NELSON BIOLOGY UNITS 3&4

FOR THE AUSTRALIAN CURRICULUM

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WITH SARAH **JONES**

NELSON
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Nelson Biology Units 3 & 4 for the Australian Curriculum
1st Edition
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PREFACE

Nelson Biology Units 3 & 4 for the Australian Curriculum has been structured to meet the requirements of the ACARA Australian Senior Secondary Curriculum – Biology. The text has been written to the A level Achievement Standard, while also presenting content in easy to understand language. The rationale and aims of the Australian Curriculum have formed the basis of the approach for all of our authors. We are proud to have gathered a team of highly experienced subject experts from research, academia and secondary teaching. They were chosen for their comprehensive knowledge of Biology and best teaching practice in Biology education at secondary and tertiary levels. They are all dedicated to science communication and lifelong learning, and together they have produced an outstanding series.

One of the goals of this text is to help students gain a wide perspective on the breadth and depth of Biology in both theory and practice. Part of the rationale for the ACARA Australian Senior Secondary Curriculum – Biology is to expose students to the wondrous beauty and interconnectivity of life. To understand the living world around us is the key role of a biologist and is a key goal of this text. It is hoped that, through the study of living things, students will gain an appreciation of the complexity of life from the cellular level to the expansive biomes of the world, that they will better understand difficult bioethics situations and better evaluate the presentation of scientific issues in the media.

Nelson Biology Units 3 & 4 for the Australian Curriculum provides students with the tools and knowledge to fully prepare for exams and future studies in the area. It will also give them a deeper understanding of the precious living world around them, whether or not they pursue further studies in the many fields of Biology.

AUTHOR AND REVIEWER TEAMS

Authors

Dr Sarah Jones – Series consultant

Sarah completed her PhD at the Walter and Eliza Hall Institute of Medical Research in Melbourne and held research positions at Harvard Medical School, Boston, and Trinity College, Dublin, before returning to Australia as a Research Fellow at Monash University. She 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 is a member and previous Project Manager for the Australasian Society for Immunology. She has authored multiple peer-reviewed scientific articles in leading journals and continues to research the causes of, and potential new treatments for, autoimmune diseases.

Pam Borger

Pam studied at Melbourne University, gaining a Bachelor of Science and Diploma of Education. She went on to complete an Honours degree in genetics while undertaking work at the Murdoch Institute at the Royal Children's Hospital. Recently, Pam completed her Masters Degree in School Leadership. She has taught Biology and developed curriculum for more than 30 years in various schools. Pam is currently working for a consortium including all Victorian universities and education jurisdictions to improve participation of low socio-economic students in higher education. She has been actively involved in VCE Biology examinations as an assessor, exam setter and expert vetter over many years.

Dr Tony Chiovitti

Tony Chiovitti attained a B.Sc. (Hons.) at the University of Melbourne in 1992. He completed a PhD at the School of Botany, University of Melbourne, in 1997, investigating the cell wall biochemistry of Australian red algae and algal evolution using gene sequences. He has 8 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 in 2007, and is now the Deputy Director. Tony has developed and delivered educational programs for students and professional learning programs for teachers on the themes of cell and molecular biology, health and disease, ecology and evolution.

Jacinta Duncan

Jacinta began her career as a molecular biologist investigating population genetics. After 4 years as a research scientist she completed her Diploma in Education at the University of Melbourne and has been teaching VCE Biology for 15 years. She worked for 2 years as a Lecturer in Science Education for the Masters of Teaching at the University of Melbourne, including coordinating Biology Method. She is now the Director of the Gene Technology Access Centre, a specialist science centre delivering programs for students and teachers in cell and molecular biology. Jacinta is currently completing a Masters of Education by Research. She was pivotal in introducing bioinformatics tasks into Victorian schools and designs educational resources that assist students in understanding Biology concepts.

Wayne Gerdtz

Wayne has been educating students and the general public about dinosaurs, fossils and all areas of natural science for more than 14 years. He is the former Curator of Melbourne Museum's award-winning Science & Life Gallery, which includes exhibitions on dinosaurs, geologic time, Earth sciences and wildlife ecology and conservation. As part of his doctoral studies, Wayne has published several academic manuscripts on fossil mammals of Victoria (particularly on the evolution of kangaroos), and has also contributed design and content for museum exhibitions, written books for children and presented short videos for the exhibitions for the Melbourne Museum, where he currently works.

Dr Patrick-Jean Guay

Patrick-Jean is an experienced zoology and genetics researcher. He obtained his PhD in Zoology at the University of Melbourne in 2008. He joined Victoria University in mid-2009 as a research fellow. His research focuses on

the ecology and conservation of Australian waterbirds. He has a long-standing interest in waterbirds and has conducted a number of studies investigating hybridisation in ducks, the ecology and conservation genetics of waterfowl and the influence of human disturbance on bird populations. Patrick-Jean is associated with both the School of Engineering and Science and the Institute for Sustainability and Innovation, and he is the deputy leader of the Applied Ecology Research Group from the Faculty of Health Engineering and Science.

Dr Genevieve Martin

Genevieve completed her medical degree at Monash University in 2013 and worked at the Alfred Hospital in Melbourne. A medallist at the International Biology Olympiad in 2007, Genevieve taught for the Australian Science Olympiads Biology Program for 5 years. Genevieve is training as a specialist in Infectious Diseases and is currently undertaking doctoral studies at the University of Oxford, UK. This research will be supported by a prestigious John Monash Scholarship and will focus on the interactions between HIV and the immune system.

Katrina Walker

Katrina has been involved in teaching science for the past 8 years in both Victoria and New South Wales. She graduated from Monash University with a major in Genetics and minor in Chemistry. She has taught in Biology and general science in both Hungary and Australia. Katrina is an accomplished science author and has contributed to various Cengage Biology and Science series.

Dr Jim Woolnough

After completing a Bachelor of Science at the Australian National University in 1976, Jim went on to do a PhD at the John Curtin School of Medical Research. He then completed a Graduate Diploma in Education and taught senior Physics and Biology for 20 years in the ACT education system. In 2000, Jim took a position as lecturer in Science Education at the University of Canberra. Jim manages the Graduate Diploma in Secondary Education Program. He also convenes a Physics teacher retraining program which is delivered under tender to the NSW Department of Education and Training. Jim has a particular interest in the conceptual basis of science teaching and the development of concept-based science curriculum.

Dr Jane Wright

Jane has been teaching Biology, Psychology and general science at the secondary and tertiary levels for more than 35 years. Her emphasis has always been on practical experiences for students, including laboratory investigations and field work. Her PhD research was in the area of Cell Biology. As a Post Doctoral Fellow, she investigated the effect of immune rejection on the sperm production and reproduction of nematodes. Jane has been President and Treasurer of the Australian Science Teachers Association and her local Science Teachers Association. As well as contributing to many association publications, she has played a very active role in the professional development of science teachers. In 2011, Jane won the Prime Minister's Prize for Excellence in Science Teaching, and in 2010 she won the National BHP Billiton Science Teacher Award.

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- Amanda Webb – John XXIII College, WA

USING NELSON BIOLOGY

Nelson Biology Units 3 & 4 for the Australian Curriculum has been purposely crafted to enable you, the student, to achieve maximum understanding and success in this subject. Each page has been carefully considered to provide you with all the information you need without appearing cluttered or overwhelming. You will find it easy to navigate through each chapter and see connections between chapters through the use of linking icons. Practical work has been integrated within the text so you can see the importance of the interconnectedness between the conceptual and practical aspects of Biology.

Each chapter begins with a **Chapter opener**. This presents the content descriptions from the Science Understanding strand of the senior Biology Australian Curriculum that will be covered in the chapter and also gives you the opportunity to monitor your own learning. The text has been authored and reviewed by experienced Biology educators, academics and researchers to ensure up-to-date scientific accuracy for users.

To improve comprehension, a number of strategies have been applied to the preparation of our text to improve literacy and understanding. One of these is the use of shorter sentences and paragraphs. This is coupled with clear and concise explanations and real-world examples. New terms are bolded as they are introduced and appear in an end-of-chapter glossary as well as a consolidated end-of-book glossary.

Throughout the text, important ideas, concepts and theories are summarised in **Important concept** boxes. This provides repetition and visual enhancement for improved assimilation of new ideas.

Important concept

Mathematical relationships are presented in context. Step-by-step instructions on how to perform mathematical calculations are shown in the **Worked examples**. The logic behind each step is explained and approximate marks allocated so that you can see that you need to show your full working out. You can then practice these steps by attempting the related problems presented at the end of the worked example.

WORKED EXAMPLE

Biology is a science and you need to be given the opportunity to explore and discover the living world through practical activities. In the text, the **Activities** feature provides the opportunity for short, hands-on tasks to clarify or reinforce a concept. The activity can be performed either individually or in groups.

ACTIVITY

The **Experiments** introduce and reinforce the Science Inquiry Skills strand of the Australian Curriculum. Experiments contain guided instruction on the materials, procedure, collection and analysis of results and discussion. In some cases, open-ended investigations are presented in the experiments. These allow you the much-needed Science Inquiry Skills practice that relates to experimental design. You have the opportunity to design and carry out your own scientific investigation, either individually or in a group. You are prompted to consider ideas for improvement and further investigation to illustrate that science is an ongoing and improving process. Further information on how to conduct a scientific investigation can be found in the Scientific Investigations chapter on page 391.

EXPERIMENT

The **Risk assessment** table occurs within the experiment boxes. The table highlights the risks of the experiment and provides suggestions on how to minimise these risks – they are not to be considered comprehensive. Teachers are expected to amend this table in the case of substitutions or in the case of any additional risks. This may mean obtaining and following Material and Safety Data Sheets (MSDS) 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.

Risk assessment

Scientific literacy

Case study

Margin note

QUESTION SET

In this age of rapid scientific change and constant access to information, it is important that you are able to understand and evaluate the information presented in the media. The **Scientific literacy** box presents a scientific text or media piece that encourages you to use evidence to evaluate the claims and conclusions presented. You can exercise your own reasoning and knowledge to construct a valid scientific argument.

The **Case study** box provides you with the opportunity to see how science is applied using an up-to-date and real-world example in context.

Full understanding of a concept is often constructed from many pieces of information. Due to the sequential nature of a book, this information cannot always be presented together as it is best placed in other chapters. Links between concepts that occur on other pages and chapters are indicated using the **Margin notes**.

Regular opportunities to recall new terms and review recent concepts are provided as short **Question sets** throughout each chapter.

The end of chapter review provides:

- a summary of the important concepts presented within the chapter. This will be a valuable tool when you are revising for tests and exams
- a glossary of all the new terms introduced within the chapter
- end-of-chapter questions that review understanding of concepts from the chapter.

Questions are ordered from lower to higher order thinking skills and also include reflection questions.

NelsonNet

NelsonNet is your protected portal to the premium digital resources for Nelson textbooks, located at www.nelsonnet.com.au. Once your registration is complete you will have access to a helpful suite of digital resources for each chapter to further enhance and reinforce learning.

Each chapter will be supplemented with the following digital resources.

- A prior knowledge activity sheet to revise content that has been covered in previous units or chapters and is considered essential to understanding the next chapter
- Activity sheets to review concepts and to practice applying understanding to new examples
- A revision sheet to further reinforce key ideas and assist studying
- A review quiz containing 20 auto-correcting multiple-choice questions to review understanding
- Links to websites that contain extra information. These are hotspotted within the ebook and they can also be accessed at http://bac3and4.nelsonnet.com.au.

Please note that complimentary access to NelsonNet and the NelsonNetBook 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.

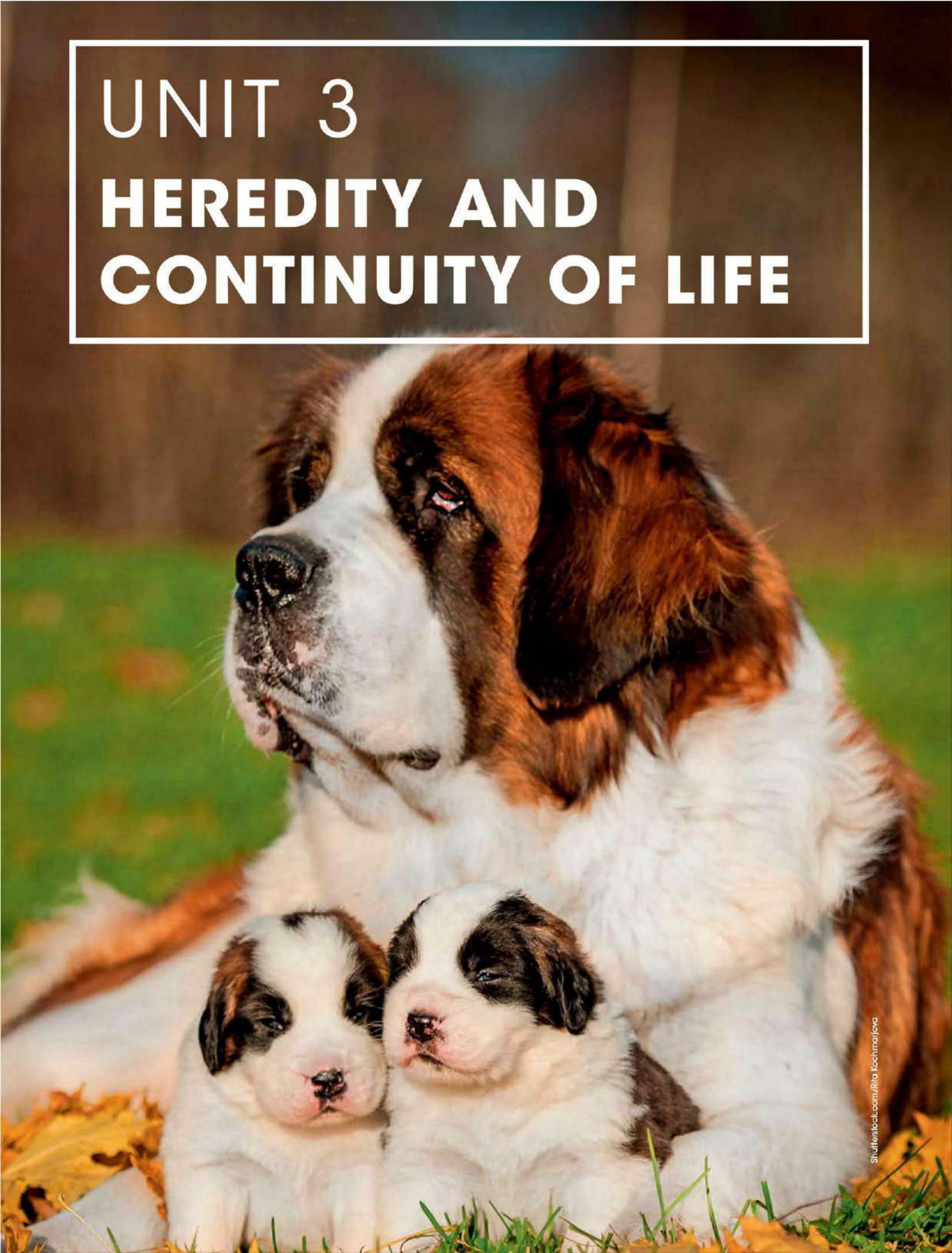
CURRICULUM GRID

		Unit 3							Unit 4					
		Chapter												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Science Inquiry Skills	Identify, research and construct questions for investigation; propose hypotheses; and predict possible outcomes (ACSBL061) and (ACSBL096)				✓	✓					✓	✓	✓	✓
	Design investigations, including the procedure/s to be followed, the materials required, and the type and amount of primary and/or secondary data to be collected; conduct risk assessments; and consider research ethics, including animal ethics (ACSBL062) and the rights of living organisms (ACSBL097)					✓								✓
	Conduct investigations, including the use of probabilities to predict inheritance patterns, real or virtual gel electrophoresis, and population simulations to predict population changes, safely, competently and methodically for the collection of valid and reliable data (ACSBL063)			✓	✓	✓								
	Conduct investigations, including using models of homeostasis and disease transmission, safely, competently and methodically for valid and reliable collection of data (ACSBL098)								✓		✓	✓		✓
	Represent data in meaningful and useful ways, including the use of mean, median, range and probability; organise and analyse data to identify trends, patterns and relationships; discuss the ways in which measurement error, instrumental accuracy, the nature of the procedure and the sample size may influence uncertainty and limitations in data; and select, synthesise and use evidence to make and justify conclusions (ACSBL064) and (ACSBL099)										✓	✓	✓	✓

		Unit 3							Unit 4					
		Chapter												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Science Inquiry Skills	Interpret a range of scientific and media texts, and evaluate models, processes, claims and conclusions by considering the quality of available evidence, including interpreting confidence intervals in secondary data; and use reasoning to construct scientific arguments (ACSBL065) and (ACSBL100)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Select, construct and use appropriate representations, including models of DNA replication, transcription and translation, Punnett squares and probability models of expression of a specific gene in a population, and including diagrams and flow charts, to communicate conceptual understanding, solve problems and make predictions (ACSBL066) and (ACSBL101)	✓	✓		✓				✓	✓	✓	✓		
	Communicate to specific audiences and for specific purposes using appropriate language, nomenclature, genres and modes, including scientific reports (ACSBL067) and (ACSBL102)	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓
Science as a Human Endeavour	ICT and other technologies have dramatically increased the size, accuracy and geographic and temporal scope of data sets with which scientists work (ACSBL068) and (ACSBL103)	✓					✓	✓	✓					✓
	Models and theories are contested and refined or replaced when new evidence challenges them, or when a new model or theory has greater explanatory power (ACSBL069) and (ACSBL104)	✓	✓				✓	✓				✓	✓	✓
	The acceptance of scientific knowledge can be influenced by the social, economic and cultural context in which it is considered (ACSBL070) and (ACSBL105)	✓		✓	✓			✓		✓		✓		
	People can use scientific knowledge to inform the monitoring, assessment and evaluation of risk (ACSBL071) and (ACSBL106)			✓					✓	✓				

		Unit 3							Unit 4					
		Chapter												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Science as a Human Endeavour	Science can be limited in its ability to provide definitive answers to public debate; there may be insufficient reliable data available, or interpretation of the data may be open to question (ACSBL072) and (ACSBL107)				✓	✓				✓				
	International collaboration is often required when investing in large-scale science projects or addressing issues for the Asia–Pacific region (ACSBL073) and (ACSBL108)	✓								✓				✓
	Scientific knowledge can be used to develop and evaluate projected economic, social and environmental impacts and to design action for sustainability (ACSBL074) and (ACSBL109)					✓		✓		✓				✓

UNIT 3
HEREDITY AND CONTINUITY OF LIFE



CHAPTER 1
DNA

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

Science Understanding

- Continuity of life requires the replication of genetic material and its transfer to the next generation through processes including binary fission, mitosis, meiosis and fertilisation (ACSBL075)
- DNA is a helical double-stranded molecule that occurs bound to proteins in chromosomes in the nucleus, and as unbound circular DNA in the cytosol of prokaryotes and in the mitochondria and chloroplasts of eukaryotic cells (ACSBL076)
- The structural properties of the DNA molecule, including nucleotide composition and pairing and the weak bonds between strands of DNA, allow for replication (ACSBL077)

Alamy/Paul Burns

Figure 1.1 ▶
Similarities can be seen between closely related individuals.

At a family gathering, we can be struck by how much we resemble some of our relatives but not others. Over the centuries, many people have been intrigued by the inheritance of particular features. Selective breeding of crops, plants, pets and domesticated animals has made use of this ability of living things to transfer particular characteristics through successive generations.

It is only since the secrets of **DNA (deoxyribonucleic acid)** were unlocked that we have been able to explain this at a cellular and molecular level. We now understand how DNA is transmitted between generations, how **genes** are controlled and how differences in genes can cause changes in the way organisms develop and behave. This knowledge has allowed us to manipulate genes, raising new ethical issues and opening up whole new fields of research into the social and ethical consequences of this technology. New technologies have also enabled us to accurately examine the interrelationships between species and account for changes that have occurred to species over time.

Chapter 4 describes Mendel's experiments and the concepts of Mendelian genetics.

Heredity is the study of inheritance. The principles of heredity and patterns of inheritance were first established by an Austrian monk, Gregor Mendel (1822–84), in the 19th century. Classical **genetics** deals with studying the mechanisms and patterns of inheritance through the transmission of coded chemical instructions from one generation to the next. Early studies focused on individual genes or groups of genes that directed the development of particular **traits** of an organism. Later research showed that proteins are the products of genes and that the inheritance of particular gene variants caused an individual to contain a specific combination of proteins. Additional research has shown that genes may code for more than one kind of protein and that genes interact in their expression, that is, in their activities.

Structural properties of DNA

What do we have in common with mice, plants, flies and bacteria? At first glance, not a lot. But all living things inherit DNA from their parents. DNA is the common genetic material for all organisms and carries the information coded in genes.

Genes are stretches of DNA, with one gene being one 'unit' of genetic information, which can be read and switched on or off independently of other genes. When a gene is switched on, the cellular machinery will 'read' the gene and produce, for example, a specific protein. The gene is then said to be 'expressed'.

DNA is the same in all organisms, but the small differences in the way the DNA molecules are put together, the DNA sequence, make individual genes different and species distinct from one another.

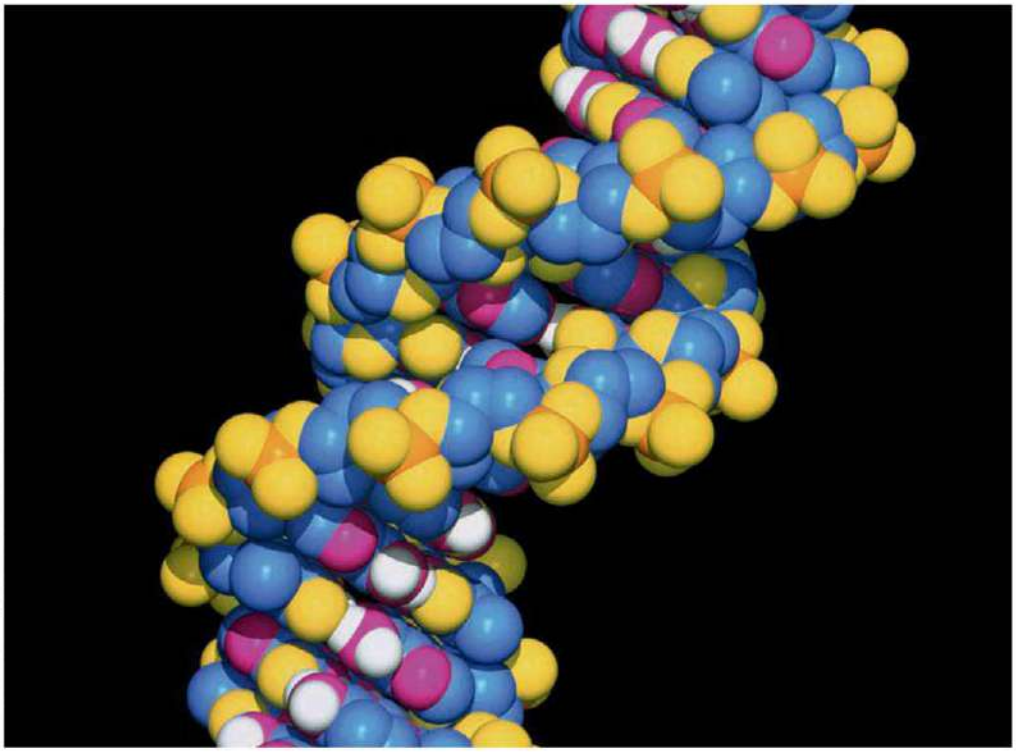
Because DNA is the same genetic material in all organisms, it must have appeared early in the evolution of species and remained essentially the same as new species evolved. This tells us that the ways that DNA carries genetic information, and the way that this information can be read to build organisms, must be a successful biological strategy. As we learn more about the structure and functions of DNA, and as scientists have learned to use DNA-based technologies, it has become clear that this special molecule is capable of holding very complex information.

DNA is the common genetic material for all organisms and carries the information coded in genes.

The discovery of DNA

Even though biologists knew since the time of Mendel that organisms inherit their physical characteristics from their parents, it has only been within the last 70 years that the mechanism of inheritance has been explained. Amazing discoveries have been made in recent years and are continuing to be made about the structure and function of genes.

The story of discovery is more than just the details of DNA structure and function. It reveals how scientists work and how scientific discoveries can be made. It shows scientists sharing what they understand and what they don't understand. Even when an experiment fails to produce the anticipated results, it may reveal interesting information that other scientists can use to answer their questions. Unexpected results may be clues used to put together the pieces of another scientific jigsaw puzzle.



Science Photo Library/Prof K Seddon & Dr T Evans, Queens University Belfast

◀ **Figure 1.2**
A model of the DNA molecule

Early clues

In 1928, a British army medical officer, Frederick Griffith, was trying to develop a vaccine against the strain of bacteria that causes pneumonia, *Streptococcus pneumoniae*. He isolated two different strains of bacteria: the harmless R strain had a rough surface and the disease-causing S strain had a smooth surface.

Griffith injected laboratory mice with both strains and found that those injected with the R strain remained healthy and that the bacterial cells in their blood died, while those mice injected with the S strain died and their blood contained living bacterial cells.

When S strain bacterial cells were killed by exposure to high temperature and injected into mice, the mice remained healthy with no living bacterial cells found in their blood. However, when heat-killed S strain bacterial cells were combined with living R strain cells and injected into mice, death occurred and both live S and R strain cells were found in their blood. This is summarised in Figure 1.3.

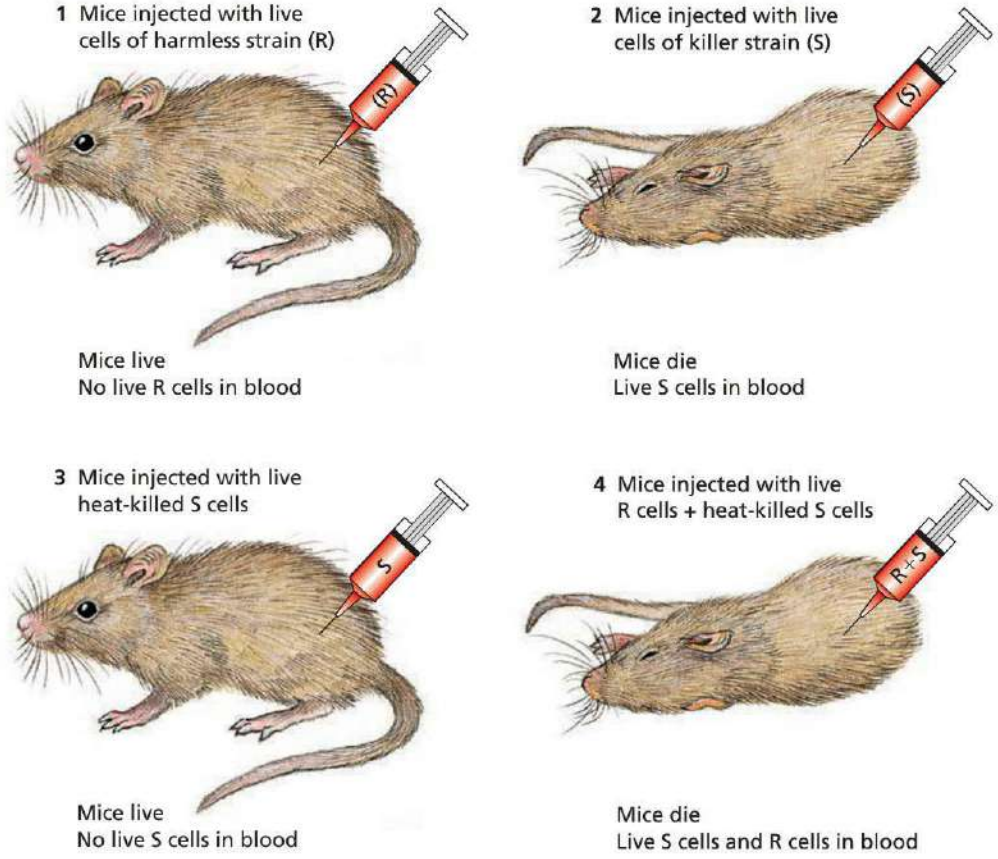


Figure 1.3 ▶ Summary of the results from Griffith's experiments with a harmless (R) strain and a disease-causing (S) strain of *S. pneumoniae*

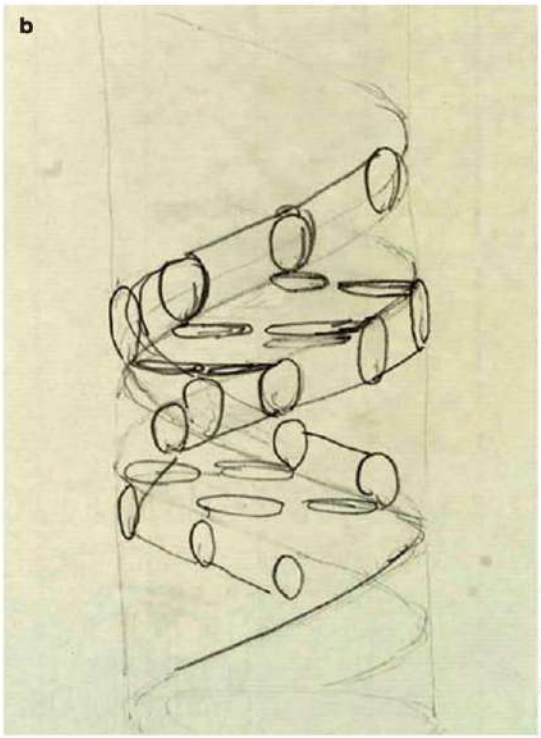
How can these observations be explained? The simplest idea is that high temperatures did not affect the hereditary material in the S strain bacterial cells and so this material was transferred to the living R cells, transforming them so they became deadly. This was supported by the observation that, many generations later, the transformed R strain bacterial cells were still killing laboratory mice.

Intrigued by Griffith's experiments, Oswald Avery of the Rockefeller Institute in New York carried out further experiments on *S. pneumoniae* bacteria and, in 1944, concluded that the hereditary material was made up of nucleic acids, possibly DNA. However, his ideas were not fully accepted until the early 1950s, when other scientists confirmed his conclusions.

Watson and Crick's model of DNA

In 1953, James Watson and Francis Crick, working in Cambridge, England, suggested a possible structure for DNA (Figure 1.4). As with many other important scientific discoveries, their model was not created alone. They based their model on the crucial contributions, discoveries and ideas of scientists before them.

Science Photo Library/A Barrington Brown



Alamy/Photo Researchers

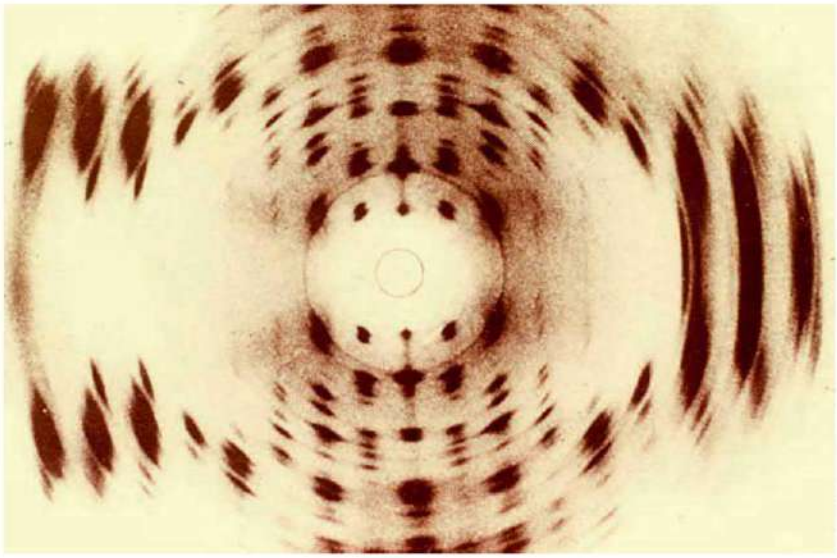
▲ **Figure 1.4**
a) Watson and Crick come up with a structure for DNA; b) Crick's doodle of a helix

Maurice Wilkins began using optical spectroscopy to study DNA in the late 1940s. In 1950, he and Ray Gosling obtained the first clear crystalline X-ray diffraction patterns from DNA fibres. Their colleague Alec Stokes suggested that the patterns indicated that DNA was helical in structure.

In 1952, Rosalind Franklin (Figure 1.5), working alongside Maurice Wilkins in his laboratory in England, obtained some clear X-ray diffraction images of DNA (Figure 1.6). This had not been easy because of the complexity and size of DNA and its reluctance to crystallise.

Alamy/Pictorial Press Ltd

▲ **Figure 1.5**
Rosalind Franklin, an expert X-ray crystallographer, worked on the structure of DNA in the early 1950s. Her work was pivotal in enabling Watson and Crick to propose their hypothesis for the structure of DNA.



Getty Images/Omikron

▲ **Figure 1.6**
An X-ray diffraction photograph of DNA. The DNA molecule was too small to see physically using conventional methods, so X-rays were used. The image produced an accurate three-dimensional shape.

Some years earlier, an American chemist, Erwin Chargaff, used a technique called chromatography to work out the ratios of **nucleotide** subunits containing four types of **nitrogenous bases**: adenine (A), cytosine (C), guanine (G) and thymine (T) (see page 10 and Figure 1.7 for further information). His results are shown in Table 1.1.

Table 1.1 Chargaff's original data on the base nucleotides in DNA from several sources

	DNA composition (approx. %)			
Source	**A**	**C**	**G**	**T**
Yeast	32	17	18	33
Avian tubercle bacilli	16	34	36	14
Ox thymus	30	18	24	28
Ox spleen	30	18	24	29
Human sperm	30	19	19	32

Using these results and other accumulated evidence, Watson and Crick suggested that DNA consisted of the now familiar two chains twisted around each other to form a double helix ladder, cross-linked by the four types of nucleotides.

This was the beginning of a further seven years of work for Maurice Wilkins and his colleagues to check and verify Crick and Watson's hypothetical model. It was for this and his original X-ray diffraction studies that Wilkins was awarded the Nobel Prize for Physiology or Medicine with Crick and Watson in 1962.

The discovery of the structure of DNA was a turning point in studies of inheritance.

EXPERIMENT 1.1

EXTRACTION OF DNA FROM FRUIT

In order to examine the inheritance of genetic traits, it is usually necessary to extract DNA from cells.

Aim

To extract DNA from fruit

Materials

- source of DNA: 1 cm^3 of each of banana, kiwifruit and strawberry
- mortar and pestle
- funnel
- 250 mL beaker
- glass stirring rod
- teaspoon
- fine mesh strainer, or filter paper
- warm saline solution (6 g salt dissolved in 200 mL water)
- dishwashing detergent
- meat tenderiser
- ethanol (ice cold) in a squeeze bottle
- hooked Pasteur pipette or paperclip

What are the risks in doing this investigation?	How can you manage these risks to stay safe?
Ethanol is highly flammable.	Keep ethanol away from naked flames or ignition sources.

In your write-up, add any more risks you can think of, as well as ways to manage them.

Procedure

1. Place your DNA source into a mortar. Add enough saline solution to cover the fruit – about 40 mL. Using the pestle, grind the fruit material and the salt solution for 20 seconds. Add more saline if the mixture is too paste-like. (Note: The physical grinding separates the cells from each other and increases the contact surface area.)
2. Use the beaker, funnel and filter paper to filter the ground mixture and collect the liquid. This removes the cell debris, but the cell nuclei should still pass through.
3. Add 1 teaspoon of detergent to the filtrate. Stir gently to mix thoroughly (about 20 seconds). Try to avoid creating foam. (Note: The detergent breaks down the oily cell membranes and proteins to get into the cell.)
4. Add a pinch of meat tenderiser to the solution in the beaker. (Note: This breaks down the nuclear membrane and the proteins binding the DNA strands to release the DNA.)
5. Add the ice-cold ethanol by running a stream carefully down the side of the beaker until you have roughly equal amounts of fruit solution and ethanol. Water is denser than alcohol, so the alcohol will rest above it. Wait 5–10 minutes. (Note: The DNA, which is not soluble in alcohol, will come out of solution at the interface of the two mixtures after a few minutes.)
6. Lift the DNA carefully out of the beaker using a hooked Pasteur pipette or paperclip.
7. Place a sample of DNA on to a microscope slide and view under low and high power.

Results

1. Describe what you saw under low and high power on the microscope slide.
2. Draw a series of annotated diagrams to illustrate how each step of the procedure affected the fruit cells to release the DNA.

Discussion

1. Explain if you would expect the DNA to look any different if you extracted it from a sample of human cells instead of from fruit.
2. The molecules of DNA, once extracted, can be manipulated by scientists to provide them with a basis for their study. Suggest how the extracted DNA from fruit cells could be used.

Conclusion

Outline how successful you were in extracting the fruit DNA. Summarise what you discovered about the appearance of DNA.

DNA EXTRACTION

DNA is extracted from human cells for a variety of reasons. With a pure sample of DNA you can test a newborn for a genetic disease, analyse forensic evidence or study a gene involved in cancer. Try this virtual laboratory to perform a cheek swab and extract DNA from human cells.

QUESTION SET 1.1

Remembering

1. List the scientists who contributed to the discovery of the structure of DNA.
2. Describe Watson and Crick's model of DNA.

Understanding

3. Explain how Frederick Griffith's observations with *S. pneumoniae* contributed to Oswald Avery's conclusion that hereditary material was made up of nucleic acids.
4. Distinguish between heredity and genetics.

Analysing

5. Use Chargaff's original data on the ratio of nucleotide subunits shown in Table 1.1 to draw conclusions about the base composition of DNA.

DNA: THE DOUBLE HELIX GAME

Your job is to make exact copies of a double-stranded DNA molecule by correctly matching base pairs to each strand and determine to which organism the DNA belongs.

DNA contains the information for life

Commonly represented by the letters DNA (deoxyribonucleic acid) and **RNA (ribonucleic acid)**, nucleic acids are large macromolecules. A molecule of DNA is composed of two long strands of subunits called nucleotides, wound around each other to form a double helix. RNA is usually composed of a single chain of nucleotides and thus forms a single strand. DNA encodes the inheritable information while RNA has functions such as in protein synthesis.

A nucleotide has three distinct chemical components:

1. a five-carbon sugar (ribose in RNA and deoxyribose in DNA)
2. a negatively charged phosphate group
3. an organic nitrogen-containing compound called a base (Figure 1.7).

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

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

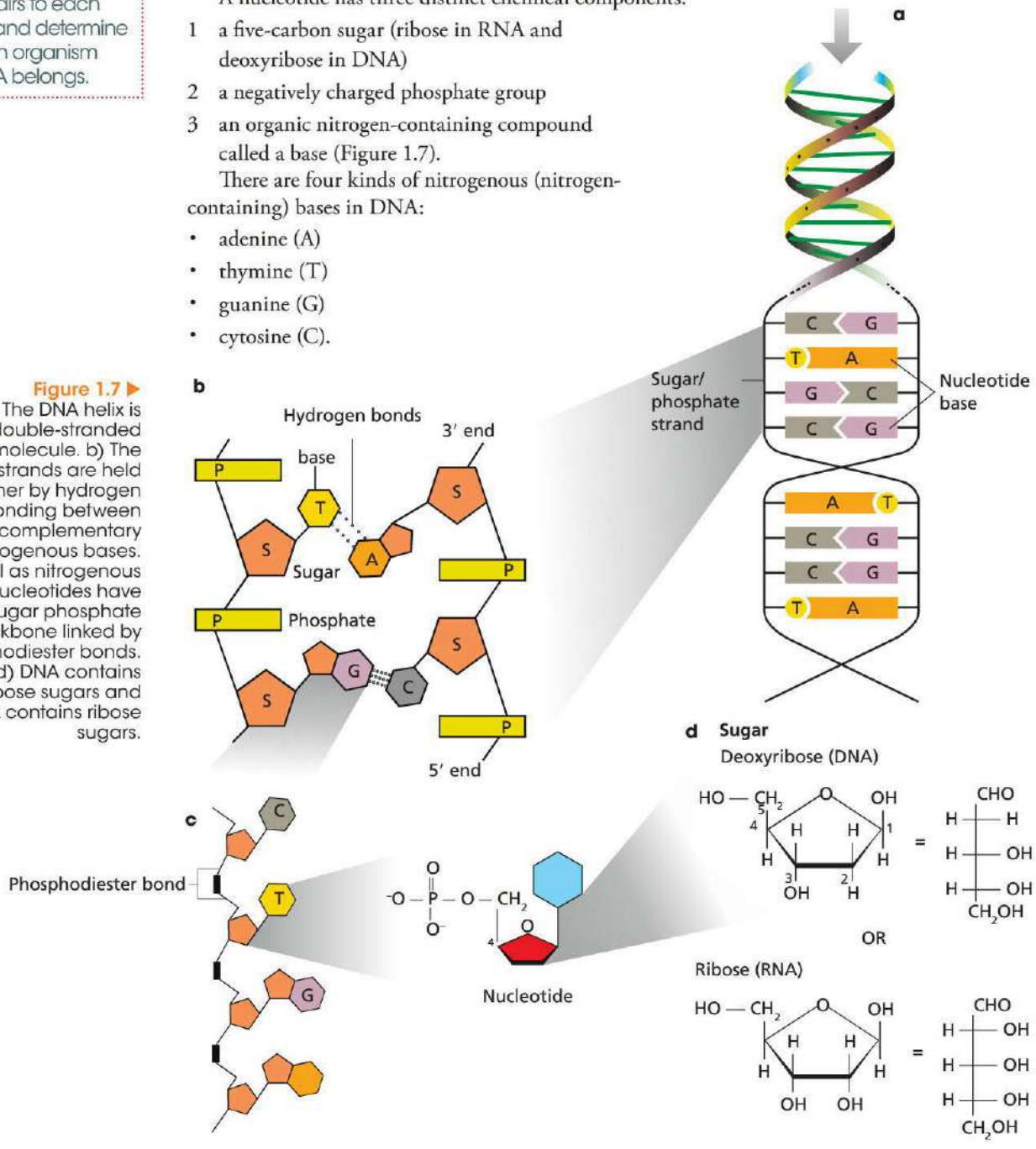


Figure 1.7 ▶ a) The DNA helix is a double-stranded molecule. b) The two strands are held together by hydrogen bonding between complementary nitrogenous bases. c) As well as nitrogenous bases, nucleotides have a sugar phosphate backbone linked by phosphodiester bonds. d) DNA contains deoxyribose sugars and RNA contains ribose sugars.

In each nucleotide strand, the sugar molecule of one nucleotide binds to the phosphate group of the next nucleotide, leaving the nitrogenous base sticking out from each sugar and opposite the nitrogenous base of the second strand. Hydrogen bonds between the opposing pairs of nitrogenous bases hold the double helix together, much like the rungs of a twisted ladder or a

spiral staircase. The bonding of the nitrogenous bases does not happen by chance: A bonds with T and C bonds with G, giving rise to the base-pairing rule (Figure 1.7).

The difference between the deoxyribose sugar of DNA and the ribose sugar of RNA is that deoxyribose has one less oxygen atom. The nitrogenous base thymine is also replaced by the base uracil (U) in RNA.

RNA and uracil are discussed in more detail in Chapter 2.

The two strands of a DNA double helix link by hydrogen bonds between complementary bases: A and T link with two hydrogen bonds, G and C link with three hydrogen bonds.

DNA replication

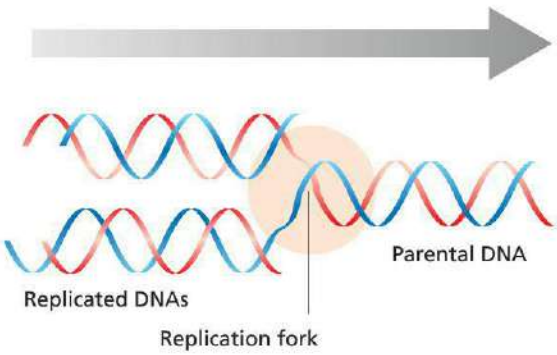
DNA is the master code that determines the very nature of cells and therefore of all life. Due to the manner in which it replicates, DNA is said to be a semi-conservative molecule that passes on this information from one generation of cells to the next and from one generation of organisms to the next.

DNA replication begins with the enzyme **DNA helicase** unzipping the long molecule or helix of double-stranded DNA by breaking the weak hydrogen bonds between the nucleotides and thus exposing the nucleotide bases. This separation of the parental DNA strands happens along a small section at a time. The hydrogen bonds that hold the two strands of the DNA molecule are weak and the enzyme is easily able to separate them.

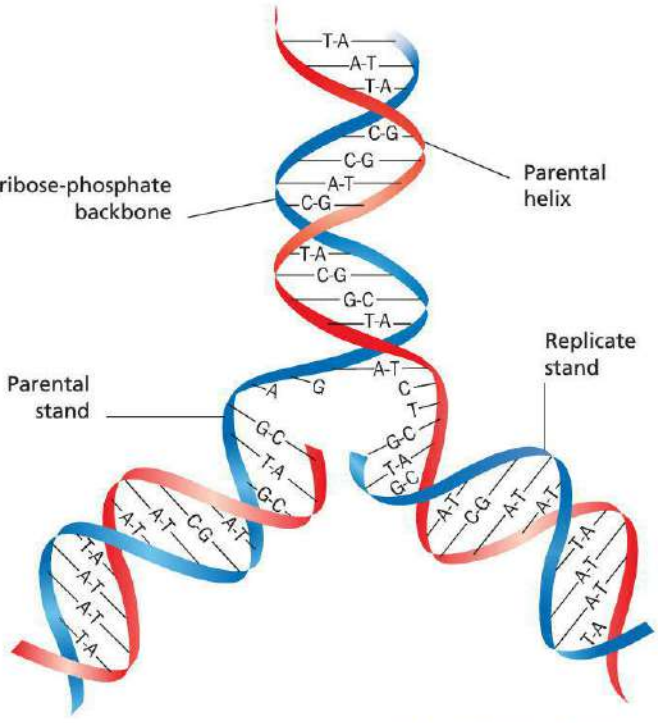
The junction between the unwound single strands of DNA and the intact double helix is called the **replication fork**. The replication fork moves along the parental DNA strand so that there is a continuous unwinding of the parental strands (Figure 1.8). Within the nucleus, stockpiles of free nucleotides attach to the exposed bases according to the base-pairing rule (Figure 1.9) with the help of the enzyme **DNA polymerase**. Another enzyme, **DNA ligase**, seals the new short stretches of nucleotides into a continuous strand that rewinds. Nucleotides link together in what is called a 5′ to 3′ direction to form long molecules.

The outcome of DNA replication is two double-helix DNA molecules, each consisting of one parental strand and one new strand. Thus, one of the two strands is conserved, or retained, from one generation to the next, while the other strand is new. Hence, this process is referred to as **semi-conservative replication**.

DNA replicates by a semi-conservative mechanism where one of the strands in the newly formed molecule is new and the other is the original strand.



▲ **Figure 1.8**
Movement of the replication fork along parental DNA causes unwinding of DNA strands and rewinding of newly replicated strands.



▶ **Figure 1.9**
Replication of DNA. The two strands of the double helix separate and free nucleotides align themselves in relation to each of the two strands. The specific relationship between A and T and between C and G ensures that the sequence of bases in the daughter DNA is exactly the same as in the parent DNA.

ACTIVITY 1.1

SIMULATION OF DNA REPLICATION

An essential property of DNA is that it should be able to replicate accurately. An attractive feature of the Watson and Crick model of DNA is that it enables us to imagine the two chains separating from each other rather like undoing a zip.

Aim

To simulate DNA replication

You will need

- 18 plastic clothes pegs of four different colours
- two 40 cm lengths of string

What to do

Each of the peg colours represents a nucleotide base. The string represents the phosphate and deoxyribose sugar backbone. Before proceeding, assign a peg colour to a particular nucleotide base. Copy Table 1.2 and enter the colours for reference.

