

intron a segment of DNA within a gene or pre-mRNA that does not code for a polypeptide and interrupts the sequence of a gene

inversion mutation a mutation resulting in the normal sequence of genes being reversed in a chromosome

isotope one of two or more atoms of the same element with the same atomic number and number of protons, but different numbers of neutrons and therefore different relative atomic masses

J

justice a moral obligation to give fair consideration to competing claims not place unfair burden on a particular group and ensure fair access and distribution of benefits of an action

K

keratin the tough, fibrous protein of the outer epidermis layer

knock-in organism an organism in which DNA has been inserted into a specific locus

knock-out organism an organism whose DNA has been modified to disable the expression or function of a gene product

Koch's postulates a set of criteria to determine the causative agent of a disease

Krebs cycle a biochemical pathway that requires oxygen and takes place in the mitochondria as part of cellular respiration; acetyl CoA, the product of glycolysis, is broken down to produce carbon dioxide, water and energy in the form of ATP

kya thousands of years ago

L

lactic acid a product of anaerobic cellular respiration in animals

lactic acid fermentation a form of anaerobic respiration (no oxygen present) that occurs in animal cells and some anaerobic bacteria; glucose is converted to lactic acid

language the system of spoken or written communication comprising distinctive words and the rules by which the words are organised and expressed

latent not active

leukocyte the general term for a white blood cell

light-dependent stage the first stage of photosynthesis; it requires light energy that is absorbed by chlorophyll to split water molecules to produce oxygen, hydrogen ions and ATP

light-independent stage the second stage of photosynthesis; through a series of reactions, carbon dioxide, hydrogen ions and ATP produce carbohydrate

limiting factor the factor that limits the rate of a reaction

lineage in evolution, a population that represents a separate line of descent from a common ancestor to modern species

loaded describes coenzymes that are attached to the specific group of atoms they transfer

lock-and-key model a model of enzyme action that suggests that the shape of a substrate molecule is an exact fit to the shape of an enzyme's active site

logbook a record of experimental investigation kept by scientists performing the investigation; it is a legal record of the investigations and their results

luminescence in absolute dating, a method for determining the age of a mineral crystal based on measuring the emission of light by electrons as they are stimulated to escape from the crystal

lymph a colourless fluid that originates from tissue fluid

lymph node an immunological organ in which antigens are trapped or delivered by phagocytes to present to lymphocytes and initiate an adaptive immune response

lymphatic system a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and removing foreign matter

lymphocyte a type of leukocyte involved in adaptive immune responses

lysis the process of a cell bursting

lysozyme an antibacterial enzyme found in tears, saliva and other body fluids

M

macrophage a large white blood cell that phagocytoses pathogens; originates as monocytes in circulation

major histocompatibility complex (MHC) protein markers found on cell surfaces that are important in distinguishing self from non-self; MHC class I is found on all cells and MHC class II is found only on antigen-presenting cells

mammal a warm-blooded vertebrate animal that has hair or fur, and the females secrete milk to nourish their offspring

mammary gland a gland in female mammals that produces milk

mandible the lower jawbone of the skull

mass extinction the extinction of many species over a relatively short (geological) period

mast cell a cell that is located in the tissues and releases granules containing histamines when activated

maternally inherited describes a genotype that is transmitted entirely from the female parent to the offspring

matrix a gel-like fluid in mitochondria, where the Krebs cycle (citric acid cycle) of cellular respiration takes place

memory cell a long-lived lymphocyte capable of responding to a particular antigen when it is reintroduced; made from B cells and T cells

messenger RNA (mRNA) RNA copied from DNA that conveys the instructions needed for polypeptide synthesis from the nucleus to the cytoplasm

method the steps taken to carry out a scientific investigation

methodology the broader framework of approach taken in the investigation to test your research question