1. On one length of string thread up to nine coloured pegs, in any order of colour, through the holes at the end of each peg.
2. On another length of string thread a complementary set of coloured pegs and then join the two complementary strands by clipping the pegs together. Copy Table 1.3 and enter both sequences of nucleotides that make up your DNA strand.
3. With a black marker pen, place a dot on to each of the pegs in the first pair and lay the threaded pegs on the table.
4. From the end facing you, unclip the pegs and then clip a spare complementary peg to the left-hand peg. Thread another piece of string through the new peg.
5. Repeat this procedure to only the one side of the strand until you have added new pegs to six of the old pegs.
6. After the left-hand side has six new pegs attached, begin the same process on the right-hand strand.
7. Now complete this process for all nine pegs in the original strand.
8. Thread the string through all nine new pegs on the right side and lay both DNA helix molecules side by side.
9. Copy Table 1.4 and enter the sequences of nucleotides that make up your new DNA strands

Table 1.2 Colour coding of bases

Base	Adenine	Thymine	Guanine	Cytosine
Colour				

Table 1.3 Sequences of nucleotides in the DNA strand

Table 1.4 Sequences of nucleotides in the new DNA strands

Strand 1				
Strand 2				

What did you discover?

1 Identify what you have made after joining the two strands together.
2 Name the type of bond represented by the joining of two pegs.
3 Describe what you noticed about the sequence of nucleotides in each DNA molecule you have made.
4 Examine the two strands of DNA you have made. Describe where the two original strands are. (Look for the strands where the black pen mark was placed.)
5 Describe what brings about the separation of the complementary nucleotides in the original strand.
6 Suggest from where in a cell the new nucleotides would come.
7 Predict if all replication is expected to produce an exact copy of the original strand. Explain your prediction.
8 Suggest why DNA replication needs to occur.
9 Describe how your model compares to real DNA replication. Discuss limitations of using a model such as this.
10 Explain how model building has assisted with your understanding of DNA replication.

Genomics

The sum of all DNA in the cell of an organism is its **genome**. Genomes differ between species although there may be similarities. The study of the genomes of organisms is termed **genomics**. This significant branch of molecular biology and its related study of **proteomics** have been made possible by advances in technologies, particularly robotics and **bioinformatics**. Automated processes in laboratories and the rapid collection and storage of data have made it possible to integrate, analyse and manipulate data at high speed. These technologies assisted the complete mapping of the human genome in 2003 by the Human Genome Project. Now the challenge is to find out what every gene actually does.

WHAT IS BIOINFORMATICS?

Learn more about bioinformatics and its role in science today.

WOW

Sequencing the human genome

In 2001, after 10 years of work and US$400 million, those involved in the Human Genome Project had completed a draft sequence of the human genome. Today, such sequencing can be completed in a couple of weeks for less than US$10 000.

QUESTION SET 1.2

Remembering

1 Draw a nucleotide, labelling the three distinct chemical components.
2 Compare the nucleotides of DNA and RNA.
3 State the rule for the pairing of nitrogen-containing bases in the DNA molecule.

Understanding

4 Describe the process of DNA replication.
5 Describe the role of the enzymes DNA polymerase and DNA ligase in DNA replication.
6 Explain why DNA replication is referred to as semi-conservative replication.

Applying

7 Predict the nucleotide sequence for the complementary strand of a fragment of a DNA chain with the nucleotide bases GCCTATTGCA.
8 The 1997 science fiction film *Gattaca* is about exploring the use of genetics to predict potential health and ability. Identify the link between the film title and DNA composition.

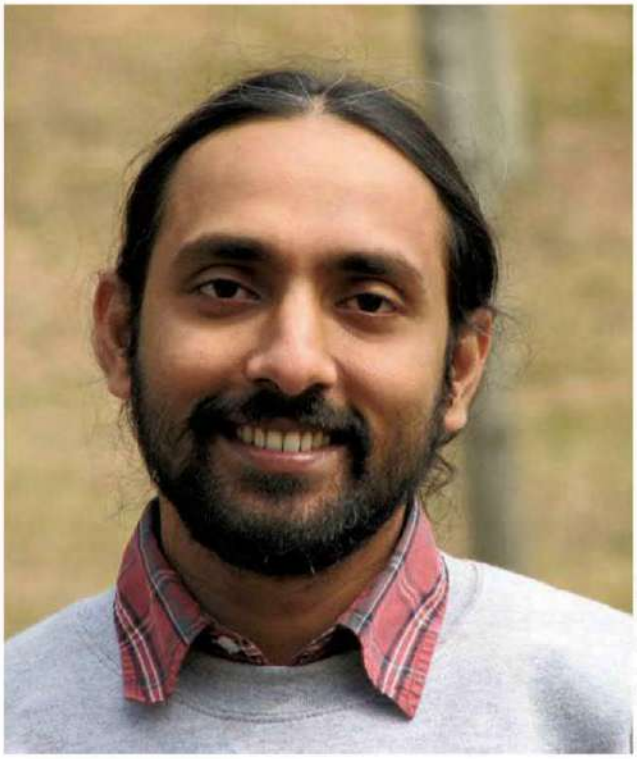
Case study

Computer power helps solve biological puzzles

The technologies to sequence DNA and determine protein structure are driving a wide range of advances in medicine, agriculture and other life sciences. Whole-genome sequencing, mass spectrometry and X-ray crystallography are just a few of the new techniques researchers are using to better understand the processes of life.

'The function of these proteins can be explained by investigating their three-dimensional structures. Knowing what causes such diseased states helps to find a cure to them and computing plays an important role in these studies,' says Dr Arun Konagurthu, of Monash University.

Robots in biology laboratories have lifted the burden of 'grunt work' from researchers. With inhuman speed, they process thousands of samples trying to find genetic markers that indicate susceptibility to disease. The result is an unprecedented deluge of digital information. The available data is so huge that if compiled in books, the data would run into 200 volumes of 1000 pages each and reading alone (ignoring the understanding factor) would require 26 years working around the clock. Making sense of so much data requires a combination of problem solving, mathematics and computer science. Dr Konagurthu has taken up the challenge to provide better tools for data analysis.



Monash University, Australia

Figure 1.10 ▲
Arun Konagurthu is a Larkins Fellow and Senior Lecturer at the Monash University School of Information Technology.

Ongoing collaboration has resulted in the development of a multiple structural alignment **algorithm**, called MUSTANG, for comparing the structures of proteins. This is available free, as an open-source program, through the Internet. Professor James Whisstock at Monash University and Professor Peter Stuckey from the University of Melbourne were also part of the MUSTANG team and continue to collaborate with Arun and his research collaborator Professor Arthur Lesk at the Hucks Institute for Genomics, Proteomics and Bioinformatics at Pennsylvania State University in the US.

Arun grew up in an academic environment. His father was a university professor of genetics. This infused him with the intellectual stimulation he needed to pursue his chosen area of research. 'There is a whole cornucopia of unsolved and fascinating problems in biology. These can be interrogated using computer science and mathematics.'

Questions

1. Explain why Arun Konagurthu's type of research is termed 'bioinformatics'.
2. List the techniques that can be used to advance our understanding of DNA and its link to proteins.
3. Describe the role Arun Konagurthu has in deciphering large quantities of digital information.
4. Discuss some biological and economic impacts of finding out the structure and function of proteins.

Scientific literacy: Exploiting redundancy and matchmaking

The rate at which genomes can be sequenced has been doubling every four months or so, whereas computing power doubles only every 18 months. Without the advent of new analytic tools, biologists' ability to generate genomic data will soon outstrip their ability to do anything useful with it.

A new algorithm that drastically reduces the time it takes to find a particular gene sequence in a database of genomes has been developed. The more genomes it's searching, the greater the speedup it affords, so its advantages will only compound as more data is generated.

This is like the data-compression that allows computer users to compress data files into smaller zip files. When you have a lot of data and if you want to store it, you would probably compress it. The problem is that eventually you have to look at it, so you have to decompress it to look at it. But the new idea is that if you compress the data in the right way, then analysis can be done directly on the compressed data. And that increases the speed while maintaining the accuracy of the analyses.

The compression scheme exploits the fact that evolution is stingy with good designs. There's a great deal of overlap in the genomes of closely related species, and some overlap even in the genomes of distantly related species.

Researchers have developed a way to mathematically represent the genomes of different species, or of different individuals within a species, such that the overlapping data is stored only once. A search of multiple genomes can thus concentrate on their differences, saving time.

If a computation is run on one genome, it takes a certain amount of time. If the same computation is run on a similar organism's genome, the fact that they're so similar means most of the work has already been done.

The new algorithm will be useful in finding out what a particular DNA sequence is similar to. Identifying microbes is one example. This could help clinicians determine causes of infections, or it could help biologists characterise collections of microbes found in animal tissue or particular microenvironments. This would be particularly useful when investigating the variations of microbes in humans that have been implicated in a range of medical conditions. It could be used to characterise the microbes in particularly fertile or infertile soil, and it could even be used in forensics, to determine the geographical origins of physical evidence by its microbial signatures.

Adapted from Massachusetts Institute of Technology. 'Searching Genomic Data Faster: Biologists' Capacity for Generating Genomic Data Is Increasing More Rapidly Than Computing Power', *Science Daily* online, 10 July 2012 <http://www.sciencedaily.com/releases/2012/07/120710132955.htm>

Questions

1. Define 'algorithm' (you can use the glossary in this chapter). List the input and the output of the new algorithm described in this article.
2. Describe how the new algorithm speeds up sequencing of genomes.
3. Explain what is meant by: 'evolution is stingy with good designs'.
4. The general public may find it difficult to engage with scientific technologies and understand their implications because of the specialised language and jargon used. List phrases from this article that you found difficult to understand and try to rephrase them in a form people with a non-scientific background could understand.
5. The time taken to sequence the genome of an organism has been dramatically reduced in the last few years. Describe some applications resulting from improved understanding of the variation and commonality of the genomes of similar species, such as bacteria.

The continuity of life

All cells originate from pre-existing cells. For life to continue, genetic information must be transferred to the next generation. Cell division processes such as **binary fission**, **mitosis** and **meiosis** as well as fertilisation facilitate this transfer.

All organisms have a life span but species continue because some members reproduce. They pass on instructions embedded in DNA for the development of characteristics that define the

species: the characteristics that appear in successive generations. Living things that originate from one parent are said to undergo **asexual reproduction**. They usually closely resemble their parent because they have only one source of hereditary information. Those organisms that reproduce via **sexual reproduction** have two sources of hereditary material, which are carried in specialised reproductive cells called **gametes**, usually from different parents. This means that in these organisms the potential for a difference in characteristics between one generation and the next is greater than in organisms that reproduce asexually.

Chromosomes of eukaryotes

Eukaryotic cells are complex cells containing membrane-bound **organelles**. In these types of cells, DNA is found in the nucleus, chloroplasts and mitochondria. In the nucleus of a eukaryotic non-dividing cell, DNA is only visible as a grainy substance without detail. Early microscopists named this seemingly grainy substance **chromatin**. We now use the term to describe the cell's DNA together with all the proteins associated with it. One DNA molecule with its associated proteins is called a **chromosome** ('chromo' = colour, 'soma' = body).

DNA and associated proteins together make up a chromosome.

When a eukaryotic cell divides, long DNA molecules appear in the nucleus as double structures each coiled around **histone** proteins (Figure 1.11) and linked at a point called a **centromere**. Chromosomes are normally visible only during cell division and only when stained. In eukaryotes, chromosomes exist in pairs, with one chromosome being inherited from each parent as explained on page 17.

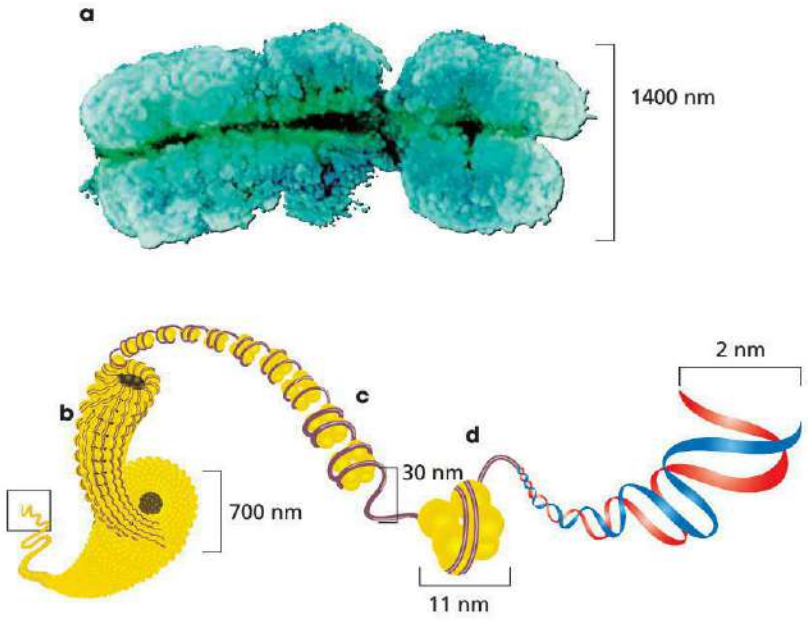
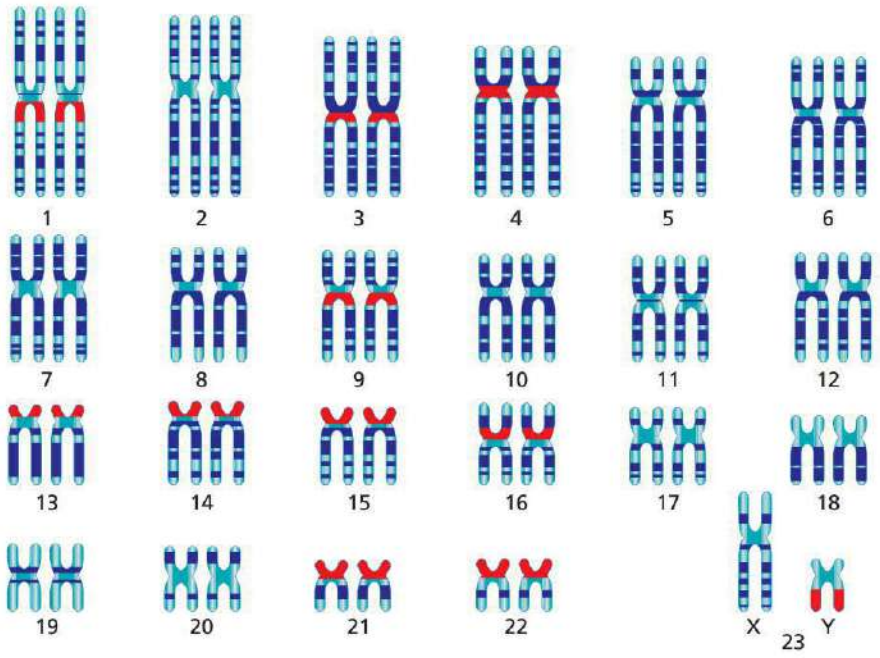


Figure 1.11 ▶ Levels of organisation in a human chromosome. a) A tightly coiled and condensed human chromosome is only visible when stained during cell division. b) Interacting proteins package loops of coiled DNA into a 'supercoil', to produce chromatin, which is organised as a cylindrical fibre. c) The loops of coiled DNA are wound around a core of eight histone proteins to produce a nucleosome. d) A nucleosome consists of a section of DNA molecule looped twice around a core of histones.

Examination of a prepared microscope slide of stained cells in the process of nuclear division reveals a jumbled cluster of chromosomes that differ in size and shape. Photographic images of chromosomes are arranged into matched and ordered pairs to create a **karyotype**: the standard form used to display and analyse chromosomes (Figure 1.12). Chromosomes can be ordered by length, from largest to smallest, and they have characteristic banding patterns. Each species of organism is characterised by a particular number of chromosomes in each cell.

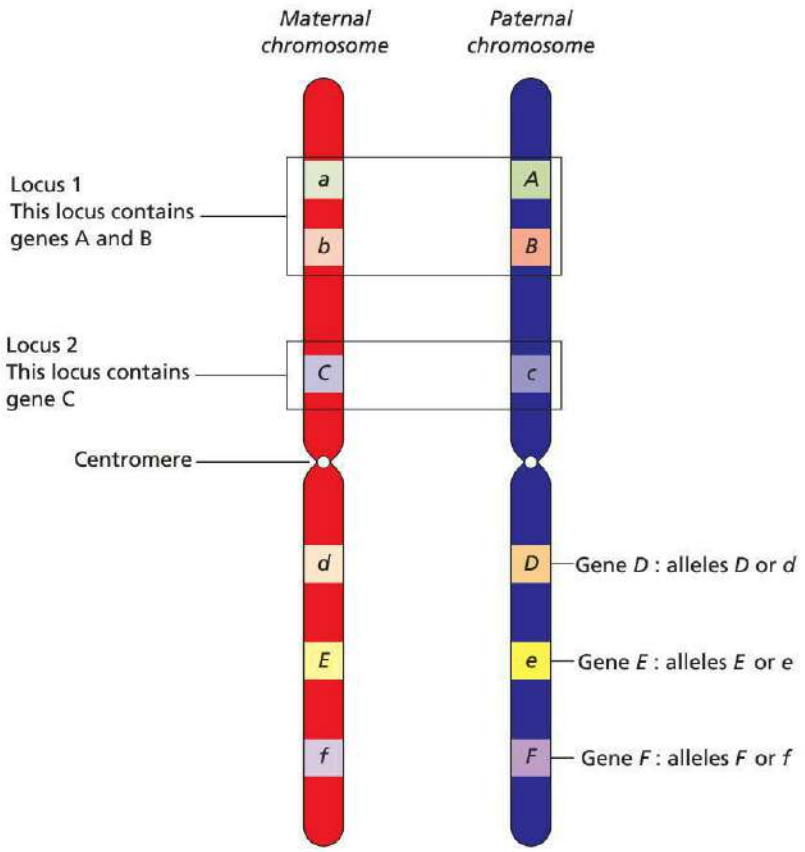


◀ **Figure 1.12**
The 46 human chromosomes divide into 23 pairs. They can be recognised individually by their size, position of centromere and banding pattern: the karyotype. The bands correspond to large groups of genes.

MAKE A KARYOTYPE

Practise making a karyotype by matching chromosomes using size, banding pattern and centromere position as guides.

The nucleus of each somatic or body cell of a human contains 46 chromosomes, which form 23 pairs, of which 22 are matched or **homologous** (Figure 1.13). One chromosome of each pair comes from the male parent via the sperm cell and the other from the female parent via the egg cell (ovum). The matched pairs are called **autosomes**, the largest of which is numbered 1 and the smallest 22. The 23rd pair, which is matched in females (XX) but unmatched in males (XY), is called a **heterosome**. Because these chromosomes determine the sex of an individual, they are also referred to as **sex chromosomes**.



◀ **Figure 1.13**
Stylised representation of a pair of chromosomes. In diploid organisms, chromosomes exist in pairs in somatic cells. One of the chromosomes comes from the male parent and one comes from the female parent.

The number of chromosomes in each **somatic cell** is called the **diploid** number and is represented as $2n$. As chromosomes occur in pairs, n stands for the number of pairs that the particular species has in each of its cells. This is called the **haploid** number. A human somatic cell, for example, has 23 pairs so its diploid number is $2n = 46$ and its haploid number is $n = 23$.

Somatic or body cells are diploid, containing 23 pairs of chromosomes; one chromosome of a pair comes from the male parent and the other chromosome of a pair comes from the female parent.

Along the length of each DNA molecule, particular regions (genes) code for different proteins that can determine particular characteristics or traits. The location of a particular gene on a chromosome is referred to as its **locus (plural loci)**. In **homologous chromosomes**, the corresponding gene is found at the same locus. Alternative forms of the same gene are called **alleles**. Alleles are versions of the same gene with slight differences. Sometimes this may be a single difference in nucleotide sequence, but this may be enough to cause large variation in the functions of a gene or the way it behaves. For example the single gene for eye colour has several alleles. One allele gives brown eyes, another allele gives blue eyes. A normal, diploid organism has two alleles for each gene, that is, one on each chromosome inherited from each parent. For the majority of genes, there is only one allele (or gene variant), but it is the different alleles that exist for some genes that give individuals distinct traits.

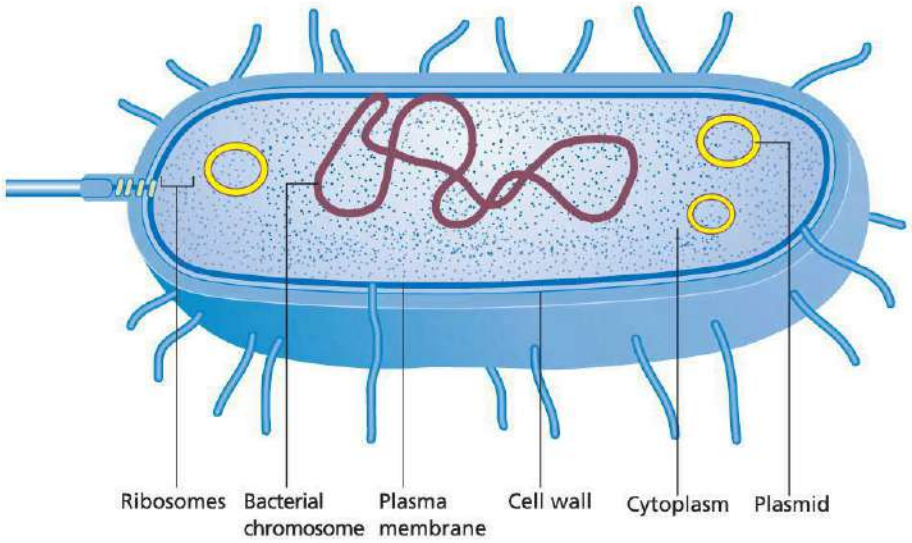
See Unit 2, Chapter 8 for information about the endosymbiotic theory.

Both mitochondrial and chloroplast DNA are similar to the single, circular chromosomes in prokaryotic cells. This feature is strong evidence for the endosymbiotic theory that proposes that eukaryote cells were formed when a bacterial cell was ingested by another primitive prokaryotic cell.

Chromosomes of prokaryotes

Membrane-bound organelles, such as the nucleus, are not present in prokaryotic cells. The DNA within these cells generally forms a single circular chromosome that lies in direct contact with the cytoplasm (Figure 1.14). Chromosomes are often joined to the plasma membrane at a single point. Although not contained by any internal structure, a chromosome can be in a distinct region of the cell called a **nucleoid**. Additional numerous small rings of DNA, called **plasmids**, may also be present in the cytoplasm. Nonessential genes are commonly encoded on these plasmids. Plasmids can replicate independently of the main chromosome and have become important tools in genetic engineering because they can be easily transferred from one bacterium to another and replicate rapidly.

Figure 1.14 ▶
DNA in a prokaryote cell



Like eukaryotic chromosomes, DNA of prokaryotic chromosomes needs to fit into a small area. This can be achieved by supercoiling where a number of proteins act together to fold and condense the DNA. Prokaryotic cells are generally haploid; they only contain one copy of each gene. Furthermore, prokaryotic DNA contains very little repetitive and non-coding DNA.

However, just as with many other examples in nature, there are exceptions to the rules. Not all bacteria have a single circular chromosome. There are some bacteria with more than one circular chromosome. Other bacteria have linear chromosomes and linear plasmids. Another notable difference between chromosomes in prokaryotes and eukaryote nuclear chromosomes is the presence of histones. Most prokaryotes do not have histones (with the exception of those species in the domain *Archaea*).

Chromosomes in prokaryotic cells are generally circular and are similar to chromosomes in mitochondria and chloroplasts.

QUESTION SET 1.3

Remembering

1 Name the place where DNA is located in a eukaryotic cell.

2 Identify the components of a chromosome from a eukaryotic cell.

Understanding

3 Draw a labelled diagram to show your understanding of the following terms.
   - a Homologous
   - b Autosome
   - c Locus
   - d Centromere

4 Discuss whether homologous chromosomes have the same number of genes or identical genes.

5 Decide whether two number 21 chromosomes in humans are homologous and justify your answer. Explain whether all human chromosomes have a homologous pair.

6 a In humans, the diploid number is $2n = 46$. State what n equals.
   b State what type of cells have the $2n$ number of chromosomes.

7 Distinguish between sex chromosomes and autosomes.

8 Explain the relationship between genes and alleles.

Applying

9 Construct a table to show the similarities and differences in chromosomes between eukaryotic and prokaryotic cells.

Analysing

10 A cell from an unknown source has been prepared and stained, and a karyotype has been displayed. Explain how you could tell if it was likely to be from a human and, if so, what sex the human was.

Cell division

Eukaryotic cells pass on their instructions for growth and development from one generation of cells to the next during nuclear division in somatic cells (mitosis) and cytoplasmic division (**cytokinesis**). Under normal circumstances cell division takes place in an orderly progression. Mitosis and cytokinesis result in the formation of two diploid daughter cells, which each contain identical sets of chromosomes.

Meiosis is a form of eukaryotic cell division that is concerned with the production of gametes and occurs in sex cells. In meiosis, nuclear division results in the formation of four

daughter cells, which each contain half the number of chromosomes of the original nucleus; that is, they are haploid. At fertilisation, two haploid gametes combine to form a diploid **zygote**.

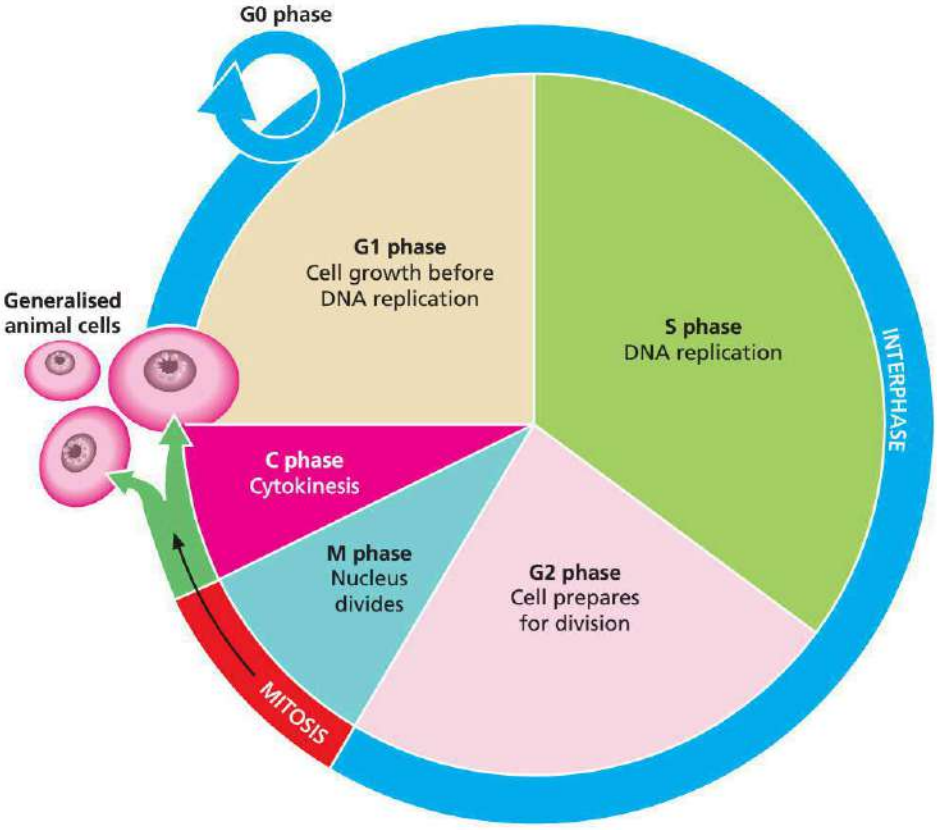
Eukaryotic cell division involves a number of phases resulting in nuclear division (mitosis and meiosis) and cytoplasmic division (cytokinesis).

The cell cycle

The sequence of events from one cell division to another is called the **cell cycle** (Figure 1.15). Even though we describe this cycle as taking place in phases, in reality it is usually a continuous process. The stage of actual nuclear division (M) is a small part of the cycle. The stage between nuclear divisions is called the **interphase**, which is a period of active growth (G1 phase), synthesis of DNA (S phase) and preparation for the next division (G2 phase).

In addition to these five phases, another phase exists. The G0 phase indicates the non-proliferating state, in which cells are undergoing an extended G1 but are not preparing to replicate DNA and divide. These cells have withdrawn from the active cell cycle. Terminally differentiated cells (that is, the most specialised cells) such as nerve cells are described as being in G0 phase. Cells in G0 can re-enter the cell cycle under certain circumstances.

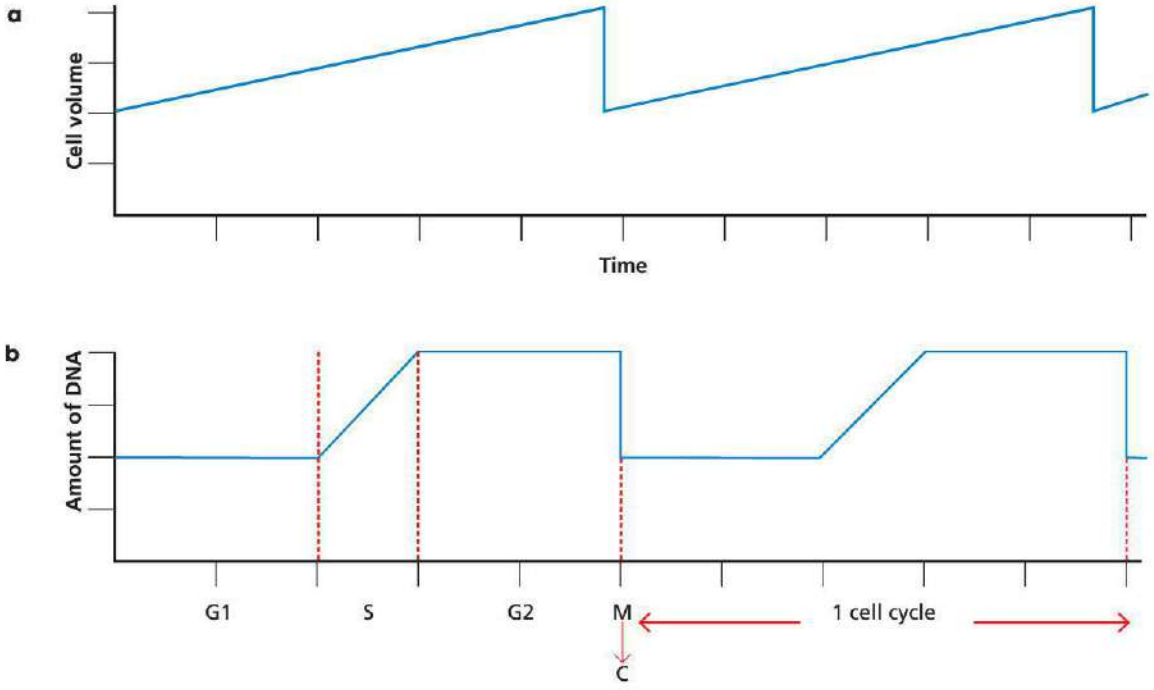
Figure 1.15 ▶
The cell cycle represents the life cycle of a cell. Most cells spend the majority of their time in interphase.



CONTROL OF THE CELL CYCLE

As a 'Cell Division Supervisor' inside the cell nucleus, you are to steer the cell division process to make sure everything happens in the right order.

The length of the cell cycle varies in different cells. Phases in the cell cycle can be identified by measuring the changes in cell volume or in the amount of nuclear DNA, which vary depending on whether the cell is in a phase of growth or DNA synthesis (Figure 1.16). But not all cells divide. Specialised cells tend not to divide, but other cells in areas of high growth or wear, such as skin cells and lining cells of the mouth or gut, tend to divide frequently to replace worn tissues. Dead cells in these organs 'slough off' due to mechanical disturbance or replacement from new cells growing below. New cells not only replace old cells but can also divide to create new organs or tissues. Cells of a growing root tip may divide every 20–24 hours.



▲ Figure 1.16
a) The cell volume and b) amount of nuclear DNA change during a cell cycle, reflecting the different phases of the cycle.

Mitosis in eukaryote cells

Interphase

During interphase, the stage between nuclear divisions described earlier, the chromosomes are not visible and cannot be clearly distinguished under a light microscope or an electron microscope. Immediately before mitosis begins, **centrioles** are visible in many animal cells. As the cell leaves interphase and begins mitosis, the chromatin threads become shorter and thicker and are visible under a light microscope (see Figure 1.17).

Prophase

During prophase, chromatin threads condense and become visible as double strands that consist of two **chromatids**, held together at a centromere. A spindle forms, made up of microtubules, originating from the centrioles and the **nucleolus** disappears from view. The nuclear membrane breaks down.

Metaphase

During the next stage, termed metaphase, chromosomes move to the centre of the cell and line up along the equator (sometimes called the metaphase plate).

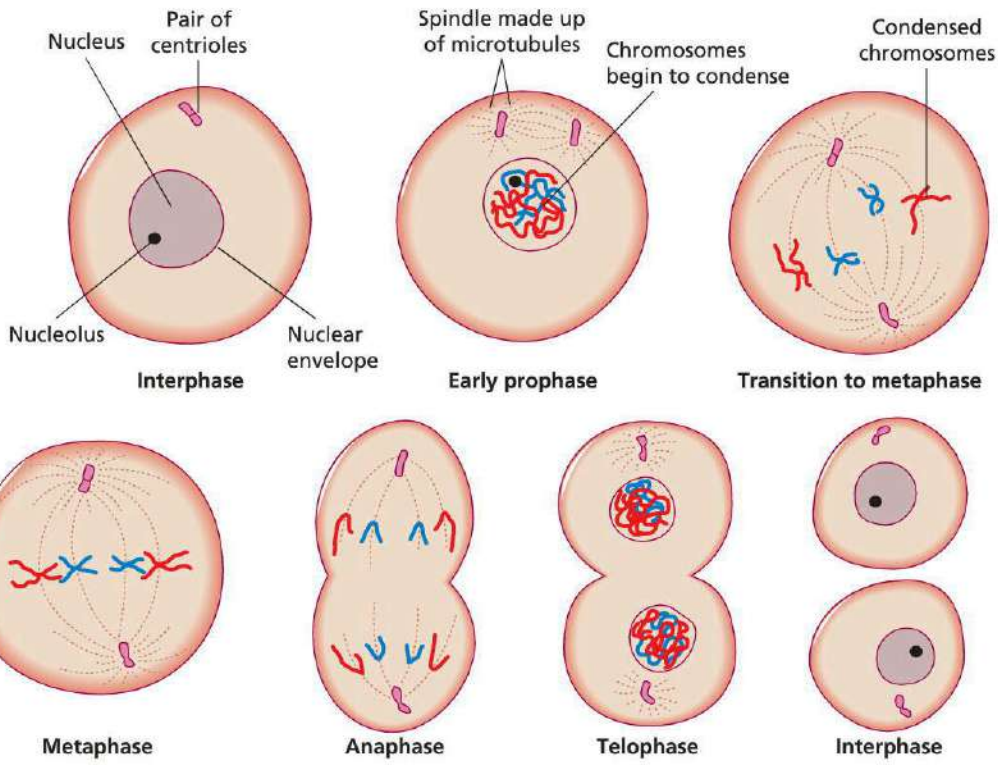
Anaphase

The next phase is called anaphase. Spindle fibres attach to the chromatids and help to pull them apart. Chromatids separate at the centromere and move to opposite poles of the spindle as the spindle fibres contract. Chromatids are now chromosomes.

Telophase

During telophase the chromosomes de-condense as the chromatin unwinds and becomes less visible. A new nuclear envelope forms, nucleoli reform and the spindle disassembles.

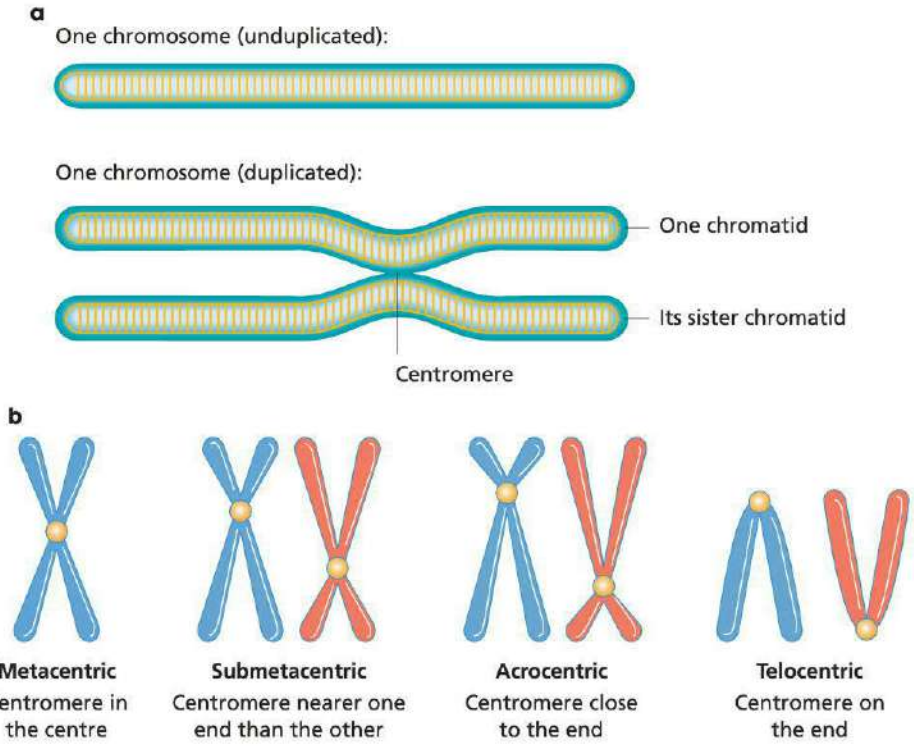
Figure 1.17 ▶
The stages of mitosis in animal cells



Centromeres

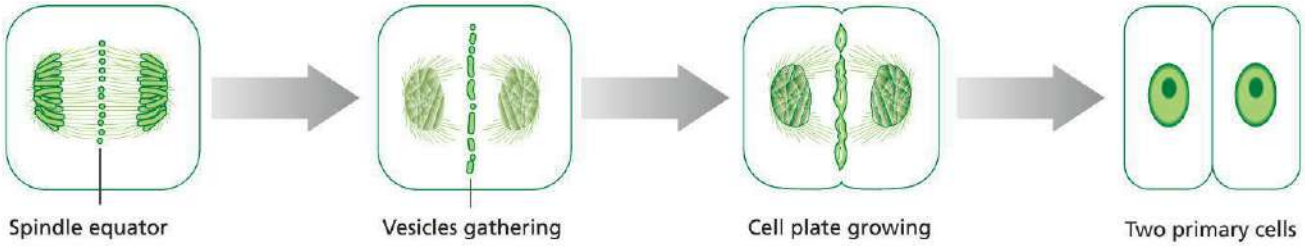
When visible and double-stranded during cell division, the chromatin of a chromosome condenses and the chromosome appears as an 'X' shape, with two chromatids attached to each other at a small region called the centromere. This is important for the attachment of chromosomes on to spindle fibres, which are generated by small organelles called centrioles, and which move the chromatids to opposite ends of the cell during division. The position of the centromere can vary in different chromosomes and descriptive names are given according to their position.

Figure 1.18 ▶
a) An unduplicated chromosome (top) and a duplicated chromosome, showing the position of the centromere (bottom); b) Different positions of centromeres along chromosomes

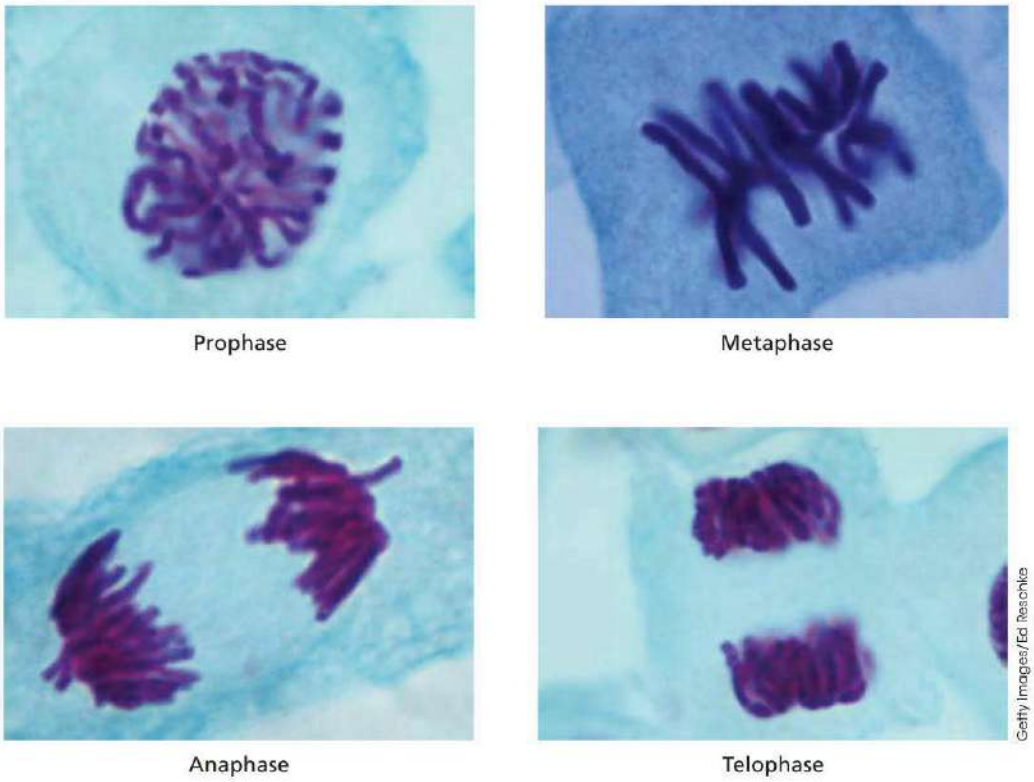


Cytokinesis in eukaryote cells

Following mitosis, cytoplasmic division, or cytokinesis, occurs. The cytoplasm of plant cells divides with the formation of a structure called a **cell plate**. Figure 1.19 shows how parts of the cell wall fuse with parts of the spindle, forming the cell plate. Cellulose is deposited at this site, forming a wall that divides the parent cell into two daughter cells, each one with a plasma membrane.



▲ **Figure 1.19**
Cytokinesis in a plant cell results from the formation of a cell plate, on which cellulose is deposited to form a cell wall around the two new daughter cells.



▲ **Figure 1.20**
The micrographs show prophase, metaphase, anaphase and telophase in onion root tip cells.

Animal cells do not have a cell wall, so division of the parent cell is less involved. In this case, the cytoplasm divides by a process known as **cleavage**. The plasma membrane around the middle of the cell draws together to form a **cleavage furrow**. The cleavage furrow continues to develop until it eventually meets and the cell is then cleaved, or split, with two new daughter cells resulting (Figure 1.21). Unequal distribution of proteins into the two daughter cells may result in the daughter cells having different fates; this normal process is termed asymmetrical cell division.

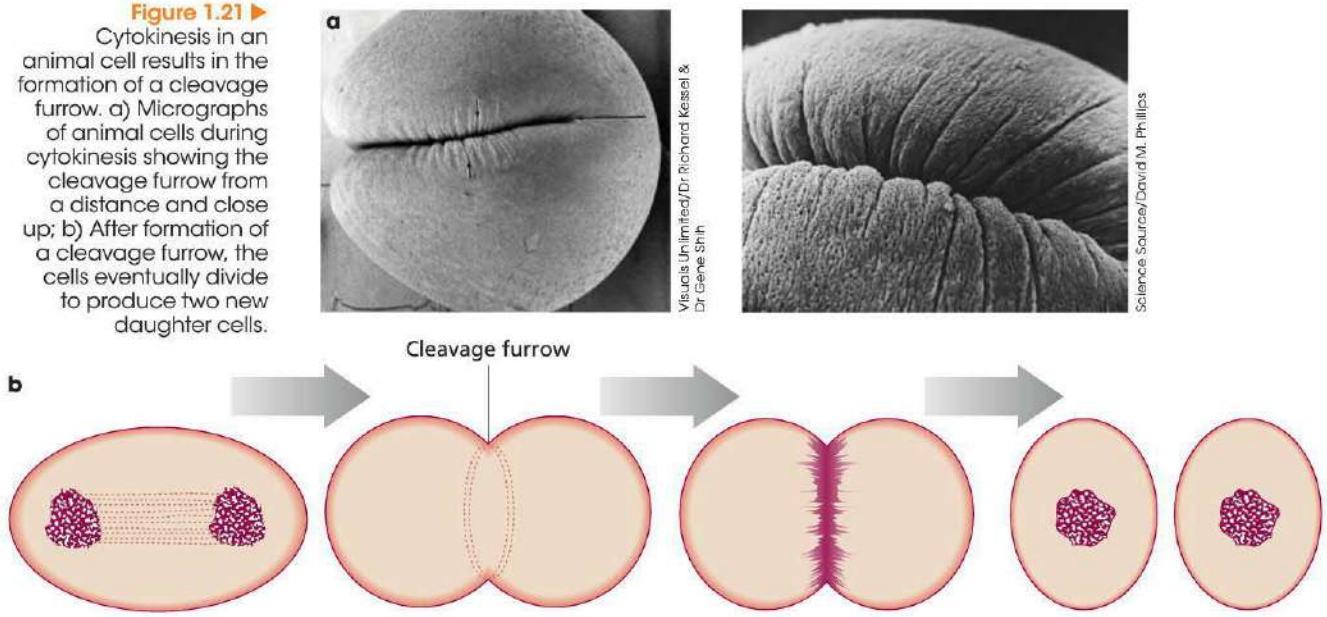
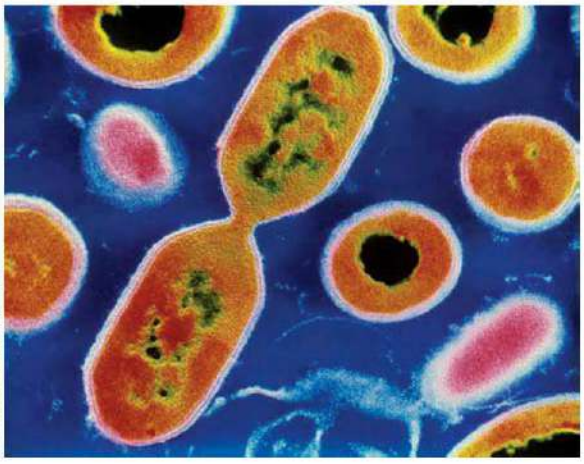


Figure 1.21 ▶ Cytokinesis in an animal cell results in the formation of a cleavage furrow. a) Micrographs of animal cells during cytokinesis showing the cleavage furrow from a distance and close up; b) After formation of a cleavage furrow, the cells eventually divide to produce two new daughter cells.

WOW

Telomeres

A region at the end of a chromosome, called a telomere, seems to prevent chromosomes sticking together. Scientists now believe they have another role in protecting chromosomes and enhancing the longevity of cells. An elderly person's telomeres are much shorter than those in a child. Each time a cell divides, part of the end of the telomere is lost. When telomeres reach a critical short length, cells die. Individuals vary in the length of telomeres they are born with and their rate of shortening. Elizabeth Blackburn, a former Tasmanian, educated in Victoria and now an American citizen, is a leading researcher in telomeres and their effect on the ageing of cells and promotion of cancer. She was awarded the Nobel Prize in Physiology or Medicine in 2009 jointly with Carol W. Greider and Jack W. Szostak for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase, which extends telomere lengths and cell lifespan as a result.



Science Photo Library/CNRI

Figure 1.22 ▲ Transmission electron micrograph of *Listeria* dividing in two by binary fission (magnification ×9800)

Binary fission

Cell reproduction is more complex in eukaryotes than in prokaryotic cells. As prokaryotes lack a nucleus and have only a single chromosome with no centromere, they cannot be properly said to undergo mitosis. They reproduce by binary fission, a process that includes DNA replication, chromosome segregation and cytokinesis (Figure 1.22). As with mitosis, binary fission also leads to the production of two daughter cells with the same number of chromosomes as the parental cell.

Prokaryotic bacterial cells simply replicate their single DNA strand. Following replication each copy attaches to a different part of the cell membrane. When the cell begins to pull apart, the replicate and original chromosomes are separated. A wall forms across the cell and divides it into two cells of identical genetic composition.

A similar process of binary fission occurs in eukaryotic cells when mitochondria and chloroplasts divide to form new organelles. They divide independently of nuclear DNA and segregate quite evenly into two daughter cells during cytokinesis.

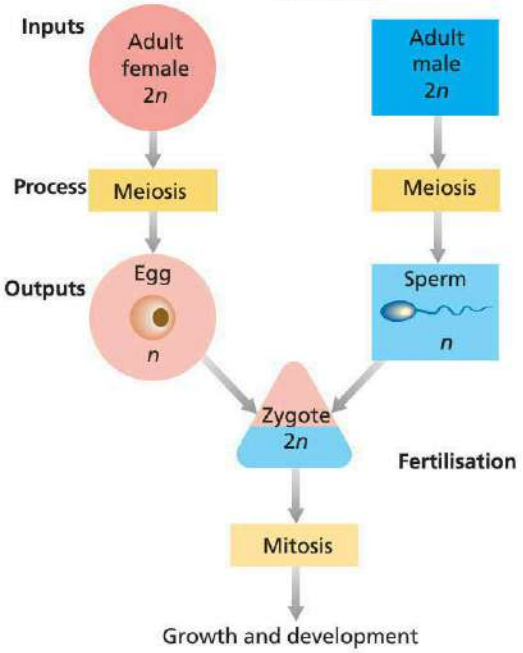
Prokaryotic cells reproduce by binary fission.

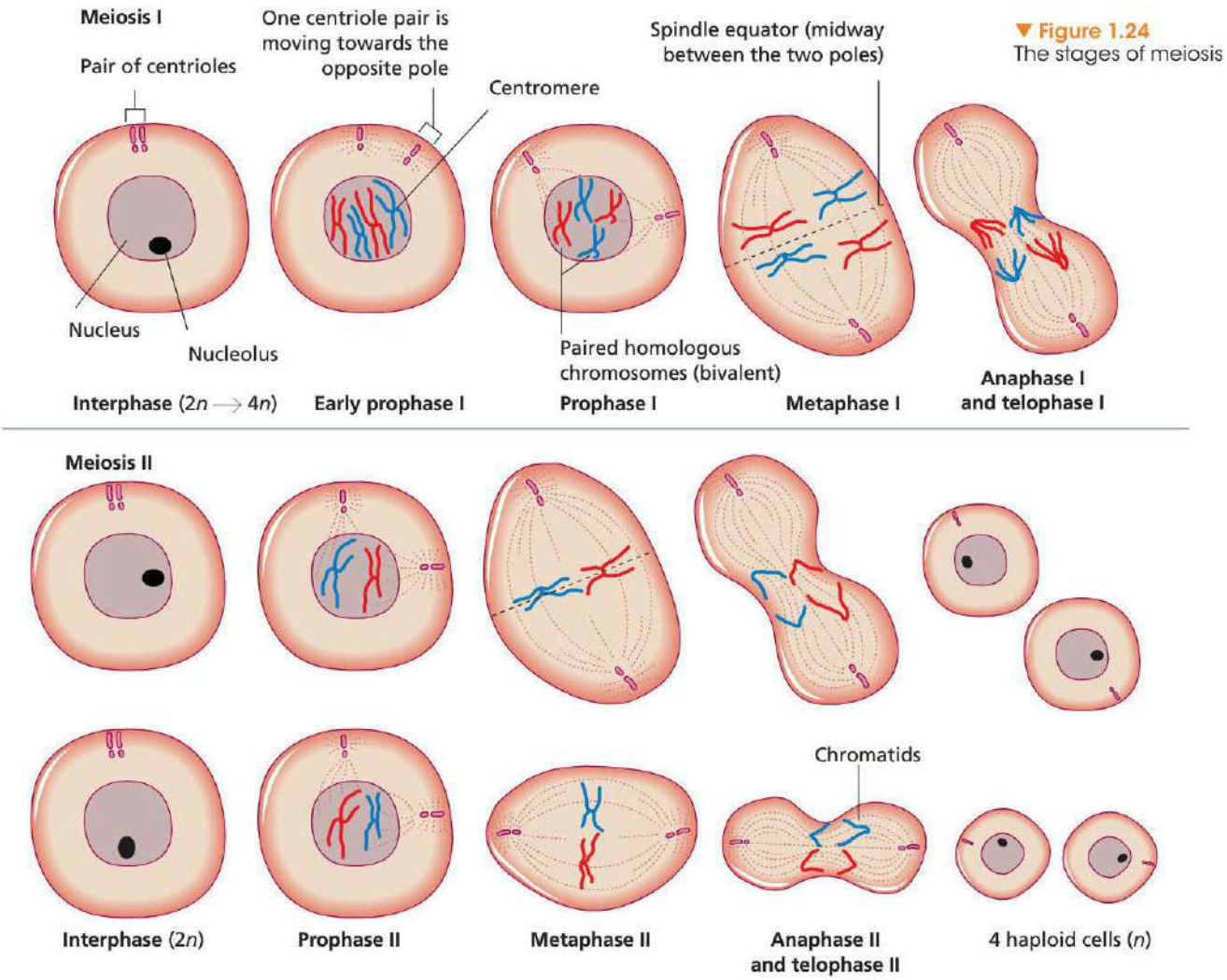
Meiosis and fertilisation

Meiosis occurs in specialised organs of sexually reproducing animals and plants, and results in the production of gametes: the sex cells, sperm and eggs (or ova). It is a form of nuclear division that prevents the doubling up of the diploid number of chromosomes at fertilisation. In meiosis, two divisions of the nucleus of the parent cell take place. In the first division, each chromosome of a pair separates and goes to each end of the cell. In the second division, the chromatids of each chromosome separate from each other. Four gametes are thus produced, each carrying half the original number of chromosomes. Gametes are therefore haploid.

As the number of chromosomes in the daughter cells has been reduced by half, meiosis is called a reduction division. During meiosis, the chromosomes of each homologous pair separate to each gamete at random. Fertilisation, the joining of the sperm and egg cells, results in the zygote gaining one of each pair of chromosomes from its parents.

▼ Figure 1.23
The inputs and outputs of meiosis





▼ Figure 1.24
The stages of meiosis

Meiosis I

Prophase I

In prophase I the chromosomes condense, the nucleolus disappears and a spindle forms with centrioles, if present, at opposite ends. Homologous chromosomes lie side by side, in a process known as **synapsis**. A pair of homologous chromosomes, one maternal and the other paternal, is now called a **bivalent**.

As prophase proceeds, homologous chromosomes may coil around each other intimately. Later, they move apart but the chromatids remain in contact at certain points called **chiasmata (singular chiasma)**.

Metaphase I

In metaphase I, the nuclear envelope breaks down and the homologous chromosomes move together to the equator of the spindle.

Anaphase I

In anaphase I, the maternal and paternal chromosomes of homologous pairs move towards opposite poles of the spindle. The separation or **disjunction** of each pair of homologous chromosomes occurs independently of other chromosome pairs.

Telophase I

In telophase I, the spindle breaks down, the cell starts to separate across its middle and nuclear envelopes form around the two new nuclei.

Cytokinesis, the division of the cell, completes the first stage of meiosis.

Interphase

At the end of meiosis I, a brief interphase usually occurs. DNA does not duplicate during this interphase.

Meiosis II

The cell then enters the second meiotic division. In prophase II, a new spindle forms at right angles to the first one. In metaphase II, the chromosomes move to the equator of the spindle. In anaphase II, the chromatids separate and move apart from each other. The chromatids become the chromosomes of the daughter cells. When they reach the poles, the cells enter telophase II. Characteristically of telophase, the spindle apparatus disappears, the chromosomes de-condense to their thread-like form and new nuclear envelopes and nucleoli form.

Meiosis is now complete. Cytoplasmic division follows so that four haploid cells form from the original single diploid parent cell. In humans, females produce an ovum containing 22 autosomes and one X chromosome, and males produce sperm containing 22 autosomes and either an X or a Y chromosome.

Table 1.5 Comparison of mitosis and meiosis

Mitosis	Meiosis
Nuclear and cell division for growth, repair and replacement of tissues	Nuclear and cell division for producing sex cells (gametes)
Takes place in somatic cells	Takes place in gonads or reproductive organs of living things (e.g. ovaries and testes of mammals, ovaries and anthers of flowering plants, spores of some plants)

(continued)

Table 1.5 *continued*

Mitosis	Meiosis
One cell division completes the process	Two cell divisions complete the process
Two cells are the outputs	Four cells (gametes) are the outputs
Each daughter cell contains the diploid number of chromosomes ($2n$)	Each daughter cell contains the haploid number of chromosomes (n)
Asexually reproducing organisms (e.g. plant cuttings, runners, bulbs) reproduce by mitotic division of cells; prokaryotes reproduce by binary fission, not mitosis	Sexually reproducing organisms reproduce by fusion of gametes, restoring the diploid number ($2n$) of chromosomes for each cell
New cells or offspring produced by this kind of reproduction do not show variation between them unless there are environmental influences or mutations; they are genetically identical to each other (i.e. clones)	Offspring produced show variation between them
Variation and diversity of offspring are narrowed	Variation and diversity of offspring are increased
Applications include for tissue culture, such as skin grafts and cloning plants	Applications include creating new varieties of organisms

Fertilisation

In the process of fertilisation, male and female haploid sex cells fuse to produce a diploid zygote. Two gametes from different individuals (usually one male and one female) of the same species need to combine to produce the new individual of that species. This is called sexual reproduction. Organisms produced by sexual reproduction will have a different combination of DNA to that of either parent.

The zygote formed is a cell with double the amount of DNA as the gamete. Therefore, meiosis halves the amount of DNA and fertilisation restores the amount of DNA to the required amount for that species. Human gametes produced by meiosis contain 23 chromosomes. Fertilisation restores the number of chromosomes to 46 ($23 + 23 = 46$), the chromosome number in somatic cells. Different species have different numbers of chromosomes.

In mammals, gametes from the male are called sperm, and gametes from the female are called ova. In flowering plants, pollen grains contain cells that are male gametes and ova contain an egg cell or female gamete.

Male or female? The role of the sex chromosomes

In humans, normally all female gametes contain 22 autosomes and an X chromosome. But 50% of male gametes contain 22 autosomes and a Y chromosome and 50% contain 22 autosomes and an X chromosome. Thus, in humans there is a 50% chance that, in fertilisation, a sperm cell bearing a Y chromosome will fuse with an egg cell, resulting in a male (XY), and a 50% chance that a sperm cell carrying an X chromosome will fuse with an egg cell, resulting in a female (XX).

In other species it is not always the male that has the unmatched sex chromosomes. For example, in birds, males are XX and females are XY. In some insects, females are XX and males are XO, with O denoting the absence of a chromosome, so the female sex cells must have two sex chromosomes but those of males have only one unmatched sex chromosome. In certain other social insects, such as honeybees, females develop from fertilised eggs and are diploid, and males develop from unfertilised eggs and are haploid.

WOW

Sex determination

A quite different system of sex determination is found in alligators and crocodiles. Here the sex of the offspring is determined entirely by environmental factors. There are no differences between males and females in terms of their chromosomes. Instead, there is a critical period in the early development of the fertilised egg during which the surrounding temperature determines the sex of the individual. In some species, high temperatures lead to males; in others, high temperatures result in females.

Not all life continues

Cells do not live forever; they are pre-programmed to age and die at a given life span. For example, some skin cells, known as keratinocytes, live for about 3 weeks. The dead cells form a surface layer that is continually shed. Keratinocytes self-destruct in an orderly and programmed manner called **apoptosis**.

Far from being detrimental to an organism, cell death by apoptosis is a vital and formative process that is essential for development, shaping organs and tissues. Apoptosis can cause some cells to die at a particular time of development. For example, dying cells enable a tadpole to lose its tail as it becomes a frog and a human embryo to lose the webbing between its fingers and toes. In fact, almost all multicellular organisms have cells that are born to die.

Figure 1.25 ▼ During mouse embryo development, programmed cell death (apoptosis) leads to loss of cells in the webbing, or interdigital tissue, between fingers to help form a paw. Hand formation in human embryos is also assisted by apoptosis.

The demise of these cells is genetically programmed and, when their time comes, death is orchestrated by the regulated expression of dozens of genes. Apoptotic cells are destroyed through a series of active, orderly events that start with enzymes shredding a dying cell's DNA into thousands of fragments. The nucleus is gradually dismantled and the membrane begins to bleb. Eventually, apoptotic bodies are formed and these are cleared by scavenging cells called phagocytes, particularly macrophages.

All cells carry a copy of the apoptotic death program and under certain conditions, such as when cells have been infected, or when a cell has reached the end of its natural lifespan after a certain amount of normal wear and tear, this program can be activated.

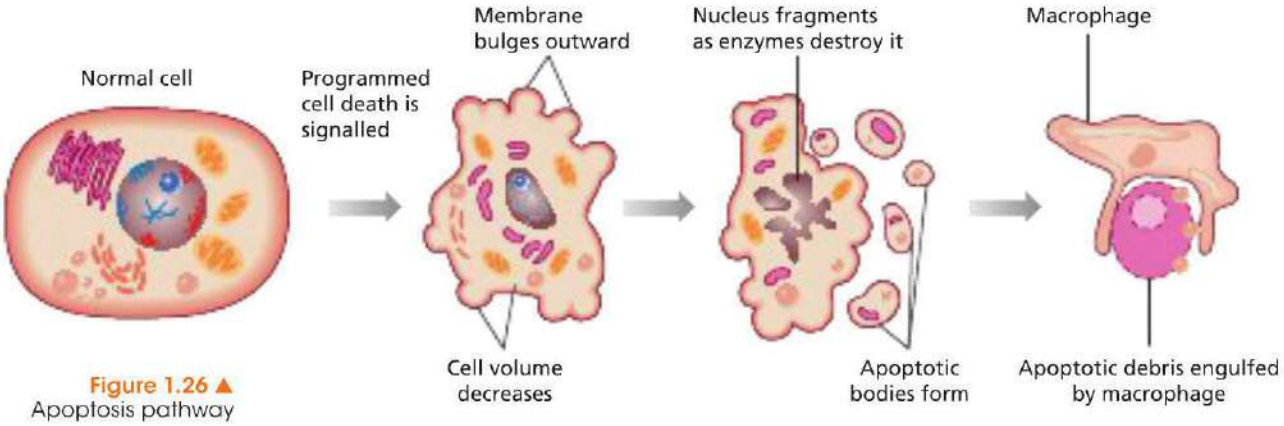
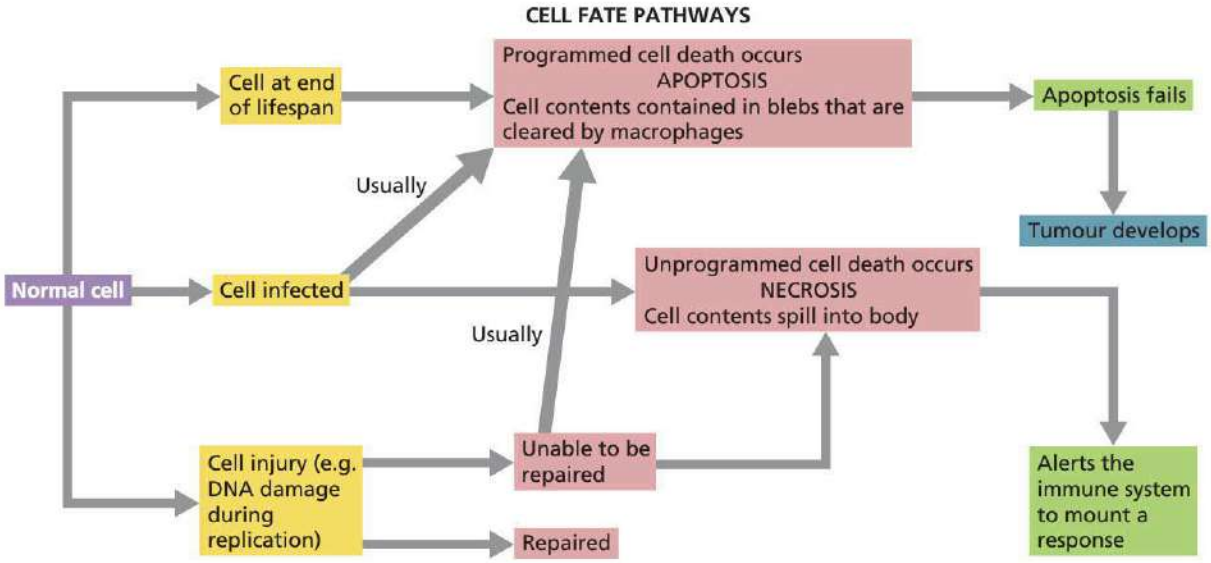


Figure 1.26 ▲ Apoptosis pathway

If apoptosis fails, all sorts of problems, ranging from developmental defects to cancer, can result. An important stage in the formation of cancer occurs when apoptosis fails and cells do not die as they should. Some tumour cells have changes to their suicide genes that make them forget how to kill themselves. Cancer is normally controlled by keeping a tight rein on cell division, particularly by preventing division when damage has occurred to DNA. When this damage cannot be repaired, apoptosis is initiated and the cell is disposed of.

Conventional cancer treatments target sick and healthy cells alike, so one treatment goal is to find drugs that trigger apoptosis only in cancer cells. Some proteins seem to act as a brake, that is, when they are not produced, apoptosis begins. Pharmaceutical companies are searching for ways to inhibit these proteins in the hope of finding drugs that will disable them only in cancer cells.

▼ Figure 1.27
Summary of potential cell fates



WOW

Cell death receptors

To die or not to die, that is the question. In the human body, around 60 billion cells die each day due to the process of apoptosis. In the immune system, programmed cell death is important for the removal of cells with DNA damaged by the sun or chemicals such as those in cigarette smoke, cells that are infected with a virus, cells that have starved and suffered damage as a result, or are immune system killer cells that have carried out their functions and cleared the infection.

Messages to trigger cell death can come from inside or outside a cell. Messages from outside the cell bind to 'death receptors'. These membrane proteins relay messages to the inner workings of the cell, which stimulate it to activate a group of 'protein-cutting' enzymes (caspases). The activated caspases break down structural and functional components of the cell. The dying cells display an 'eat me' signal that is recognised by a passing macrophage, which disposes of the cells by engulfing them.

QUESTION SET 1.4

Remembering

1 Name the process responsible for killing up to 60 billion cells in our bodies each day.

2 Distinguish between:

- a mitosis and cytokinesis.
- b mitosis and meiosis.
- c gamete and zygote.

Understanding

3 Explain why offspring produced from asexual reproduction resemble their parent whereas offspring produced from sexual reproduction are different to their parents.

4 Draw an annotated graphic that summarises the cell cycle.

5 Suggest why it is important for DNA to replicate before cell division.

6 Explain why apoptosis is important for maintaining life.

Applying

7 Draw a Venn diagram to illustrate the relationship between cell division, nuclear division and cytokinesis.

CHAPTER SUMMARY

- Heredity includes the study of inheritance patterns and mechanisms through generations.
- Many groups of scientists have contributed to the discovery of the structure and function of DNA.
- James Watson and Francis Crick are credited with the discovery of DNA structure in 1953.
- DNA is composed of four different types of nucleotides; each nucleotide has a deoxyribose sugar, phosphate group and one of four different types of nucleotides: adenine (A), thymine (T), guanine (G) and cytosine (C).
- The two strands of a DNA double helix link by hydrogen bonds between complementary bases: A links with T, G links with C.
- RNA has nucleotides with ribose sugar and uracil (U) instead of thymine.
- DNA replicates by a semi-conservative mechanism where one of the strands in the newly formed molecule is new and the other is the original strand.
- The enzymes DNA helicase and DNA polymerase facilitate DNA replication.
- All of the DNA in the cell of an organism is its genome.
- Technology used in areas such as bioinformatics is used to analyse large amounts of DNA and protein data.
- DNA and associated proteins together make up a chromosome.
- Eukaryotic chromosome structure consists of DNA coiled around histone proteins to form nucleosomes.
- When matched and ordered, eukaryotic chromosomes are displayed in a karyotype and different chromosome sizes, centromere positions and banding patterns can be observed.
- Somatic or body cells have pairs of homologous chromosomes; each chromosome of a pair comes from the male parent and the other chromosome of a pair comes from the female parent.
- Sex chromosomes that determine an individual's sex are generally matched in one sex (e.g. XX) and unmatched in the other sex (e.g. XY).
- Homologous chromosomes have the same genes at the same position (locus) but these genes may have alternative forms of the gene called alleles.

- A diploid number ($2n$) of chromosomes is found in somatic cells; a haploid number (n) of one of the pair of homologous chromosomes is found in gametes.
- Chromosomes in mitochondria and chloroplasts are similar to prokaryotic chromosomes.
- Chromosomes in prokaryotic cells are generally circular and found in a region of the cell called the nucleoid.
- Small rings of DNA called plasmids may also be present in prokaryotic cells.
- The sequence of events in cell division is called the cell cycle.
- Eukaryotic cells form new cells through the process of mitosis and meiosis.
- Eukaryotic cell division involves a number of phases resulting in nuclear division (mitosis and meiosis) and cytoplasmic division (cytokinesis).
- Cells formed by mitosis have the same genetic material as their parent cells.
- Prokaryotic cells reproduce by binary fission.
- Cells formed by meiosis are called gametes and join together in sexual reproduction to form a new cell with genetic material from two different parent cells.
- Apoptosis is important for development, removing unwanted cells and protecting an organism from ill health.

CHAPTER GLOSSARY

algorithm a method expressed as a list of instructions for calculating a function; the instructions describe a computation from the start through a sequence of steps to the final output

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

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cell by various enzymes including caspases

asexual reproduction a form of reproduction in which offspring are produced from a single parent

autosome chromosomes that are the same in both males and females of a species; they do not include sex chromosomes

binary fission the division of a cell into two without mitosis; a prokaryotic cell splits to form two daughter cells

bioinformatics the science of managing and analysing biological data using advanced computing techniques; it is especially important in genomics research because of the large amount of complex data this research generates

bivalent visible bodies in a cell during prophase I of meiosis, which are made up of two homologous chromosomes joined together

cell cycle the sequence of events from one cell division to another

cell plate the structure produced by dividing plant cells where the new cell wall is to be formed

centriole a minute rod-shaped body present in many resting cells just outside the nuclear membrane; it doubles before mitosis, moving apart to form the poles of the spindle; usually absent in plants

centromere the waist-like constriction in a chromosome required for the movement of chromosomes during cell division

chiasmata (singular chiasma) the point of contact between homologous chromosomes during prophase I of meiosis

chromatid daughter strands of a duplicated chromosome that are joined together by a centromere

chromatin a complex of proteins and DNA in eukaryotic chromosomes

chromosome a structure composed of DNA and protein that contains along its length linear arrays of genes carrying genetic information; prokaryotes have one circular chromosome whereas eukaryotes have a number of linear chromosomes

cleavage division of the cytoplasm in an animal cell

cleavage furrow a shallow, ring-like depression that forms at the cell surface of an animal cell undergoing cytokinesis as contractile microfilaments pull the plasma membrane inward; it defines where the cytoplasm will be cut in two

cytokinesis division of the cytoplasm

diploid ($2n$) describes a cell or organism that has a genome comprising two copies of each chromosome, represented by $2n$

disjunction the moving apart of homologous chromosomes during anaphase of meiosis

DNA (deoxyribonucleic acid) an information molecule that is the universal basis of an organism's genetic material; it contains instructions, written in a chemical code, for the production of proteins by the cell

DNA helicase an enzyme that helps the two strands of the DNA double helix unwind and separate

DNA ligase an enzyme used to catalyse the formation of a bond between two pieces of DNA

DNA polymerase an enzyme capable of making exact copies of fragments of DNA

gamete a cell produced in sexual reproduction, which combines at fertilisation; in humans, the gametes are ova and sperm cells; in flowering plants, pollen grains contain male gametes and ova contain a female gamete

gene a unit of heredity that transmits information from one generation to the next; a segment of DNA that codes for polypeptide

genetics the study of the mechanism and patterns of inheritance through the transmission of coded chemical instructions from one generation to the next

genome all of the genetic material contained in an organism or a cell; includes the chromosomes within the nucleus and the DNA in mitochondria and chloroplasts

genomics the study of the genome – how genes interact with each other and the environment and the resultant proteins produced; it requires a knowledge of an organism's entire DNA sequence so studies rely on powerful technologies and bioinformatics

haploid (*n*) describes a cell or organism that has a genome that contains one copy of each chromosome, represented by *n*

heredity the study of inheritance; the genetic transmission of characteristics from one generation to another

heterosome non-identical chromosomes pairing up at meiosis (e.g. the XY chromosomes in human males)

histone a protein around which DNA winds in eukaryotic cells

homologous chromosomes a pair of chromosomes that have the same size, shape and genes at the same locations

interphase the stage between nuclear divisions

karyotype a display of the number and appearance of the chromosomes of an organism or cell observed at metaphase

locus (plural loci) the position a gene occupies in a chromosome

meiosis a two-phase type of cellular division in which the chromosome number of a cell is halved to the haploid number; meiosis is the basis of gamete formation in animals and spore formation in plants

mitosis a type of nuclear division that maintains the parental number of chromosomes for daughter cells; it is the basis of bodily growth and asexual reproduction in many eukaryotic species

nitrogenous bases a structural component of nucloetides, DNA has adenine (A), cytosine (C), guanine (G) and thymine (T); in RNA thymine is replaced with uracil (U)

nucleoid the region within a prokaryotic cell that contains the genetic material

nucleolus a structure found within the nucleus of a non-dividing cell; a site in which the protein and RNA subunits of ribosomes are assembled

nucleotide the basic building block of nucleic acids (DNA and RNA) linked together by phosphodiester bonds; each nucleotide is made up of a five-carbon sugar, a phosphate group and a nitrogenous base

organelle a specialised part of a cell, with its own specific function; 'little organ'

plasmid a small circular piece of DNA, found in bacteria, which is able to replicate independently of the cell's chromosomes; plasmids carry antibiotic resistance markers

proteomics the study of the entire protein content expressed by a cell.

replication fork the junction between the unwound single strands of DNA and the intact double helix during replication

RNA (ribonucleic acid) a molecule consisting of a single strand of nucleotides; it plays an essential role in protein synthesis (as messenger RNA and transfer RNA) and as a structural component of ribosomes

semi-conservative replication the production of two new DNA double helix molecules, each consisting of one parental strand and one daughter strand

sex chromosome chromosomes that affect sexual traits; one sex has homologous sex chromosomes, the other sex has a dissimilar set

sexual reproduction a form of reproduction in which offspring are produced from two parents

somatic cell a normal body cell, as compared with a germ-line cell from which a gamete (i.e. sperm or ovum) is derived

synapsis the pairing of homologous chromosomes

trait a heritable characteristic

zygote the first cell of a new individual, which is formed by fusion of a sperm and ovum at fertilisation

CHAPTER REVIEW QUESTIONS

Remembering

1. State the type of chemical bond that holds strands of DNA together.
2. Describe how a karyotype is made and identify how chromosomes are ordered.
3. Describe the composition of an organism's genome.

Understanding

4. The amount of nuclear DNA in any given cell can be measured quite accurately during the cell cycle. Predict at what stages throughout the cell cycle you would expect to see changes in the amount of nuclear DNA.
5. Compare binary fission to mitosis.
6. Relate the concept of a chromosome to the concept of a gene.
7. Explain why each of the following statements is incorrect and rewrite it as a correct statement.
   - a. One strand of the DNA helix ladder is maternal and the other strand is paternal.
   - b. Different organisms have different types of DNA because they are very different from each other.
   - c. Each chromosome is made of more than one DNA molecule.
   - d. The different cell types (skin, muscle, cartilage, etc.) found in a given individual's body contain different DNA.
   - e. In sexually reproducing organisms, half of the organism's body cells contain DNA from the mother and half contain DNA from the father.
   - f. Every person's DNA is unique.

Applying

8. Predict what would happen if cytokinesis did not occur during a cell cycle.
9. Find the meaning of the prefixes used in the stages of cell division: pro-, meta-, ana-, telo-. Relate their meanings to the events occurring.
10. Explain how nucleotides join together to form a polynucleotide chain. Using knowledge of the chemistry of a nucleotide, explain what is meant by the 5' and 3' direction.
11. Find out what 'Chargaff's rule' is and explain how it helped Watson and Crick discover the nature of DNA.
12. Explain how the weak hydrogen bonding between nucleotides and complementary base pairing in a DNA molecule allows semi-conservative DNA replication to occur.

Analysing

13. When animals of different species are kept together in captivity they sometimes mate and produce offspring. A donkey is known to have a diploid number of 62 and a zebra has a diploid number of 44.
    - a. Name cells in the donkey that would be expected to contain 31 chromosomes.
    - b. Name cells in the zebra that would be expected to contain 44 chromosomes.
    - c. Estimate how many chromosomes are expected to be in the somatic cells of the donkey.
    - d. If a 'zonkey' (a hybrid formed by the fertilisation of a female donkey egg with a zebra sperm) is produced, predict the $2n$ number.
    - e. Describe how a zonkey karyotype would differ from the karyotype of a zebra.
    - f. Suggest problems that might occur when the zonkey produces gametes.
    - g. Explain why most hybrid animals are infertile.
14. Cell B was found to contain twice the amount of DNA compared with a normal body cell C. Name the phase of the cell cycle cell B is likely to be in. If cell C has 16 chromosomes, calculate how many chromatids you would find in cell B.
15. Draw a simple table that compares the inputs, process and outputs of mitosis and meiosis.
16. Write the full names of DNA and RNA. Find out and explain why these names were used with respect to their chemistry and the history of their discovery.
17. Scientists from the Australian Museum and Queensland University of Technology have sequenced the koala genome. Analysis of the data should reveal between 12 000 and 20 000 genes on the 16 chromosomes.

a Name the nucleotide expected to have approximately the same number as the adenine nucleotides sequenced.

b Explain whether you would expect the sequence of DNA to be exactly the same in all members of the koala species.

c Estimate how many chromosomes a baby koala would get from its mother.

d State a koala's diploid number and its haploid number.

e Find out how the knowledge gained by sequencing the genome can help manage koala populations.

Evaluating

18 If Watson and Crick had not discovered the structure of DNA in 1953, do you think the structure would have remained unknown for much longer? Show how collaboration of different groups of scientists contributes to expanding our scientific knowledge.

19 'DNA is self-replicating.' Discuss whether you think only DNA itself is needed for replication.

20 All chromosomes are double stranded and linear in shape. Do you agree with this statement? Justify your answer.

21 'Cells produced by meiosis only contain half the amount of DNA compared to their parent cells. This means DNA does not replicate during meiosis.' Do you agree with this statement? Justify your answer.

22 Explain why meiosis is more appropriate for gamete formation compared to mitosis.

23 A group of cells being studied were never observed to undergo division. Predict if this means the cells were dead. Justify your answer.

24 Prokaryotes divide by means of binary fission and produce identical cells. Complex organisms produce sex cells that combine to form a new individual. Identify the advantages and disadvantages of asexual and sexual reproduction.

Creating

25 Paclitaxel is a drug that stops microtubules such as spindle fibres from disassembling.

a Predict what effect this would have on mitosis.

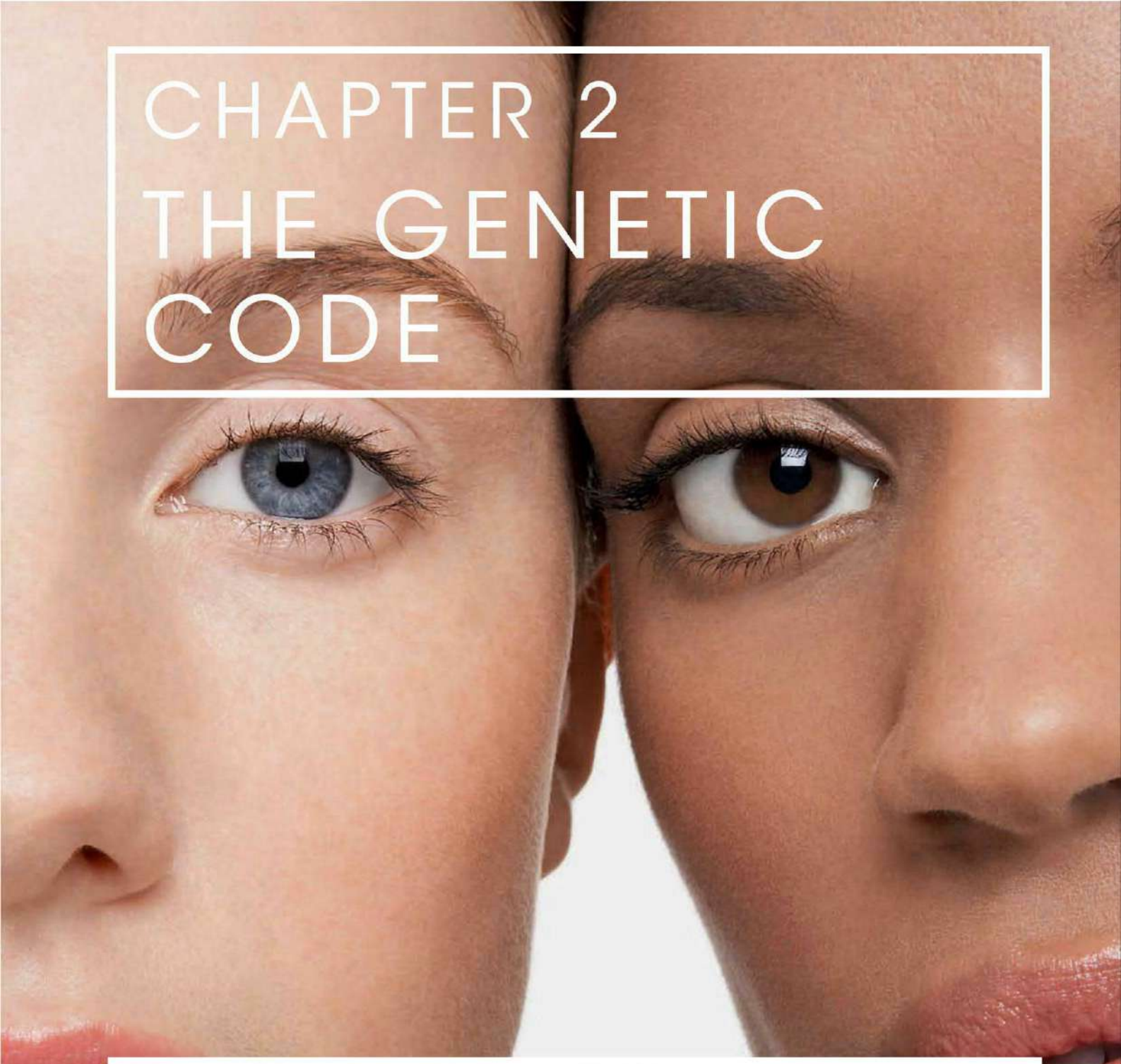
b Write a press release for Paclitaxel to communicate its benefits for treating cancer.

26 Design an activity where students act out the process of mitosis and meiosis. This can be in the form of a physical movement activity or even a dance.

Reflecting

27 Watson and Crick used the contributions of scientists before them as a basis for their hypothetical model of DNA. Reflect on how your understanding of new ideas is built on the knowledge and skills you have gained in your previous years of science study.

CHAPTER 2
THE GENETIC CODE



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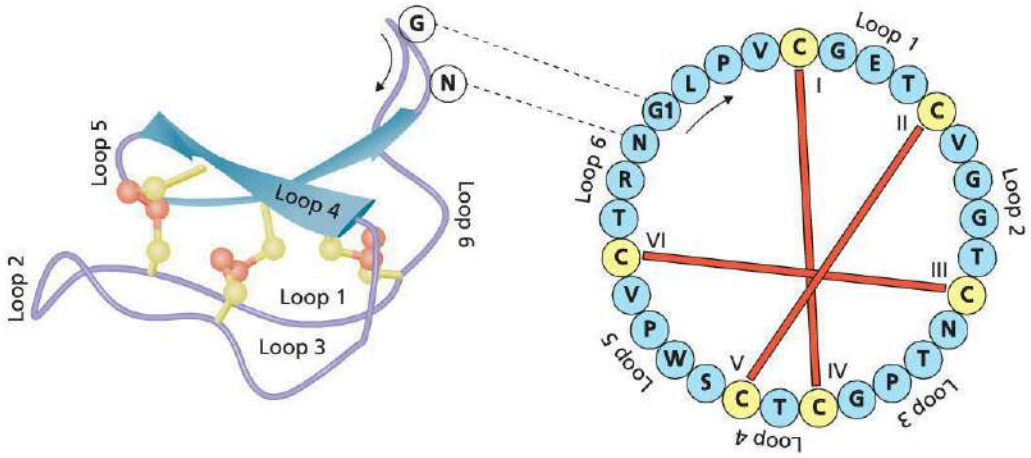
By the end of this chapter you will have covered the following material.

Science Understanding

- Genes include 'coding' and 'non-coding' DNA, and many genes contain information for protein production (ACSBL078)
- Protein synthesis involves transcription of a gene into messenger RNA in the nucleus, and translation into an amino acid sequence at the ribosome (ACSBL079)
- Proteins, including enzymes, are essential to cell structure and functioning (ACSBL080)
- The phenotypic expression of genes depends on factors controlling transcription and translation during protein synthesis, the products of other genes, and the environment (ACSBL081)

Figure 2.1 ▶
Kalata B1 is a protein with special properties and a cyclic peptide structure.



Residents of the Republic of Congo have a novel use for the perennial weed *Oldenlandia affinis*. They boil a handful of the dried plant in a litre of water and the resulting green brew is given to women in labour. This results in stronger contractions and a shortened delivery time: good news for both mother and baby. Scientists have become interested in the uteroactive ability of this brew and have scientifically analysed it. The uteroactive agent was found to be a small protein, only 29 amino acids long, called kalata B1. Apart from stimulating uterine contractions in female humans, kalata B1 also has powerful insecticidal and antimicrobial properties. This protein has a bracelet-like structure, making it a circular, or cyclic peptide. The structure of proteins varies and with this, their function also varies. Cyclic peptides are highly stable and have a range of built-in features that make them ideal for applications in agriculture and pharmaceuticals.

Proteins

Proteins are essential to cell structure and functioning. There are many types of proteins that exist. The kalata family of proteins is just one type. They are small, circular proteins that are very hardy and resistant to boiling. Some proteins have structural roles in cells; others are purely functional, including enzymes. All the different protein family members have different, specialised functions that are effective and highly suited to their 'task'.

The structure and function of each individual cell is dependent upon the suite of proteins produced within it. Essentially all cell types require specialised proteins to carry out their particular function. For example, a red blood cell during development must accumulate the haemoglobin protein but it has no need for keratin protein. Muscle cells need to produce large amounts of actin and myosin proteins, arranged in highly ordered arrays, for their contractile function. A gland cell needs to be able to produce the appropriate hormone and it needs to produce it only for brief periods when it is required.

Everything a cell does – what it develops into, what it synthesises and how it operates – is determined, more than anything, by the proteins it expresses. The characteristics of a cell or organism, determined by its protein expression, are called its **phenotype**.

Enzymes

Enzymes are one of the most important groups of proteins. Enzymes are able to speed up the rate of chemical reactions (catalyse them) without undergoing any change themselves. Without enzymes the chemical reactions that occur in living organisms would be so slow as to hardly proceed at all; this would be incompatible with the maintenance of life. The sum of the thousands of chemical reactions that occur constantly in each living cell is known as cellular metabolism. The metabolic reactions that occur in cells do not take place haphazardly; all are controlled and regulated to maintain cell functions and to meet the energy needs of the cell. The rate of cellular metabolism varies among organisms, and they need to occur at a rate that allows the cell to function. Each step in the pathway is controlled by an enzyme.

Proteins control many cell processes including the rate of cellular metabolism.

Protein synthesis

Proteins provide the essential link between DNA and the functioning cell. It is not surprising, therefore, that the central role of DNA is to determine what proteins the cell makes. A protein consists of subunits of amino acids that vary from each other in size and charge. It is the order in which the amino acids are arranged and their relative abundance that give a particular protein its individuality and functionality. DNA, with its four different nucleotide bases, determines in what sequence the 20 different types of amino acids are put together in the protein. But how can a four-letter code, for that is what DNA amounts to, specify a protein that at any given point consists of one of 20 different amino acids?

Clearly, a single base cannot specify a single amino acid, for then only four different amino acids could be coded for and proteins containing only four kinds of amino acids would be formed. Nor could just two bases specify a single amino acid, since only 16 amino acids could be coded for ($4 \times 4 = 16$). But three bases are sufficient: with these a total of $4 \times 4 \times 4 = 64$ bases can be specified, more than enough to account for the 20 different amino acids commonly found in cells. This allows for some overlap, or redundancy, within the code.

Thus, a minimum combination of three bases is needed to code for one amino acid. This suggestion was first put forward by Francis Crick on purely theoretical grounds but since then a firm body of experimental evidence has been established to support it. This 'triplet' of bases is known as a **codon**; many codons, or triplet codes, form the basis of the genetic code.

WOW

Kalata B1

Professor David Craik from Queensland is a world expert on proteins such as kalata B1. He determined the circular structure of kalata B1 in the 1990s. Circular proteins have no ends so they can survive digestive enzymes that normally attack the ends of proteins. Because of this they can pass through the digestive system unharmed and travel to their target, making them an ideal template for drugs.

DNA communicates with the cytoplasm via messenger RNA

How does a eukaryotic cell act on the instructions in each codon? DNA carries the sets of instructions in the chromosomes in the nucleus, but it is **ribosomes** in the cytoplasm that make proteins. Therefore, the information held by the codons in the DNA has to be conveyed from the nucleus through the nuclear membrane to the sites of protein synthesis in the cytoplasm.

There are really only two possible ways that this might take place. One way is that the DNA itself, or part of it, moves out from the nucleus into the cytoplasm. The other way is that the DNA stays in the nucleus and another molecule, acting as a go-between or messenger, carries instructions from the DNA to the cytoplasm. The first hypothesis was discounted on the grounds that chromosomal DNA was never detected in the cytoplasm of non-mitotic cells. This left biologists with the second possibility. We now know that DNA remains in the nucleus and is the basis or template for the production of another sort of nucleic acid, called **messenger RNA (mRNA)**, which conveys the instructions needed for protein synthesis from the DNA in the nucleus to the cytoplasm. Like DNA, mRNA consists of a string of nucleotides. However, RNA differs from DNA in four ways, as shown in Table 2.1.

Table 2.1 Comparison of RNA and DNA

RNA	DNA
Contains the sugar ribose	Contains the sugar deoxyribose
Single-stranded	Double-stranded
Contains uracil, cytosine, guanine and adenine	Contains thymine, cytosine, guanine and adenine
Shorter than DNA (usually fewer than 4000 nucleotides)	Much longer than RNA

Transcription: from gene to mRNA

Transcription of DNA generates a single-stranded RNA molecule that is almost identical in sequence with one of the strands of the double helix. The difference is that instead of the base thymine (T) pairing with adenine (A), the base uracil (U) does.

The first step in the process of transcription is DNA in the region of a gene unwinding, and then unzipping, exposing the nucleotide bases of both strands. Only one of these strands is used to direct the synthesis of mRNA; this is called the **template strand**. The other strand, of DNA origin, has the same sequence as the mRNA (except for T bases instead of U) and is called the **non-template strand**, or complimentary strand.

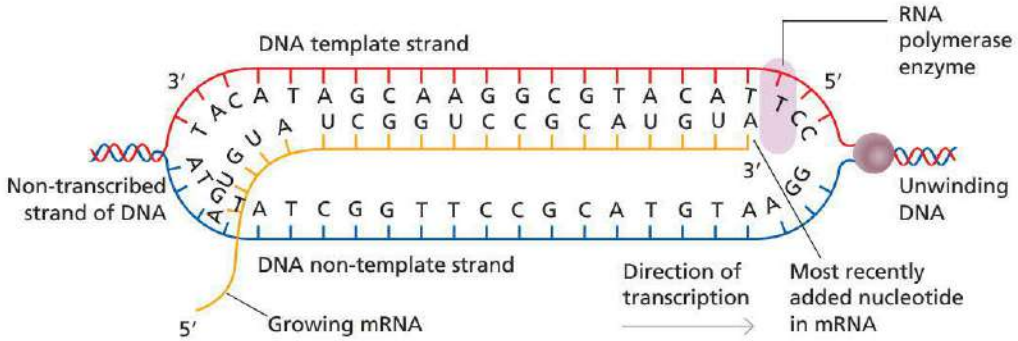


Figure 2.2 ▶ Diagrammatic representation of the synthesis of mRNA from DNA template. The DNA molecule unwinds and RNA nucleotides pair with one of the exposed strands of DNA, which serves as a template. These nucleotides are then joined together by the enzyme RNA polymerase, resulting in mRNA.

A particular nucleotide sequence at the beginning of the gene, called a **promoter**, signals the start of a gene. Proteins position RNA polymerase on to the DNA to bind with the promoter. Complementary RNA nucleotides are progressively joined together by RNA polymerase moving along the length of DNA. A base sequence at the end of the gene serves as a stop signal and the mRNA is released as a single strand. The DNA zips up and twists itself back into a double helix again once the mRNA has peeled off.

In transcription of DNA, single-stranded mRNA molecules are generated in the nucleus.

Finishing touches to mRNA

The mRNA strand at this stage is called **pre-mRNA**. Before it leaves the nucleus it is modified by the addition of a **methylated cap** at the 5′ end and about 100–200 adenine nucleotides at the 3′ end, which is then called the **poly-A tail**. These additions are important for the stability of the mRNA molecule and its nuclear export and translation (Figure 2.3).

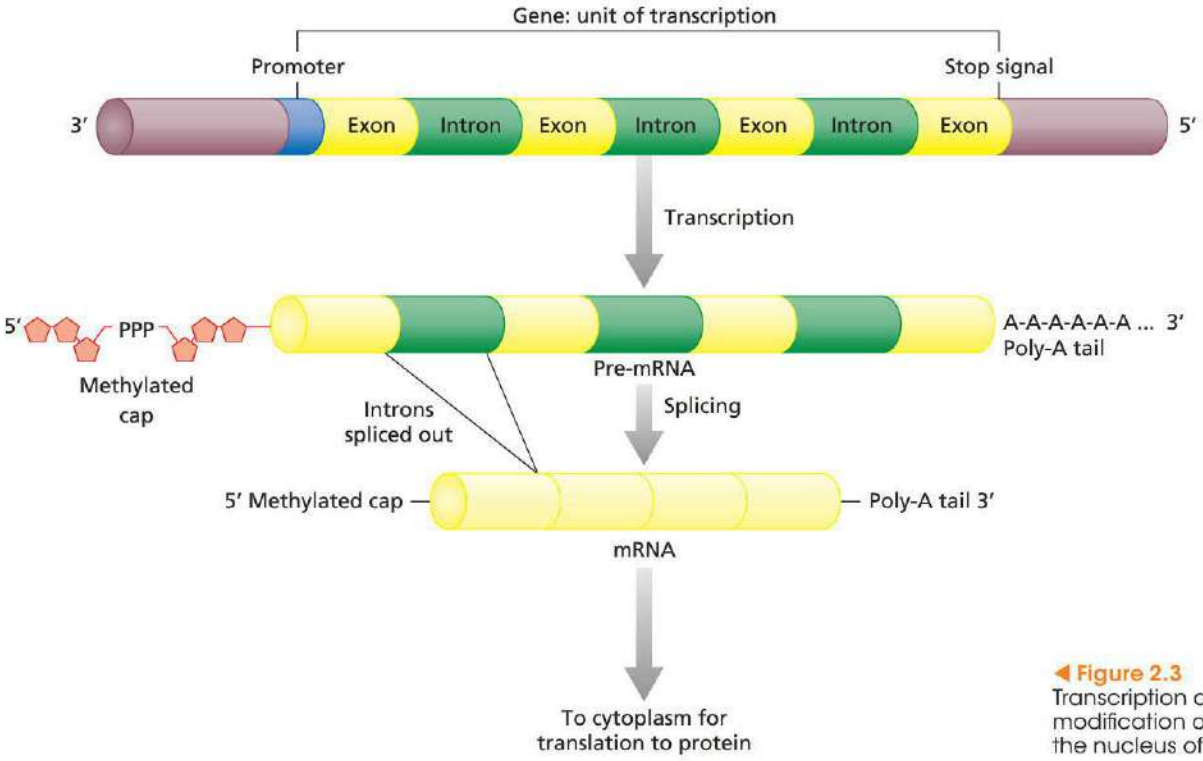
After these additions to the ends of the mRNA strand, some segments are removed. Most eukaryotic genes have regions of base sequences that are not translated into the amino acids of proteins. These regions are called **introns** and are interspersed with regions of DNA called **exons**, which are coding regions and contain the actual information for protein formation.