MHC restriction refers to the fact that T cells can only recognise antigens that are presented on MHC proteins

microbiome the bacteria, viruses and fungi that live in the gut plus their released metabolites and nucleic acids that exist in a specific environment

microbiota a community of micro-organisms, including fungi and bacteria, that live in or on another organism

microflora *see* microbiota

midden a pile of discarded waste left by humans in the past

mineralisation the process by which minerals from sediments have replaced the biological matter in a deceased organism, making it prone to become a fossil

missense mutation a mutation that results in one amino acid being replaced by another amino acid in the encoded protein

mitochondrion an organelle within the cytoplasm that is the site of aerobic cellular respiration, which releases energy for the cell

model a representation of a system or phenomenon that explains the system or phenomenon; a model may be mathematical equations, a computer simulation, a physical object, words or some other form

molecular clock the number of substitutions that have accumulated in the amino acid sequence of a polypeptide or the nucleotide sequence of a gene in a given lineage; the rate of the molecular clock is used to estimate the time since two species diverged

molecular homology the similarity of patterns in the nucleotide sequences of DNA or amino acid sequences of polypeptides as evidence for a common evolutionary origin

molecular size marker a set of pieces of DNA of known length that is used to estimate the size of other DNA fragments in a gel

monoclonal antibody a laboratory-produced molecule that serves as a substitute antibody to fight cancerous cells

monoculture the practice of cultivating a single genetically uniform breed of plant or livestock

monocyte a white blood cell that circulates in the blood and matures into a macrophage when it moves from the blood into the tissues

monophyletic describes a taxonomic group of species that have all descended from the same common ancestor

morphological species concept usually applied to fossils, defines a species by measurable anatomical criteria and characteristics

mucosal-associated lymphoid tissue (MALT) an extensive system of lymphoid tissue deposited all over the body; initiates immune responses along mucosal areas such as gastrointestinal tract, eyes and lungs

mucous membrane a mucus-secreting membrane that lines the respiratory, excretory and reproductive tracts

multidrug resistance when a bacterium becomes resistant to two or more antibiotics

mutagen an agent capable of inducing mutations

mutation when a gene or chromosome has undergone a change relative to the original gene or chromosome; it may also refer to the process of generating such changes

mya millions of years ago

N

NAD^+ the unloaded form of the nicotinamide adenine dinucleotide, a coenzyme that has a role in cellular respiration

NADH the loaded form of nicotinamide adenine dinucleotide, a coenzyme that has a role in cellular respiration

$NADP^+$ the unloaded form of nicotinamide adenine dinucleotide phosphate, a coenzyme that has a role in photosynthesis

NADPH the loaded form of nicotinamide adenine dinucleotide phosphate, a coenzyme that has a role in photosynthesis

natural killer cell a circulating leukocyte that kills body cells infected with a virus or transformed by cancer

natural selection the process whereby individuals with certain heritable traits survive and reproduce more successfully than other individuals

necrosis cell death that results from tissue damage or infection when the plasma membrane is breached; results in inflammation

neutral mutation a mutation that has no effect on the organism's chances of survival and reproduction

neutralisation the process by which antibodies prevent toxins from acting by binding to them and blocking them from binding to their targets

neutrophil a phagocytic leukocyte found in the blood and tissues

niche an organism's ecological role; the way the organism lives and functions in its environment

node a junction point in a phylogenetic tree that represents the common ancestor of the lineages that diverge from it

non-cellular pathogen a disease-causing pathogen that is not made of living cells; for example, viruses and prions

non-coding region DNA that does not encode a protein sequence

non-template strand the DNA strand that has the same sequence of nucleotides as the mRNA (except it has T instead of U)

non-competitive inhibitor a molecule that binds to an enzyme at a site other than the active site; this changes the shape of the enzyme so that the substrate can no longer bind to the active site

non-maleficence an ethical concept that involves avoiding harm or ensuring that harm caused by action is proportionate to the benefit gained from the action

non-self antigen a molecule that is not recognised by the immune system as being part of the organism itself

non-specific response a response that is the same regardless of the type of antigen

nonsense mutation a mutation in which a codon for an amino acid is changed to one that codes for a stop codon, terminating translation

nucleic acid a large, linear polymer built from nucleotide monomers bonded together; includes DNA and RNA

nucleosome a histone with a length of DNA coiled around it

nucleotide the monomer, or building block, of DNA and RNA, consisting of sugar, phosphate and a nitrogen base

O

obligate parasite a parasite that cannot complete its life cycle without a suitable host; without a host, the parasite cannot reproduce

observation acquisition of information through your senses

operator a segment of DNA to which a protein binds, usually to switch off gene expression

operon a group of genes that are expressed as a single unit

opsonisation the process in which a pathogen is coated with antibodies and/or complement and marked for phagocytosis

optically stimulated luminescence a luminescence technique that stimulates electrons to escape a mineral crystal when the crystal is exposed to coloured light

optimum pH the pH at which an enzyme works fastest

optimum temperature the temperature at which an enzyme works fastest

Out of Africa hypothesis *see* recent single origin

outlier a data point that does not fit the pattern shown by the other measured data points

oviparous an animal that lays eggs that are expelled from the body and from which juveniles hatch

P

pairwise comparison in evolutionary studies, a comparison between two polypeptide sequences, two DNA sequences or two genomes to determine how similar they are

palaeoanthropology the field of study concerned with fossil hominins

pandemic the spread of a disease across the world

passive immunity immunity characterised by the transfer of antibodies from one individual to another; does not generate immunological memory

pathogen an organism foreign to the body and capable of causing disease

peptide bond a chemical bond that links two amino acids in a chain

personal error a mistake or miscalculation due to human error

phagocyte a cell that is capable of phagocytosis; includes macrophages, dendritic cells and neutrophils

phagocytosis a process by which phagocytes engulf a particle or cell

phagolysosome a membrane-bound vesicle formed from the fusion of a phagosome and lysosome

phagosome a membrane-bound vesicle formed around a particle during phagocytosis

phenotype the actual form taken by a specific feature in a particular individual based on their genotype; can be used in reference to particular traits or characteristics or to the overall form of an individual

phosphodiester bond a chemical bond that links two nucleotides in a growing chain

photoautotroph an organism that makes its own food from inorganic substances, using light as its primary energy source

photorespiration the process in which plants take up oxygen and release carbon dioxide

photosynthesis the anabolic reaction in which light energy is captured by chlorophyll molecules and used to split water molecules, releasing oxygen and hydrogen atoms, which are joined to carbon dioxide to form glucose

phylogenetic tree a branching diagram showing the evolutionary relationships between species; groups joined together in the tree are believed to have descended from a common ancestor

phylogeny the evolutionary relationships that exist between species, often expressed as a tree-like diagram or represented by taxonomic classification

phylogram a type of phylogenetic tree with branch lengths scaled to represent the number of nucleotide or amino acid changes that have occurred during the evolution of each lineage

pigment a molecule that absorbs certain wavelengths of light and reflects all others

placental describes an animal in which the foetus develops inside the mother while attached to an organ that provides nutrients and oxygen and removes wastes

plasmid a small, circular DNA structure independent of the chromosome in prokaryotic cells

platelet a cell fragment found in the blood involved in blood clotting

point mutation a mutation that affects a single base-pair position within a gene

poly-A tail a chain of 100–200 adenine nucleotides added at the 3′ end of an mRNA strand

polymerase chain reaction (PCR) a cyclical reaction in which DNA polymerase is used to copy a DNA template, making millions of copies of the same piece of DNA

polymorphism a variation in DNA sequences among individuals

polypeptide a linear polymer built from amino acid monomers

polyribosome a chain of ribosomes formed by attaching to and translating from a single mRNA strand

population a group of individuals of the same species that live in the same area and interbreed, producing fertile offspring

population genetics the study of allele frequencies in populations and how they change over time

postcranial all of the skeleton, except the skull

pre-mRNA an unprocessed RNA strand that is transcribed directly from the DNA

precise how closely together measurements are to one another

precision grip a grip defined by the tips of the thumb and fingers pressing together to finely manipulate an object

prefrontal cortex the portion of cerebral cortex that covers the front part of the brain

prehensile capable of curling and grasping

primary data data that you have measured or collected yourself

primary host an organism in which a pathogen reproduces

primary lymphoid organ the bone marrow and thymus; responsible for the production and maturation of immune cells

primary response the response generated when an antigen is encountered for the first time; contrasted with the secondary response

primary source an original source of information, created by the author and usually including primary data

primary structure the linear sequence of amino acids that makes up a polypeptide chain

primate a member of the order Primata; includes lemurs, lorises, tarsiers, monkeys, apes and modern humans

primer a single-stranded DNA molecule that acts as the start of the amplification process

prion an infectious protein that can cause other unaffected prion proteins in the brain to take the affected form, causing transmissible spongiform encephalopathies

product the outputs of a chemical reaction that are formed from the reactants or inputs

prognathism a condition in which the jaws protrude from the plane of the face

promoter region a segment of DNA to which RNA polymerase binds to begin transcription

protein a polymer made up of amino acid monomers; may consist of a single polymer chain or many polymers bonded together into a functional molecule

protein secretory pathway the pathway through which cells package proteins into vesicles for release into the extracellular environment

proteome the complete set of proteins produced by a cell, a tissue, or an organism

proteomics the study of proteomes

protist a unicellular eukaryotic organism

pyruvate a three-carbon molecule that is the end product of glycolysis

Q

quadrupedalism a type of locomotion in which an organism walks on four limbs

qualitative data a measurement with descriptive or nonnumerical results

quantitative data a measurement with numerical values

quarantine restricting the mobility of person or persons to a certain area so they reduce contact with other people in order to stop the spread of a pathogen

quaternary structure the structure formed when two or more polypeptides associate into a mature protein