Both the exons and introns are transcribed into pre-mRNA, but introns are removed in a process called splicing before the mature mRNA leaves the nucleus. Once the pre-mRNA has been modified in this way, it is ready to leave the nucleus and move into the cytoplasm, where translation of the nuclear code into proteins is carried out by ribosomes. The average mature mRNA strand is about 1000–2000 bases long, including the methylated cap and 100–200 adenine bases in the poly-A tail.

When different exons from the same RNA are spliced together, different forms of the mRNA are created and are translated into different versions of the protein. Variations in expressed proteins are therefore generated by this alternative splicing.

The sequences for guiding the splicing are largely contained within the introns of the immature RNA transcript. These sequences are usually recognised by proteins within the cell that direct the splicing machinery to cut up and reassemble the transcript into the mature mRNA. The patterns of alternative splicing often vary between different cell types so that any particular tissue contains unique versions of the protein encoded by a single gene. The

proportion of alternatively spliced genes differs between organisms but it is estimated that more than 95% of human genes are processed this way.



◀ **Figure 2.3**
Transcription and modification of mRNA in the nucleus of a cell

Comparing transcription and DNA replication

The process of transcription is very similar to DNA replication in that the DNA molecule unwinds and free-floating nucleotides bind to the strand. The sequence is complementary to the DNA strand, as shown in Figure 2.2.

The process of transcription, however, differs from DNA replication in a number of respects.

- Only a small part of a DNA strand is used as the template for the RNA strand. This **coding region** of the DNA is a gene. DNA replication, on the other hand, involves the whole DNA molecule as the template.
- The enzymes involved in joining the RNA nucleotides together are **RNA polymerases** rather than the DNA polymerases involved in DNA replication.
- Only single-stranded RNA is produced in transcription and it is found both in the nucleus and cytoplasm in eukaryotic cells, whereas DNA formed by replication is double-stranded and found only in the nucleus.

QUESTION SET 2.1

Remembering

1 Identify three important functions of proteins in a cell.
2 Define 'cellular metabolism'.
3 List four different types of proteins.

Understanding

4 Outline the role of enzymes in metabolic reactions.
5 Compare pre-mRNA and mature mRNA.
6 Distinguish between transcription of DNA and replication of DNA.
7 Define 'gene', 'exon' and 'intron'. Write one sentence describing transcription using all three terms.

8 Explain why scientists chose the term 'template strand' for only one of the two strands in a DNA molecule.
9 Describe the outcome of alternative splicing and explain its significance in cell structure and function.

Applying

10 A DNA template strand has the nucleotide sequence AGGTCCTAG.
   a Suggest the sequence of the mRNA sequence transcribed from the DNA.
   b Suggest the DNA sequence of the non-template strand.

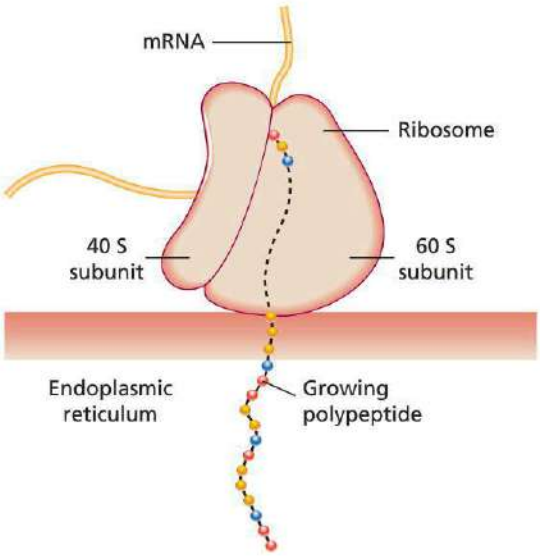


Figure 2.4 ▲
A ribosome attached to the endoplasmic reticulum is synthesising a polypeptide.

Translation of mRNA into proteins

When mRNA moves into the cytoplasm, it attaches itself to a ribosome, where amino acids then assemble in a particular order. The resulting chain of amino acids forms a specific **polypeptide**. This process by which a ribosome synthesises polypeptides from specific coded mRNAs is known as **translation**. Polypeptides join together to form the many and varied proteins found in cells.

Ribosomes are made up of two subunits, one small called a 40 S subunit and one large called a 60 S subunit (S is a unit of size). The nucleolus in the nucleus assembles them both from **ribosomal RNA (rRNA)** and proteins. The subunits move from the nucleus into the cytoplasm, where they combine to form the functional units of translation.

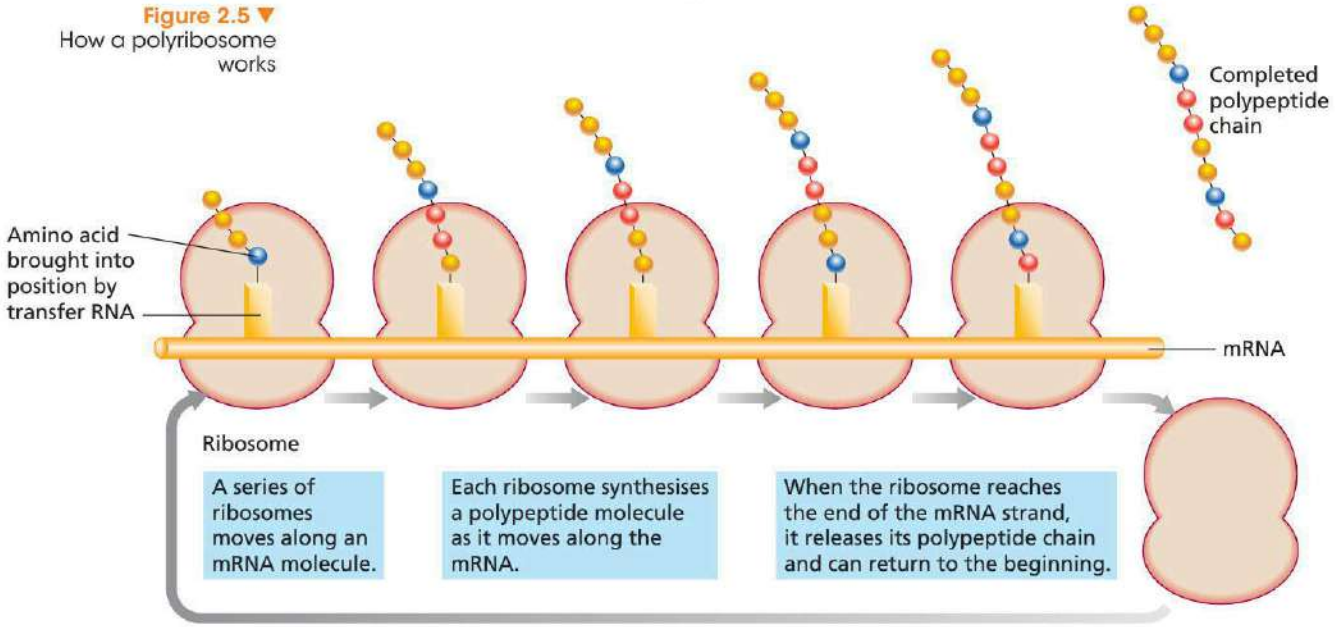
Figure 2.4 shows a eukaryotic ribosome bound to rough endoplasmic reticulum. Unbound ribosomes are also found throughout the cytoplasm. Generally, proteins that enter the secretory pathway after production, to be secreted by cells, are synthesised on endoplasmic reticulum-bound ribosomes, whereas those that remain in the cytosol are made on free ribosomes.

Ribosomes are also found in prokaryote cells and in mitochondria and chloroplasts. They are smaller than the ribosomes found in the cytoplasm of eukaryotic cells, although they also consist of two subunits and are involved in protein synthesis.

Polyribosomes

Ribosomes that occur in chains are called **polyribosomes** or polysomes, all binding to a single mRNA strand. This is shown in Figure 2.5.

Figure 2.5 ▼
How a polyribosome works

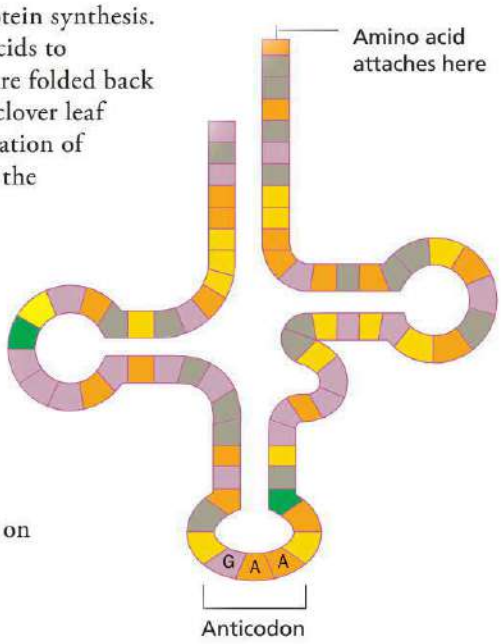


The advantage of polyribosomes is that they allow a large number of polypeptides to be made from a single mRNA strand in a comparatively short time. For example, it has been calculated that, in red blood cells, the time required for a single ribosome to travel the full length of an mRNA strand and produce a completed polypeptide chain is about 1 minute. By having 10 or more ribosomes at any one time making proteins from a single mRNA strand, the rate of protein synthesis is greatly increased. In bacterial cells protein synthesis happens even more rapidly. This is because prokaryotes lack a nucleus and protein synthesis can begin even before mRNA synthesis is complete.

Transferring amino acids

Another type of RNA, called **transfer RNA (tRNA)**, is also needed for protein synthesis. tRNAs take their name from their function: they transfer or carry amino acids to ribosomes. They exist as free-floating molecules within the cytoplasm and are folded back on themselves to form a compact three-dimensional structure rather like a clover leaf (Figure 2.6). Note the three nucleotide bases at the bottom of the representation of the molecule, called the **anticodon**, and the **amino acid binding site** at the top of the representation of the molecule.

The anticodon of the tRNA is complementary to the codon of the mRNA. Cells possess more than 20 different types of tRNAs: more than enough for the different types of amino acids. The type of amino acid picked up by RNA is related to the sequence of the anticodon. To find out which anticodon on the tRNA joins with a particular amino acid, first find the complementary nucleotides of the mRNA. For example, a tRNA molecule with the anticodon ACG will pick up a cysteine amino acid on its amino acid binding site. A tRNA with anticodon UGA will carry a threonine amino acid on its binding site. The codon AUG is called the **start codon** and it acts as a translational start site on the mRNA.



▲ **Figure 2.6**
Structure of transfer RNA. The diagram shows the molecule as a flat molecule shaped like a clover leaf. The three bases shown at the bottom illustrate the mRNA binding site, or anticodon. The amino acid binding site is shown at the top of the molecule.

Stages of translation

A small ribosome subunit loaded with an initiator tRNA (one that can start the process) recognises an mRNA strand as it leaves the nucleus and travels to the cytoplasm. The ribosome subunit binds to the methylated cap on the mRNA and moves along it 'scanning' for an AUG start codon. Once found, a large ribosomal subunit joins with the small one. The ribosome then passes along the mRNA strand and, as it passes each codon in the mRNA, a tRNA, carrying the appropriate amino acid, moves to the ribosome. The three bases in the mRNA binding site (codon) bond to the complementary three bases (anticodon) in the tRNA molecule.

The ribosome then moves on to the next codon of the mRNA strand, another tRNA molecule with a complementary anticodon sequence joins the codon and another amino acid is drawn into position, and so on. As the ribosome moves along the mRNA strand, more and more amino acids are added to the growing polypeptide chain until a **stop codon** is reached. In this way, the amino acids are linked in an order corresponding to the sequence of nucleotide base codons in the mRNA. As this is determined by the sequence of base codons in the original DNA, it follows that the base sequence in the DNA determines the order in which amino acids line up.

Once aligned, **peptide bonds** form between adjacent amino acids, resulting in the formation of a polypeptide chain. As the amino acids join up, the completed polypeptide chain peels off from the tRNA molecules. Once the job of the tRNAs is complete, they detach

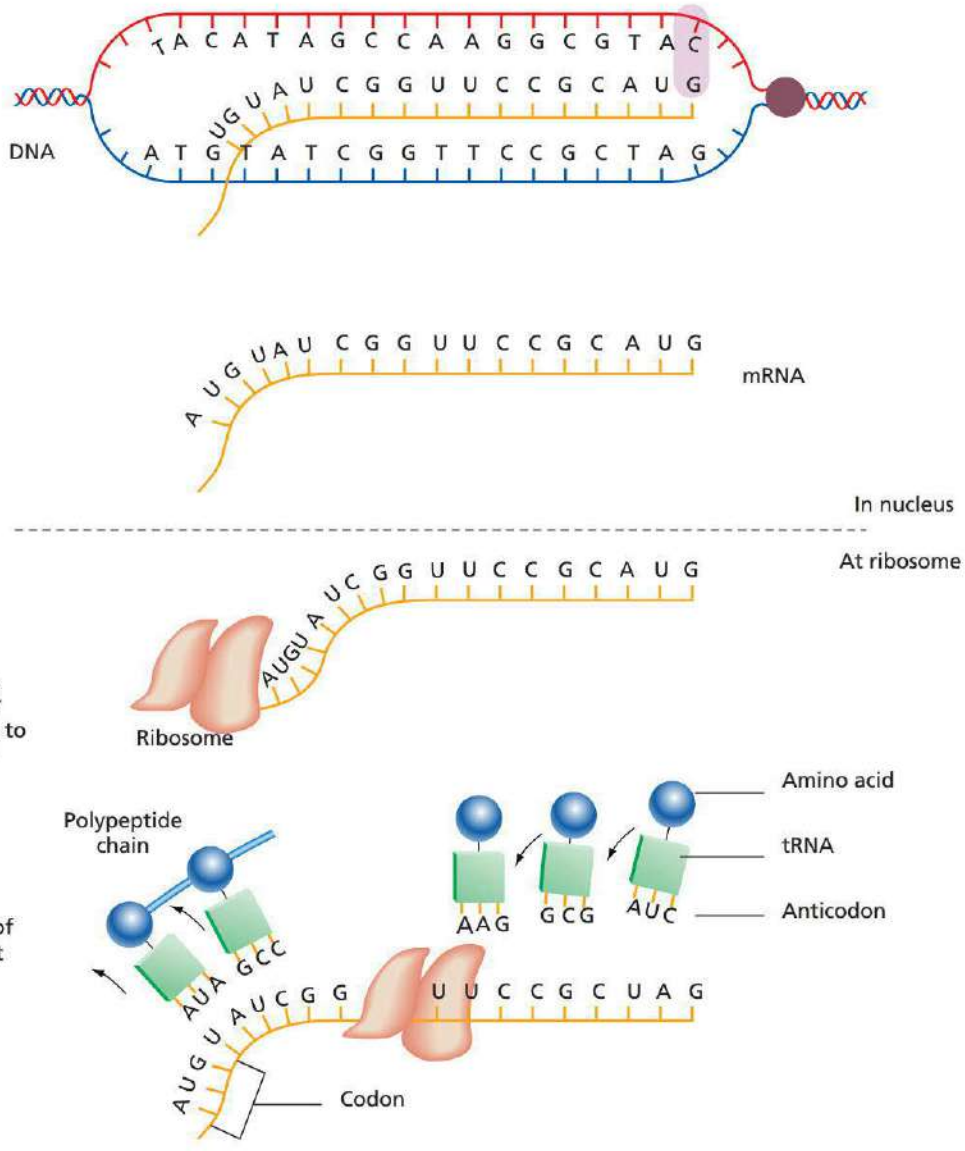
from the mRNA and return to the pool of tRNAs in the cytoplasm, from where they can be drawn upon to pick up specific amino acids again when required. This process is shown in Figure 2.7.

Meanwhile other ribosomes are carrying out the same process, moving along the mRNA strand simultaneously, each synthesising a polypeptide chain as it goes. On reaching a stop codon the ribosome releases the mRNA strand and the newly synthesised polypeptide chain. The mRNA strands are broken down by the cell releasing the nucleotides to be re-used in transcription. A protein molecule is made up of one or more polypeptide chains joined together to make a three-dimensional structure.

In translation, amino acids are assembled in the order prescribed by mRNA at the ribosomes.

The process of protein formation requires energy, which is provided by adenosine triphosphate (ATP) and related compounds. A large proportion of the fuel that our bodies burn up is needed for protein synthesis. Enzymes are involved at various stages of protein synthesis, for example, to attach the amino acids to the tRNAs and to join the adjacent amino acids together.

Figure 2.7 ▶
The process of protein formation



Through the action of tRNA, the mRNA dictates the order in which amino acids link up to form the polypeptide chain.

The anticodons at the ends of tRNA molecules complement the codons in the mRNA.

The genetic code

In 1966, the complete genetic code (Figure 2.8) was determined; this was a triumph of modern biology. The genetic code shows the relationship between the codons in mRNA and the amino acids that are translated from the mRNA code. From this, it is possible to work out the relationship between the bases in the original DNA and the amino acids that result.

Most of the amino acids are coded for by more than one codon. Three of the codons do not actually code for an amino acid. Instead they stop the polypeptide chain at that point, acting as termination signals. These **stop codons** play an essential role in the cell, allowing polypeptides of precisely the right length to be produced.

The genetic code is also known as the 'universal genetic code' because all known organisms use it as a code for DNA, mRNA, tRNA and amino acids. However, all rules have their exceptions, and such is the case with the genetic code. Small variations exist in the DNA code in mitochondria and certain bacteria. Nonetheless, it should be emphasised that these variations represent only a small fraction of known cases, and that the genetic code applies broadly to all forms of life. DNA is said to be a universal indicator of life.

MAKING PROTEINS – TRANSCRIPTION AND TRANSLATION

This is an animation of how the genetic code is transcribed and translated into proteins.

The universal genetic code is the sequence of nucleotides in DNA or RNA that determines the specific amino acid sequence in the synthesis of proteins in nearly all organisms.

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: Stop; 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 2.8
The genetic code. The mRNA codons correspond to the 20 amino acids made by translation on the ribosomes. Three codons act as stop codons and under certain conditions the codon AUG initiates protein synthesis.

ACTIVITY 2.1

PROTEIN SYNTHESIS

The nucleus of eukaryotic cells is packed with DNA, the molecule that is the template for all the proteins produced by the cell. Ribosomes, the site of synthesis of proteins, are found in the cytoplasm outside the boundary of the nucleus. DNA is unable to leave the nucleus, so in order to produce a protein, a message must be sent from the nuclear DNA to the ribosome.

To do this, two processes take place:

1. transcription of the message from the DNA into an mRNA molecule
2. translation of the mRNA into a specific amino acid sequence at the ribosome.

The molecule of mRNA is transcribed from the template DNA strand using the complementary sequences. The only exception to this is that the thymine present in DNA is replaced with uracil in RNA. The complementary sequences in RNA are A-U and G-C.

Translation of the mRNA occurs at ribosomes where the sequence of nitrogen bases in the mRNA is read in groups of three called a codon. The tRNA molecules contain an anticodon, which is complementary to the codon of the mRNA and each tRNA binds a specific amino acid (Figure 2.9). The tRNA molecules bring these amino acids to the ribosome where they are bonded together, forming a long chain of amino acids in a specific sequence according to the sequence of the mRNA being translated.

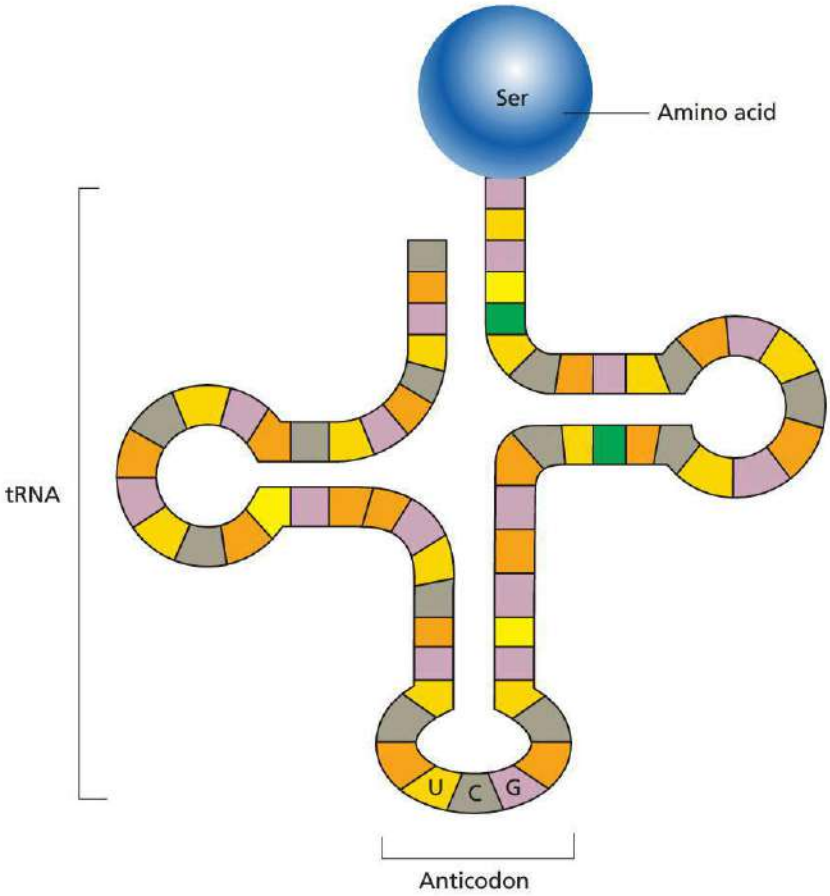


Figure 2.9 ▲
The amino acid serine being carried by a tRNA molecule

Aim

To simulate the production of a protein from a sequence of DNA

You will need

- A3 paper
- coloured pencils

What to do

The sequence of nucleotides below belongs to a protein, the enzyme lysozyme, which will be used in this activity.

ATGACCCATGCGTTAGGC

Refer to Figure 2.8 on page 43 to complete this activity.

1. Divide a piece of A3 paper into six sections.
2. In the first section of the A3 paper, draw a nucleus containing the DNA strand using the sequence above as the template for the complementary strand.
3. In the second section of the A3 paper, show the process of transcription of the template DNA into mRNA.

4 In the third section of the A3 paper, show the movement of mRNA out of the nucleus.
5 In the fourth section of the A3 paper, show the process of translation of the message.
6 In the fifth section of the A3 paper, describe in words, the process of protein synthesis that you have just completed by diagrams.

What did you discover?

1 Describe in your own words the processes of transcription and translation. Include an explanation of where in the cell these processes take place and which other molecules are involved in each process. Explain why the cell needs each process.
2 Describe how the hydrogen bonds are rejoined between complementary DNA nucleotides during transcription.
3 State the sequence of nucleotides in the transcribed mRNA sequence of lysozyme.
4 Not all of the transcribed DNA contains codes for a protein during the production of mRNA. These non-coding sections get broken down to nucleotides for re-use in the nucleus. Name these sections.
5 State the anticodon sequence for the lysozyme protein. Explain the importance of anticodons in these processes.
6 State the final amino acid sequence for the sequence of DNA you are working with.
7 Explain the role of uracil in the process of transcription.

QUESTION SET 2.2

Remembering

1 a Describe the structure of a eukaryotic ribosome.
  b Compare the ribosomes of prokaryotes to those in eukaryotes.
2 Discuss the advantages of polyribosomes to a cell.

Understanding

3 Describe the relationship between rRNA, the nucleolus and ribosomes.
4 'Each mRNA codon codes for a specific amino acid.'
  a Explain what this statement means.
  b Identify any exceptions to this statement.
  c Explain how a specific amino acid can be coded for by more than one codon. Give an example to illustrate your answer.
5 Describe the relationship between a protein, polypeptide and amino acid.

Applying

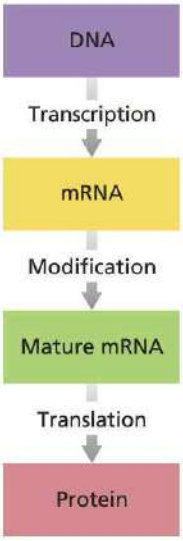
6 There are three nucleotide bases (a codon) of DNA code for each separate amino acid.
  a Estimate how many combinations of codons are possible.
  b Explain why it is not possible for two nucleotide bases to code for one amino acid.
7 Refer to Figure 2.8, which shows the genetic code, to answer the following questions.
  a Name the start codon.
  b Name the termination codons.
  c Identify the sequence of codons on mRNA when DNA contains the following sequence of bases: AGC TAT CGA GTC AAA.

The phenotypic expression of genes

During the progressive development of an individual – such as from a fertilised egg, asexual spore or juvenile to an adult – many different changes to cells and tissues occur. Cells can become specialised to carry out particular functions, and biochemical activities can change at various times. If all cells have the same DNA content, and the DNA of a cell specifies its activities and characteristics, why aren't all cells of an individual the same?

Cells do not express all the genes of their genome at the same time. Rather, cells must have some genes (for specific proteins) active and other genes (for unnecessary proteins) inactive. This ensures the cell does not waste energy and resources producing unwanted proteins, as well as ensuring the cell does not produce proteins whose functions may interfere with the cell properly performing its specialised role.

Even when expressed, there are controls over how fast specific genes are transcribed and translated. The expression of genes seems to depend on the type of cell, its stage of development and conditions within and around the cell.



A cell does not express all the genes of its genome at the same time and same rate.

As with other studies in molecular biology, insights into the action of gene expression and regulation have been advanced by the generation and analysis of mutants. Yeast, fruit flies and roundworms have been instructive model organisms in this regard and experiments with other organisms indicate that there are common mechanisms for gene expression among all eukaryotes.

Prior to the publication of the human genome sequence in 2003, estimates for the number of genes it would contain centred on around 100 000. This was a reflection of the number of the different types of proteins produced in the human body. When the sequence was finally published the estimate fell to around 25 000 genes. How could such a comparatively small genome code for so many more proteins? Considering the large number of functional proteins synthesised in human cells, it now seems likely that our DNA–RNA–protein definition has to be questioned.

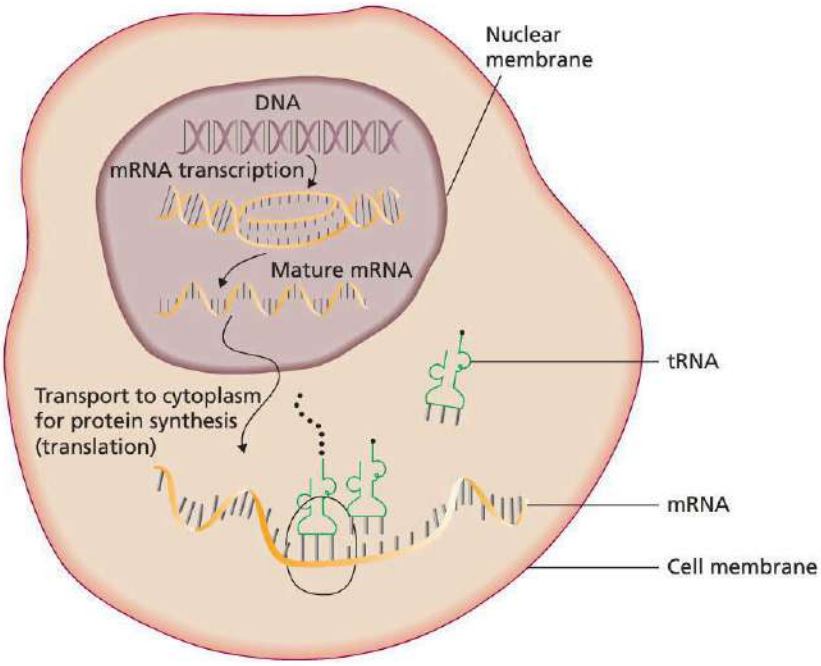


Figure 2.10 ▲ The classic view of the central dogma of molecular biology

Central dogma of molecular biology

Originally stated by Francis Crick in 1958, the term 'central dogma of molecular biology' is used to describe the one-way sequence of information where DNA acts as a template for its own replication, the transcription to RNA, the modification to mature mRNA and finally as the template for translation into a protein. There are many examples to show this one-way sequence of information is no longer the only possibility. For example, a group of viruses called retroviruses use the enzyme reverse transcriptase to produce DNA from its RNA genome. Human immunodeficiency virus (HIV) is an example of a retrovirus.

Scientific literacy: The blueprint of life?

The anniversary of the discovery of DNA's molecular structure rightly celebrates how Francis Crick, James Watson and their collaborators launched the 'genomic age' by revealing how hereditary information is encoded in the double helix. Yet the conventional narrative — in which their 1953 *Nature* paper led ultimately to the Human Genome Project and the dawn of personalised medicine — is as misleading as the popular narrative of gene function itself, in which the DNA sequence is translated into proteins and ultimately into an organism's observable characteristics, or phenotype. Today, the very definition of 'gene' is hotly debated. We do not know what most of our DNA does, or how, or to what extent it governs traits. In other words, we do not fully understand how evolution works at the molecular level.

Describing genes as the 'blueprint' or 'book' of life is misleading and distorted. The usual tidy tale of how 'DNA makes RNA makes protein' is sanitised to the point of distortion.

While specialists debate what the latest findings mean, the rhetoric of popular discussions of DNA, genomics and evolution remains largely unchanged, and the public continues to be fed assurances that DNA is as much a blueprint as ever.

Difficult questions are raised by a number of discoveries. These discoveries include the fact that only a tiny fraction of the genome produces protein; that chemical modification of DNA can affect expression of genes; and that genes work together in networks.

When the structure of DNA was first realised, it seemed to supply the final part of a beautiful puzzle. The simplicity of that picture has proved too alluring. We should do DNA a favour and lift some of the awesome responsibility for life's complexity from its shoulders.

Adapted from Ball, P. (2013). 'DNA: Celebrate the unknowns', *Nature, 496* (April), pp. 419–20 and Salleh, A. (2013) 'Genes – the "book" of life?', *ABC Science* online, 25 April

Questions

1. Outline the main point the authors are trying to make.
2. When this commentary was published, many experts responded and defended the 'central dogma of molecular biology'. Relate what the authors say about the relevance of this dogma.
3. The authors imply that there are dangers in telling a simple story. Suggest some advantages and disadvantages of embracing complexity in public communication about genetics.
4. The authors write that 'Describing genes as the "blueprint" or "book" of life is misleading and distorted.' Justify this statement.

The genome consists of coding and non-coding DNA

Eukaryotic genomes are comparatively very large. The human genome for example, consists of 3.1 billion nucleotide pairs. However, only a small fraction of the genome actually codes for protein. Coding DNA sequences transcribed into mRNA and then translated into proteins only make up less than 2% of the entire human genome. This coding DNA is the most well-known part of the genome formed by exons, called the **exome**. The vast majority is **non-coding DNA**.

Table 2.2 Comparison of genome sizes

Organism	Size of genome (base pairs)
Human	3 100 000
Fruitfly (*Drosophila*)	168 700
Nematode worm	100 300
Yeast	12 200

Much of the non-coding DNA is made up of repetitive sequences. A considerable proportion of these repetitive sequences is transcribed into RNA, although the function of these repetitive sequences is something of a mystery. Some scientists argue the repetitive DNA and its transcribed RNA have no function, that it is 'junk'. In this case, it's a bit like

the clutter that builds up in your bedroom over time if you do not tidy up. It takes less effort to leave it there than to clear it away because, among all that junk, there may be some useful things you want to access at some point in the future.

It is estimated that at least 80% of the human genome is transcribed at one time or another. As only about 1–2% of the human genome codes for protein, however, the vast majority of transcribed RNA is not mRNA destined for protein translation by ribosomes. The purpose of the transcribed but untranslated RNA is an ongoing area of research.

At least some sections of the non-coding DNA have specific functions in switching on or switching off gene expression. **Gene expression** refers to a gene being transcribed into mRNA and translated into a protein. **Gene regulation** refers to the processes within a cell that enable a gene to be expressed in specific cells and at specific times.

The National Human Genome Research Institute is coordinating and funding the Encyclopedia of DNA Elements, or ENCODE, project. The main goal of the ENCODE project is to determine which parts of the DNA affect protein coding gene expression and how they influence gene transcription. The initial analysis revealed that at least 80% of the genome serves some purpose. Genes for non-coding RNA (e.g. rRNA, tRNA), regulatory DNA and introns are just some of the types of non-coding DNA that control how genetic instructions are interpreted.

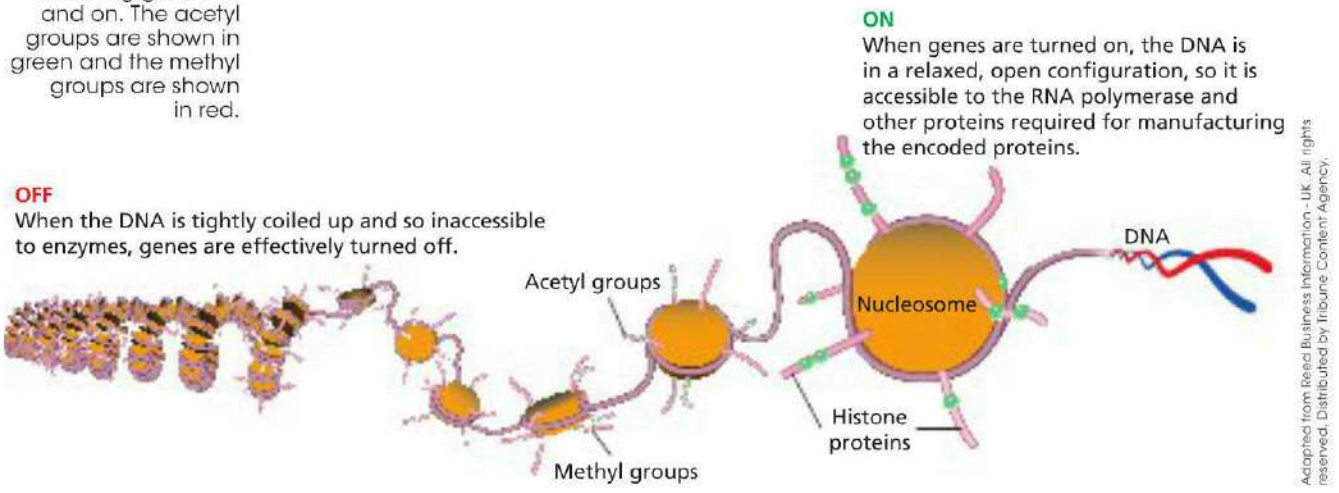
Turning genes on and off

Chapter 1 described how DNA is packaged with proteins to form the DNA-protein complex referred to as chromatin (see Figure 1.15, page 20). The most fundamental unit of chromatin is the nucleosome, in which the double-helical DNA is wound up around histone proteins. Nucleosomes, formed from eight histone proteins that associate with each other in a ball shape, form the scaffold for the packaging of DNA molecules. Each of the histones has a tail that extends out from the nucleosome. Nucleosome formation is assisted by interactions between the negatively charged DNA and the positively charged histones (see Figure 2.11).

If DNA is packaged into chromatin, the genes within the DNA are not available for expression. This is because RNA polymerase is unable to access the DNA in the chromatin to begin transcription. In essence, such genes are 'switched off' and the corresponding proteins are not made.

In eukaryotic cells, it seems the default state of gene expression is 'off'. Some genes that encode proteins that serve to maintain basic cellular processes are continually expressed at some level to maintain cell function. We tend to call these **housekeeping genes**. Genes that express proteins for glycolysis or for biosynthesis of macromolecules, for example, are housekeeping genes. It is only when specific DNA sequences are in an open configuration on the chromatin, and accessible for specific proteins to bind to, that gene expression is 'on'.

Figure 2.11 ▼ Switching genes off and on. The acetyl groups are shown in green and the methyl groups are shown in red.



During protein synthesis, the expression of genes depends upon factors that control transcription and translation.

QUESTION SET 2.3

Remembering

1 List reasons why cells do not express all genes in their genome at the same time.
2 Distinguish between coding and non-coding DNA.
3 List three examples of non-coding DNA.
4 Distinguish between gene expression and gene regulation.
5 Outline the aims of the ENCODE project.

Understanding

6 All cells of a multicellular organism have the same DNA but they don't always have exactly the same structure and function. Explain why.
7 Outline how the packaging of DNA affects transcription.
8 The default state of expression of many genes in eukaryotic cells is said to be 'off'. Explain what this means.
9 Discuss why it was a surprise to scientists to realise humans had fewer genes than the number of different types of proteins.

Chemical modification influences the phenotypic expression of genes

Chromatin can be 'remodelled' to allow segments of DNA containing genes to become exposed. Hence, chemical modification seems to exert control over the phenotypic expression of genes.

One of the first observations of chemical modification was in the 1970s when scientists noticed a particular pattern in DNA. Some cytosine bases in DNA had a methyl group attached. When this modified base appears, the DNA is said to be methylated. Wherever genes were active, DNA **methylation** was not found. Convincingly, when the control region of a gene was methylated, the gene could not be turned on. The gene is said to be silenced. It is now known that methylation switches off gene expression because the methyl group projects out from the DNA and blocks the RNA polymerase from binding or transcribing the gene. In addition, methyl groups are positively charged and can interact with the negatively charged phosphate groups of DNA, allowing the DNA to condense and hence restricting access by RNA polymerase.

One classic example of DNA methylation is X-inactivation. DNA methylation is used to inactivate one of the two X chromosomes in female mammals. In tortoiseshell cats (Figure 2.12) one X chromosome carries the orange allele of the fur colour gene, and the other X chromosome carries the black allele of the fur colour gene. Depending on which of the X chromosomes is inactivated early in embryonic development, patches of fur descending from these original cells only express the colour from the X chromosome that has not been inactivated.

Alamy/Ken Barber

▲ **Figure 2.12**
The fur of a tortoiseshell cat is a result of X-inactivation.

Imprinting is a version of gene silencing. This process involves silencing one of the two alleles of a gene inherited from each parent. In some cases the paternal copy is always silenced and in other cases it is the maternal copy that is silenced. The silencing lasts for the life of the cell and is passed on to new cells. Only some genes are affected in this way.

Beckwith–Wiedemann syndrome is an overgrowth disorder. Infants are larger than normal with a predisposition to tumour development. Normally, the father's copy of the gene is expressed and the mother's gene is silenced or 'imprinted'. In some cases of children with the disorder, there is no evidence of DNA sequence changes. It is possible the mother's gene is not properly silenced and too much gene product leads to the symptoms of the disorder.

X-INACTIVATION

Investigate how the X chromosome is inactivated.

However, not all modifications silence genes. In the 1960s several research groups discovered that the histone proteins could have methyl and acetyl groups attached. Scientists observed that when the acetyl groups were added to histones, the genes in the surrounding regions were likely to be active. If the histone tails do not carry many of the chemical tags, the DNA is wound tightly around the histones and the local genes are not expressed. On the other hand, if the histone tails are rich in these chemical tags, the DNA is essentially loosened from the nucleosome and the associated genes become exposed. RNA polymerase can transcribe mRNA from the exposed genes and gene expression is activated.

After the importance of DNA methylation had been recognised, there have been discoveries of many other chemical modifications affecting gene expression. We now know there are more than 150 chemical modifications that can be made to histones or directly to the chromosomal DNA. These include addition of methyl groups, phosphate groups and small proteins. The signals these convey for gene expression are an active area of research in molecular biology.

What is also intriguing is that these chemical signatures can be altered by environmental factors and they can sometimes be passed on through germ-line cells from parent to offspring. These modifications therefore represent an alternative form of inheritance apart from the specific alleles offspring may receive from their parents. The study of the acquisition and inheritance of these modifications without involving changes in DNA sequences underpins the field of **epigenetics**. Epigenetic mechanisms include DNA methylation, histone modification, imprinting, non-coding RNA and post-translational modifications.

The **epigenome** consists of chemical compounds that modify the genome. The chemicals are not part of the genome but, as seen in this section, affect expression of the genes.

GENE CONTROL

Turn the control knob to alter the epigenetic tags and notice what happens to the mRNA and protein levels.

WOW

Epigenetics and cancer

Epigenetic changes have been suspected as the cause of some cancers. Anti-cancer drugs that target epigenetic processes have been used. Some chronic leukaemias have been successfully treated using a de-methylating drug.

The products of other genes influence the phenotypic expression of genes

The previous section described how the transcription of genes can be influenced by the chemical modification of the genome. Transcription can also be influenced by the genome itself.

Regulatory proteins are products of genes that regulate gene expression. Specific regulatory proteins that bind to the DNA are called **transcription factors (TFs)**. Most of these are activators but some are repressors. **Activators** are regulatory proteins that bind to DNA to activate gene expression. In essence, they switch a gene on. Activators normally recognise a 6–10 base pair non-coding segment in the promoter region of eukaryotic genes. They enable the DNA to unwind from histone proteins and expose the gene for transcription. Activators also assist the binding of RNA polymerase to promoters to begin transcribing the gene. In effect, these activators enhance the expression of specific genes. They may also bind to **enhancer regions** and even to introns of a gene. Some activating TFs can turn on multiple genes at the same time.

Repressors are regulatory proteins that bind to DNA in order to switch off gene expression. For example, a **repressor protein** may bind to the promoter of a specific gene to block the RNA polymerase from binding and prevent transcription.

Regulatory proteins normally consist of multiple parts, or **domains**, with separate functions, one of which is usually capable of binding to DNA: the DNA-binding domain. Regulatory proteins bind to specific sequences of DNA in the neighbourhood of the genes they activate or repress. Sequence specificity ensures that the regulatory proteins precisely target the genes whose expression they are meant to control. Regulatory proteins also contain a functional domain responsible for carrying out the activation or repression of gene expression. In addition, regulatory proteins contain domains that can interact with other proteins. These other proteins include signalling molecules, such as hormones, that relay information about the physiological or developmental state of the organism.

Hormones and other signalling molecules will be explored in more detail in Chapters 9 and 10.

A regulatory protein that serves as an activator for one gene may act as a repressor for another gene. Ultimately, the timing and patterns of gene expression is determined by the action of activators and repressors in concert with one another and through their interactions with other proteins.

The products of other genes can influence the phenotypic expression of genes.

Case study

Prostate cancer and gene activation

An Australian study at Sydney's Garvan Institute of Medical Research has shown that roughly 2% of a person's genome is epigenetically activated in prostate cancer. Regions activated contain many prostate cancer-specific genes, including PSA (prostate specific antigen) and PCA3, the most common prostate cancer markers. A previous study from these researchers showed that similarly large regions of the prostate cancer genome are also epigenetically silenced, demonstrating a structured rearrangement of the cancer epigenome.

The study showed that the epigenetic process known as 'methylation' can activate genes, often by changing the gene start site, overturning the prevailing dogma that DNA methylation can only silence genes.

The team at the Garvan Institute used gene expression profiling data and genome-wide sequencing technology from prostate tumour cells to determine which parts of the genome were epigenetically activated in prostate cancer. They then examined the mechanisms behind activation.

Gene start sites have dense clusters of cytosine-guanine DNA nucleotide base pairs very close to them. This is unlike other parts of the genome. These cytosine-guanine (CG) clusters, known as 'CpG islands', are where methylation occurs. The team showed that if you methylate CpG islands that are very close to transcription start sites, but not exactly on top of them, then it's possible to turn genes on. Neighbouring genes were also being coordinately activated in cancer.

The findings have extensive implications for cancer diagnosis and treatment. Epigenetic-based gene therapies that are aimed at promoting gene activation rather than suppressing cancer gene expression may be a promising area of research.

Source: Based on materials provided by the Garvan Institute of Medical Research (2012). (Journal ref: Bert, S. A., Robinson, M. D., Strbenac, D., Statham, A. L., Song, J. Z., Hulf, T., Sutherland, R. L., Coolen, M. W, Stirzaker, C. & Clark, S. J. (2012) Regional Activation of the Cancer Genome by Long-Range Epigenetic Remodeling. *Cancer Cell*, doi: http://dx.doi.org/10.1016/j.ccr.2012.11.006.)

Questions

1. Suggest why it is useful to identify common prostate cancer markers.
2. List three new findings about prostate cancer generated from this research.
3. Apply your knowledge of gene expression to predict what would happen in a non-cancer cell if the start sequence of a gene was methylated.
4. Evaluate the significance of the findings of the research for general cancer research.

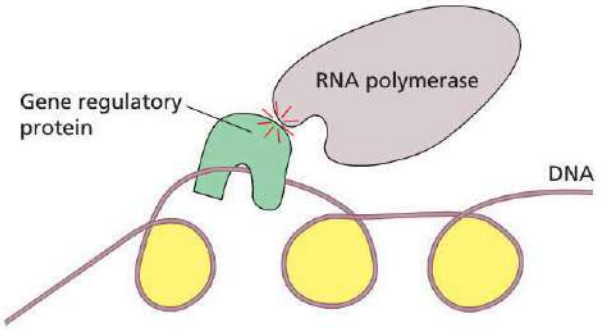


Figure 2.13 ▲ A gene regulatory protein blocks RNA polymerase from attaching to and copying a gene.

Regulation of translation

A further level of regulation can be achieved after a gene is transcribed and processed into mRNA. In this type of regulation, the mRNA is prevented from being translated into protein. This is another example of repression in which gene expression is switched off. It can be achieved in a couple of different ways.

First, mRNA-binding proteins attach to the mRNA and block it from being translated. These proteins bind to specific RNA sequences. The sequences normally occur in the segments of the mRNA before the first AUG (the Start codon). Upon binding, the protein blocks the ribosome from assembling around the mRNA and translating it into protein. The specific sequences these repressor proteins bind to are found in the mRNA of different genes, so these repressor proteins may shut down the expression of a number of genes simultaneously.

Second, in plants and animals, repression of translation may be achieved by another class of RNA called **microRNAs (miRNAs)**. These miRNAs are specifically transcribed and processed into short segments of around 20 nucleotides in length. The miRNAs are complementary to sequences within mRNA. When miRNAs base-pair with mRNA sequences, double-stranded RNA molecules form that prevent translation. In many cases, particularly in plants, double-stranded RNA is a trigger for the cell to digest and destroy the RNA molecules. In these cases, the mRNA is prevented from being translated.

Gene expression in prokaryotes

Expression of genes differs between prokaryotes and eukaryotes. Unlike highly evolved eukaryotes where only a fraction of a genome's products are required in a particular cell at a particular time, most genes are turned on in prokaryotes. They need to be repressed when transcription products are not needed.

Considerable evidence has supported the idea of prokaryotic genes being switched on and off. During the late 1950s, François Jacob and Jacques Monod carried out a series of experiments on the genetic control of enzyme synthesis in the bacterium *Escherichia coli*. This led them to put forward a theory explaining how the genes responsible for the production of enzymes that break down lactose are regulated.

The bacterium *E. coli* inhabits the mammalian gut, living on sugars and other nutrients. During infancy, mammals feed on milk from their mothers. This milk contains the sugar, lactose. But the preferred source of food for *E. coli* is glucose since it can be directly used in cellular respiration, whereas lactose must be converted first. Thus, if both glucose and lactose are present, the bacterium turns off lactose metabolism in favour of glucose metabolism.