R

radioactive decay a process by which the nucleus of an unstable isotope splits and emits energy in the form of radioactivity

radiometric dating a method for determining the age of a rock or fossil based on the predictable rates of decay of naturally occurring radioactive isotopes present

random coil a secondary protein structure in which the polypeptide chain does not fold into a specified arrangement

random error an unpredictable variation in measurement; can be improved by taking multiple measurements and calculating an average

reactant the inputs of a chemical reaction that are required to form products or outputs

recent single origin a hypothesis that modern humans evolved in Africa and subsequently migrated out and colonised the other continents; also known as Out of Africa hypothesis

recombinant DNA technology the process of transferring a gene from a cell of one species to the cell of a different species

recombinant plasmid a plasmid with foreign DNA inserted into it

references a list of all the sources that have been used in the write-up of a scientific investigation

regulatory gene a gene whose product switches on or switches off expression of one or more other genes

regulatory T cell (T_{reg} cell) a class of lymphocyte that helps to negatively regulate the immune response; also called a regulatory T lymphocyte

relative dating the process of determining the age of rocks and the fossils they contain relative to each other, allowing an estimation of 'oldest to youngest' without assigning an actual age in years

reliable highly likely to be a trustworthy source of information or reproducible data

repeatable an investigation that can be conducted again by the same investigator under the same conditions to generate similar results

replicates independent samples that allow you to take multiple measurements, increasing the reliability of your data

reporter gene a gene that enables visualisation or quantification of gene expression

repressor protein a protein that binds DNA to prevent RNA polymerase attaching or transcribing essentially shuts off gene expression

reproducible giving the same result within uncertainty limits; when repeated measurements are made by a different investigator

reproductively isolated when sexual reproduction can no longer occur freely among any adult members of the population

research question a specific question that a particular investigation is attempting to answer

respect an ethical concept that considers the rights of an individual or a group; e.g. respect for animals considers their welfare

restriction digest reaction a reaction in which restriction enzymes are incubated with DNA to cut the DNA into fragments at specific restriction sites

restriction endonuclease (restriction enzyme) an enzyme that cuts DNA at a specific restriction site

restriction fragment a short fragment of DNA generated after the cutting of a longer DNA fragment by a restriction enzyme

restriction site a specific nucleotide sequence (usually 4–8 bp) that is recognised as a cleaving site for a restriction enzyme

ribonucleic acid (RNA) a type of nucleic acid consisting of a single strand of nucleotides; has essential roles in protein synthesis

ribosomal RNA (rRNA) an RNA strand that serves as a structural component of a ribosome

ribosome a small structure consisting of RNA and proteins where amino acids are joined to form polypeptides

risk assessment a process of evaluating potential risks of an investigation

RNA polymerase the enzyme that catalyses the synthesis of RNA

robust of sturdy build

rough endoplasmic reticulum endoplasmic reticulum with ribosomes attached

S

sagittal crest a prominent raised bony ridge along the midline of the skull

sagittal keel a thickening of bone along the midline of the skull

sebaceous gland a gland in the skin that secretes oils that lubricate and protect patches of skin and hair

second line of defence non-specific immune responses including fever and inflammation

secondary data data that has been measured and collected by someone other than you

secondary host an organism in which the immature pathogen becomes mature

secondary lymphoid organ an organ that provides an environment for the initiation of the immune response; includes lymph nodes, spleen and mucosal-associated lymphoid tissue

secondary metabolite an organic compound produced by bacteria, fungi or plants; its role is to sustain functional and homeostatic health of cells within organs by assisting cells to excrete wastes and toxic substances

secondary response the response generated when the body encounters a pathogen to which it has previously generated an immune response; involves reactivation of memory lymphocytes and occurs more rapidly and with greater magnitude than the primary response

secondary source a source of information that has been obtained from another source and/or summarised, e.g. a popular science magazine

secondary structure the localised folding of a polypeptide chain when neighbouring amino acids bond to each other to form α-helices, β-pleated sheets or random loops

selection pressure a factor that favours the survival of some individuals over others within a population

selective breeding *see* artificial selection

self-antigen an antigen or a molecule that is a normal body component

self-tolerance the deletion or inactivation of lymphocyte clones that can bind to self-antigens to prevent an immune response to these antigens

semi-conservative replication the replication of DNA in which the product contains one original and one newly made strand

sensitisation initial exposure to an allergen resulting in an adaptive immune response that generates IgE

sequence alignment a display in which homologous polypeptide or DNA sequences are positioned against each other to identify patterns of conserved sequence

sexual dimorphism where males and females of a species

short tandem repeat (STR) a short non-coding region of DNA of up to five bases that is repeated many times in the genome of an organism; the number of times an STR is repeated is variable and can be used in DNA profiling

silent mutation a mutation in which the DNA codon for one amino acid becomes another DNA codon for the same amino acid; also referred to as a synonymous mutation

somatic describes a body cell that will not pass its genes on to the next generation

speciation the evolution of one or more new species from an ancestral species

species a group of similar organisms capable of breeding and exchanging genes with one another and whose offspring are capable of doing the same; also describes the lowest formal taxonomic rank and forms the second part of an organism's scientific name

specific response an adaptive immune response directed against a particular antigen that retains immunological memory of that antigen

spleen an abdominal organ that stores white blood cells

sporadic seen infrequently in a small number of people

stereoscopic describes vision that has a sense of depth

sterile inflammation inflammation resulting from the detection of damage- or danger-associated molecular patterns released during tissue injury in the absence of infection

sticky end the end of a DNA fragment that is created following cleavage by a restriction enzyme that cuts DNA at different positions on each strand

strata (singular: stratum) successive layers of sedimentary rocks; each layer represents a unique age range and contains a unique collection of fossils

stroma the jelly-like, semifluid interior of a chloroplast

structural gene a gene that codes for tRNA, rRNA or a polypeptide other than a regulatory molecule

structural morphology the study of the physical structure and form of organisms

subspecies the level of classification below species, referring to races of a species that are geographically isolated from each other

substitution mutation a mutation in which a single nucleotide is swapped for another in the original gene sequence

substrate the substance that an enzyme acts on

subunit a distinct component of a biological particle; in proteins, it refers to each polypeptide that contributes to the quaternary structure

superfamily a taxonomic rank immediately superior the traditional rank of family; a superfamily may contain multiple taxonomic families

superposition the principle that strata are deposited in a time sequence, with the oldest at the bottom and the youngest at the top

suspensory locomotion a type of locomotion in which an organism hangs or moves beneath the limbs of trees

sweat gland a gland in the skin that secretes sweat

sympatric speciation when two species evolve from an ancestral population while still inhabiting the same geographical area

synonymous mutation *see* silent mutation

systematic error a predictable deviation in data, e.g. as a result of the equipment used

T

T cell receptor (TCR) a protein receptor found on the surface of T cells; binds to antigens presented on major histocompatibility complex proteins

T-cell transfer therapy a therapy that boosts the ability of T cells to fight cancer cells

taxonomy a system of scientific conventions for naming and classifying organisms

template a pattern that can be used for making many more copies

template strand a strand of DNA that is copied during DNA or RNA synthesis

tertiary structure the overall three-dimensional shape of a completely folded polypeptide

tetrapod a 'four-footed' vertebrate animal; includes amphibians, reptiles and mammals

theory a collection of models and concepts that explains specific systems or phenomena; scientific theories allow predictions to be made and hence are falsifiable

thermoluminescence a luminescence technique that stimulates electrons to escape a mineral crystal when the crystal is heated

thylakoid membrane the interconnected, folded membrane within a chloroplast

thymus the gland in the upper chest where T cells mature

trace fossil a fossil produced by the organism's activities, such as fossil footprints or burrows

transcribe to copy DNA into mRNA

transcription the process by which DNA is copied into mRNA

transcription factor a protein that binds to DNA to control the rate of transcription from a gene

transfer RNA (tRNA) an RNA molecule that transports an amino acid to the ribosome for assembly into a polypeptide

transformation the process by which the genetic material of an organism is changed by the addition of new genetic material.