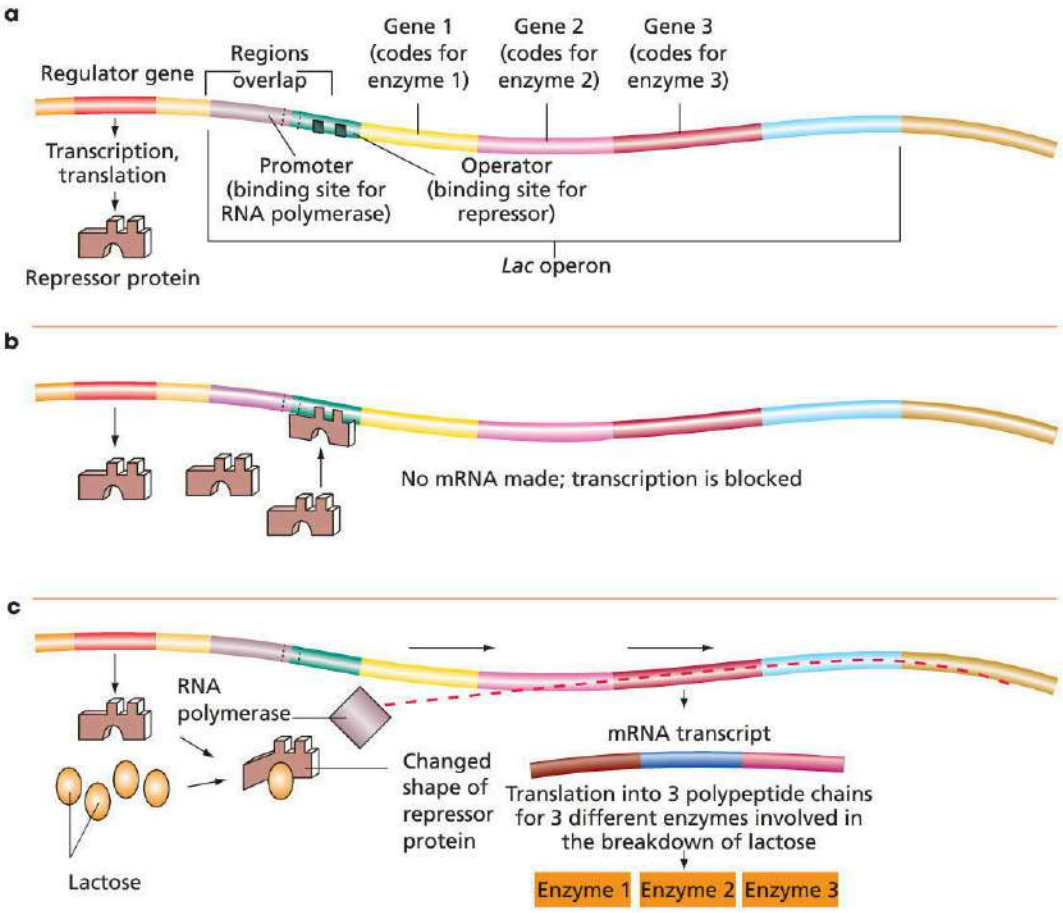
If only lactose is present, *E. coli* produces enzymes to break it down. But if lactose is absent, these enzymes are not produced. If lactose is added to a medium, and if glucose is not present, then, and only then, will the bacteria start synthesising the enzymes.

Negative control by the *lac* operon

To explain how this occurs we need to consider non-coding DNA regions and genes other than the genes that code for enzymes that control the breakdown of lactose. *E. coli* uses three enzymes (β-galactosidase, β-galactoside permease and β-galactoside transacetylase) in this process. The coding regions for each of these three enzymes appear next to each other on the *E. coli* genome and are translated into a single mRNA strand. This set of genes is called the *lac* operon.

Our story starts with the **regulator gene**, a sequence of the DNA that codes for a repressor protein (Figure 2.14a). If no lactose is available, the repressor protein binds to two sections of non-coding DNA, called **operators** (see Figure 2.14b). Operators are binding sites that are situated around a promoter. Promoters are non-coding base sequences that signal the start of a gene (Figure 2.14a). RNA polymerase binds to the promoter region, thus activating the gene and causing it to transcribe the mRNA that, for example, in *E. coli* is then translated into β-galactosidase and two associated enzymes (Figure 2.14c).

When lactose is absent, the repressor protein binds to the set of operators causing the part of the DNA with the promoter to loop outwards, as shown in Figure 2.14b. This prevents RNA polymerase attaching to the promoter, hence blocking the transcription of the structural genes.



◀ **Figure 2.14**
a) The *lac* operon consists of an operator, a promoter and three genes that code for enzymes involved in lactose breakdown. b) If lactose is absent, or low, the repressor protein binds to the operator, covering part of the promoter. RNA polymerase cannot bind to the promoter and transcription of the three genes is blocked. c) If lactose is present, it binds to the repressor, altering its shape so that it cannot bind to the operator. The three genes are able to be transcribed to form the enzymes.

QUESTION SET 2.4

Remembering

1 Describe the effect of methylation of cytosine bases in DNA on gene expression. Recount the observations of scientists that led to the discovery of the effect.
2 Provide an example of 'imprinting'.
3 List three epigenetic mechanisms.
4 Recall the role of TFs.

Understanding

5 Explain why the fur of a tortoiseshell cat provides evidence for the inactivation of one of the sex chromosomes (X-inactivation).
6 Explain how a regulator gene prevents transcription of a gene coding for a structural protein.
7 Distinguish between gene activation and gene silencing.

Applying

8 a Draw a flow chart that shows the events leading to the prevention of the production of enzymes that break down lactose in *E. coli*.
b Draw an equivalent flow chart to show events if lactose is present.

The environment influences the phenotypic expression of genes

Even though identical twins have the same genes, they often show variations in their characteristics. They may have slightly different weights and heights, for instance. How can we explain this if there is a direct relationship between genes and their expression?

It seems that environmental conditions affect gene expression. A well-known case of fur colour in the Himalayan rabbit demonstrates this. The Himalayan rabbit has a white body with black ears, nose, feet and tail (Figure 2.15). At first glance, it seems that this pattern is simply under genetic control but a simple experiment shows that this is not the case. If a cold pad is fixed to the rabbit's back, left in position for a few weeks and kept cold, black hair starts to develop beneath the pad.

Shutterstock.com/Lynn Currie

Figure 2.15 ▶ The Himalayan rabbit normally has black hair only on its long ears, nose, tail and lower leg limbs. In one experiment, a patch of a rabbit's white fur was removed, and then an icepack was secured over the hairless patch. Black hairs grew back where the colder temperature had been maintained.

What seems to be happening is that the heat prevents the development of the black pigment. Black fur only grows in those parts of the body that are cool enough, that is, the extremities. The same thing happens in seal-point Siamese cats. Owners of such cats sometimes find that, in winter, the black areas enlarge, only to get smaller in warmer weather.

The phenotypic expression of genes can be influenced by the environment.

There are many other cases of the environment influencing an organism's development. For example, in plants chlorophyll will only develop if light is available, and flowers will only appear if the day length is of certain duration and the temperature suitable, and so on. It is easy to underestimate the importance of the environment on the development of organisms and to assume that everything is under genetic control. In reality, development is the result of a subtle and complex interaction between heredity and the environment.

WOW

Survival advantage of dark extremities

The dark coloured fur in the extremities of Himalayan rabbits provides a survival advantage. The extremities are usually the coldest part of the rabbit due to less blood flow. A gene that is activated by cold temperatures below 35°C actively produces pigment, making these parts of the animal black. The darker the colour, the more light energy and consequently heat that can be absorbed. This would help keep their extremities warmer, warding off frostbite that would potentially kill the rabbit in the very harsh, cold environment of the Himalayas.

Influence of the environment on the epigenome

Consider the food we eat. How valid is the statement, 'You are what you eat'? We are often warned about the dangers and benefits of certain foods. Can factors in our lifestyle influence the way our genes are expressed? The answer to these questions lies with our understanding of how our genes are regulated. There is a great deal of evidence that environmental contaminants such as arsenic, heavy metals and some organic pollutants affect gene expression.

In some instances the environment has been found to influence DNA methylation. Studies have shown that stress and diet influence epigenetic changes. We know the food we eat is broken down and used for our body functioning. But there is now evidence that some food can also affect the way our genes behave.

The agouti gene is found in a range of animals including humans and mice. In an agouti type mouse, coat colour of offspring normally ranges from yellow to dark brown. If pregnant mice are given a diet high in methyl groups, they give birth to more brown mice of normal weight and size. The agouti gene was methylated. When methylation is low, the agouti gene has a high expression of its protein product. In this situation, the mainly yellow mice produced are obese, with a higher risk of cancer and diabetes. In this case, not only is the saying 'You are what you eat' true, but also 'You are what your parents eat'.

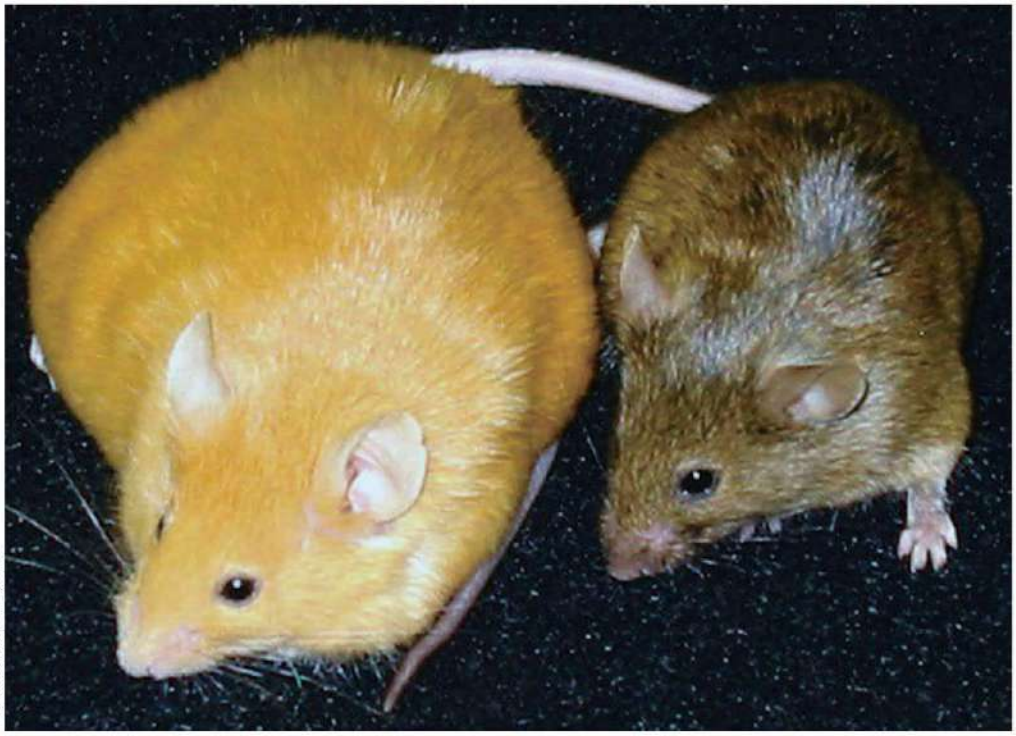


Photo courtesy Randy Jirtle

◀ Figure 2.16
These inbred agouti mice are genetically identical. The mother of the mouse on the left ate a normal mouse diet. The mother of the mouse on the right ate a diet supplemented with methyl donors (choline, folic acid, betaine and vitamin B12). Instead of giving birth to a pup similar to its parents – yellow, fat and prone to obesity, diabetes and cancer – the pup was a normal size and brown in colour. Methylation altered the epigenome of the mouse.

Influence of the environment on bacteria

There is ample evidence that a bacteria's environment affects gene expression. The amount of an amino acid tryptophan in the environment, for example, is known to affect gene expression. *E. coli* can synthesise its own tryptophan. Five genes are required to code for enzymes needed for its synthesis. When tryptophan levels in the environment are low, transcription of the genes occurs and tryptophan is synthesised. When tryptophan in the environment is plentiful, tryptophan molecules bind to a regulatory protein repressor and activate it. This activated repressor blocks transcription of the enzymes and thus tryptophan is not synthesised by the cell. This mechanism reduces unnecessary energy use by the cell.

ACTIVITY 2.2

LICK YOUR RATS

Aim

To run a simulation to test the effect of nurturing on epigenetics of offspring

What to do

1. Go to the weblink and test a low, moderate and high amount of licking.
2. Summarise your findings in three sentences. Be sure to discuss the effect and implications for the pup during its lifetime.

LICK YOUR RATS

Change the amount of time a mother rat spends licking and grooming her pup and find out the effect on the pups' epigenome.

QUESTION SET 2.5

Remembering

1. Recount the effect of temperature on the colour of Himalayan rabbits.
2. List two environmental conditions that affect plant characteristics.
3. List three environmental factors that affect gene expression.

Understanding

4. Describe evidence to show that gene expression is affected by the surroundings.
5. Some genes are common to many different species. They remain active regardless of the environment. Predict the type of function of these genes.
6. Construct a diagram showing the feedback effect of levels of tryptophan in the environment on *E. coli* tryptophan production.

CHAPTER SUMMARY

- Proteins, like enzymes, control many processes including the rate of cellular metabolism and are essential to cell structure and function.
- DNA determines the order of amino acids in a protein hence determining the protein's structure and function.
- Three DNA nucleotides are required to code for one amino acid.
- In the transcription of DNA, single-stranded mRNA molecules are generated in the nucleus.
- mRNA transcription starts when RNA polymerase joins to a promoter region on the template DNA strand of a gene.
- Complementary RNA nucleotides join until a stop signal is reached to form the single-stranded pre-mRNA.
- Before the mRNA leaves the nucleus it is modified by the addition of a 5′ methylated cap and adenine nucleotides forming a poly-A tail at the 3′ end of the mRNA molecule.
- Splicing follows the modification where introns are removed and exons remain.
- During translation, amino acids are assembled in the order prescribed by mRNA at the ribosomes.
- Polyribosomes speed up the rate of protein synthesis.

- tRNAs carry specific amino acids to ribosomes.
- Each three mRNA nucleotides called a codon join with the complementary nucleotide bases (anticodon) of a tRNA molecule.
- Amino acids are joined together with peptide bonds forming a polypeptide.
- The universal genetic code is the sequence of nucleotides in DNA or RNA that determines the specific amino acid sequence in the synthesis of proteins in nearly all organisms.
- All cells do not express all the genes of their genome at the same time or same rate.
- The central dogma of molecular biology describes the unidirectional sequence where DNA is transcribed to mRNA and then translated into a protein.
- Coding DNA is translated into proteins.
- Non-coding DNA does not encode proteins but much of it serves some purpose.
- In eukaryotic cells, the default state of gene expression is 'off' and transcription cannot occur.
- The phenotypic expression of genes depends on factors controlling protein synthesis.
- Many chemical modifications affect gene expression, such as DNA methylation, which usually silences gene expression, and acetylation, which is usually associated with active gene expression.
- Epigenetics is the study of changes in gene expression not involving changes in DNA sequences.
- Regulatory proteins affect gene expression such as TFs that bind to DNA.
- In prokaryotic cells, the default state of gene expression is 'on' and genes need to be repressed if the transcription product is not needed.
- Environmental conditions also affect gene expression.
- The products of other genes can affect gene expression.

CHAPTER GLOSSARY

activator a regulatory protein that binds to an enzyme or DNA, causing a change of conformation so that enzymes become active, or activating gene expression

amino acid binding site the site of attachment of an amino acid to a tRNA molecule

anticodon a sequence of three nucleotide bases on a tRNA molecule that pairs with the complementary bases of an mRNA strand during translation at the ribosome

coding region the small part of a DNA strand used as a template for synthesis of an mRNA strand; also known as a gene

codon a series of three adjacent nucleotide bases in mRNA; each codon specifies a particular amino acid to be added to a polypeptide; a stop codon indicates the termination of the polypeptide chain

domain the functional region or portion of a protein

enhancer region regions found in eukaryotic DNA that act as binding sites for some activator proteins

epigenetics the study of chemical modifications to gene function that are not due to DNA sequence changes; DNA methylation is an example

epigenome chemical compounds that modify the genome

exome all the genome's exons

exon the region of DNA or RNA transcript that encodes a protein sequence

gene expression the process of information from a gene being transcribed into mRNA and translated into a protein

gene regulation the processes within a cell that control gene expression; it controls what genes are turned on and off, when and where

housekeeping gene a gene that encodes proteins that are required to maintain basic cellular processes

imprinting an epigenetic process where one allele of a gene is methylated and hence 'silenced'

intron a section of DNA or pre-mRNA that does not encode a protein sequence; an intron is removed ('spliced') from pre-mRNA to form a mature mRNA molecule

messenger RNA (mRNA) a ribonucleic acid formed in the nucleus during gene transcription, its sequence being complementary to DNA exons; it travels to the cytoplasm, where its information is translated by the ribosomes to add amino acids together to form proteins

methylated cap a modified guanine nucleotide that has a methyl group and a phosphate group bonded to it, which is added to pre-mRNA at the 5′ end; also known as the 5′ cap

methylation the attachment of a methyl group to nucleotides or histone proteins

microRNA (miRNA) a small non-coding segment of RNA that plays a role in regulating gene expression at the post-transcription level

non-coding DNA all of the DNA sequences within a genome that are not found within RNA-coding exons; examples include introns, promoters and enhancers of genes

non-template strand a strand that is complementary to the template strand; it does not guide the synthesis of complementary polynucleotides

operator a region of DNA situated around a promoter that interacts with a specific repressor; when bound with a repressor protein, it prevents transcription of the structural gene

peptide bond a bond that forms between two amino acid monomers with the elimination of a water molecule

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

poly-A tail an untranslated feature of mRNA that enhances its stability; consists of about 100–200 adenine nucleotides added to the 3′ end of pre-mRNA

polypeptide the polymer of many amino acids linked by peptide bonds; forms a protein or part of a protein

polyribosome many ribosomes forming into chains along an mRNA strand

pre-mRNA unmodified, 'immature' RNA containing introns and without the 5′ methyl cap and 3′ poly-A tail

promoter a short stretch of DNA usually at the start of the gene to which RNA polymerase can bind and start transcription; specifies the timing and location of gene transcription

regulator gene a gene that codes for the production of a repressor protein that inhibits the action of an operator gene, thereby preventing transcription of a structural gene

regulatory protein a protein that binds DNA to switch on or switch off expression of a gene

repressor protein a protein coded for by the regulator gene that binds to an operator gene, which inhibits transcription of a structural gene

ribosomal RNA (rRNA) a folded molecule of RNA that is formed in the nucleolus of eukaryotic cells, which combines with proteins to form ribosomes

ribosome the site of protein synthesis in all cells; a ribosome consists of two rRNA subunits that lock onto an mRNA molecule; the ribosome moves along mRNA to translate its code and link amino acids, forming a polypeptide

RNA polymerase an enzyme involved with adding RNA nucleotides together

start codon the first codon of a messenger RNA transcript translated by a ribosome

stop codon the codon that stops the synthesis of a polypeptide chain

template strand polynucleotide (DNA or RNA) that serves as a guide for making a complementary polynucleotide

transcription the formation of mRNA by the complementary nucleotide base pairing of the template strand of DNA in the nucleus

transcription factors (TFs) regulatory proteins whose function is to activate or to inhibit transcription of DNA by binding to specific DNA sequences

transfer RNA (tRNA) an RNA molecule, shaped similar to a clover leaf, that picks up amino acids from the cytoplasm and brings them to the ribosome to match up with specific mRNA codons

translation the joining of amino acids in a specific order, resulting in the formation of a polypeptide, when the information in mRNA is read by ribosomes

CHAPTER REVIEW QUESTIONS

Remembering

1. Outline the role of proteins in a cell.
2. Name the format of instructions used by a cell to determine the type of proteins it makes.
3. Describe how the genetic code gets out of the nucleus.
4. Define 'housekeeping genes'. Suggest why this name is used.

Understanding

5. Describe the function of DNA, mRNA, tRNA and rRNA.
6. Construct a flow chart showing the main steps in protein synthesis.
7. Suggest an advantage to a eukaryotic cell of having some of its ribosomes on the endoplasmic reticulum and some free in the cytosol.
8. Determine which of the following statements are true and justify your answer.
   a. Thymine is a nucleotide base found in RNA.
   b. Adenine is complementary to uracil, cytosine is complementary to guanine.
   c. The DNA sequence AGGTCA would be complementary to a strand containing GCCAGT.

d DNA of one species differs from that of other species by its sequence of nucleotide bases.
e An RNA molecule is single-stranded.
f mRNA is produced by translation.
g Anticodons pair with DNA codons.
h Transcription occurs in the nucleus.

9 Explain why each of the following is incorrect and rewrite it as a correct statement.
a Each cell contains only the specific genetic information required for its function.
b Amino acids provide instructions for making proteins in an organism.
c The actions of protein molecules do not affect an organism's metabolism.
d Four nucleotides are needed to code for one amino acid.

10 Describe the physical state of chromatin necessary for gene transcription. Explain why chromatin must be packaged in this way for transcription to occur.

11 Describe two ways in which mRNA translation can be prevented.

Applying

12 Explain the advantages to a cell when:
a mRNA is transcribed from a particular gene in different amounts at different times.
b mRNA travels to the cytosol to be translated away from the nucleus.

13 In the construction of a house, a carpenter refers to the house plans and uses the plans to construct a kitchen. The carpenter uses wood, hammers and nails. In this analogy, match each of the elements to the process of DNA transcription and translation. Provide reasons for your decisions.

14 Explain why the ability to control which genes are being expressed is important in specialised cells.

15 Predict how the findings of the agouti gene experiments (see Figure 2.16, page 55) could benefit people suffering from type 2 diabetes bought on by obesity.

16 In many reptile species, the sex of offspring is determined by the temperature of nests that eggs hatch in. In some species warmer nests are more likely to have females hatching and cooler nests are more likely to produce males. Predict the likely effect of global warming on the sex ratio of hatchlings. Predict some long-term effects on a particular species if average temperatures increase.

Analysing

17 A section of nucleic acid is isolated and examined. Describe features you would look for to determine whether it is DNA or RNA. List other information that would be useful in your determination.

18 A section of DNA transcribed into mRNA is measured. Compare its size with the mRNA formed immediately after transcription. Compare this to the size of the mRNA that leaves the nucleus. Outline reasons for your answer.

19 A particular protein has 100 amino acids.
a Suggest how many codons there would be in the mRNA strand that codes for this.
b Suggest how many nucleotide bases there would be in the DNA strand that coded for the mRNA.
c If the template DNA strand was known to contain 63 adenine nucleotides, predict how many thymine molecules the non-template strand would be expected to have.
d Discuss if it would be possible to predict how many guanine molecules were in the non-template strand.
e Name the organelle in the cell that assembles amino acids into protein.

20 Samples of DNA from monozygotic (identical) twins were taken and analysed. In some sets of twins, different patterns of methylation in the same DNA region were found. Predict possible effects this would have on the difference of gene expression in these twins.

21 Build a model and demonstrate the processes involved in *E. coli* protein synthesis when:
a both lactose and glucose are available.
b lactose is available but glucose is not.
c lactose and glucose are both absent.

Evaluating

22 All the cells of a multicellular organism have a complete copy of the organism's DNA rather than just the sections they need for their own functioning. Explain how it is possible for the specialisation of cell types to arise.

23 Cells from unicellular organisms are more likely to give rise to new individuals than cells from adult multicellular organisms. Suggest why this is.

24 Describe the central dogma of molecular biology. Discus the accuracy of this idea using examples to illustrate your answer.

25 Suggest a possible reason that most genes are turned 'on' as the default in prokaryotes.

26 Give your opinion on whether you feel there should be limits to the money spent on researching epigenetics.

Creating

27 Draw a labelled diagram to show how miRNA could prevent translation of mRNA.

28 Create a table summarising the factors that can affect the phenotypic expression of genes.

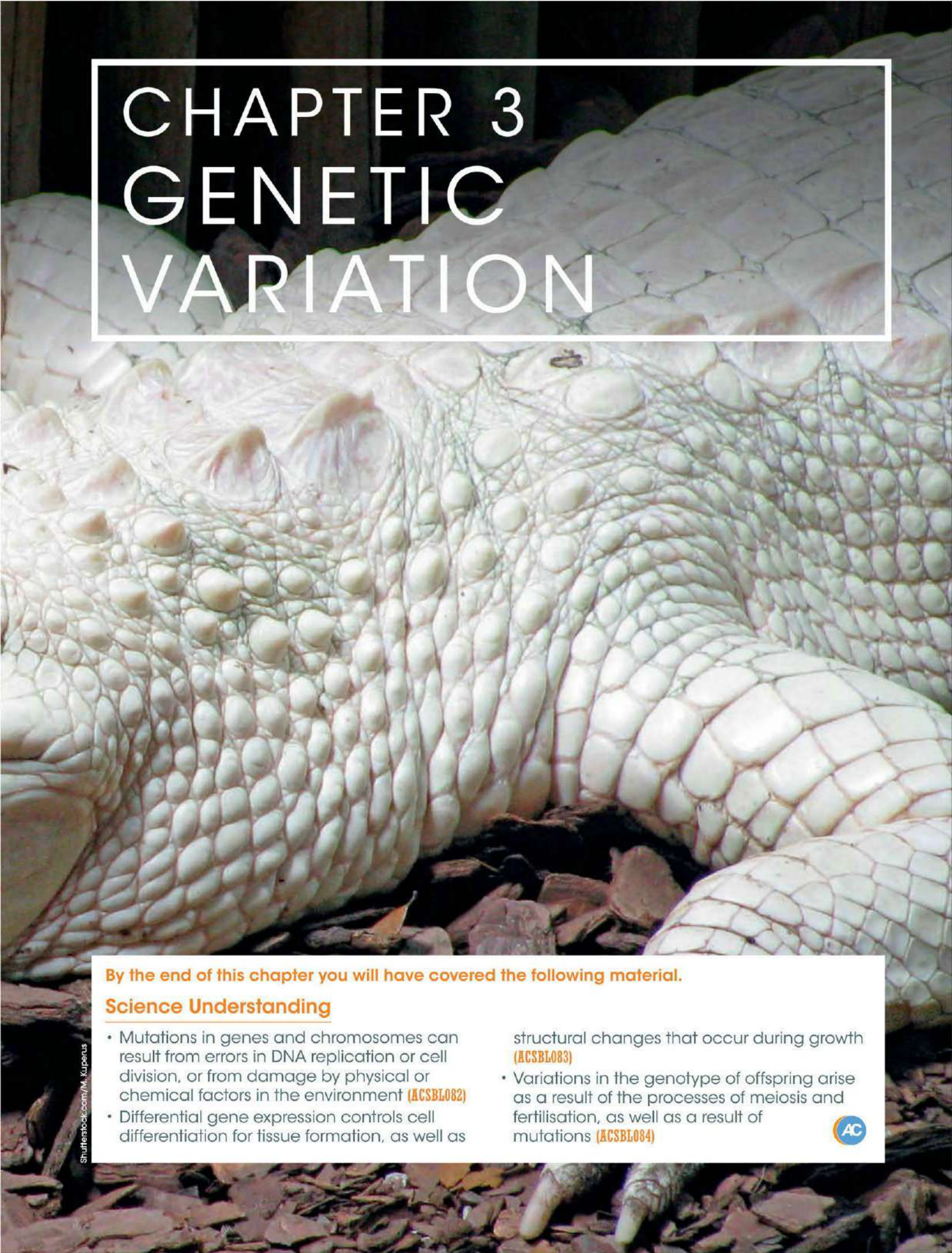
29 Discuss whether you think your future has been mapped out for you in your genes at conception.

Reflecting

30 Discuss what you have learned about gene regulation from Beckwith–Wiedemann syndrome.

31 Commercial products such as '23andMe' provide information about your genetic susceptibility to hundreds of complex diseases and traits. Suggest why this is expressed only as a chance and not a certainty. Consider whether you would want to take this test. Discuss reasons for your decision.

CHAPTER 3 GENETIC VARIATION



Shutterstock.com/M. Kuperus

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

Science Understanding

- Mutations in genes and chromosomes can result from errors in DNA replication or cell division, or from damage by physical or chemical factors in the environment (ACSBL082)
- Differential gene expression controls cell differentiation for tissue formation, as well as structural changes that occur during growth (ACSBL083)
- Variations in the genotype of offspring arise as a result of the processes of meiosis and fertilisation, as well as a result of mutations (ACSBL084)

Nature Picture Library/Wolmsley

Figure 3.1 ▲ Genetic variation occurs within these mallard duck siblings.

Two eggs warm side by side in an incubator. One is white and relatively small, less than 2 cm in length. The other is a creamy grey and around 6 cm long. Although the two eggs have experienced the same conditions of warmth and humidity, when they at last hatch, a Gouldian finch will emerge from the smaller egg and a mallard duck will come from the other one. There will be no mistaking the two. Consistent differences among organisms provide useful diagnostic features with which to distinguish between **species**. The environment does not alter these outcomes.

However, even within a single species, the characteristics of the individuals are not uniform. There are similarities but each is uniquely different. Some of these, such as physical or morphological variation, are obvious. Many others are subtle but have profound consequences for the survival of the individual. Variation in characteristics is determined in a large part by the unique genetic make-up of each organism. In this chapter we explore how genetic variation is generated anew, the mechanisms of turning genetic variation into variation in **phenotypes**, and the impacts those variations have on the survival, growth and development of organisms.

A colourful example of variation: the Gouldian finch

A strikingly coloured bird with vivid splashes of yellow, lilac and green hops onto a branch and tips her black-topped head from side to side. She is a Gouldian finch (*Erythrura gouldiae*) and she is surveying the other birds for a suitable mate. Who does she prefer and why does it matter?

The Gouldian finch is a native inhabitant of the tropical grasslands of northern Australia, as well as a popular aviary bird. There are three distinctive forms distinguishable by head colour: red, black or yellow (Figure 3.2). The colour variation is associated with a suite of other differences in the birds. For example, the red-headed birds tend to be the most aggressive and frequently establish themselves at the top of the pecking order. By contrast, black-headed birds

Figure 3.2 ▶ The three forms of Gouldian finch

Alamy/Petra Wegner

tend to be more inquisitive and are more likely to explore novel features in their environment. There are physiological differences, too. The red-headed birds are comparatively sensitive to starvation if food becomes scarce and, during breeding, they respond by reducing the number of eggs they lay. Under the same conditions, however, black-headed birds continue to lay the same number of eggs and work harder to find food.

Females primarily seek a mate whose head colour matches their own. The head colouration is a mark of genetic compatibility. If a black-headed female mates with a red-headed male, comparatively few of their hatchlings survive to maturity. Around 60% of the sons and less than 20% of the daughters from such a pairing survive.

The contribution of alleles to phenotypic variation

The Gouldian finch offers insights into the nature of variation. Variations in many features are observed between members of the same species. This is referred to as **intraspecific variation**. The form that any particular feature takes in an individual organism is described as a phenotype. Phenotypic variations can be classified according to how they are detected in the organism's appearance, chemical make-up or function (Table 3.1).

Table 3.1 Different types of phenotypic variation in the Gouldian finch

Type of variation	Description	Example
Morphological	Variation in the shape and structure of the organism, including that of the organism's internal anatomy	Bird's size and shape
Biochemical	Variation in the chemical structure and composition of organisms, including differences in specific types of proteins, lipids and carbohydrates, as well as other types of molecules, such as pigments	Expression of enzymes creating pigments and resulting colour of head feathers
Physiological	Variations in the way individuals carry out metabolism and maintain their bodily processes	Starvation stress: control of egg production
Behavioural	Differences in the ways individuals perceive, think and react. Includes their mental processes (**cognition**) and the way they translate thoughts and corresponding emotional responses into action (**behaviour**)	Aggression, inquisitiveness, mate selection

What is the cause of the variation observed among individual birds? In essentially every case, the phenotype is shaped by the presence or absence of specific proteins and the activity of those proteins. For example, whether a bird's head feathers are yellow, black or red depends on the presence of specific enzymes that generate the pigments that colour the feathers. A bird's response to starvation is dependent upon the types and activities of the hormones and metabolic enzymes it has to sustain it with an altered diet. As proteins are the products of genes, it is a straightforward conclusion that each phenotype has an underlying genetic basis.

Recall that eukaryotic cells have two copies of every gene, each copy residing on one of a pair of **homologous chromosomes**. However, each copy of a particular gene is not necessarily identical. There are often small differences in the DNA sequence of the gene from one copy to another. These different versions of the same gene are called **alleles**. For instance, essentially all cells of an individual Gouldian finch carry the same chromosomes. Each bird therefore possesses two alleles for any particular gene, and the two alleles may be the same or they may be different. If a single gene determines the colour of the head feathers, it is the combination of alleles the bird has (the **genotype**) that determines whether that colour will be yellow or black or red (the phenotype).

The colour of the head feathers, the position occupied in the social hierarchy, the response to environmental challenges, mate selection and many other features are, in a large part, an outward expression of the alleles each bird possesses. The Gouldian finch also demonstrates, however, that variation is not entirely explained by the alleles each individual has. The size a bird grows to and the physiological state of the animal are influenced by the availability of food. Breeding behaviour and outcomes are influenced by the availability of potential mates. To a greater or lesser extent, the organism's environment also plays a part.

The two-step shuffle: phenotypic variation dances to the beat of alleles

The genetic component of variation is predominantly determined by alleles. Alleles are transmitted from generation to generation through the production of gametes by **meiosis** and the union of those gametes through **fertilisation**. During meiosis, homologous chromosomes pair and then move to different daughter cells independently of each other. This results in gametes with different combinations of parental chromosomes and therefore different combinations of parental alleles. When homologous chromosomes separate and recombine in the first division of meiosis, they sometimes exchange segments with one another. This **crossing over** further rearranges the combinations of alleles available on each homologous chromosome. Fertilisation comes about by the union of two random gametes from each parent, providing further variation in the possible combinations of alleles offspring inherit.

Refer to Chapter 1 for more on meiosis and fertilisation, and Chapter 4 for more about predicting the inheritance of alleles in offspring.

Meiosis and fertilisation shuffle existing alleles into different combinations in each individual from one generation to the next. However, if that was all there was to variation, species would remain unchanged forever. This is not the case. Occasionally, new variations within a species appear. Indeed, new species appear over time, an outcome of both the available genetic variation and the environmental circumstances experienced by individuals. For new variations to appear, new alleles must be created. In effect, there must be changes in the DNA.

See Chapter 7 to learn more about the processes of evolution.

Genetic variation is driven by sexual reproduction, facilitated by random segregation of alleles into gametes during meiosis and subsequent fertilisation of random gametes.

WOW

Medicine or mediocre?

Sensitivity to different medications is an example of physiological variation in humans. What causes this variation? Between 70 and 80% of all drugs in clinical use are modified by enzymes in the liver, either being converted into their active form to exert their effect or becoming deactivated for excretion by the kidneys. An important class of these enzymes are the cytochrome P450 enzymes. Variations in the number and the types of cytochrome P450 enzymes contribute to each individual's ability to respond to particular drugs. The enzyme variants are encoded by the different alleles each individual person inherits.

QUESTION SET 3.1

Remembering

1 Define the following terms.
   a Intraspecific variation
   b Phenotype
   c Genotype
   d Allele

2 Name four types of phenotypic variation.

3 Describe how alleles contribute to phenotypic variation.

Understanding

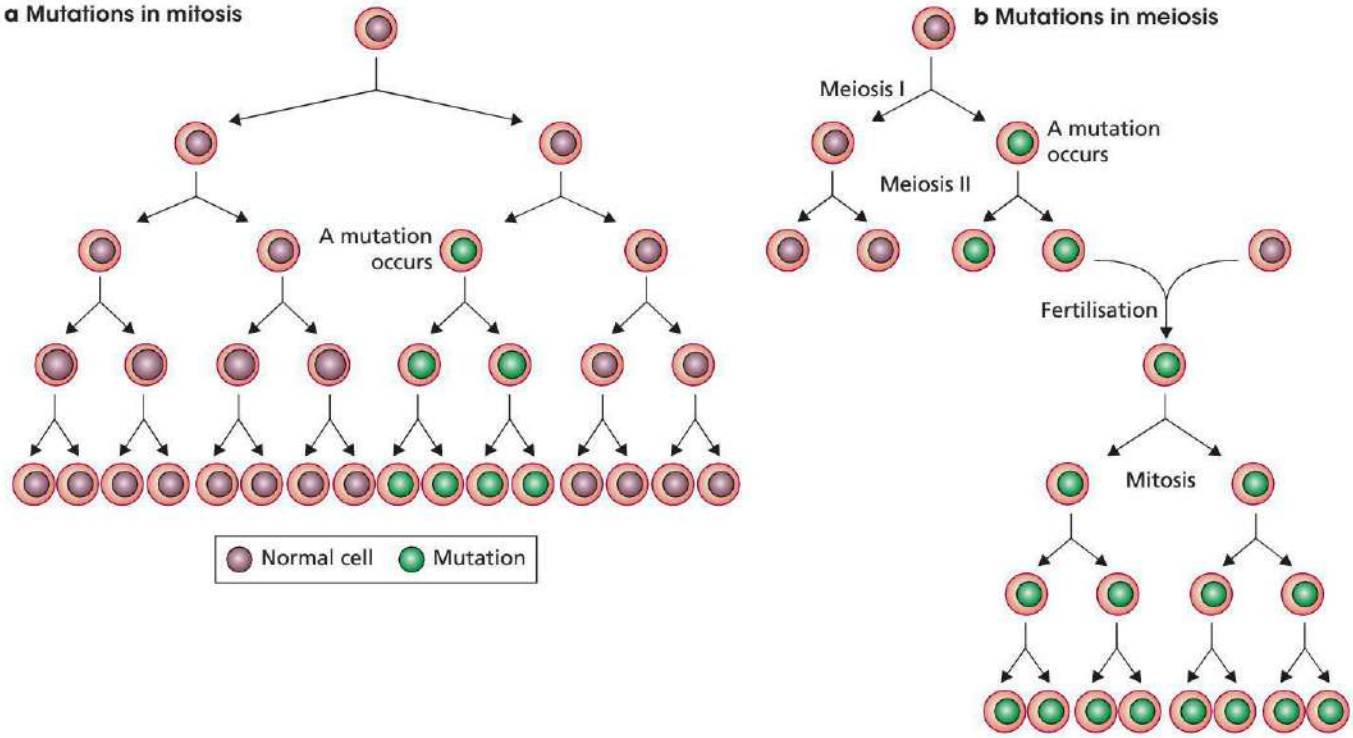
4 Draw an annotated diagram that shows how gamete formation and fertilisation contributes to phenotypic variation.

Mutations are the source of novel genetic variation

Changes to DNA are termed **mutations**. Mutations may arise spontaneously during cell division, or they may be induced by physical or chemical **mutagens**, or through the action of biological agents. Mutations that occur in genes often affect the translated proteins they code for. These effects are sometimes subtle. More often, they are severe with potentially catastrophic effects for the survival of the organism that bears them. Rarely, they can enhance the function of the protein or make it better suited to the environment the organism inhabits. The effect of a mutation depends upon whether it has occurred in non-reproductive (body, or **somatic**) cells or in the reproductive (**germ-line**) cells (Figure 3.3).

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 a salient consequence 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 germ-line cells affect gametes and have the potential to be inherited, or passed on to the next generation so that they are incorporated into every cell of the offspring. Often, the germ-line mutation results in developmental abnormalities that cause the affected embryo or foetus to be spontaneously aborted. If carried through to birth, the germ-line 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.



▼ **Figure 3.3**
a) Mutations in somatic cells affect only the cell in which it occurred and all its daughter cells.
b) Mutations in germ-line cells affect all body cells of the individual who inherits them.

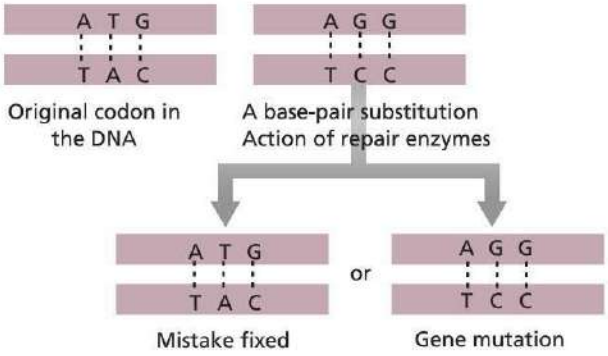


Figure 3.4 ▲ Base-pair substitution results in either the mistake being fixed or a mutation.

DNA DAMAGE AND REPAIR

View the animation on DNA damage and repair.

Errors in cell division

Spontaneous mutations occur during the S phase (synthesis) of the cell cycle when the DNA is exposed for replication and vulnerable to damage. These mutations arise because the exposed nitrogen bases convert backwards and forwards between different chemical forms, one that defines its usual structure in DNA and another that is atypical. Adenine, for example, normally base pairs with thymine but may spontaneously undergo a chemical change that makes it resemble a guanine, which pairs with cytosine. During DNA replication, the identity of the chemically different form of adenine may be mistaken and a guanine is introduced into the DNA sequence instead.

Errors may also be introduced into DNA sequences by highly corrosive chemicals containing oxygen. These chemicals, termed reactive oxygen species, may be generated naturally by the cell's own metabolism or by the action of mutagens. Enzymes in the cell, such as catalase, remove many of these chemicals but if there is, for any reason, an excess of them, they readily react with DNA to cause damage to the DNA structure.

During the G2 phase of the cell cycle, DNA is proofread and any errors that are detected are repaired. Repair often depends on one of the DNA strands being intact. The intact strand serves as a template for proofreading and restoration of the damaged complementary strand. However, if a mutation is not repaired or it is repaired improperly, the mutation becomes part of the DNA sequence and persists through subsequent cell divisions (Figure 3.4).

The DNA repair mechanisms are usually highly effective, so mutations are comparatively rare. **Mutation rates** vary, however, for different organisms (Table 3.2). The precise rate is also variable across the **genome** of each organism since genes at different loci have different mutation rates. Low mutation rates bear witness to the tremendous accuracy with which DNA is replicated.

Table 3.2 Estimated mutation rates for different organisms

Organism	Genome size (nucleotide pairs)	Mutation rate (mutations per genome per cell replication)
Roundworm	97×10^6	0.018
Fruit fly	180×10^6	0.058
Mouse	2600×10^6	0.49
Human	3100×10^6	0.16

Low mutation rates made it difficult for geneticists to investigate mutations until the discovery in 1927 by an American biologist, H. J. Muller, that the mutation rate in fruit fly (*Drosophila melanogaster*) can be greatly accelerated by irradiation with X-rays. Since then it has been found that other environmental mutagens speed up the mutation rate. The discovery of mutagens made it easier to study the cause and transmission of mutations. Bacteria and plants are used in most experiments, although scientists also perform experiments on animal cells using tissue cultures.

From these studies, three main ideas have emerged. First, mutations arise spontaneously and are in no sense 'directed' by the environment. Environmental influences can greatly affect the mutation rate but they cannot induce a particular mutation to occur. Second, mutations are persistent. They tend to be transmitted through many cell divisions without further change, although there is always the possibility that they may mutate again, either producing another new feature or reverting to the original condition. Third, the majority of mutations confer disadvantages on the organisms that inherit them. The premature death of organisms with harmful mutations (before reproductive age) prevents harmful mutations accumulating in populations. The occurrence of a useful mutation is an extremely rare event.

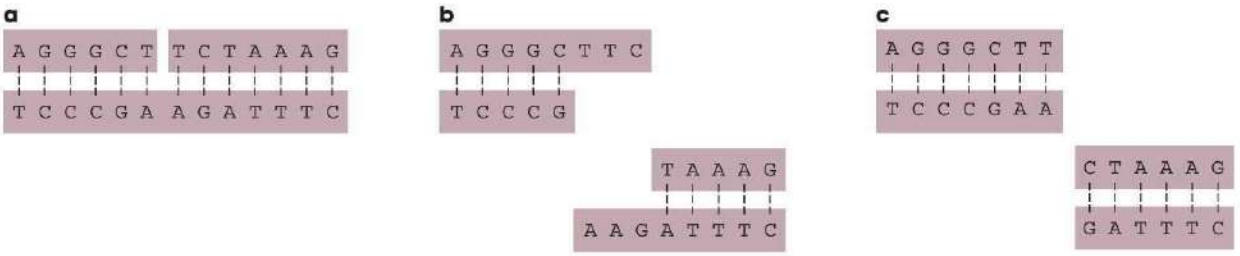
Physical mutagens

Physical mutagens are various types of radiation that cause DNA damage (Table 3.3). One example is ultraviolet light (UV light), a natural component of sunlight. Public awareness campaigns have drawn attention to the risks of excessive exposure to UV light, such as increased risk of skin cancer. Physical mutagens often affect the nitrogen bases causing distortions in the double helix, and UV light, for example, fuses adjacent thymines or cytosines in the DNA sequence. Ionising radiation, such as X-rays, cause the loss of adenine and guanine bases, although the DNA backbone remains intact, creating gaps in the double helix. These aberrations disrupt complementary base pairing. Ultimately, incorrect bases may be inserted in their place during DNA replication.

Table 3.3 Some physical mutagens and their effects

Physical mutagen	Effect
UV light	Structural distortion by cross-linking neighbouring nucleotides
X-rays	Gene and chromosome aberrations
Nuclear radiation	Breaks in DNA strands

Physical mutagens frequently also cause **double-strand breaks**, which are essentially complete breaks in the chromosomes (Figure 3.5). Sometimes the broken ends leave single-stranded overhangs that are complementary to one another. This enables them to bind one another and facilitate repair of the broken ends. However, sometimes the double-strand break has no overhangs, or the DNA at the fragment ends is damaged so that these ends no longer match. In such cases, mistakes can occur during repair and the consequences may be especially hazardous to the cell. Broken ends can be rejoined inappropriately to the wrong fragments of DNA. Intervening segments of broken DNA can be lost. These kinds of anomalies result in chromosomal rearrangements. A cell can put the brakes on during cell division to give it time to repair breaks and mutations before it divides, but an accumulation of double-stranded breaks upon intense exposure to physical mutagens is often lethal to the cell. Apoptosis of the cell in this situation is a mechanism to guard against cancer formation.



▼ **Figure 3.5** Compare a single-stranded break in DNA a) with double-stranded breaks (b, c). The double-stranded break in c) is the most difficult of the three for the cell to repair.

Chemical mutagens

The mechanisms by which chemical mutagens exert their effects vary (Table 3.4); however, a common outcome is the substitution of one nitrogen base for another.

Some chemical mutagens, such as 5-bromouracil, act directly as a substituting base. The 5-bromouracil resembles thymine and can become incorporated in place of it during replication. However, unlike thymine, the incorporated 5-bromouracil can form hydrogen bonds with either adenine or guanine. The ambiguous pairing affects DNA replication during subsequent cell divisions, leading to a C-G pair being swapped for the original T-A pair.

Some chemical mutagens change the structure of the existing nitrogen bases in the DNA so that an A-T pair may replace the original G-C pair during DNA replication. Other compounds can readily slip in between the nitrogen bases of DNA, distorting the packing of the double helix. This distortion leads to either loss of a nucleotide or incorporation of an extra nucleotide during subsequent DNA replication.

Table 3.4 Some chemical mutagens and their effects

Chemical mutagen	Effect
Acridine orange	Addition and/or removal of bases in DNA
Aflatoxin A, Ethidium bromide	Disrupts packing of DNA by slipping between nitrogen bases
Polycyclic aromatic hydrocarbons	Crosslink with adenine and guanine to block DNA repair
2-aminopurine, 5-bromouracial	Nucleotide substitution
Colchicine	Prevents spindle formation in mitosis and so doubles chromosome number
Cyclamate	Chromosome aberrations
Ethyl methanesulfonate, Nitrosoguanine	Chemical modification of nitrogen bases
Mustard gas	Guanine in DNA replaced by other bases
Nitric acid	Adenine in DNA is deaminated so it behaves like guanine

Biological agents

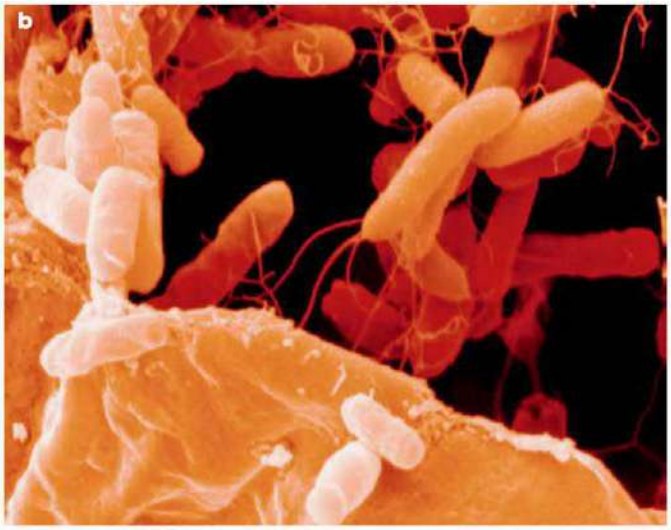
Genetic mutations sometimes arise because of the action of invasive pathogens, such as bacteria and viruses. Occasionally, the DNA of these pathogens becomes permanently integrated into the host cell's DNA, causing mutations in subsequent generations.