transgenic organism an organism that has been modified by incorporating a piece of foreign DNA into its genome

transitional fossil a fossil that bears features of both an older ancestral life form and a younger descendant

translation the process of turning the nucleotide sequence of mRNA into the amino acid sequence of a polypeptide

translocation a mutation occurring when a section of one chromosome breaks off and reattaches to another chromosome

transmitted when an infection is passed from one person or organism to another

transport vesicle a small membrane-bound sac containing protein that is transported from the Golgi apparatus to the plasma membrane for release into the extracellular environment

tribe a taxonomic rank inserted between family and genus

triplet a set of three nucleotide codes

true value a value obtained in an ideal measurement

tumour a mass of abnormally growing cells

U

uncertainty a range of values that the true value falls within

unloaded can accept protons, electrons or chemical groups that are released from catabolic reactions

V

vaccination the administration of a vaccine to protect someone from a disease

valid describes results that are affected by only a single independent variable and hence are reproducible

variable something that can change or be changed, as distinct from a constant, which does not change

vasodilation widening of blood vessels, particularly arterioles

vector a vehicle used to transfer DNA sequences from one organism to another

vestigial structure a structure found in organisms that has lost most, if not all, of its original function in the course of evolution; in ancestral organisms, the structures served a purpose, but in their descendants, the structures become atrophied or rudimentary

virulence the capacity of a pathogen to cause severe disease within its host

virus an obligate intracellular pathogen that can use the host cell's machinery to replicate itself; usually consists of a nucleic acid surrounded by a protein coat

viviparous an animal that gives birth to live young

W

wild type the genotype or phenotype that is most common, or standard, in natural conditions, in contrast to an atypical or mutant form

Z

zoonotic describes a disease that can be transmitted from animals to humans

Answers

Unit 3 Area of Study 1 review

Multiple choice

1 A
2 A
3 D
4 D
5 C
6 A
7 A
8 B
9 A
10 D
11 A
12 A
13 D
14 B
15 A

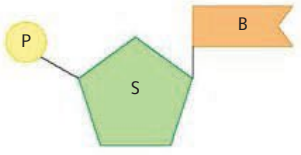
Short answer

1 a RNA polymerase
b To carry a copy of the instruction for polypeptide/protein production from the DNA in the nucleus to the ribosome in the cytosol.
c Processing (any two of): intron removal or exons joined; addition of a methyl cap/guanine cap; addition of poly-A tail; add 5' cap.
d Different factors expressed by regulator genes could lead to production of the different proteins OR Alternate splicing of the pre mRNA means that exons are joined in a different order, creating a different base sequence that would lead to the translation of a different protein.
e The genetic code being universal

2 a The ribosome binds to or reads the mRNA; tRNA brings in specific amino acids; or the tRNA anticodon is complementary to the mRNA codon. The amino acids are joined by condensation polymerisation.
b Any three of the following:

Organelle	Role
Rough endoplasmic reticulum	Transports trypsin within cell, including to the Golgi apparatus
Golgi apparatus	Packages trypsin into vesicles for export from the cell
Vesicles	Carries trypsin to plasma membrane where it fuses and releases the trypsin from the cell
Plasma membrane	Vesicles fuse with it and are released by exocytosis
Mitochondria	Provides the energy required e.g. for packaging

3 a mRNA: C A C U G U A A U G A G
b 30%
c i ribosomes
ii rRNA (ribosomal RNA) associates with proteins to from ribosomes
d Histidine, cysteine, asparagine, glutamic acid
e



Unit 3 Area of Study 2 review

Multiple choice

1 C
2 B
3 C
4 A
5 D
6 B
7 B
8 D
9 D

10 B **12** B **14** B
11 D **13** A **15** D

Short answer

1 **a** Oxygen levels decreased as the yeast cells were respiring aerobically
b Anaerobic respiration.
c Oxygen levels would stay the same as yeast cells would switch over to anaerobic respiration. Ethanol levels would rise as ethanol is a by-product of anaerobic respiration.

2 **a** Light-independent stage or Calvin (Benson) cycle
b Light-dependent stage; Input: light, water
Role: to provide energy to generate energy molecules such as ATP from ADP + Pi
Input: ADP or $NADP_+$ provides H+ needed to produce NADPH
Role: to carry energy from the light-dependent reaction to the light independent reaction

3 **a** Rubisco
b To carry energy needed for the reactions in the light independent reactions to occur. NADPH also provides the hydrogen ions needed to make glucose
c Glucose

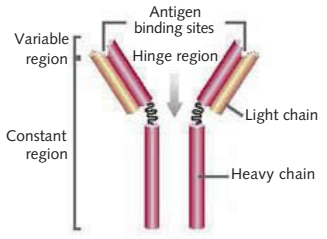
Unit 4 Area of Study 1 review

Multiple choice

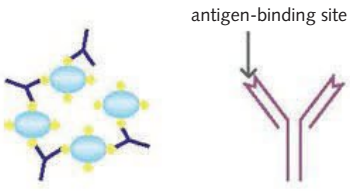
1 C **6** B **11** C
2 B **7** C **12** B
3 C **8** C **13** B
4 D **9** A **14** C
5 B **10** A **15** D

Short answer

1 **a** A pathogen is an contagious/infectious disease causing agent 1 mark.
b 2 marks



c **i** 2 marks



ii The antigen–antibody complex either immobilises the pathogen, agglutinates the pathogen or allows phagocytes to engulf the pathogen.

2 **a** Thick waxy cuticle on leaves and stems or thick bark on trees that act as a barrier to the entry of pathogens. Thorns or hairs to deter insects (2 × 1 mark)
b Two of the following: Intact skin; nose lined with thick, sticky mucous; fine nasal hairs to trap pathogens (2 × 1 mark)
c Macrophages engulf pathogens; dendritic cells engulf pathogens, process them and present them to other cells of the immune system. (2 × 1 mark)

3 **a** Lymph or lymphatic fluid (1 mark)

b Filter the lymph for foreign particles and invading pathogens; activation of white blood cells causing the influx of more white blood cells; and enlargement of lymph node (2 × 1 mark)

c B cells produce antibodies; rough endoplasmic reticulum produces proteins that make up antibodies. (2 × 1 mark)

Unit 4 Area of Study 2 review

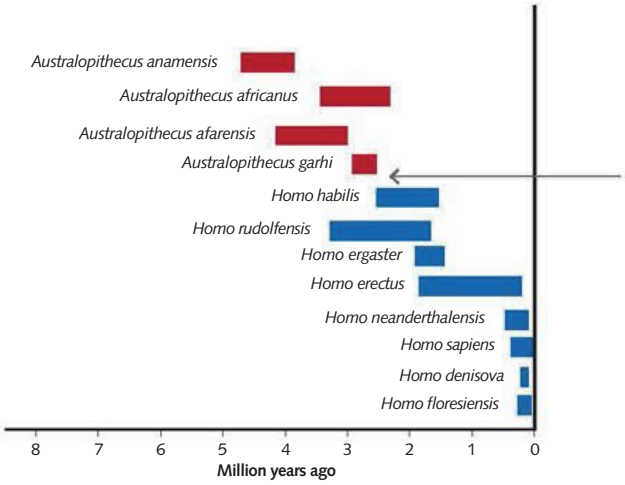
Multiple choice

1	B	**6**	B	**11**	B
2	D	**7**	D	**12**	A
3	C	**8**	A	**13**	A
4	B	**9**	D	**14**	C
5	C	**10**	A	**15**	A

Short answer

1 **a** Two of the following suggested answers.
The skull shows: a flatter face, a more parabolic jaw, a relatively larger brain case or teeth of a more uniform shape. The skeleton shows upper limbs/forelimbs shorter and hind limbs/legs longer. The features needed to be comparative.

b



2 **a** Sedimentary rock

b Organism must have hard body parts; is covered quickly; high pressure to promote mineralisation; low oxygen conditions to stop decay (1/2 mark each)

c Correct. Layers of sediment build up over time; the oldest fossils are found in the lowest stratum; the youngest at the highest stratum

3 **a** Human 1, Human 2, Neanderthal, Chimpanzee 2, Chimpanzee 1

b **i** Skeletal structure/morphology may be used to assist in determining the evolutionary relationship of Neanderthals with humans and chimpanzees.

ii The fossil is only 25,000 years old, it would be more accurate to use carbon dating to date the fossil. Carbon dating compares the ratio of carbon 14 to carbon 12 and the known rate of carbon 14 decay can be used to find the age of the fossil.

iii Absolute dating is more accurate than relative dating. Absolute dating uses know decay rates of parent isotopes to daughter isotopes to find the absolute age of a fossil. Relative dating uses rock layers to determine if a fossil is older or younger than other fossils or the presence of index fossils to give an age range only.

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