Bacteria and viruses getting horizontal

Bacteria of the genus *Agrobacterium* cause crown gall disease in the stems of plants of several species (Figure 3.6). The bacterium achieves this by inserting a **plasmid**, called a Ti plasmid, into a cell of the host plant. The Ti plasmid contains genes which code for enzymes that cut the host plant's DNA and integrates a segment of the Ti plasmid into it. The cell of the host plant thus becomes modified by **horizontal gene transfer**. The integrated bacterial DNA contains additional genes that essentially hijack the host plant cell machinery to produce nitrogen- and carbon-rich compounds that the bacterium uses as a nutritional source. The infected cell is also induced to produce hormones that stimulate the plant cells to rapidly divide and grow. The increased cell divisions result in the formation of the distinctive tumour-like gall that is, in

Figure 3.6 ▼
a) Crown gall disease of a rose bush caused by the bacterium *Agrobacterium tumifaciens*; b) *Agrobacterium tumefaciens* at the surface of a leaf

Nature Picture Library/Visuals Unlimited



Alamy/Custom Medical Stock Photo

effect, a food factory that sustains the expanding population of bacteria. The capacity to carry out horizontal gene transfer has made specially engineered strains of *Agrobacterium* a valuable **cloning vector** for genetically modifying plants.

See Chapter 5 to learn more about plasmids and cloning technology.

A number of viruses are also capable of horizontal gene transfer. Notable among them is the human papillomavirus (HPV), which infects epithelial cells of human skin and mucosal membranes. There are over 100 different subtypes of HPV. A dozen or so HPV subtypes cause over 99% of cervical cancers, the second most common form of cancer in women worldwide. The viral genome is transferred to the host nucleus where it is copied dozens of times and inserts into the host cell DNA. HPV genes shut down DNA repair so that insertions of HPV DNA and other aberrations of the normal cellular DNA are retained, rather than removed, by the host cell. At the same time, increased replication of cells results in increased replication of the virus. The human epithelial cells therefore divide out of control and any mutations that arise during subsequent cell divisions, even those not associated with HPV, are allowed to accumulate. Such an infection often progresses to cancer.

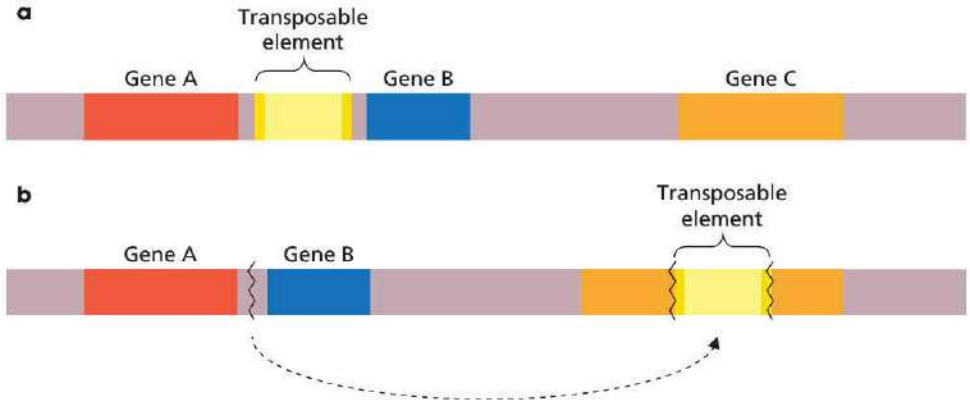
Jumping genes

HPV achieves horizontal gene transfer of somatic cells. Other types of viruses have presumably achieved this in germ-line cells. **Transposable elements**, also referred to as 'jumping genes' are a likely example of this. Transposable elements are believed to originate from ancient viral infections that have become permanently established as non-coding sequences in eukaryotic genomes. Transposable elements are capable of replication and spontaneous relocation ('jumping') in the genome (Figure 3.7). Consequently, the transposable elements generate changes in the sequences of the DNA in which they insert themselves and continue to be a source of genetic change with consequences for the function and evolution of organisms.

Genome sizes among eukaryotic organisms vary widely. This observation is intriguing because sometimes even related organisms have astonishingly different genome sizes. For example, rice and barley are related cereal grasses with a similar number of genes in their respective genomes (~ 32 000), yet the genome of barley is 10 times bigger than that of rice (5 billion and 420 million nucleotide pairs, respectively). The difference is largely accounted for by the quantity of **repetitive DNA** in the **non-coding DNA** of the two species. These resemble sequences of transposable elements that have multiplied and expanded the genome. Indeed, repetitive DNA makes up the bulk of the genomes of eukaryotic organisms. More than half the human genome is composed of repetitive DNA.

The vast majority of transposable elements in the human genome have become inactive and are recognised today as sequence repeats in non-coding DNA. There are, however, a few that can still 'jump', leading to the generation of new alleles with adverse phenotypes. For example, there are at least 11 such 'jumping' events responsible for mutating a gene located on the human X chromosome that is involved in the pathway for blood clotting. The mutations result in an inactive protein that prevents blood clotting and causes haemophilia.

Mutations in DNA may be caused by chemical or physical mutagens or by biological agents, such as viruses or bacteria.



◀ Figure 3.7
a) A transposable element is at first sitting between two genes, then b) it 'jumps', inserting itself into another gene and interrupting the gene's sequence.

QUESTION SET 3.2

Remembering

1 Define 'mutation'.
2 Define 'double-strand break'.
3 Describe the different effects on the individual resulting from mutations in somatic and germ-line cells.
4 Describe the difference between a physical and chemical mutagen.

Understanding

5 Classify the following as to whether a chemical or physical mutagen or a biological agent is responsible.
   a The withdrawal of furylfuramide as a food preservative in Japan owing to it causing cancers in test laboratory animals
   b The transmission of genes conferring antibiotic resistance between bacteria
   c The proposed risk of mutations induced by exposure to cosmic radiation during space flight
   d The use of radiotherapy to treat a primary tumour
6 Select one of the chemical mutagens from Table 3.4. Draw an annotated diagram to show a plausible sequence of events leading from exposure to this mutagen during S phase of the cell cycle to the mutation incorporated in the DNA by the end of G2 phase.

Applying

7 A unique segment of DNA consisting of 2907 nucleotide pairs first appeared in the genome of wild fruit fly (*Drosophila melanogaster*) in the mid-20th century. Since then, it has spread throughout wild populations and increased in copy number within individual flies. Discuss what might account for these changes in the fruit fly DNA over the last half century.

Getting to the point: types of gene mutations

SINGLE POINT MUTATIONS
View the video clip on single point mutations.

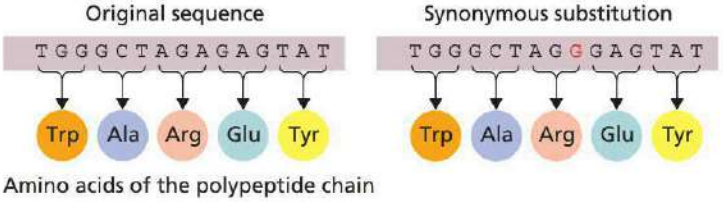
The simplest form of mutation is a **point mutation**, in which just a single nucleotide within the original DNA sequence is affected. Differences between sequences in the nucleotides at one position are called **single nucleotide polymorphisms** (SNPs, often pronounced as 'snips'). If the point mutation occurs in a gene, the mutated gene sequence can be transcribed and translated into a protein that is 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** are a source of novel SNPs and have a number of possible effects on the translated protein.

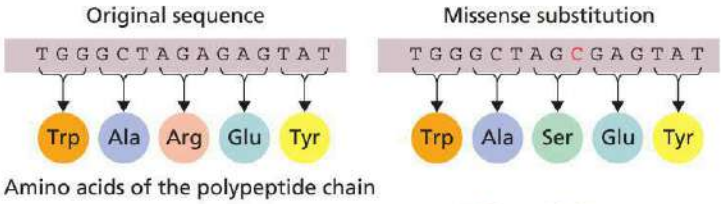
A **synonymous mutation**, also referred to as a **silent mutation**, occurs when the substituted base results in a **codon** (also known as a triplet) that codes for the same amino acid as the original codon. For example, AGA and AGG both specify for the addition of an arginine amino acid in the polypeptide chain (Figure 3.8). The protein encoded by the mutated gene is therefore identical to that encoded by the original gene. Synonymous mutations are possible because a level of redundancy is built into 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. A **missense mutation** arises when a single nucleotide substitution changes the

Figure 3.8 ▼
Synonymous mutation

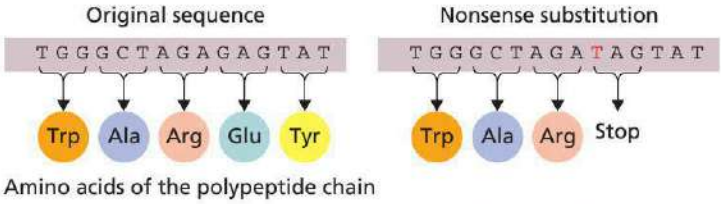


amino acid. For example, substitution in an AGA codon to generate an AGC codon results in a serine amino acid being added to the polypeptide instead of the original arginine (Figure 3.9).

A **nonsense mutation** occurs when a single point mutation creates a new stop codon within the original gene sequence (Figure 3.10). This leads to early termination of translation of the transcribed gene sequence. As the remaining sequence downstream of the new stop codon is not translated, the result is the production of an incomplete polypeptide.



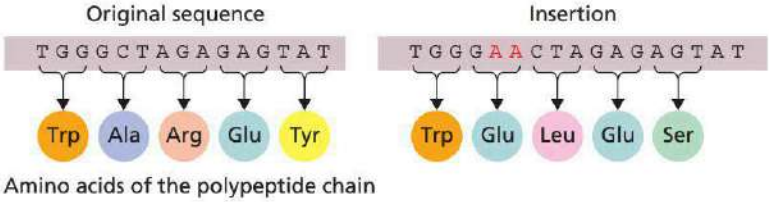
▲ **Figure 3.9**
A missense mutation in the gene sequence leads to one amino acid being substituted for another in the polypeptide chain.



▲ **Figure 3.10**
A nonsense mutation in the gene sequence results in premature termination of translation.

Insertions and deletions

As the name suggests, an **insertion mutation** is the addition of one or more nucleotides at a site within the original gene sequence. A **deletion mutation** is the loss of nucleotides from a site within the original gene. Collectively these are referred to as 'indels'. The effect of the indel is frequently a **frameshift mutation**, in which the reading frame for the corresponding amino acids has been nudged 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 3.11). Under such circumstances, even a single nucleotide insertion or deletion can have a profound effect on the corresponding protein.



▲ **Figure 3.11**
An insertion in the gene sequence results in a frameshift mutation.

Point mutations can cause changes in a DNA sequence by either 1) substitution of a nucleotide or 2) insertion or deletion of a nucleotide. Substitutions can be classified as synonymous, missense or nonsense mutations. Insertions and deletions are classified as frameshift mutations.

Effects of mutations on survival

A protein's function is dependent upon its structure. Mutations that change a protein's structure can have consequences for protein function with potential impacts on the organism's survival. Mutations can therefore also be classified according to whether the effect of the mutation on the protein's function and the organism's survival is unchanged, changed for the worse, or changed for the better.

Neutral mutations

In the case of synonymous mutations, the protein product is unchanged compared with the original, so the organism's survival is unaffected by the change. This is said to be 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 and causes the amino acid, glutamic acid, to be swapped for an aspartic acid. Both amino acids are negatively charged, however, 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

A living organism can be compared with a complex product of engineering, such as an aeroplane, in which the components are so intricately integrated that an indiscriminate change to any component harms the overall operation of the aircraft and makes it unfit to fly. Similarly, random mutations may disrupt the function of the encoded protein, undermining the organism's overall ability to carry out its basic processes and survive. Such mutations are referred to as **deleterious mutations**. The majority of 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 the functional version of the protein. The deleterious mutation is thus masked within the phenotype of the organism. If the organism is unfortunate enough to have only 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.

Transposable elements are abundant in the non-coding DNA of eukaryotic organisms but evidently much less so in genes. What might explain this observation? Insertion of a transposable element into a gene is usually a deleterious mutation that reduces the organism's chances of survival. Presumably, individuals with such insertions died prematurely and the mutation was eliminated from the population. By contrast, insertions of transposable elements into non-coding DNA tend to be less harmful and, if they occur in germ-line cells, the mutations persist from generation to generation. Over many generations, these insertions accumulate in the non-coding regions of the genome.

Beneficial mutations

Occasionally gene mutations lead to the generation of 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.

See Chapter 12 to learn more about the function of the adaptive immune system.

The human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS). Without treatment, AIDS is fatal in essentially all cases. A few individuals have been exposed to the virus but have proved to be resistant to infection. These individuals have a nonsense mutation that results in the elimination of one of the surface proteins required by HIV to enter cells. This deletion confers resistance to HIV infection.

Mutations can be categorised as neutral, beneficial or deleterious depending upon their effect on the survival of the individual.

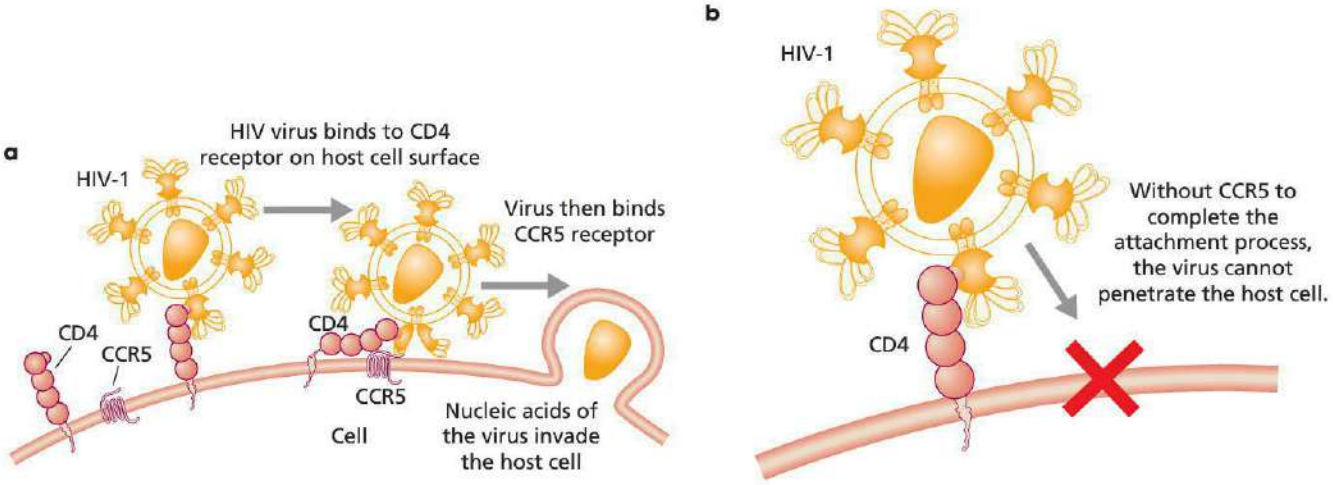


Figure 3.12 ▼
a) Invasion of a host cell by HIV depends upon attachment to two surface protein receptors, CD4 and CCR5. b) A nonsense mutation in the CCR5 gene results in cells that resist HIV infection.

Scientific literacy: Forget patents, human gene back in public domain, US court rules

Human genes will no longer be controlled by private companies in America after a landmark ruling by the country's highest court.

The decision overturns thousands of US gene patents, and may have ramifications for an Australian case challenging the so-called breast cancer gene patent.

Cancer advocates, lawyers and pathologists are celebrating the US decision, and have called on the Australian government to amend the Patent Act to reflect it.

Rebecca Gilsenan, the principal lawyer at the firm fighting the Australian patent, Maurice Blackburn, said the US decision was exciting and encouraging.

'The Australian court is not bound by what the US Supreme Court has decided; however, I expect that an Australian court will be very interested in what [it] has decided and the reasons it had, and will take notice of that', she said. 'It's a very significant development by a very significant court.'

About 41% of the human genome is subject to American patents.

In February, Maurice Blackburn lost a Federal Court case challenging the granting of a patent to US firm Myriad Genetics on a mutation in the BRCA1 gene that drastically increases a person's risk of cancer. In early August it will appeal on the grounds that Justice Nicholas erred in finding that simply isolating a gene outside the body made it patentable.

'The US Supreme Court has held up the essence of the argument we have put forward in our case, that genes are a naturally occurring substance that are not patentable,' Ms Gilsenan said.

Cancer Voices Australia said the government should immediately amend the Patent Act, to 'reflect the wishes of the Australian people. Our genes are not invented by companies like Myriad Genetics, so we are very pleased that this has been legally confirmed,' spokeswoman Sally Crossing said.

Cancer Council head Ian Olver also said the Patent Act should be amended, as there was 'nothing in Australian law to prevent commercial interests trying to monopolise the use of genetic materials'.

In Australia, Myriad has granted an exclusive patent licence to Genetic Technologies Limited. It tried to enforce the patent in 2008, threatening pathology and cancer centres with legal action, but backed down after a public backlash. It has not actively defended the case in Australia with Myriad.

The Royal College of Pathologists of Australasia's Graeme Suthers said the threat to enforce the patent was disturbing. He said the US decision was 'game-changing' and would ensure patients there got better-quality tests.

Corderoy, A. (2013) 'Forget patents, human gene back in public domain, US court rules', *The Age*, 15 June.

Questions

1. Does the BRCA1 gene in its own right dramatically increase a person's chances of having cancer?
2. Describe the kind of data that would be required to determine if a particular mutation in the BRCA1 gene increased a person's risk of having cancer.
3. Suggest two concerns that are raised by a company owning the patent to a human gene sequence.
4. If the argument is accepted that naturally occurring genes should not be patented, under what circumstances might a patentable gene come about?
5. Do you agree or disagree with the US Supreme Court decision? Outline two reasons in support of your view.

Case study

Genetic testing gets personal

'Knowledge is power', commends the website offering personalised genetic testing for just $99. Spit into a tube, send the sample off and within 4–6 weeks you too can have a report outlining your genetic predisposition to over 240 health conditions. What are the consequences of receiving this information? How would you respond if you discovered you had an elevated risk of developing diabetes, heart disease or cancer?

These are the kinds of issues that engage Associate Professor Sylvia Metcalfe (Figure 3.13), Group Leader of Genetics, Education and Health Research at the Murdoch Childrens Research Institute in Melbourne. 'My role as an educator made me recognise the importance of being able to make informed decisions about genetic testing, as well as the impact of genetics on families.'

Courtesy Sylvia Metcalfe

Figure 3.13 ▲
Associate Professor Sylvia Metcalfe

Professor Metcalfe cautions against jumping to conclusions about the risks associated with alleles that predispose people to certain conditions. The majority of common health conditions are influenced by complex interactions between countless genes, the environment and lifestyle factors. Genetic testing does not necessarily equate to sequencing the whole genome. Current tests rely on examining small regions of a person's DNA and detecting SNPs that are characteristic of specific alleles for particular genes. The emergence of more rapid and more affordable sequencing technologies, however, raises the prospect of routinely screening a newborn's whole genome. For many years already, essentially all newborn babies in Australia have been routinely screened for about 20 metabolic conditions, including PKU, and for cystic fibrosis. We asked Sylvia if she thought there is any merit in genome sequencing as an integral part of every individual's lifetime health management.

'My own view is that it is too early to introduce genome sequencing of newborns. One of the considerations of sequencing genomes is that we have a long way to go before we can interpret the massive amount of information that is generated.'

Sylvia points out that sequencing is revealing thousands of alleles that cannot yet be determined as being disease causing or not. 'Right now we're finding these variants in both healthy people and in people with particular genetic conditions, so it is challenging to predict the effect of having these variants in a healthy newborn.' She believes that interpretations will improve as more research is done but adds, 'There is no doubt that, in children with undiagnosed conditions, genome sequencing is playing a very important role in discovering the genetic basis of their condition.'

Questions

1. Is a person's genome a reliable predictor of cancer?
2. Contrast the kind of knowledge that can be gained from screening the genome of a healthy individual compared with one who has a disease of unknown genetic cause.
3. Screening an individual's genome to inform lifestyle decisions is an example of personalised medicine. How effective might this be compared with traditional approaches that are based on public policy derived from population-wide health statistics?
4. People taking out life insurance are obliged to disclose their family history of certain types of disease, such as diabetes or heart disease. Insurance premiums are adjusted accordingly. Discuss how genetic screening compares as a source of such information and whether it is to the advantage or the disadvantage of the individual.
5. A friend of yours is considering having their genome screened and they turn to you for your opinion. Outline the advice you would give them. Would your advice be different if, during the conversation, your friend revealed they came from family with a history of cancer?

QUESTION SET 3.3

Remembering

1 Define 'single nucleotide polymorphism (SNP)'.
2 Describe the following types of genetic mutations.
   a Substitution
   b Insertion
   c Deletion
3 Describe the effect of the following mutations on a coded protein.
   a Synonymous mutation
   b Missense mutation
   c Nonsense mutation
   d Frameshift mutation

Understanding

4 Classify the following mutations as neutral, deleterious, or beneficial to an organism's chances of survival.
   a An indel 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 synonymous 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.

Re-weaving the genetic threads: variation in chromosomes

Genetic variations can also be driven by wholesale changes to the chromosomes. Alterations to chromosomes contrast with single point mutations as they can affect many genes simultaneously. Some of the variations that occur with chromosomes, such as chromosome number, are quite natural in certain situations and are therefore integral to the functioning and continuity of the species. Others arise because of anomalies that occur during the formation of the gametes.

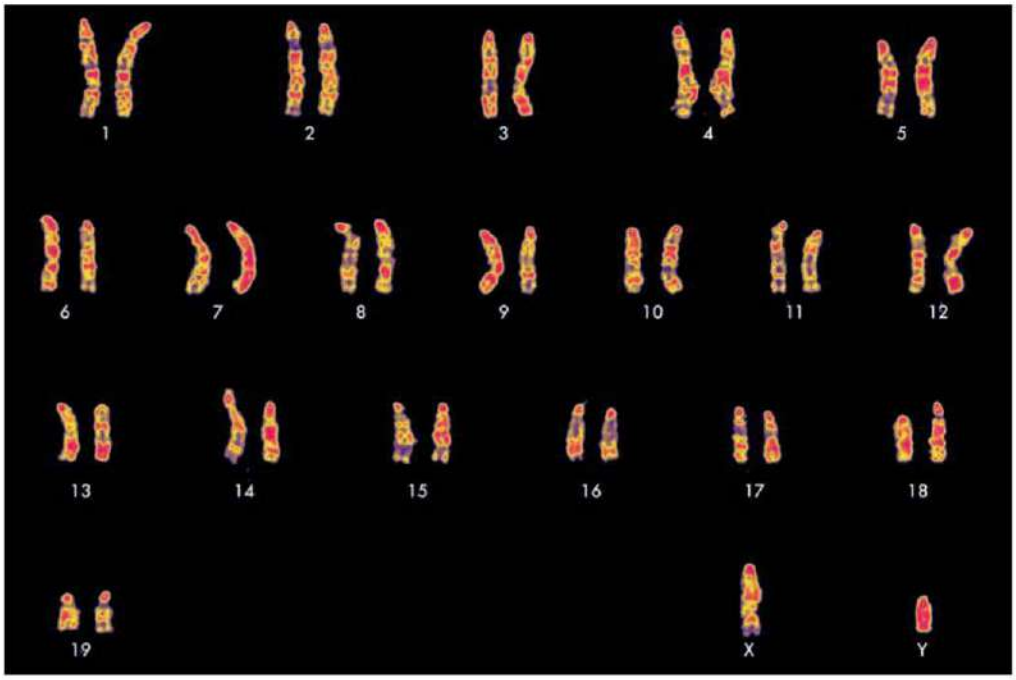
Chromosome alterations can be observed and analysed by examination of a prepared microscope slide of stained cells in the process of nuclear division. This reveals a jumbled cluster of chromosomes that differ in size, shape and banding. Photographic images of chromosomes are rearranged into matched and ordered pairs to create a **karyotype**, the standard form used to display and analyse chromosomes (Figure 3.14 and Chapter 1, Figure 1.12). Chromosomes are ordered by length, from largest to smallest, and they have characteristic banding patterns. Each species of organism is characterised by a particular number of chromosomes in each cell.

As with the genetic mutations discussed above, chromosomal anomalies often have dire consequences for the organism that has them. Occasionally, however, the anomaly is a fortunate accident that confers a survival advantage upon the recipient.

KARYOTYPES

Construct a karyotype and determine the sex of the individual.

Figure 3.14 ▶
A karyotype of a mouse



Variations in chromosome number

In many eukaryotic organisms, the somatic cells are **diploid (2*n*)**: the cells contain two sets of chromosomes, one set inherited from each parent. The gametes are **haploid (*n*)**. There are consequences for organisms when the complement of chromosomes in the somatic cells varies from the usual diploid state.

Monoploidy

Common to many colonial insects such as ants, bees and wasps, the males of such species are **monoploid (1*n*)**. By contrast, the females, including the queen, are diploid (Figure 3.15). The males are not haploid in the sense of the gametes of regular diploid animals. Their chromosomes represent a single complete and operational set and the males function as conventional, multicellular animals. By contrast, in haploid gametes, the chromosomes represent half the complete set and are packaged in a dormant state awaiting the fertilisation event that will activate them. In these insects, the queen produces eggs by meiosis, whereas the males produce sperm by mitosis. Fertilisation results in diploid female offspring. The males are instead produced by **parthenogenesis**, a process by which the entire organism is regenerated from a single egg cell without the need for fertilisation.

Figure 3.15 ▼
Bees maintain their colony structure with diploid females and monoploid males.

Shutterstock.com/Lehrer

Many fungi and algae are also monoploid and, as well as insects, there are examples of monoploid fish, amphibians and reptiles. Monoploidy seems economical because only one set of chromosomes are required, so why are diploid organisms so much more common? The advantage for diploid organisms is that any defective alleles that arise can be masked by a functional allele on the corresponding chromosome. In monoploid organisms, any defective allele is the only allele available for a particular gene and the consequences are likely to be deleterious.

Polyploidy

Sometimes the cell divisions that give rise to haploid gametes fail altogether, so that half the gametes contain two copies of each chromosome (diploid, $2n$) and the rest have none. If a diploid gamete fuses with a normal haploid gamete, the resulting individual is triploid ($3n$): it has three of each type of chromosome. If two diploid gametes fuse, a tetraploid ($4n$) individual will be produced. It is therefore possible for an organism to acquire one or more complete extra sets of chromosomes, a phenomenon called **polyploidy**.

Polyploidy is particularly common in flowering plants, ferns and green algae. Approximately half of all flowering plant species are polyploid and many varieties of commercial fruit and cereals, for example, are generated polyploids (Figure 3.16). Polyploidy is often associated with advantageous features, such as increased size and greater hardiness, although such advantages are sometimes offset by reduced fertility. Polyploidy also occurs in fungi and in some fish and amphibian species.

Polyploidy is lethal in humans. In the rare situation of a pregnancy continuing to a live birth (1% of human polyploids), the newborn dies within a month.

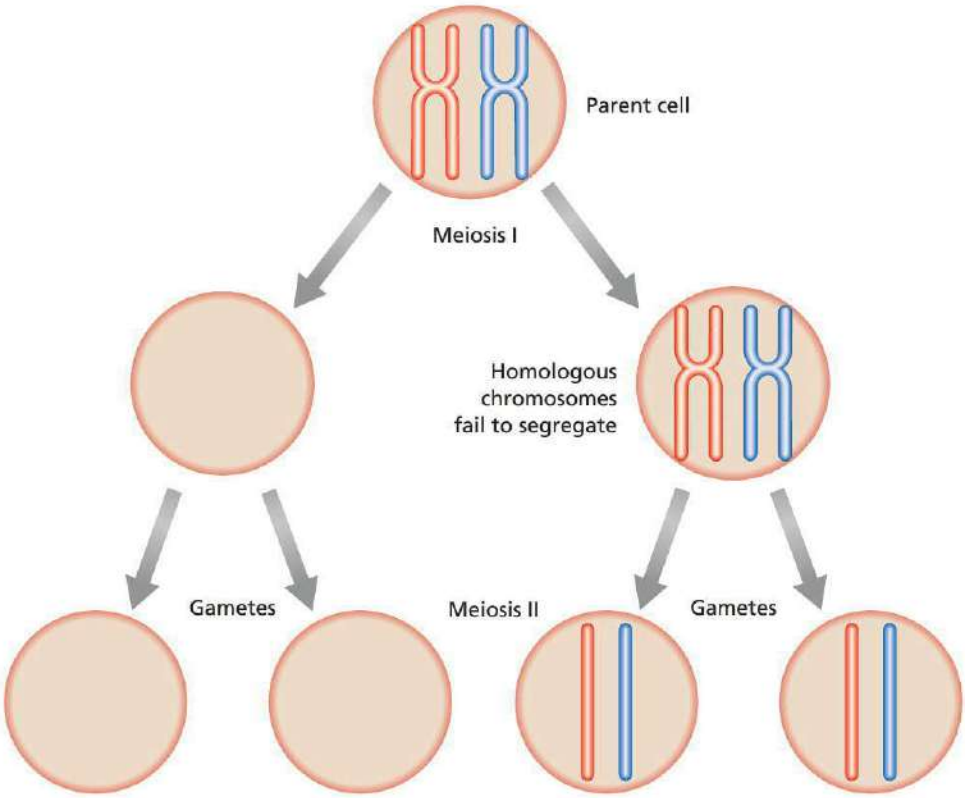
Shutterstock.com/Mau Horng

▲ **Figure 3.16**
Polyploid varieties of fruit are bigger and have bigger cells than regular diploid varieties. The application of polyploidy to creating infertile fruit, such as these seedless grapes, is of considerable commercial significance.

Aneuploidy

Aneuploidy is the condition in which there is an addition or loss of one chromosome from a cell (i.e. $2n + 1$ or $2n - 1$). Occasionally more than one chromosome may be affected. Reproductive failure by miscarriage is common and it has been found that many of these embryos are aneuploids. To understand how this comes about, consider the process of meiosis. Normally in meiosis, identical chromosomes come together and then segregate into separate cells, so that the gametes finish up with only one of each pair of chromosomes. Occasionally, however, the two identical chromosomes, instead of separating, go into the same cell. This phenomenon is known as **non-disjunction**. It results in the formation of two types of gametes in equal proportions, but one type has two copies of a particular chromosome and the other type has none (Figure 3.17).

Refer back to Chapter 1 to revise the process of meiosis.



◀ **Figure 3.17**
In non-disjunction, the chromosomes fail to segregate, so half the gametes contain two chromosomes of a pair (bivalent) each and the other half contain no chromosomes at all. Generally, non-disjunction only takes place with one pair of homologous chromosomes, while the rest behave normally. It can occur during either the first or the second meiotic division.

The fusion of a gamete containing both homologous chromosomes with a normal gamete containing one of the chromosomes gives a zygote with three such chromosomes; the normal pair plus an extra one. This condition is called **trisomy**. Fusion of a gamete with none of the homologous chromosomes with a normal gamete gives an individual with only one of this particular type of chromosome in each cell This condition is called **monosomy**.

Some types of aneuploidy survive in humans. Down syndrome is caused by the presence of an extra chromosome 21 (one of the smallest chromosomes) in every cell, thus giving three copies of chromosome 21. Children with Down syndrome vary in their symptoms but most show moderate-to-severe delayed development, characteristic almond-shaped eyes and round face with shortened body parts, loose joints, and weak muscles and muscle reflexes. About 40% develop heart defects and they are more susceptible to infections, both of which often cause their lives to be shorter. On the other hand, they are usually affectionate, cheerful people, often deriving great pleasure from music and dancing.

Down syndrome is an example of autosomal trisomy as a non-sex chromosome is added (Figure 3.18). It is the most common autosomal trisomy in humans. Its incidence increases with increasing age of the mother (Table 3.5). Older men are also more likely to father a Down syndrome child but their age has less of an effect on the chances of having a Down syndrome baby than does the age of the mother.

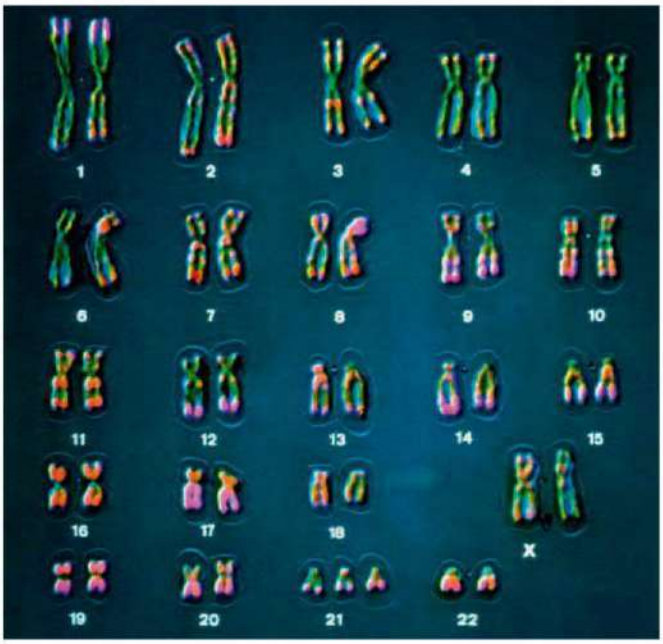


Figure 3.18 ▶ False-colour karyotype from a Down syndrome female. The syndrome is the result of there being three copies of chromosome number 21. This condition is also know as Trisomy 21.

Table 3.5 The risk of a child being born with Down syndrome increases with the age of the mother.

Maternal age at birth of child (years)	Risk of child having Down syndrome
20	1 in 1925
25	1 in 1205
30	1 in 885
35	1 in 365
40	1 in 110
45	1 in 32
50	1 in 12

Non-disjunction also causes various sex chromosome abnormalities in humans. For example, approximately two in every thousand men have the genetic constitution XXY, which is known as Klinefelter syndrome. This may result either from the fusion of a Y sperm with an XX egg or from the fusion of an XY sperm with an X egg. Although XXY individuals are phenotypically

men, they have very small genitals and are infertile. In addition, they may develop breasts, but testosterone therapy at puberty can often help alleviate the symptoms.

Turner syndrome is due to the absence of one of the sex chromosomes. Foetuses with 22 normal pairs of autosomes and a single Y chromosome never survive to birth. However, children may be born with 22 normal pairs of autosomes and a single X chromosome. Such individuals have the genetic constitution XO. They are females and occur with an incidence of approximately 0.4 per thousand live-born girls. The phenotypic effects of Turner syndrome are relatively minor but the person is infertile. Individuals are usually shorter than normal with a characteristic webbed neck. Oestrogen replacement therapy can allow normal pubertal development and growth can be stimulated with growth hormone.

Genetic variation is also caused by changes in chromosome number, such as monoploidy, polyploidy or aneuploidy.

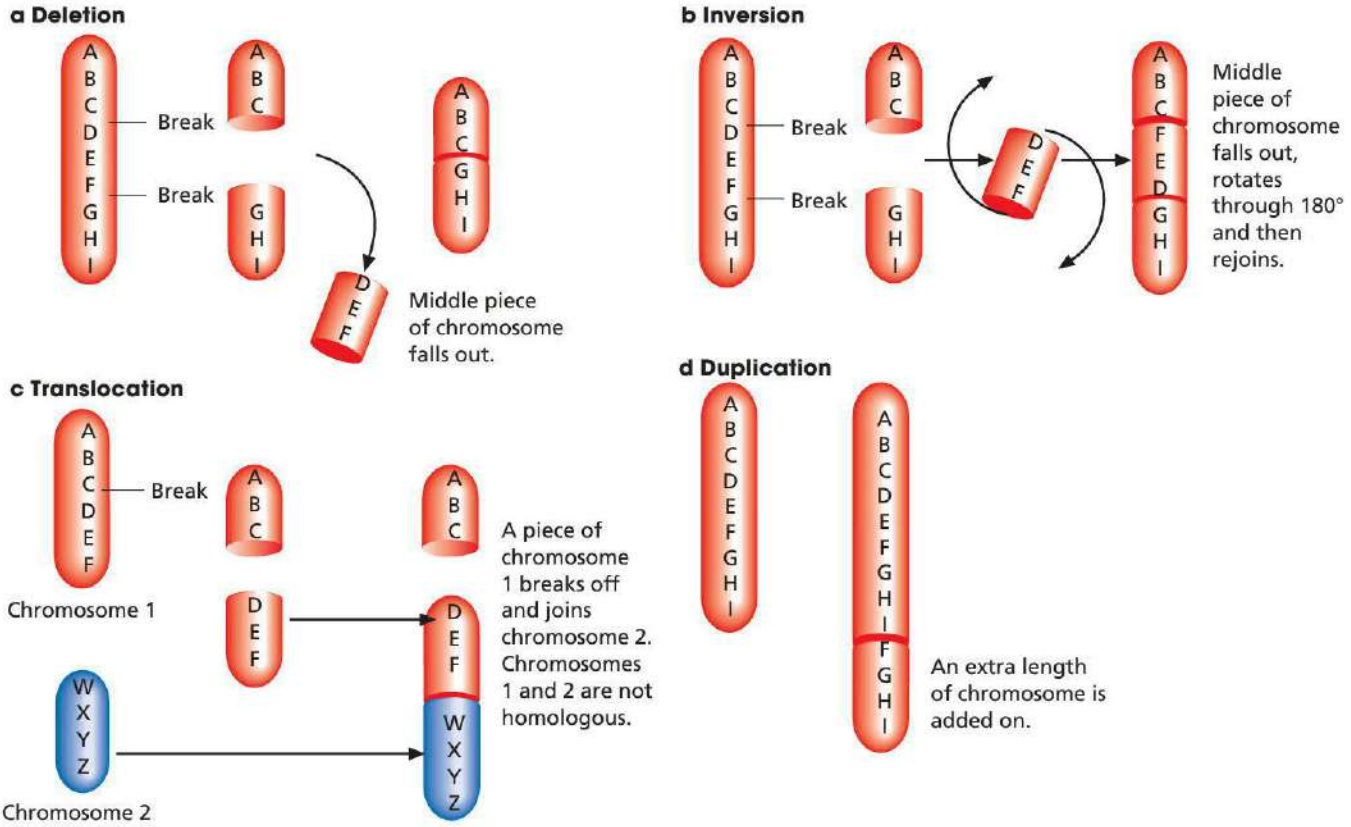
Variations in chromosome structure

Changes to chromosome structure largely come about by the occurrence of two or more double-strand breaks in chromosomes and the rearrangement of the broken segments of chromosomes. Some of these breaks occur naturally during meiosis as the chromosomes entangle around one another, cross over and move apart. Others occur because of exposure to mutagens, which accelerate the rates of double-strand breaks. The breaks are normally repaired but occasionally mistakes are made in the way the segments are relocated in the repaired chromosomes. There are several classes of these chromosomal rearrangements.

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 in between. This is called a chromosome deletion (Figure 3.19a) and, as it leads to an absence of certain genes, it can have a profound effect on the development of an organism. All but the shortest deletions are usually fatal and the few that survive are associated with adverse affects.

▼ **Figure 3.19**
Abnormalities caused by chromosomal mutations may arise by deletion, inversion, translocation or duplication.



The Image Works/Peter Hvizdak

Figure 3.20 ▲ A person with Williams syndrome is characterised by an 'elfin' facial appearance and low nasal ridge.

Williams syndrome is an example of a condition that arises because of a deletion event that affects about 1 in 10 000 people. Patients are characterised by certain physical (Figure 3.20) and temperamental features, including an unusually cheerful and affectionate disposition, and an exaggerated predilection for music and dance. The syndrome is associated with an unusual development of the nervous system, developmental delay and life-threatening cardiovascular problems. Williams syndrome is caused by a deletion of around 1.5 million nucleotide pairs from one copy of chromosome 7. The DNA segment carries between 15 and 25 genes of known and unknown function. The syndrome demonstrates that both copies of one or more of these genes are required for normal development.

Inversions

Another kind of chromosomal rearrangement occurs if a chromosome breaks in two places and the segment in the middle rotates through 180° before being re-joined within the chromosome, reversing the normal sequence of genes (Figure 3.19b). This 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. The inversion may, however, disrupt a gene through which it occurs or cause two different genes to become fused together. Also, if the chromosomes do not align properly for meiosis, the affected individual may have reduced fertility.

Translocations

Sometimes a section of one chromosome breaks off and re-attaches to another chromosome. This is known as translocation (Figure 3.19c). An example of translocation in humans is when a segment of chromosome 8 ends up with chromosome 14, or vice versa. 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 either into the same chromosome or into another chromosome (Figure 3.19d). Gene sequences can be replicated several to many times, 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 may be advantageous. The various genes that control the different haemoglobins produced in human red blood cells are thought to have arisen by duplications.

Changes to chromosome structure such as inversion, deletion, duplication and translocation of chromosome segments are another cause of genetic variation.

ACTIVITY 3.1

STARING MUTATIONS IN THE FACE: EXPLORING THE CHROMOSOMAL ANOMALIES OF THE DEVIL FACIAL TUMOUR DISEASE

As the world's largest living carnivorous marsupial, the Tasmanian devil (*Sarcophilus harrisii*) is an Australian icon. Since the mid-1990s, however, a sizeable proportion of the wild population has succumbed to a rare and typically fatal form of transmissible cancer called the devil facial tumour disease (DFTD, Figure 3.21).

The cancer is spread between animals when they bite each other, often during courtship. Analysis of DFTD demonstrated that the tumours have essentially the same karyotype even when isolated from genetically different animals in different locations. Transmission relies on the recipient of the DFTD failing to mount an immune response against the foreign DFTD cells. The lack of resistance is largely because Tasmanian devils have essentially no genetic diversity in the immune molecules that would normally distinguish between individuals in other mammal species.


Corbis/Dave Watts

▲ **Figure 3.21**
A Tasmanian devil infected with DFTD

The cancer that causes DFTD is caused by an infectious pathogen; the fact that it can be transmitted is considered rare in relation to cancer. For more information on DFTD and how scientists are working to control the spread of the disease, see Chapter 10, page 295.

Aim

To construct and compare two karyotypes for the Tasmanian devil, one for healthy cells and the other for cells taken from the DFTD and suggest what mutations may have occurred to give rise to DFTD

You will need

- pencil
- scissors
- glue
- blank sheet of paper
- photocopy of the chromosome images in Figure 3.22.

What to do

Start with the chromosomes from the healthy cells.

1 Cut out each chromosome and assemble into a karyotype by pairing the chromosomes from biggest to smallest.

2 Orientate the sheet of paper in landscape format. Secure the karyotype by gluing the chromosome pairs onto the top half of the paper, leaving a space of approximately 10 cm on the right hand side. The biggest chromosomes should be on the left, the smallest on the right.

3 Label the chromosome pairs by numbering the biggest pair '1' and continuing to label sequentially down to the second smallest pair. The two smallest chromosomes are the sex chromosomes, so label them accordingly.

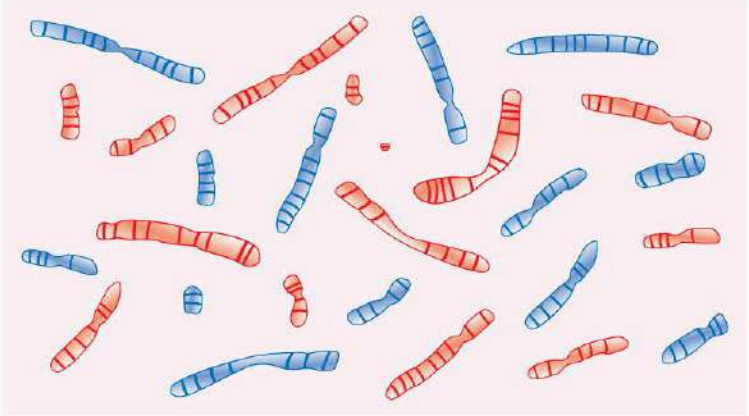
Now work on the chromosomes from the DFTD cells.

4 Cut out each chromosome from the DFTD and pair matching chromosomes. Leave aside any that cannot be paired.

5 Compare the paired chromosomes from the DFTD with those in the karyotype of the healthy cells. Arrange the paired chromosomes from the DFTD underneath the corresponding chromosomes from the healthy cells and glue them onto the paper.

6 Compare the unpaired chromosomes from the DFTD with those of the healthy cells. If any DFTD chromosomes are comparable to those in the healthy karyotype, glue them onto the paper underneath the corresponding chromosomes.

7 You will be left with unpaired chromosomes from the DFTD that cannot be identified from the healthy karyotype. Arrange these from largest to smallest and glue them onto the page in the space to the right hand side of the DFTD chromosomes.



▲ **Figure 3.22**
Chromosomes from a healthy cell (red) and a DFTD cell from a Tasmanian devil (blue)

8 Label the identifiable DFTD chromosomes, including any unpaired chromosomes, with the same labels as for the healthy cells. Label the remaining DFTD chromosomes to the right-hand side 'M1', 'M2' etc.

What did you discover?

1 What features of the chromosomes did you use to match corresponding pairs?
2 What is the diploid and haploid number for the healthy cells?
3 What is the sex of the animal the healthy cells were taken from? How do you know?
4 What differences are there between the karyotypes of the healthy cells and the DFTD cells?
5 Discuss what changes might have occurred to the chromosomes of the Tasmanian devil to give rise to DFTD.

QUESTION SET 3.4

Remembering

1 Define 'karyotype'.
2 Describe the relationships between:
   a haploid and diploid.
   b monoploid and haploid.
   c monoploid, diploid and polyploid.
   d diploid and aneuploid.
3 Describe four types of structural rearrangement that result in chromosomal abnormalities.

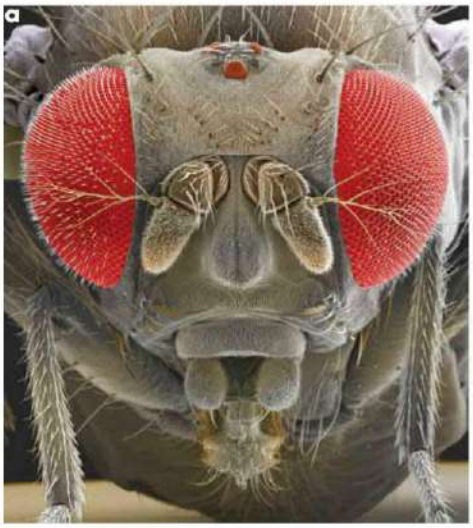
Understanding

4 Draw an annotated diagram of a diploid cell with four chromosomes undergoing meiosis and show two ways that non-disjunction can occur. Indicate the kind of chromosome anomalies that can arise in a zygote formed by fertilisation with each of the resulting gametes and one normal gamete.
5 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.
6 Discuss why parental age might be a factor in the increasing incidence of mutation in the offspring.
7 Defend or refute the statement, 'Aneuploidy is always deleterious' and explain your reasoning.

Variation in gene expression for growth and development

Understanding how genes regulate growth and development is a long-standing and still active area of research. Early clues to the influence of individual genes on growth and development came with late 19th and early 20th century observations of naturally occurring **mutants**, such as fruit flies that developed well-formed legs in place of their antennae (Figure 3.23). Research in the field accelerated with the application of mutagenesis experiments in the middle to late 20th century. It continues to unfold today with ever more sophisticated biochemical methods for analysing genes and the availability of whole genome sequences for organisms.

These studies have demonstrated how a single mutation can dramatically alter the structural development of an organism. The altered features in the mutants often are recognisable body structures located in the wrong position. These observations have provided insights into how different



Science Source/Andrew Syred

Visuals Unlimited/Science VU/Dr F Rudolph Turner

◀ **Figure 3.23**
a) A normal fruit fly (*Drosophilia melanogaster*) and b) a fruit fly showing abnormal development of legs at the sites where antennae should be

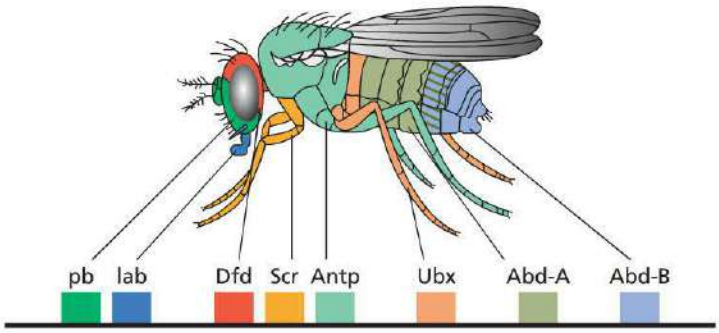
body parts are controlled by similar genetic processes. Furthermore, studies of model organisms have shown that the same sorts of processes are at work in most eukaryotic organisms, including humans.

Homeobox genes

It turns out that embryonic development is regulated by a small subset of genes. For example, of the 13 000 genes in the genome of a fruit fly, as few as eight key genes are responsible for determining its fundamental body structure. A few dozen more are involved in determining the body's axes of symmetry when viewed from the front, rear, top or bottom, or when left and right sides are compared. These genes are referred to as **homeobox genes**, or toolkit genes. Each one of the toolkit genes is expressed precisely in particular parts of the embryo during development (Figure 3.24). Two questions arise from this. First, what causes these genes to be expressed where they are? Second, what do the corresponding proteins do once they are expressed?

HOMEOBOX GENES

View the video about the role of homeobox genes in evolution.



◀ **Figure 3.24**
Eight genes regulate the formation of the fruit fly body plan. Each is expressed in a precise location within the animal during development.

To answer the first question, specific proteins are produced in the egg before fertilisation. These proteins (called maternal-effect proteins) diffuse across the egg, forming concentration gradients that convey positional information in the egg (Figure 3.25a). For example, the zone of highest concentration of one of these proteins, called Bicoid protein, identifies the future embryo's anterior (front) end. The lowest concentration of Bicoid protein will become the embryo's posterior (rear) end. Other such proteins create concentration gradients that mark positions within the egg (Figure 3.25b). These concentration gradients of the proteins are preserved during the early rounds of cell division after fertilisation so their positional information is retained by the daughter cells in the developing embryo. These proteins interact with non-coding regions immediately adjacent to the homeobox genes to either activate or repress gene expression. For example, high concentrations of Bicoid protein will activate homeobox genes that initiate development of the front half of the animal but repress homeobox genes that are required for development of the rear part of the animal. Each homeobox gene is therefore activated by specific combinations of positional proteins. The pattern of expression of

the homeobox genes ultimately reflects the concentrations of the positional proteins within the developing embryo as the fertilised egg undergoes cell division.

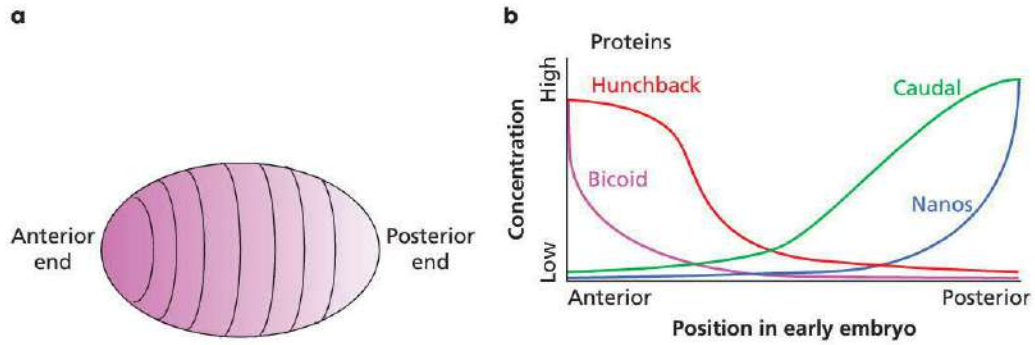


Figure 3.25 ▶ a) Bicoid protein in a fruit fly egg diffuses to create a concentration gradient. The highest concentration marks the future embryo's anterior end. b) Relative concentration of four proteins conveying positional information in the developing embryo

Once expressed, the product of each homeobox gene is a protein that binds to DNA to activate gene expression. These proteins activate expression of collections of many other genes within the genome. The genes that are expressed are those required for the formation of the intended organ or body part at that position within the developing embryo. These homeobox proteins are thus **regulatory proteins** with wide-ranging effect.

See Chapter 2 for more about gene regulation.

Determination for sex: the role of the Y chromosome

In mammals, gender is determined by the sex chromosomes. Typically, an individual with two X chromosomes is female, whereas an individual with one X and one Y chromosome is male. It's a straightforward observation, but how do the sex chromosomes affect development?

The Y chromosome clearly must have genes that set the organism on the path to developing into a male. The single most influential gene in this process is the sex determining region Y (SRY) gene, located on the p (small) arm of the Y chromosome. The gene encodes a protein that, like homeobox genes, binds to DNA to activate the expression of many genes. The SRY protein is a regulatory protein that primarily launches the genetic program for testes development. As the testes develop, they produce hormones, including testosterone, that steer the embryo towards the differentiation of male features, ultimately including formation of the penis and other components of the male reproductive system.

Dealing with the X factor: X chromosome inactivation

If the individual lacks a Y chromosome and is unaffected by the SRY gene, they begin the course towards developing into a female. Proper female development relies on the presence of both X chromosomes to direct the formation of fully functional ovaries.

The occurrence of two X chromosomes in every somatic cell presents its own issues, however. If both X chromosomes are active, twice the required concentrations of proteins are being produced inside each cell. This creates a biochemical imbalance that affects the normal functioning of the cell. To compensate, the cells shut down one of the X chromosomes. This is referred to as X chromosome inactivation. The inactivation is achieved by changes to the **chromatin** structure of the X chromosome. X chromosome inactivation occurs when the embryo comprises less than a hundred cells. In each cell, the inactivation of an X chromosome is initially random. The information to shut down the same X chromosome, with its suite of alleles, is transmitted to each daughter cell by subsequent rounds of mitosis. Any region of the body descended from one particular embryonic cell is influenced by gene expression from the one active X chromosome. The patchy colouring of female tortoiseshell cats is an example of random X chromosome inactivation in the embryo.

See Chapter 2 for more about chromatin structure and the role of histone proteins.

An organism's growth and development is governed by the activation of homeobox genes in the embryo.

QUESTION SET 3.5

Remembering

1 Define 'homeobox gene'.
2 Define the 'SRY gene'.
3 Describe the process of X chromosome inactivation.

Understanding

4 Draw an annotated diagram to show how homeobox genes are activated in the segment bearing the second pair of legs of the developing fruit fly embryo.
5 Draw an illustrated flow diagram that shows the sequence of events leading from random fertilisation of human gametes to sex determination in males and females.

CHAPTER SUMMARY

- Phenotypic variation:
  - can be described as morphological, biochemical, physiological or behavioural
  - is underpinned by genetic variation; phenotypes are determined to a large extent by the particular combination of alleles an individual possesses
  - is also affected to some extent by the environment.
- Sexual reproduction shuffles combinations of alleles in the offspring through:
  - random assortment of chromosomes and crossing over of homologous chromosomes in meiosis I during gamete formation
  - fertilisation of random gametes.
- Gene mutations are a source of novel alleles. Mutations arise through the action of:
  - cell division, owing to spontaneous mutations
  - the action of physical or chemical mutagens
  - horizontal gene transfer from one organism to another
  - transposable elements 'jumping' from within the genome.
- Gene mutations can be classified as:
  - substitutions, which are a source of SNPs
  - insertions or deletions (indels)
  - synonymous, missense, nonsense or frameshift mutations, depending upon the effect they have on the protein the gene codes for
  - neutral, harmful or beneficial, depending upon the effect they have on the survival of the organism.
- Chromosomal anomalies can be observed by examining a karyotype and may arise by:
  - non-disjunction of chromosomes during meiosis, leading to aneuploidy
  - changes in chromosome number, classified as aneuploidy, monoploidy or polyploidy
  - rearrangements of chromosome structure, including deletions, inversions, translocations and duplications.
- Variations in gene expression direct embryonic development. The variation is coordinated by a small number of genes. These genes code for proteins that activate expression of a large number of other genes required for particular developmental programs. These genes include:
  - homeobox genes, which direct the development and organisation of the organism's body plan
  - the SRY gene of the Y chromosome, which directs development of male features in mammals.

- X chromosome inactivation restores the proper concentration of proteins within cells of females, and the process leads to further variation in gene expression.

CHAPTER GLOSSARY

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

aneuploidy describes a genome that varies from the conventional by the loss or addition of one or just a few chromosomes

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cell by various enzymes including caspases

behaviour responses and reactions of an organism in particular situations

beneficial mutation a mutation that increases an organism's chances of survival and reproduction

chromatin a complex of proteins and DNA in eukaryotic chromosomes

cloning vector in cloning, the DNA molecule that is used to carry the cloned piece of DNA

codon a series of three adjacent nucleotide bases in mRNA; each codon specifies a particular amino acid to be added to a polypeptide; a stop codon indicates the termination of the polypeptide chain

cognition mental processes relating to sensing, perceiving, thinking and remembering

crossing over an event during meiosis in which homologous chromosomes exchange segments with one another

deleterious mutation a mutation that decreases an organism's chances of survival and reproduction

deletion mutation a mutation in which nucleotide pairs have been lost from a segment of DNA

diploid (2*n*) describes a cell or organism that has a genome comprising two copies of each chromosome, represented by 2*n*

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

fertilisation the union of male and female gametes

frameshift mutation a mutation that dislocates the translational reading frame

genetic code a system by which each combination of three DNA nucleotides in a gene sequence determines a specific amino acid in the protein

genome all of the genetic material contained in an organism or a cell; includes the chromosomes within the nucleus and the DNA in mitochondria and chloroplasts

genotype a specific combination of alleles for a particular gene locus belonging to an individual or cell

germ-line the cell line in eukaryotic organisms from which the gametes are derived

haploid (*n*) describes a cell or organism that has a genome that contains one copy of each chromosome, represented by *n*

homeobox gene a gene that codes for proteins that regulate body formation and patterning in the developing embryo

homologous chromosomes a pair of chromosomes that have the same size, shape and genes at the same locations

horizontal gene transfer a 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

intraspecific variation differences between individuals of the same species

karyotype a display of the number and appearance of the chromosomes of an organism or cell observed at metaphase

meiosis a two-phase type of cellular division in which the chromosome number of a cell is halved to the haploid number; meiosis is the basis of gamete formation in animals and spore formation in plants

missense mutation a gene mutation that results in one amino acid being replaced by another amino acid in the encoded protein

monoploid (1*n*) a cell or organism that has a functional genome consisting of one copy of each chromosome, represented by 1*n*

monosomy the condition in which somatic cells contain one copy of a particular chromosome

mutagen an agent capable of inducing mutations

mutant a cell or organism that bears a mutation

mutation a gene or chromosome that has undergone a change relative to the original gene or chromosome; it may also refer to the process of generating such changes

mutation rate the number of changes per gene copy in a population over a period of time

neutral mutation a mutation that has no effect on an organism's chances of survival and reproduction

non-coding DNA all of the DNA sequences within a genome that are not found within RNA-coding exons; examples include introns, promoters and enhancers of genes

non-disjunction the failure of sister chromatids in mitosis or homologs in meiosis to separate and go to opposite poles

nonsense mutation a mutation in which a codon for an amino acid is changed to one that codes for a stop codon, terminating translation

parthenogenesis the production of offspring from a female gamete without the requirement for fertilisation

plasmid a small circular piece of DNA, found in bacteria, which is able to replicate independently of the cell's chromosomes; plasmids carry antibiotic resistance markers

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

polyploidy a cell or organism with a genome comprising three or more copies of each chromosome, represented by 3*n*, 4*n*, 5*n*, 6*n* etc.

regulatory protein a protein that binds DNA to switch on or switch off expression of a gene

repetitive DNA DNA sequences that are present in very many copies in the genome, usually regarded as non-functional DNA

silent mutation see *synonymous mutation*

single nucleotide polymorphism (SNP) nucleotide difference that occurs at a given position in the genomes of two or more individuals

somatic a body cell that will not pass its genes onto 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

spontaneous mutation a mutation occurring in the absence of exposure to mutagens

substitution mutation a mutation in which a single nucleotide is swapped for another in the original gene sequence

synonymous 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 'silent' mutation

transposable element a piece of eukaryotic DNA that is capable of cutting itself out of one position in the genome and inserting itself into another position in the genome; also referred to as 'jumping genes'

trisomy the condition in which somatic cells contain three copies of a particular chromosome

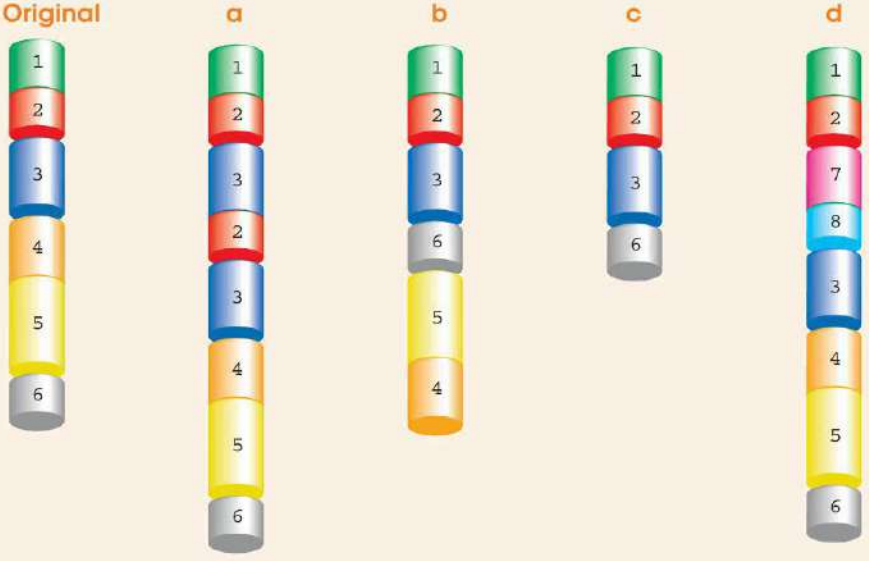
CHAPTER REVIEW QUESTIONS

Remembering

1 Identify which kind of phenotypic variation is represented by each of the following.
   - **a** You enjoy solving mathematical puzzles, whereas your friend is frustrated by them.
   - **b** The daily amount of milk produced by different individuals of a particular variety of cow (*Bos taurus*)
   - **c** The girth of mature Mountain Ash (*Eucalyptus regnans*) trees.
   - **d** Some cowpea (*Vigna unguiculata*) grow better in acidic soils than others.
   - **e** Presence or absence of a hydroxyl (OH) group on the anthocyanin pigment produced by different individuals of corn (*Zea mays*).

Understanding

2 The images below show segments of chromosome with genes numbered along their lengths. Identify the mutation that has occurred in each of these structural rearrangements from the original for each of **a** to **d**.



◀ **Figure 3.26**
Chromosomal mutations

3 Discuss the relationship between SNPs, substitutions, synonymous, missense and nonsense mutations.

Applying

4 What might account for the fact that most forms of aneuploidy are rarely, if ever, observed in humans?

5 A transposable element consisting of precisely 270 nucleotide pairs lands in the intron of a functional gene without affecting the splicing sites for the transcribed RNA.

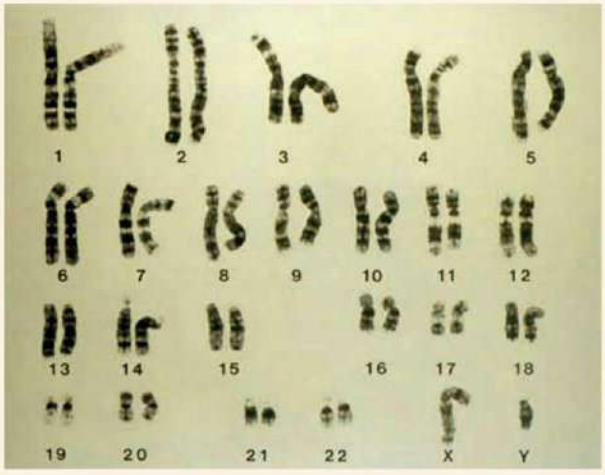
- a What kind of genetic mutation does this represent?
- b Describe what the effect would be on the protein sequence.
- c Would this likely be a neutral, beneficial, or deleterious mutation?

6 'Krüppel' is a protein whose concentration defines the posterior end of the developing embryo in the fruit fly *Drosophila melanogaster*. Draw a diagram of the developing embryo, label the anterior and posterior ends, and shade in the concentration gradient of the Krüppel protein. Determine which of the genes shown in Figure 3.24 is likely to be activated by the Krüppel protein and explain what the effect of that activation is likely to be.

7 Compare the four human karyotypes **i** to **iv** in Figure 3.27 below.

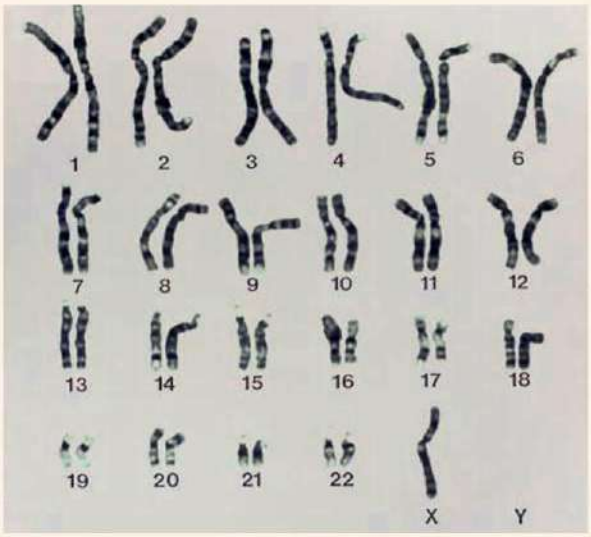
- a Determine which of the four is a normal karyotype and identify the sex of the individual.
- b Determine the aberration in each of the other three karyotypes.
- c Explain how the three aberrations could have come about.

i



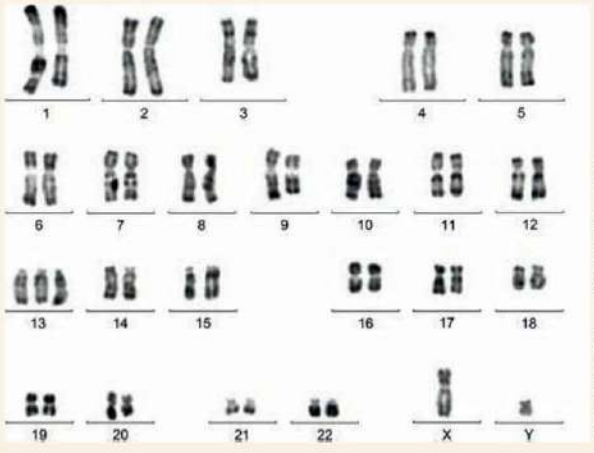
Science Photo Library/Dept of Clinical Cytogenetics, Addenbrookes Hospital

ii



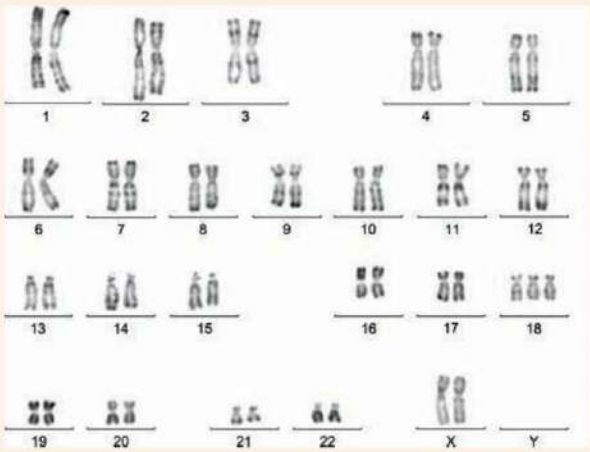
Science Photo Library/Dept of Clinical Cytogenetics, Addenbrookes Hospital

iii



Science Photo Library/ISM/Dr Philippe Vago

iv



Science Photo Library/ISM/Dr Philippe Vago

▲ **Figure 3.27**
Four human karyotypes

8 Copy and complete the following table using information provided in Table 3.6. Note that more than one type of mutation may describe the effect on the protein.

Genetic mutation	Amino acid	Type of genetic mutation	Effect on protein
GTCCCA ↓ GTCCCT	Valine-Proline ↓ Valine-Proline	Substitution	Synonymous
TCAATA ↓ TAATA	Serine-Lysine ↓		
AGAGGT ↓ AGATGT	Arginine-Glycine ↓		
GCAAGA ↓ GAAAGA	Alanine-Arginine ↓		
CAGTAC ↓ CACGTAC	Glutamine-Tyrosine ↓		

Table 3.6 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
Positively charged, hydrophilic	Aspartic acid	GAT, GAC,
	Glutamic acid	GAA, GAG
Negatively charged, hydrophilic	Histidine	CAT, CAC
	Lysine	AAA, AAG
	Arginine	CGT, CGC, CGA, CGG, AGA, AGG
	STOP	TAA, TAG, TGA

Analysing

9 List all the codons that could result from a synonymous mutation of GGG. What observation can you make about which of the three nucleotides in the codon is most prone to being mutated?

10 An exceptionally large plant with enlarged fruit grows among a natural population. Discuss what genetic change might have occurred in this individual and describe how you could test it to find out.

11 'X-ray imaging of a newborn child and a full grown adult are equally risky.' Consider whether you agree or disagree with the statement and explain your reasoning.

Evaluating

12 Polycyclic aromatic hydrocarbons are a diverse collection of compounds produced by combustion, such as in cigarette smoke. With reference to effects, mutations, mutation rates and level of exposure, provide an explanation for the statistical link between cigarette smoking and the increased incidence of lung cancer.

13 Trypsin and chymotrypsin are proteases (enzymes that digest proteins) with strikingly 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 same original gene.

14 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, and explain your reasoning. Discuss how, if at all, your interpretation of 'neutral', 'beneficial', and 'deleterious' is influenced by the individual's environment.

Reflecting

15 What are some of the potential mutagens you encounter in your daily life, and how might you reduce your exposure to some of them?

CHAPTER 4 MENDELIAN GENETICS

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

Science Understanding

- Frequencies of genotypes and phenotypes of offspring can be predicted using probability models, including Punnett squares and by taking into consideration patterns of inheritance, including the effects of dominant, autosomal and sex-linked alleles and multiple alleles, and polygenic inheritance **(ACSBL085)**

Getty Images/Time Life Pictures

Figure 4.1 ▲
Mendel discovered key principles of inheritance in his pea garden.

Can you roll your tongue into a U-shape? When you clasp your hands together, is your left or right thumb on top? Look at a classmate's ears. Are the lobes attached or free? These and many more of your characteristics are inherited from your parents. Because you have two sets of homologous chromosomes, one from each parent, you have two copies of the **genes** for each characteristic. If genes are expressed differently, alternatives are possible. Either you can roll your tongue or you cannot. Either your left thumb or your right thumb is on top when you clasp your hands together. Either your ear lobe is attached or it is free.

To study patterns of **inheritance**, we will turn back the clock to examine the innovative experiments and observations of an Austrian monk, Gregor Johann Mendel. Unfortunately, the significance of his painstaking work was not realised during his lifetime. Today his contribution is recognised by the term used to describe the patterns of inheritance he discovered: Mendelian genetics.

From peas to clues: interactions between alleles

The principles of **heredity** and patterns of inheritance were first established by Gregor Mendel (1822–84) in the 19th century. Gregor Mendel (Figure 4.1) was unique in his time. He spent 2 years studying mathematics at the University of Vienna. He was also somewhat of an expert in agricultural practices, being a member of the regional agricultural society and reading the available literature to keep up-to-date with developments in breeding experiments. In about 1856, Mendel carried out a number of breeding experiments on pea plants in the garden at his monastery. The conclusions he drew from his studies form the foundations on which the study of heredity is built.

Mendel's peas

In the early stages of his work, Mendel studied the inheritance of seven pairs of contrasting characteristics in pea plants. These included variations such as yellow or green pea pods, round or wrinkled seeds and tall-stemmed or dwarf-stemmed plants. Pea plants were ideal for his work: the characteristics, or variables, had no intermediate forms, pea plants self-pollinated and therefore self-fertilised, and the characteristics that he studied were largely unaffected by environmental factors.

In one such experiment, he took a **purebreeding** tall pea plant and crossed it with a purebreeding short pea plant. Purebreeding plants are ones that, when crossed among themselves, always give rise to offspring that are like the parents. The way Mendel crossed the plants was to take pollen grains containing sperm cells from the anthers of one plant and dust them onto the stigma of another plant, having first removed the anthers of this second plant to ensure that it could not self-pollinate (Figure 4.2).

Figure 4.2 ▼
Hand pollination involves taking the pollen from the anther of one plant and dusting it on to the stigma of another plant after removal of the second plant's anthers to ensure no self-pollination occurs.

Alamy/Edward Parker

Mendel collected the seeds that resulted from the crosses between the tall pea plants and the short ones, and sowed these. He found that the seeds, once they had germinated and grown into adult plants, always developed into tall offspring (Figure 4.4). This was the case whether pollen grains from tall plants were placed on to the stigmas of short plants, or pollen grains from short plants were placed on to the stigmas of tall plants. In these crosses, we refer to the original purebreeding parent plants as the **parental generation (P)** and the offspring belong to what we call the **first filial generation (F_1)**.

Mendel then took the tall F_1 plants and self-pollinated each of them, again taking precautions to prevent them being pollinated by any other kind of pollen. The resulting seeds were sown and the offspring, belonging to the **second filial generation (F_2)**, were examined. Mendel found that some of these F_2 plants were tall and

some were short. Overall, he counted 1064 plants. Of these, 787 (74%) were tall and 277 (26%) were short. It seemed as though approximately three-quarters of the F_2 generation were tall and one-quarter were short. In other words, the ratio of tall to short plants was approximately 3:1.

The relationship between genes and traits

What conclusions can we draw from Mendel's results? The first striking fact to notice is that in the F_1 and F_2 generations there are no medium-sized plants; that is, there are no plants that are intermediate between the tall and the short parents. From this, we conclude that inheritance is not necessarily a process in which features of two parents blend together to produce an intermediate result, like the mixing of black and white paints to produce grey. Rather, definite factors, which may or may not show themselves in the outward appearance of the organism, pass from parents to offspring. That such factors exist is borne out by the observation that, despite its absence in the F_1 generation, the short form reappears in the F_2 generation.

The second conclusion is drawn from the observation that there were no short plants in the F_1 generation, despite the fact that one of the parent plants was short. Short plants reappeared, however, in the F_2 generation. From this we can conclude that, although the F_1 plants are tall, they must receive a factor for shortness from their short parent, which remains 'hidden' in the F_1 plants and does not reveal its presence until the F_2 generation.

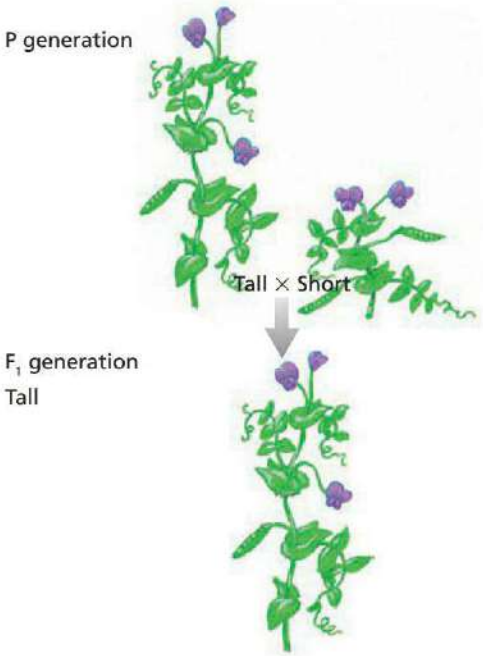
A third conclusion is that the factor for shortness, which fails to show itself in the F_1 generation, must be masked in some way by the factor for tallness. Only in the absence of this factor will the factor for shortness show itself in the outward appearance of the plant. In other words, the factor for tallness is **dominant** to the factor for shortness. Shortness is described as **recessive**.

Although Mendel knew nothing of chromosomes and genes, he suggested that the factors he described pass from parents to offspring via the gametes. If we are right in assuming that the F_1 plants contain factors for shortness as well as for tallness, it is reasonable to suppose that each F_1 plant receives one factor for tallness from its tall parent and one factor for shortness from its short parent via the gametes. That is, the gametes contain only one of the two factors for size, while the plants to which these gametes give rise contain a pair of such factors.

▼ **Figure 4.3**
Tall and short pea plants: two different phenotypes for plant height

Visuals Unlimited/Nigel Cattlin

▼ **Figure 4.4**
Mendel found that tall pea plants crossed with short pea plants always gave rise to tall pea plants.



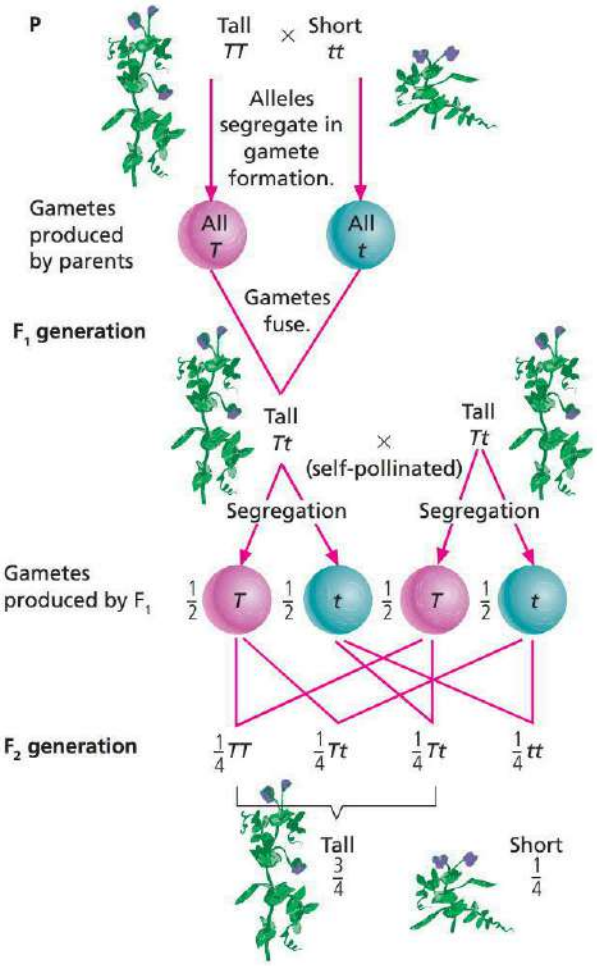


Figure 4.5 ▲
Summary of what happens when a purebred tall pea plant is crossed with a purebred short pea plant. Gametes are circled. This is an example of a monohybrid cross.

Figure 4.5 shows a summary of what Mendel discovered. Instead of 'factors' we use the term gene. As Mendel observed, the gene controlling height in the pea plant exists in two forms, which we now call **alleles**. One allele functions in a certain way and is responsible for producing a tall plant. The other influences development in such a way that, if two copies of this allele are present together, a short plant is produced.

In Figure 4.5 the allele for tallness is represented by *T*, and the allele for shortness by *t*. We shall assume that each parent plant (or, more strictly, each somatic cell of each parent plant) contains a pair of identical alleles: *TT* in the case of the tall parent, *tt* in the case of the short parent. When an organism contains identical alleles like this it is said to be **homozygous**. In making this statement, we are describing the genetic make-up of the parent plants, or at least the part of it that determines the size of the plant. The genetic composition of an organism is known as its **genotype**. In essence, the genotype describes the alleles that a cell or organism has at a particular gene locus for a particular **trait**. The way genes are expressed in the outward appearance of the organism is known as its **phenotype** (Figure 4.3). In the case of the parent generation of pea plants described earlier, plant height is the phenotype. Pea plants with the 'tall' phenotype have the genotype *TT*. The pea plants with the 'short' phenotype have the genotype *tt*.

The *T* allele is present in each of the gametes produced by the tall parent and the *t* allele is present in each of the gametes produced by the short parent. Fertilisation brings the *T* and *t* alleles together, so that all the F_1 offspring have the genotype *Tt*. Phenotypically they are all tall, as tallness is dominant to shortness. When an organism contains two dissimilar alleles, it is said to be **heterozygous**. In this particular instance, the *T* allele expresses itself in the phenotype and the expression of the *t* allele is being masked by the expression of the *T* allele. A dominant phenotype is expressed whether it occurs in the homozygous or the heterozygous condition. However, a recessive trait is only expressed when in the homozygous condition.

Science Photo Library/Adrian Thomas

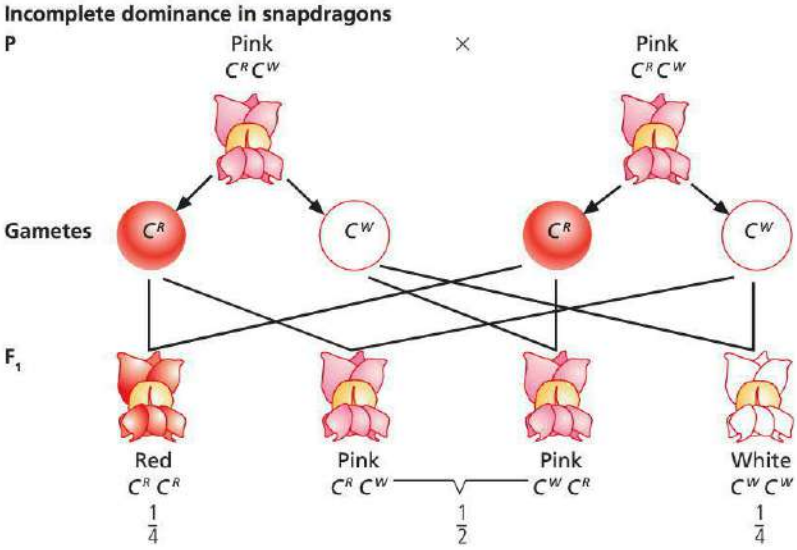
Figure 4.6 ▲
In purebreeding snapdragons, incomplete dominance of the red flower colour and white flower colour results in a pink flower colour.

Dominance is not always clear cut

Mendel's experiments were based on characteristics that were not only determined by genes found on different chromosomes but all showed one phenotype dominant over another phenotype. However, the expression of genes is not always this straightforward. Phenotypic variations can result when the characteristics of offspring show both parental characteristics or are a combination of parental characteristics.

If a purebreeding red snapdragon plant is crossed with a purebreeding white snapdragon plant, as shown in Figure 4.6, the F_1 offspring all have pink flowers. When these F_1 pink snapdragons are crossed, the F_2 offspring have flowers in the ratio of 1 red : 2 pink : 1 white. This is known as **incomplete dominance** or **partial dominance** because one trait is not fully dominant over its partner and the heterozygous phenotype (pink) is intermediate between the homozygous parental phenotypes (red and white).

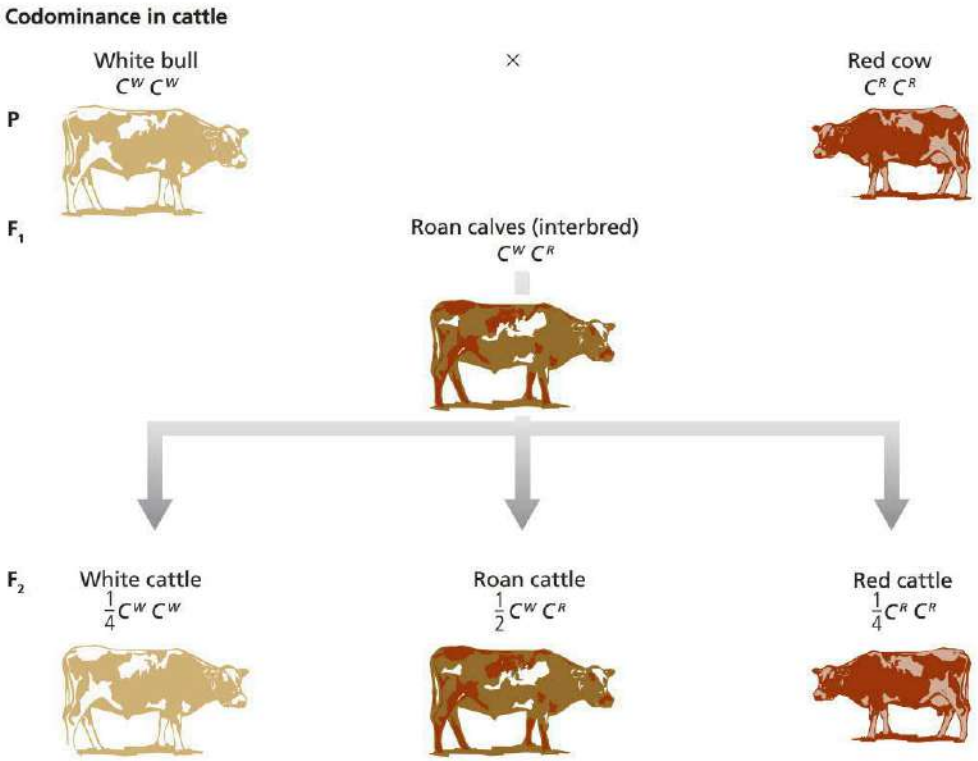
A special notation is used to indicate inheritance of partially dominant traits. A suitable upper-case letter designates the gene for the trait (e.g. *C* for colour) and upper-case superscript letters indicate the alleles (e.g. C^R = red colour, C^W = white colour). Figure 4.7 shows a diagram using the appropriate notation to describe inheritance of colour in snapdragons.



◀ **Figure 4.7**
Diagram showing the result of crossing snapdragons with pink flowers among themselves. A phenotypic ratio of approximately 1 red : 2 pink : 1 white results.

The study of certain coat colours in horses and cattle reveals another type of dominance relationship. In this case, both alleles in the genotype are fully expressed in the heterozygote. Such traits are said to be **codominant**. In shorthorn cattle, alleles for coat colour are inherited in this way and the two alleles are expressed as red (C^R) and white (C^W). This is similar to the incomplete dominance shown in snapdragons but, in this case, the offspring of purebreeding red and white parents have roan coats (C^WC^R), which are a mixture of red and white hair. The codominant inheritance of coat colour in shorthorn cattle is shown in Figure 4.8. An outline of the differences between the types of dominance relationships is given in Table 4.1.

An organism's phenotype is largely due the alleles that comprise its genotype. The genotype may be homozygous or heterozygous, and the alleles interact to express phenotypes that are dominant, recessive, partially dominant or codominant.



◀ **Figure 4.8**
In shorthorn cattle, codominant inheritance results in a roan coat colour in the offspring of pure-breeding red and white parents.

Table 4.1 Differences between the types of dominance relationships

	Complete dominance	Incomplete (partial) dominance	Codominance
Parents	*BB*, *bb*	C^BC^B, C^WC^W	C^BC^B, C^WC^W
Gametes	(B) (b)	(C^B) (C^W)	(C^B) (C^W)
F_1 genotype	*Bb*	C^BC^W	C^BC^W
Phenotype	Black	Grey	Black and white patches
Gametes	(B) (b) × (B) (b)	(C^B) (C^W) × (C^B) (C^W)	(C^B) (C^W) × (C^B) (C^W)
F_2 genotype	*BB*, *Bb*, *bb*	C^BC^B, C^BC^W, C^WC^W	C^BC^B, C^BC^W, C^WC^W
Phenotype	Black White	Black Grey White	Black Black and white patches White
Heterozygote	Same as dominant	Intermediate between homozygous parents	Properties of both homozygous parents

Note: *B* and C^B = alleles for black coat colour; *b* and C^W = alleles for white coat colour.

QUESTION SET 4.1

Remembering

1 Describe 'purebreeding'.
2 Distinguish between a gene and an allele.
3 Describe 'genotype' and 'phenotype'.
4 Define the P, F_1 and F_2 generations.
5 Define 'heterozygous' and 'homozygous'.

Understanding

6 How many different genotypes are possible for the tall and short traits of Mendel's peas? List them and classify them according to whether they are homozygous or heterozygous and whether they result in a dominant or recessive phenotype.
7 The petal colour of carnations is determined by a single gene with two alleles: one for white, one for red. Describe the phenotypes you would expect in the F_1 generation of two purebreeding parents, one white, one red in the following circumstances.
   a White is dominant to red.
   b The two traits are codominant.
   c The two traits are partially dominant.

Inheritance of a single autosomal gene

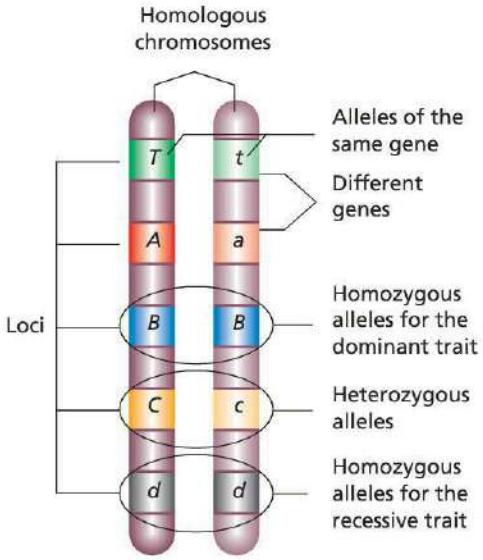
Mendel's studies at the University of Vienna helped him to explain the outcome of breeding experiments between tall and short pea plants in mathematical terms. The 3:1 ratio he predicted in the F_2 generation was based on observations of many different crosses using a variety of pea plant characteristics (Table 4.2). Mendel could not explain why he observed this ratio because he had no knowledge of meiosis.

Table 4.2 Results of some of Mendel's crosses on the garden pea (*Pisium sativum*)

Purebreeding parental phenotypes	F_1 phenotypes	F_2 phenotypes	F^2 ratio
Tall plants × short plants	All tall	787 tall, 277 short	2.84:1
Purple flowers × white flowers	All purple	705 purple, 224 white	3.15:1
Green pods × yellow pods	All green	428 green, 152 yellow	2.82:1
Yellow peas × green peas	All yellow	6022 yellow, 2001 green	3.01:1
Round peas × wrinkled peas	All round	5474 round, 1850 wrinkled	2.96:1

We can relate the behaviour of chromosomes at meiosis to the 3:1 ratio obtained. In meiosis, homologous chromosomes separate from each other, so that haploid gametes receive only one of each type of chromosome instead of the two chromosomes present in diploid cells. In diploid cells, alleles occur in pairs, one of each pair being located on one of two homologous chromosomes (Figure 4.9). When homologous chromosomes separate in meiosis, the alleles are separated and thus each gamete receives only one of a pair of alleles: just as they only receive one of a pair of homologous chromosomes (Figure 4.10). Historically, it was the striking similarity between the segregation of Mendel's factors in inheritance and the separation of homologous chromosomes in meiosis, as observed under the light microscope, that provided evidence that genes are carried on chromosomes.

Inheritance for alleles of a single autosomal gene can be analysed using a monohybrid cross. If the P generation is purebreeding, the proportion of dominant to recessive alleles in the F_2 generation is typically 3:1.



▲ **Figure 4.9** Homologous chromosomes contain alleles in pairs. This diagram shows the various combinations of alleles possible.

Equipped with an understanding of meiosis, the outcomes of several different types of crosses can be predicted. We will now explore how this is done, beginning with the simplest type of cross, the **monohybrid cross**.

Taking it one gene at a time: the monohybrid cross

A monohybrid cross is a cross that involves one pair of contrasting phenotypes. The cross between the tall and short pea plants studied by Mendel is an example of a monohybrid cross. The parental generation had the genotypes *TT* (tall) and *tt* (short). Segregation of the alleles into the gametes can be explained in terms of meiosis. If homologous chromosomes contain *TT* or *tt* alleles, each gamete produced after meiosis must contain only a *T* (from a *TT* parent) or a *t* (from a *tt* parent). If the parent has one homologous chromosome containing a *T* and

its pair containing a *t* (*Tt*), then half of the gametes will be expected to contain a *T* and the other half a *t*. When these gametes fuse at fertilisation, the offspring will have two homologous chromosomes, one from each parent. One way of showing how this comes about is illustrated in Figure 4.11.

Figure 4.10 ▶ The segregation of alleles in inheritance corresponds to the segregation of homologous chromosomes in meiosis.

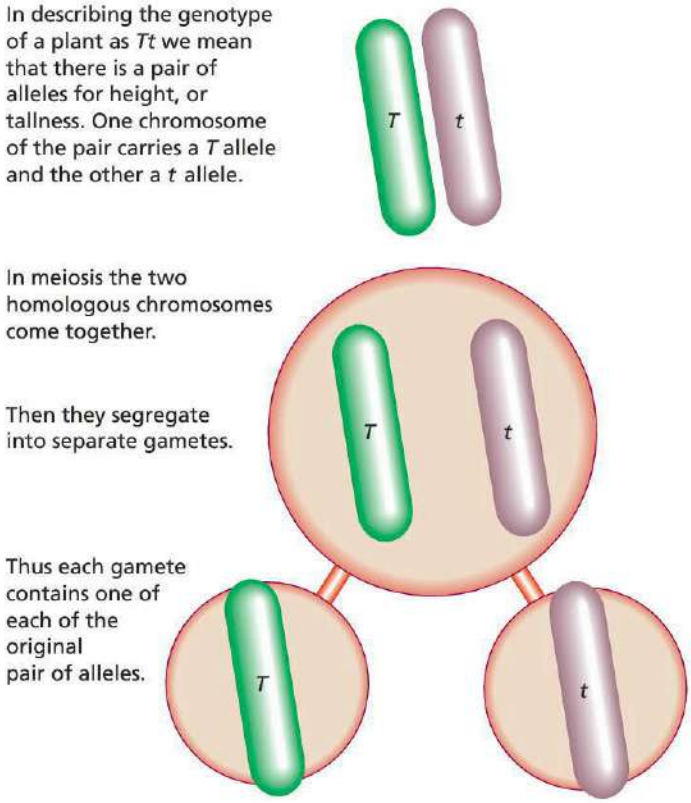
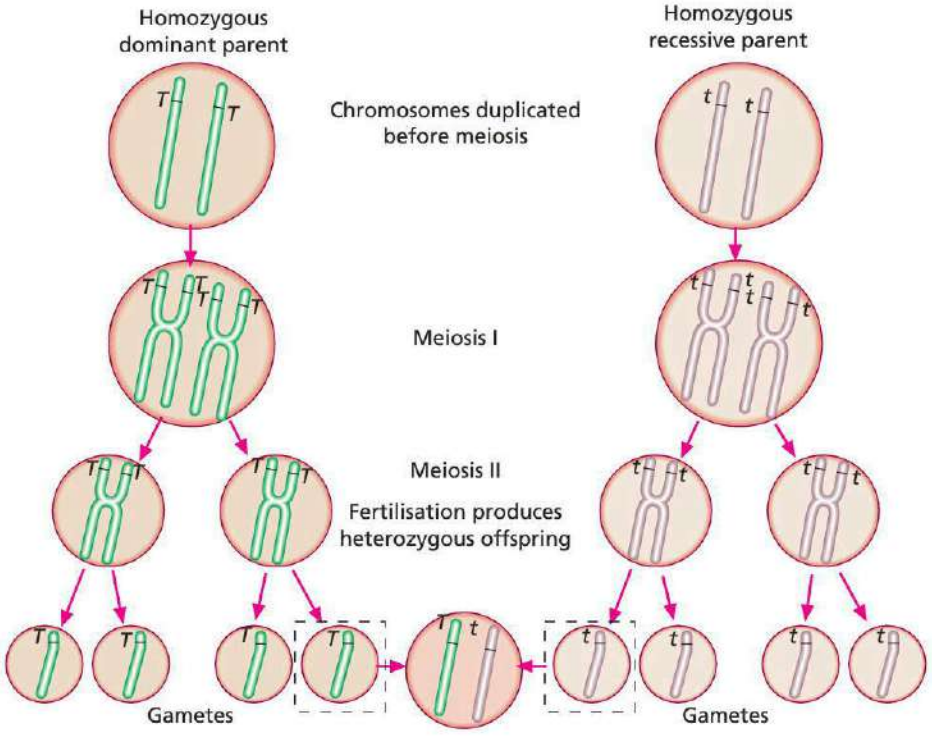


Figure 4.11 ▶ The segregation of chromosomes in a monohybrid cross. Two homozygous parents with different phenotypes can only produce heterozygous offspring.



The most straightforward way of showing how this arises is with a **Punnett square**, conceived by Sir Reginald Punnett. In a Punnett square, the alleles in the gametes of one parent are written along the top of a series of boxes and the alleles in the gametes of the other parent are written down the side. The products of the various fusions are written in the appropriate boxes and their relative numbers can be estimated (Worked example 4.1).

WORKED EXAMPLE 4.1

A pea plant that is purebred for green pea pods is crossed with a pea plant purebred for yellow pods (Figure 4.12). Their offspring all have green pods. If two of the F_1 generation plants are crossed, work through the stages to predict the proportion of the F_2 generation that will also produce green pea pods.

a Assign the alleles. (1 mark)

b Draw the Punnett square and enter the female and male gametes. (1 mark)

c Determine the genotypes of all the possible offspring. (2 marks)

d Determine the proportions of the phenotypes of the offspring. (2 marks)

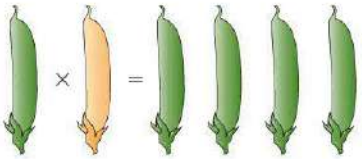


Figure 4.12
A purebreeding pea plant with green pods is crossed with a purebreeding pea plant with yellow pods, giving offspring that all have green pods.

Answer

a G = green pods
g = yellow pods

Logic

If the plants of the parental generation are purebreeding, each must be homozygous for their respective trait. 1 mark

As both the parents are homozygous, all the offspring must be heterozygous (Figure 4.11) and they carry one allele for the green phenotype and one allele for the yellow phenotype.

All the heterozygous F_1 plants have green pods, proving that the green phenotype is dominant.

Choose a capital letter to represent the allele for the dominant phenotype. In this case, G is appropriate for 'green'.

The corresponding lower case letter is used to represent the allele for the recessive phenotype. In this case, g is appropriate for 'yellow'.

b

		Female gametes	
		$\frac{1}{2}G$	$\frac{1}{2}g$
Male gametes	$\frac{1}{2}G$		
	$\frac{1}{2}g$		

All the F_1 plants have the genotype Gg. The alleles segregate on their homologous chromosomes during meiosis (Figure 4.10), so half their gametes acquire the G allele and the other half acquire the g allele. 1 mark

In effect, the top row and left column display the haploid gametes.

c

		Female gametes	
		$\frac{1}{2}G$	$\frac{1}{2}g$
Male gametes	$\frac{1}{2}G$	$\frac{1}{4}GG$	$\frac{1}{4}Gg$
	$\frac{1}{2}g$	$\frac{1}{4}Gg$	$\frac{1}{4}gg$

All the remaining squares of the Punnett square represent the product of the fertilisation events of each of the gametes. These will be filled with the genotypes of the diploid offspring. 2 marks

Start with the empty top left square. This square is the intersection of the female column headed $\frac{1}{2}G$ and male row headed $\frac{1}{2}G$. As half the female gametes possess a *G* allele and half the male gametes possess a *G* allele, the proportion of offspring inheriting these alleles together is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$. The same logic applies to the alleles themselves: $G \times G = GG$. The empty square must therefore be filled with the product of the two gametes: $\frac{1}{2}G \times \frac{1}{2}G = \frac{1}{4}GG$.

The next square to the right is the intersection of the female column headed $\frac{1}{2}g$ and male row headed $\frac{1}{2}G$. The empty square must be filled with the product of these two gametes: $\frac{1}{2}g \times \frac{1}{2}G = \frac{1}{4}Gg$.

Note: By convention, the allele for the dominant phenotype is always written first.

Continue through each square until the Punnett square is complete.

d Three-quarters, or 75%, of the F_2 offspring are predicted to be green.

Each genotype with at least one *G* allele must express the dominant phenotype. The Punnett square shows that three of the four squares for the offspring have at least one *G* allele. 2 marks

In other words, $\frac{1}{4}GG + \frac{1}{4}Gg + \frac{1}{4}Gg$, a total of $\frac{3}{4}$ of the offspring will show the dominant phenotype, green. This solves the original problem.

Only the *gg* genotype will express the recessive phenotype. The Punnett square shows that just one of the four squares for the offspring has this genotype. In other words, $\frac{1}{4}$ of the offspring will show the recessive phenotype, yellow.

Try these yourself

Using the alleles *T* and *t*, draw Punnett squares to show the ratio of tall to short plants among the offspring from the following crosses.

1. A purebreeding tall pea plant crossed with a purebreeding short pea plant, yielding all tall plants.
2. A cross between two pea plants of the F_1 generation from the parental cross in question 1.

Accepting the chances: probability in genetic ratios

It is important to clarify the statement that three-quarters of the F_2 generation will be tall and one-quarter short. This is what is predicted based on probability and this outcome will most likely only be seen when there is a large number of F_2 individuals. Another way of putting it is to say that if an F_2 plant is selected at random, there is a 3 in 4 chance of it being tall and a 1 in 4 chance of it being short. In other words, the *probability* of it being tall is $\frac{3}{4}$, or 75%.

Now, even if we looked at a large number of the F_2 plants, we should not expect the ratio of tall to short individuals to be exactly 3:1. Rather, we would expect approximately three-quarters

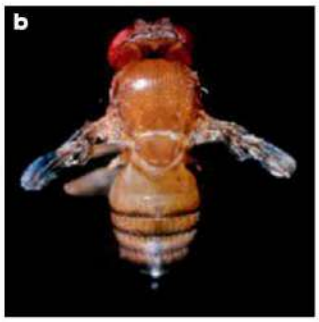
of the F_2 plants to be tall. It is true that, based on probability, half of the gametes (or potential gametes) should have *T* alleles and half *t* alleles. However, many of these gametes will fail to develop, die or fail to give rise to a zygote.

Another point is that even if exactly half of the gametes that gave rise to the F_2 plants had *T* alleles and exactly half had *t* alleles, the random fusion of gametes would mean that we could end up with all the offspring being heterozygous and so all being tall.

For all these reasons, the actual ratios obtained in genetic crosses only approximate to the expected ratios. However, the more individuals that are counted, the closer the observed ratios come to the expected ones. This is why Mendel looked at more than 1000 pea plants. Only then was he convinced that the ratios he observed were the ones he expected.

▼ **Figure 4.13**
The fruit fly *D. melanogaster* may have a) long wings or b) vestigial wings.

Science Source/Biology Pics

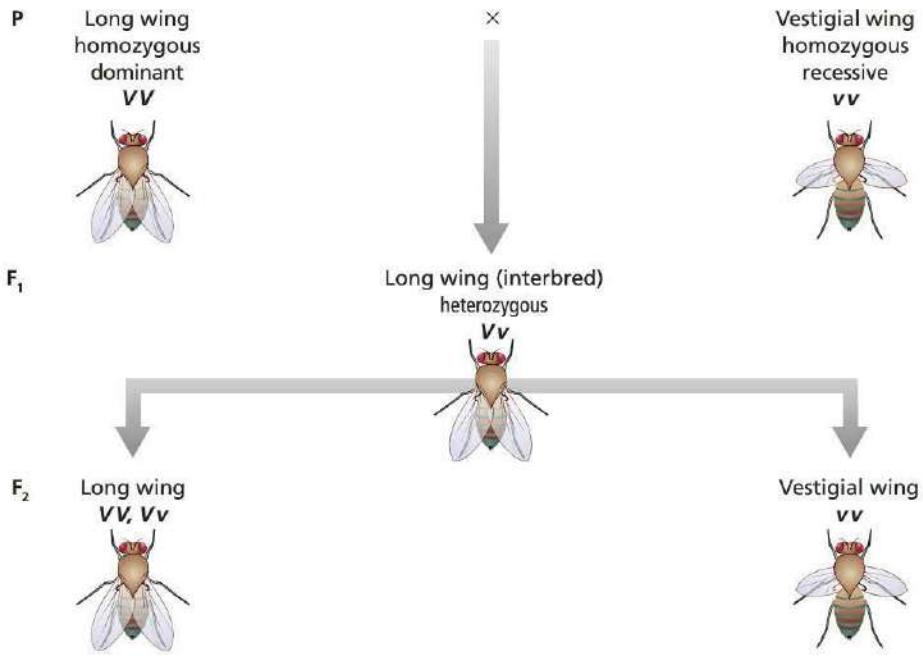


Science Source/Carolina Biological Supply Co.

The test cross

How do we determine whether an organism is homozygous dominant or heterozygous at a particular locus if the organism is incapable of self-fertilisation, which is the case for most animals? A technique used by geneticists is to cross the individual whose genotype is unknown with an individual that is homozygous recessive at the locus in question. This is called a **test cross**. We can illustrate this by reference to an animal that is used in many genetic experiments, the fruit fly *Drosophila melanogaster*.

D. melanogaster is known to exist in a large number of variants or forms. For instance, most individuals have red eyes but some have white eyes. Similarly, most individuals have long wings, but some have small or 'vestigial' wings (Figure 4.13). The long-winged condition (*V*) is dominant to vestigial wing (*v*). Accordingly, if a purebred long-winged fly (*VV*) is mated with a vestigial-winged fly (*vv*) the F_1 individuals are all heterozygous at this locus (*Vv*) and have long wings. If two of these F_1 flies mate with each other, a mixture of long-winged and vestigial-winged flies are produced in a ratio of approximately 3:1 (Figure 4.14).

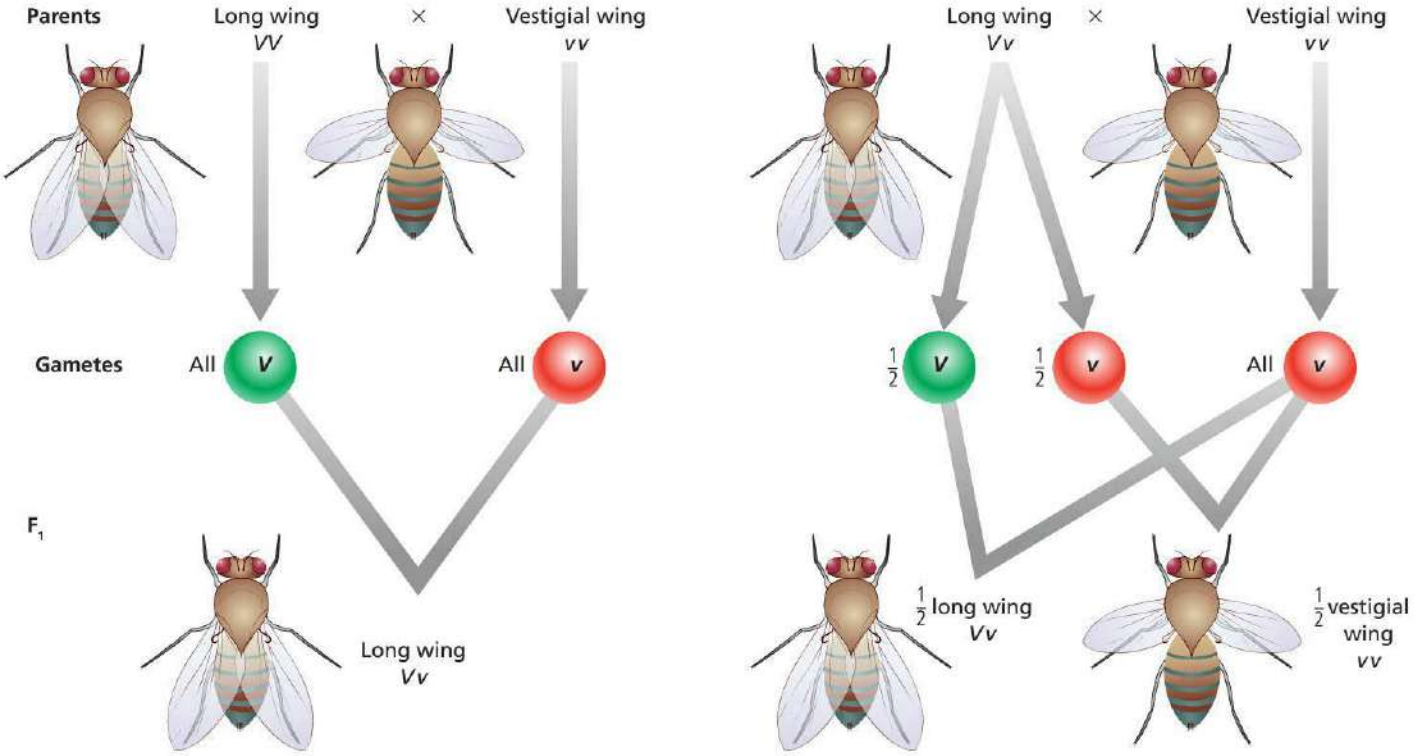


◀ **Figure 4.14**
Summary of a monohybrid cross in fruit flies

This is expected for a monohybrid cross. But how can we decide whether a given F_2 long-winged fly is homozygous dominant (*VV*) or heterozygous (*Vv*)? The simplest way is to cross it with a vestigial-winged fly. We know that a vestigial-winged fly must be *vv* (homozygous recessive); it cannot be anything else. If the long-winged fly whose genotype we wish to

Figure 4.15 ▼
A test cross to determine whether a fruit fly is homozygous dominant or heterozygous for long wings

determine is *VV*, then crossing it with a vestigial-winged fly will give nothing but long-winged flies. If, however, the unknown fly has the genotype *Vv*, then the cross will give a mixture of long and vestigial-winged flies in approximately equal numbers. This is summarised in Figure 4.15.



FRUIT FLIES IN THE LAB

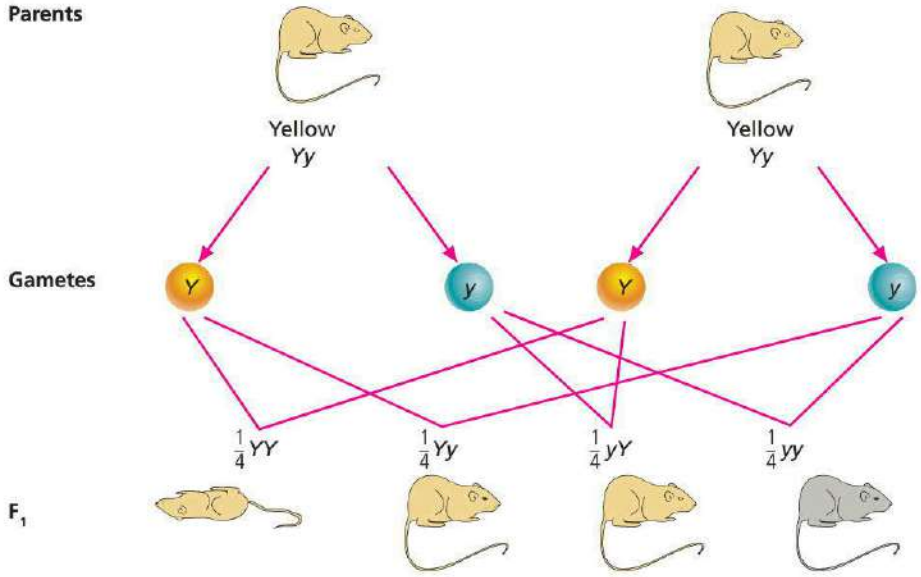
Order fruit flies with selected phenotypes, cross them and analyse the phenotypic ratios of the offspring to determine inheritance patterns.

Test crosses with individuals that are known to be homozygous recessive at the locus in question are a routine method of establishing an organism's genotype.

Deadly combinations and lethal phenotypes

Experiments looking at fur colour in mice demonstrate that yellow (*Y*) is dominant to grey (*y*). In a cross between two heterozygous yellow mice, offspring are produced in the following proportions: 23 yellow, 13 grey, a ratio of approximately 2:1. For a total of 36 offspring, the expected observation should have been approximately 27 yellow and 9 grey (3:1), so what is happening? One explanation is that mice homozygous for the *Y* allele die before birth. In other words, the genotype *YY* presents a **homozygous lethal phenotype**. This would explain why the proportion of yellow offspring is reduced (Figure 4.16). Alleles for lethal phenotypes are known to exist in a wide range of organisms.

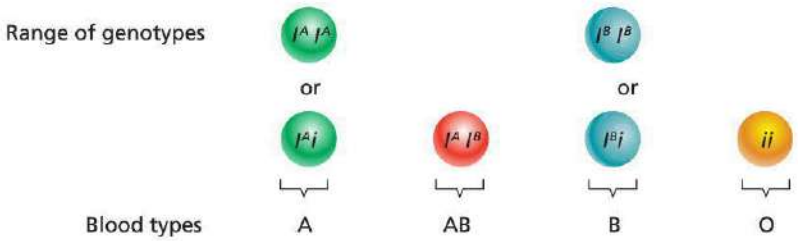
Certain dominant phenotypes are so important for normal development that an individual homozygous for a mutant recessive form of the trait cannot survive. The alleles for the mutant recessive phenotype can be perpetuated in the population by heterozygotes. In most cases, lethal phenotypes are recessive. Occasionally, though, the presence of an allele in a heterozygous genotype is sufficient to cause death. Such alleles are expressed as dominant lethal phenotypes. An example of a medical condition in humans caused by a dominant lethal allele is Huntington's disease, which is characterised by deterioration of the nervous system. Here, individuals with a single copy of the malfunctioning allele always develop the disease. Such dominant lethal phenotypes usually only persist in a population if they cause death after the reproductive age.



◀ **Figure 4.16**
Mice that are homozygous *YY* for fur colour die before birth, so a cross of two mice with the *Yy* genotype fails to produce the 3:1 ratio typical of monohybrid crosses.

From one to many: multiple alleles for one gene

Sometimes there are more than two types of alleles for a gene. In any one individual, of course, only two alleles are normally present. A multiple allele system is present when three or more alleles of a gene exist among the members of a population. An example of this is seen in the ABO blood group system in humans. In the human population, the phenotype expressed by allele I^A, which is codominant with I^B, produces molecular markers on red blood cells. The phenotype expressed by the third allele i is recessive to both I^A and I^B and produces no marker (O). Figure 4.17 summarises the range of genotypes and the resulting phenotypes (blood groups).



◀ **Figure 4.17**
Different blood types are found in humans due to combinations of multiple alleles.

The fact that there are more than two alleles responsible for determining the blood group makes no difference to their transmission, which takes place in a normal Mendelian fashion. Thus, a child whose parents are both blood group O must be blood group O. However, consider what happens when two people, one of whom is blood group A and the other blood group O, have children. The genotypes of the children depend on the genotype of the A parent. If he or she is homozygous with the genotype I^AI^A, the children can only have the genotype I^Ai and be blood group A. However, if the group A parent is heterozygous with the genotype I^Ai, each child has a 50% chance of being blood group A (I^Ai) or blood group O (ii).

QUESTION SET 4.2

Remembering

1 Define 'monohybrid cross'.

2 Describe the purpose of a test cross and how it is achieved.

Understanding

3 Explain how offspring may have a different genotype at a particular locus to either of their parents.

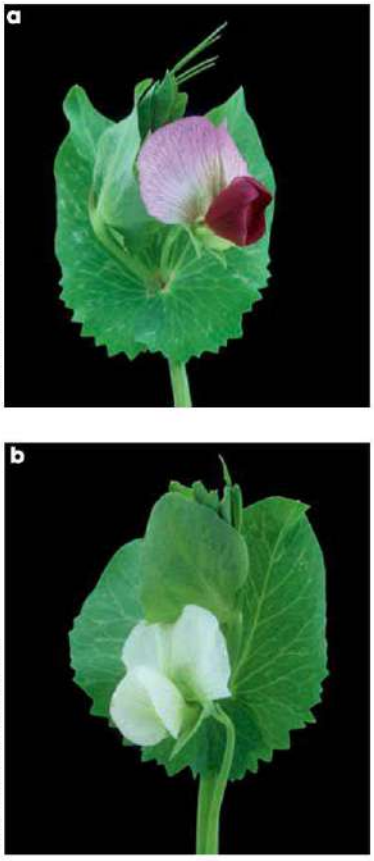
4 Draw a Punnett square to represent each of the crosses depicted in Figure 4.15.

Applying

5 Some strains of yeast can occur as either red or cream coloured cells. If a purebred red yeast is mated with a purebred cream yeast, the resulting offspring are cream. Assign the alleles for cell colour and use a Punnett square to predict the outcome of the cross between members of the F_1 generation.

6 'Xolo' is miniature breed of hairless dog. The hairless phenotype is dominant to hairy. Assign the alleles for hairless and hairy and use a Punnett square to demonstrate the expected phenotypes for a litter of four pups born to two heterozygous Xolo parents.

7 Following from Question 7 above, experimental data show that litters from Xolo crosses typically include hairless and hairy pups in the ratio of approximately 2:1 and hairless Xolo dogs are never purebreeding. What might account for this observation?



Visuals Unlimited/Wally Eberhart

Visuals Unlimited/Wally Eberhart

Figure 4.18 ▲
Pea plant flowers may be a) purple or b) white.

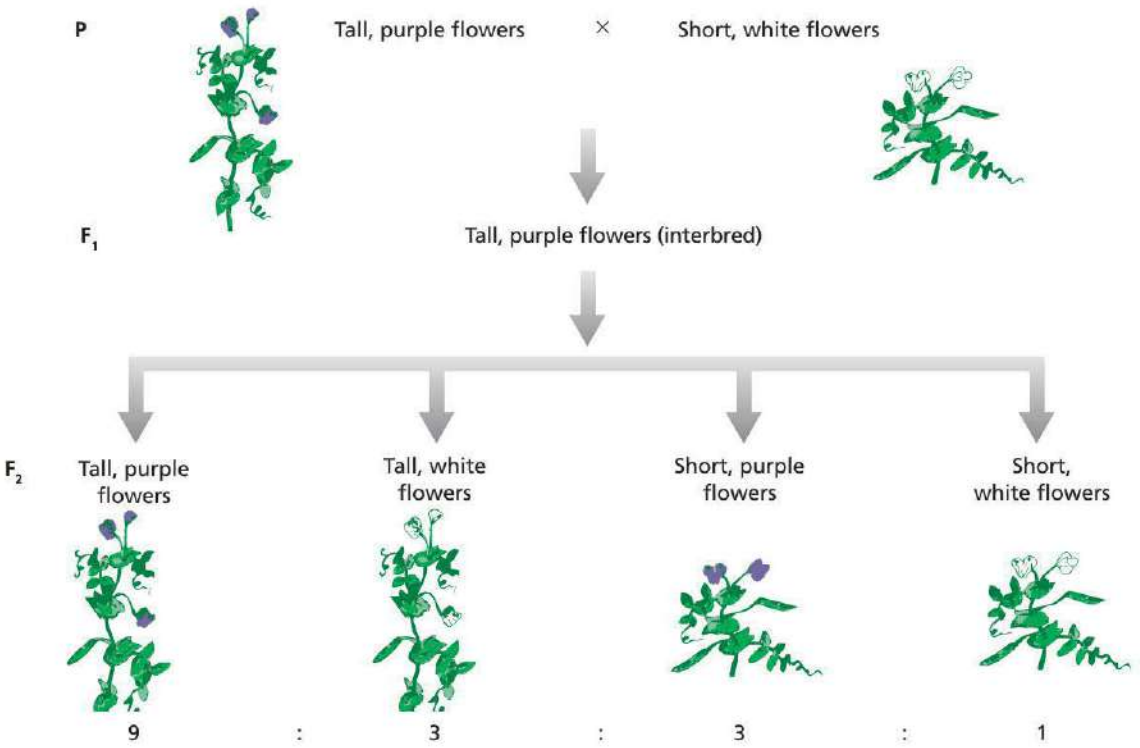
Inheritance of multiple autosomal genes

So far, we have considered the inheritance of only one pair of contrasting characteristics. However, Mendel also studied **dihybrid inheritance**, which is the inheritance of two pairs of contrasting characteristics.

Slipping into a pair of genes: the dihybrid cross

In one experiment, Mendel crossed a purebred tall pea plant possessing purple flowers with a short plant possessing white flowers (Figure 4.18). In the F_1 generation, all the plants produced were tall and had purple flowers. These were then self-pollinated. In the F_2 generation, there were four different phenotypes: tall plants with purple flowers; tall plants with white flowers; short plants with purple flowers; and short plants with white flowers. In other words, the offspring showed the two pairs of characteristics (tall, short; purple, white) combined in every possible way.

As before, Mendel counted the different types of plants and found 96 tall purple plants, 31 tall white plants, 34 short purple plants and 11 short white plants, giving a ratio of approximately 9:3:3:1. The experiment is summarised in Figure 4.19. What conclusions can be drawn from these results? First, the observation that all the F_1 plants are tall with purple flowers confirms that tall is dominant to short and purple flower is dominant to white flower. This is as expected from the results of the monohybrid crosses.



▲ Figure 4.19
Summary of Mendel's dihybrid cross

Figure 4.20 shows how the alleles are transmitted in this **dihybrid cross**. *T* represents the allele for tallness, *t* for shortness, *P* for purple flowers and *p* for white flowers. Mendel always started his experiments with purebred plants, so the parent plants must be homozygous for both genes. The genotype of the tall plant with the purple flowers is, therefore, *TTPP*, and that of the short plant with white flowers is *ttpp*. From Mendel's earlier work, we know that the gametes produced by the parent plants are *TP* from the tall purple parent and *tp* from the short white parent. All the F_1 offspring will, therefore, have the genotype *TtPp*, heterozygous for both genes.

The next step in the argument is crucial. If all four possible combinations of characteristics are to show up in the F_2 generation, we must conclude (as Mendel did) that the F_1 plants produce four kinds of gamete: *TP*, *Tp*, *tP* and *tp*. The Punnett square in Figure 4.20 shows the different ways these gametes can fuse, together with the genotypes of the F_2 offspring. To be tall, the genotype of the plant must contain at least one *T* allele; to be purple, it must contain at least one *P* allele. From the Punnett square we can see that there are 16 possible combinations. Of these, 9 give tall purple plants, 3 tall white plants, 3 short purple plants and 1 short white plants. The observed 9:3:3:1 ratio can be accounted for if all the possible combinations occur with equal likelihood.

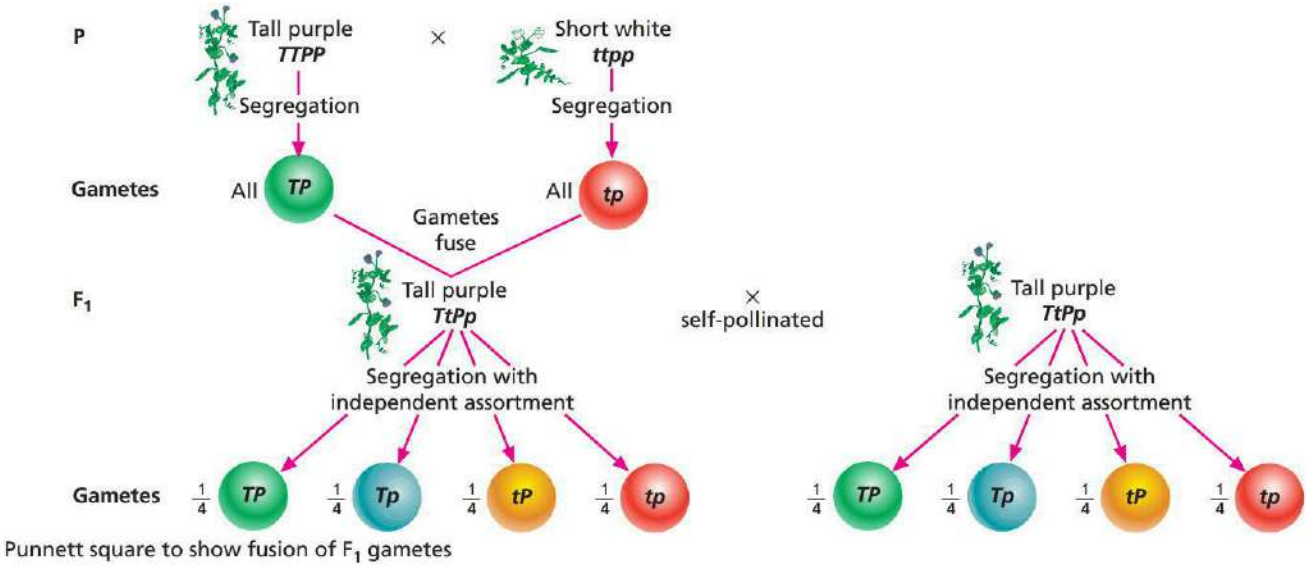
What general conclusion emerges from all this? The main one, surely, is that the alleles of the two genes are transmitted independently of each other from parents to offspring and, therefore, 'assort freely'. In other words, each of the alleles of one gene may combine with each of the alleles of another gene in equal probabilities. This is known as **independent assortment**.

By contrast, two alleles may be inherited together 80% of the time rather than 50% of the time. This is not independent assortment because these alleles are situated near each other on the same chromosome and are said to be **linked**.

When expressed in terms of probability, transmission of the genes determining stem height and flower colour in the garden pea are independent events. If we consider the alleles for height on their own, the probability of any one F_2 plant being tall is $\frac{3}{4}$ and of it being short is $\frac{1}{4}$. This is shown in Figure 4.21; $\frac{9}{16}$ of the plants are tall and have purple flowers and $\frac{3}{16}$ are tall and have white flowers. Combining these numbers, we can see that a total of $\frac{12}{16}$, that is, three-quarters, of the F_2 plants are tall.

Similarly, if we consider the flower colour alleles alone, the probability that an F_2 plant will be purple is $\frac{3}{4}$ and that it will be white is $\frac{1}{4}$. What, then, is the probability of an F_2 plant being both tall and purple? Assuming that the alleles are transmitted independently, and that the probability of being tall is $\frac{3}{4}$, the answer is $\frac{3}{4} \times \frac{3}{4} = \frac{9}{16}$. This means that the chance of any F_2 plant, chosen

at random, being both tall and purple is 9 out of 16, which is slightly more than 50%. It also means that in a large random sample of F_2 plants, approximately 9 out of 16 can be expected to be tall and purple.



Punnett square to show fusion of F_1 gametes

		Male gametes: 1/4 TP	1/4 Tp	1/4 tP	1/4 tp
Female gametes	1/4 TP	1/16 TTPP Tall purple	1/16 TTPp Tall purple	1/16 TtPP Tall purple	1/16 TtPp Tall purple
	1/4 Tp	1/16 TTPp Tall purple	1/16 TTpp Tall white	1/16 TtPp Tall purple	1/16 Ttpp Tall white
	1/4 tP	1/16 TtPP Tall purple	1/16 TtPp Tall purple	1/16 ttPP Short purple	1/16 ttPp Short purple
	1/4 tp	1/16 TtPp Tall purple	1/16 Ttpp Tall white	1/16 ttPp Short purple	1/16 ttpp Short white
	F_2	9/16 Tall purple	3/16 Tall white	3/16 Short purple	1/16 Short white

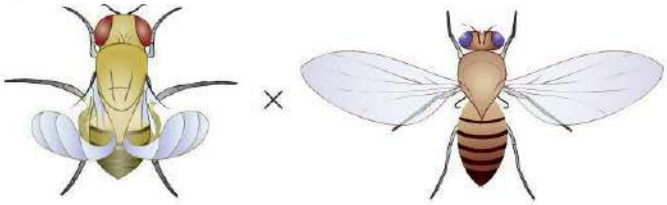
Figure 4.20 ▲ Dihybrid cross of a purebred tall pea plant with purple flowers with a short pea plant with white flowers.

We can apply similar reasoning to the other possible combinations of characteristics. The probability of an F_2 plant being tall and white is $\frac{3}{4} \times \frac{1}{4} = \frac{3}{16}$, short and purple is $\frac{1}{4} \times \frac{3}{4} = \frac{3}{16}$, and short and white is $\frac{1}{4} \times \frac{1}{4} = \frac{1}{16}$. These numbers agree with those obtained by the Punnett square method (Worked example 4.2) and with the results of Mendel's experiments (Figure 4.20). They can be explained by postulating that the two pairs of alleles are transmitted independently and assort freely.

Inheritance for two unlinked autosomal genes can be analysed with a dihybrid cross. If the P generation are purebreeding with respect to all traits, the F_2 generation typically shows a ratio of 9:3:3:1.

WORKED EXAMPLE 4.2

A purebred fruit fly that has curly wings and red eyes is crossed with a purebred fruit fly that has straight wings and purple eyes (Figure 4.21). Their offspring all have curly wings and red eyes. Two of the F_1 generation flies are crossed. If the alleles for wing shape and eye colour assort independently, predict the phenotypes of the F_2 generation and the proportions of each phenotype.



◀ **Figure 4.21**
Purebreeding fruit flies with curly wings and red eyes are crossed with purebreeding fruit flies with straight wings and purple eyes.

a Assign the alleles. (1 mark)

b Draw the Punnett square and enter the haploid gametes. (2 marks)

c Determine the genotypes of all the possible offspring. (4 marks)

d Determine the corresponding phenotypes for the genotypes. (4 marks)

Answer

a C = curly wing
c = straight wing
R = red eyes
r = purple eyes

b

		Female gametes			
		$\frac{1}{4}CR$	$\frac{1}{4}Cr$	$\frac{1}{4}cR$	$\frac{1}{4}cr$
Male gametes	$\frac{1}{4}CR$				
	$\frac{1}{4}Cr$				
	$\frac{1}{4}cR$				
	$\frac{1}{4}cr$				

c

		Female gametes			
		$\frac{1}{4}CR$	$\frac{1}{4}Cr$	$\frac{1}{4}cR$	$\frac{1}{4}cr$
Male gametes	$\frac{1}{4}CR$	$\frac{1}{16}CCRR$	$\frac{1}{16}CCRr$	$\frac{1}{16}CcRR$	$\frac{1}{16}CcRr$
	$\frac{1}{4}Cr$	$\frac{1}{16}CCRr$	$\frac{1}{16}CCrr$	$\frac{1}{16}CcRr$	$\frac{1}{16}Ccrr$
	$\frac{1}{4}cR$	$\frac{1}{16}CcRR$	$\frac{1}{16}CcRr$	$\frac{1}{16}ccRR$	$\frac{1}{16}ccRr$
	$\frac{1}{4}cr$	$\frac{1}{16}CcRr$	$\frac{1}{16}Ccrr$	$\frac{1}{16}ccRr$	$\frac{1}{16}ccrr$

Logic

The P generation flies are purebreeding, so they are homozygous for wing shape (curly or straight) and eye colour (red or purple). 1 mark

It follows that all the F_1 generation must be heterozygous with respect to both wing shape and eye colour.

All the F_1 generation have curly wings and red eyes. This indicates that curly wings are dominant to straight wings and red eyes are dominant to purple eyes.

The F_1 cross is between heterozygous fruit flies, that is $CcRr \times CcRr$. For each individual, half the gametes will receive the C allele and half will receive the c allele; half will receive the R allele, half will receive the r allele. 2 marks

The alleles for each trait (Cc and Rr) assort independently of one another, so four equally likely combinations of alleles are present in the gametes of the F_1 generation: $\frac{1}{4}CR$, $\frac{1}{4}Cr$, $\frac{1}{4}cR$ and $\frac{1}{4}cr$.

As with the monohybrid cross, each square is filled with the product of the intersecting female and male gametes. For example, $\frac{1}{16}CCRR$ is entered into the first vacant square, which represents the product of $\frac{1}{4}CR$ (female) and $\frac{1}{4}CR$ (male). The next vacant square to the right is filled with $\frac{1}{16}CCRr$, which is the product of $\frac{1}{4}Cr$ (female) and $\frac{1}{4}CR$ (male). Continue working through until all sixteen squares are filled. 4 marks

d

Male gametes \ Female gametes	$\frac{1}{4}CR$	$\frac{1}{4}Cr$	$\frac{1}{4}cR$	$\frac{1}{4}cr$
$\frac{1}{4}CR$	$\frac{1}{16}CCRR$	$\frac{1}{16}CCRr$	$\frac{1}{16}CcRR$	$\frac{1}{16}CcRr$
$\frac{1}{4}Cr$	$\frac{1}{16}CCRr$	$\frac{1}{16}CCrr$	$\frac{1}{16}CcRr$	$\frac{1}{16}Ccrr$
$\frac{1}{4}cR$	$\frac{1}{16}CcRR$	$\frac{1}{16}CcRr$	$\frac{1}{16}ccRR$	$\frac{1}{16}ccRr$
$\frac{1}{4}cr$	$\frac{1}{16}CcRr$	$\frac{1}{16}Ccrr$	$\frac{1}{16}ccRr$	$\frac{1}{16}ccrr$

The dihybrid cross gives a 9:3:3:1 ratio of fruit flies.

curly wings, red eyes : curly wings, purple eyes : straight wings, red eyes : straight wings, purple eyes

Nine of the 16 squares show offspring with at least one *C* and one *R* allele, and these offspring will have both dominant phenotypes, curly wings and red eyes. Three of the 16 squares show offspring with one *C* allele for curly wing shape and two *rr* alleles for purple eye colour.

Three of the 16 squares show offspring with two *cc* alleles for straight wings and at least one *R* allele for red eyes.

Just one of the 16 squares shows offspring with the genotype *ccrr* for both recessive phenotypes, straight wings and purple eyes. 4 marks

Try these yourself

1. A purebred fruit fly that has curly wings and long legs is crossed with a purebred fruit fly that has straight wings and short legs. Their offspring all have curly wings and long legs. Two of the F_1 generation flies are crossed. If the alleles for wing shape and leg length assort independently, predict the phenotypes of the F_2 generation and the proportions of each phenotype.
2. A purebred pea plant with yellow wrinkled peas was crossed with a purebred pea plant bearing green round peas. All the F_1 offspring have yellow round peas. If two of the F_1 pea plants are crossed, predict the possible combinations of pea phenotypes with respect to colour and shape and the proportions in which they are likely to occur.

Test crosses and dihybrid ratios

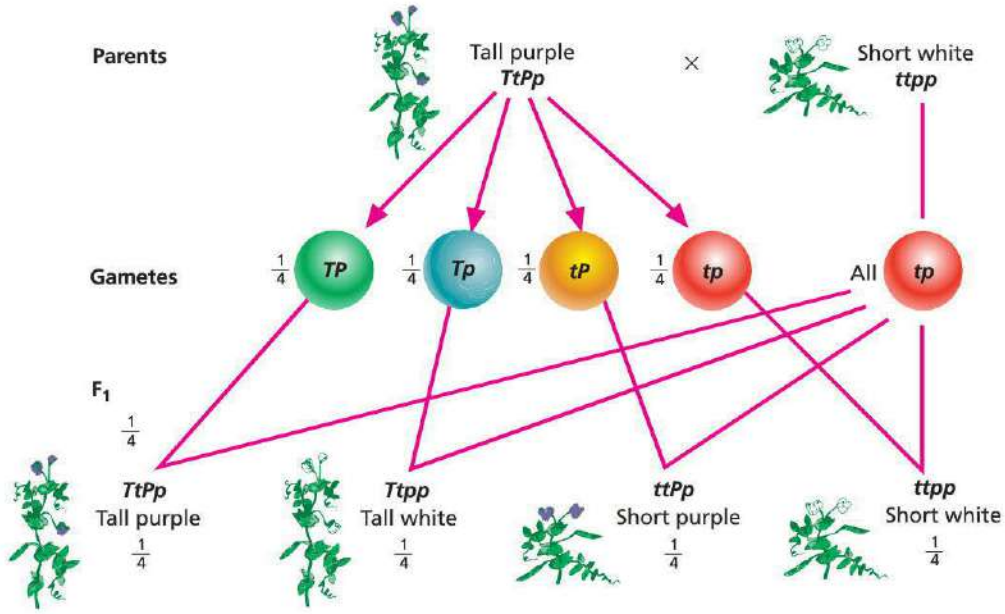
It is clear from the Punnett squares in Figure 4.20 and Worked example 4.2 that the same phenotype may result from several different genotypes. For example, a tall purple plant may have one of four possible genotypes: *TTPP* (homozygous for both genes), *TTPp* (homozygous tall, heterozygous for colour), *TtPP* (heterozygous for height, homozygous purple) or *TtPp* (heterozygous for both genes).

The easiest way of establishing the genotype of a tall purple plant is to cross it with a short white one (*ttpp*), that is, to perform a test cross. Thus, if the plant with the unknown genotype is homozygous for both characteristics, all the offspring from the test cross will be tall and purple.

Now consider the outcome if the unknown plant happens to be heterozygous for both genes. In this case, it will produce four types of gametes: *TP*, *Tp*, *tP* and *tp*. The short white plant, however, produces only one type of gamete: *tp*. The fusion of the gametes is shown in Figure 4.22, from which it is clear that four types of offspring should be produced in approximately equal numbers: tall purple, tall white, short purple and short white. Mendel carried out this experiment and this is precisely what he found; he obtained 47 tall purple, 40 tall white, 38 short purple and 41 short white plants.

There are two other possible genotypes for a tall purple plant: *TTPp* and *TtPP*. If *TTPp* was test crossed with a short white plant (*TTPp* × *ttpp*), then tall purple (*TtPp*) and tall white (*Ttpp*) plants would be expected in equal numbers. If *TtPP* was similarly test crossed with a short white plant (*TtPP* × *ttpp*), then tall purple (*TtPp*) and short purple (*ttPp*) plants would also be expected in equal numbers.

A test cross is performed to determine whether an unknown dominant genotype is homozygous or heterozygous by crossing it with a homozygous recessive genotype.



◀ **Figure 4.22**
In a test cross of a pea plant that is heterozygous for both height and flower colour with a homozygous recessive plant, four possible offspring may result.

Punnett square to show fusion of gametes in test cross

Gametes		1/4 TP	1/4 Tp	1/4 tP	1/4 tp
Gametes	tp	1/4 *TtPp* Tall purple	1/4 *Ttpp* Tall white	1/4 *ttPp* Short purple	1/4 *ttpp* Short white

Explanation of dihybrid ratios

The observation that characteristics such as height and flower colour are inherited independently of each other is known as independent assortment. The explanation lies in the behaviour of the chromosomes at meiosis, just as was the segregation of alleles that Mendel observed in his monohybrid crosses.

Independent assortment requires that the genes concerned be carried on different chromosomes; for example, the alleles of the gene for flower colour are located on one pair of chromosomes and the alleles of the gene for height on another pair of chromosomes (Figure 4.23). In metaphase I of meiosis, homologous chromosomes line up side by side on the spindle prior to separating at anaphase I. In doing this, different pairs of homologous chromosomes behave independently of each other; the way one pair of homologous chromosomes arranges itself on the spindle and subsequently separates has no affect whatsoever on the behaviour of any other pair of chromosomes.

The consequence of the independent behaviour of non-homologous chromosomes in meiosis is shown in Figure 4.23, which illustrates how the four different types of gametes (*TP*, *Tp*, *tP* and *tp*) can be formed from a plant that is heterozygous for height and flower colour (*TtPp*). We can summarise the situation by saying that the alleles for height and flower colour segregate and assort independently because they are carried on separate chromosomes, which themselves segregate and assort independently in meiosis.

All for one (and one for all): polygenic inheritance

So far, we have only dealt with cases where a characteristic is controlled by the alleles of one gene. Sometimes, however, a single characteristic is controlled by the alleles of two or more genes interacting with one another. A characteristic controlled by more than one gene is known as a polygenic characteristic, and its transmission is called **polygenic inheritance**.

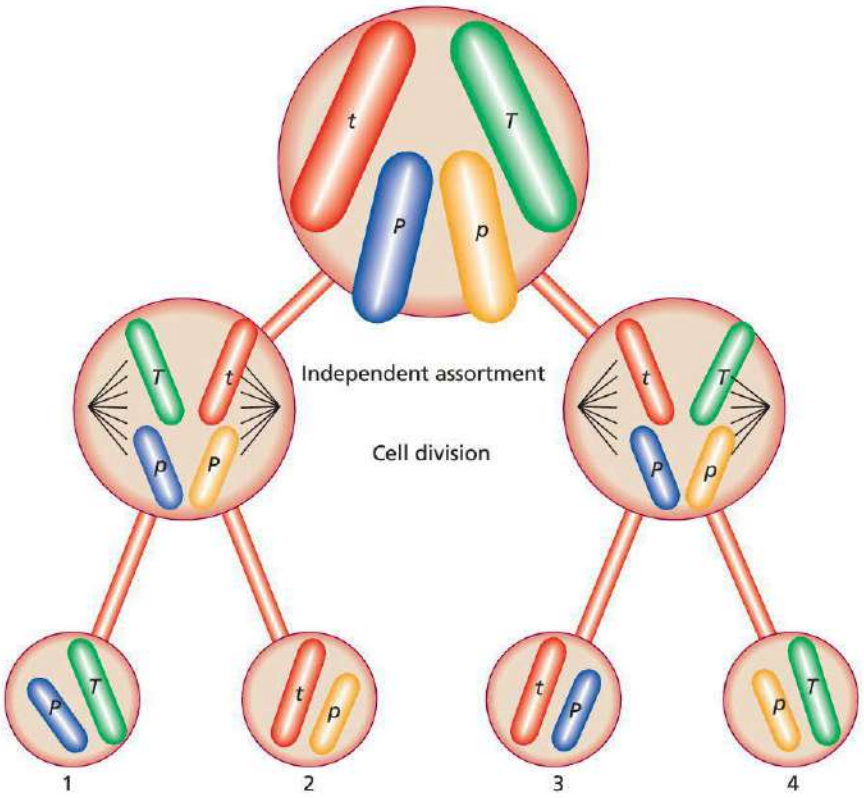


Figure 4.23 ▶ Meiosis provides the explanation for the independent assortment that Mendel found in his dihybrid crosses. The independent assortment of alleles in inheritance corresponds to the free assortment of chromosomes during meiosis.

An example of polygenic inheritance is human height. Unlike Mendel's pea plants, which were either tall or short, humans have a range of heights with a smooth gradation from one extreme to another. This can be seen when you line up for your school photographs each year. Whether it is a form or level group photograph, most students are different in height to others. According to the Australian Bureau of Statistics' Health Survey of 2011–12, the average adult height in Australia is 161.8 cm for women and 175.6 cm for men. In the last 40 years, many genes contributing to height have been identified by association with short stature or overgrowth. More recently, studies of the human genome have identified many more, with the total now exceeding 200 genes.

Clearly, height is a complex trait, the outcome of many genes, the majority of which have just a modest effect. Some genes, however, have a great influence. For example, short stature can be caused by mutations that abolish the contribution of a single gene, such as the gene encoding the growth hormone, GH1. On the other hand, because GH1 is produced by the pituitary gland in the brain, cancers of the pituitary gland can overstimulate production of GH1 leading to gigantism with patients attaining heights of up to 2.5 m.

See Chapter 9 to learn more about the action of hormones.

The condition of showing a range of phenotypes is called **continuous variation**. Traits that show continuous variation are controlled by two or more genes (**polygenes**). The greater the number of genes and the greater the influence of environmental factors (e.g. nutrition and the standard of medical care), the greater the expected distribution of all phenotypes for height.

Discontinuous variation occurs when only one gene is involved and results in a small number of phenotypes, such as pea plants with either purple flowers or white flowers, but no colours other than these.

EXPERIMENT 4.1

A DIHYBRID CROSS IN MAIZE

The popular edible grain, corn (*Zea mays*), also known as maize, is a member of the grass family (Poaceae). It was first domesticated in Central America and later transferred to Europe following Columbus' expeditions there in the late 15th century. Today, there are dozens of varieties of domesticated corn (Figure 4.24), which have been generated by **selective breeding**, which is also known as **artificial selection**. This approach to cultivating new varieties depends upon crossing individuals with desirable phenotypes.

Corn is a useful organism for investigating patterns of inheritance because the offspring of the same parents are represented as kernels on a single cob. This means that examining a single cob provides data on several hundred offspring of the same parents. Each kernel is made up of an embryo surrounded by nutritive tissue called the endosperm and an outer covering called the aleurone layer (Figure 4.25).

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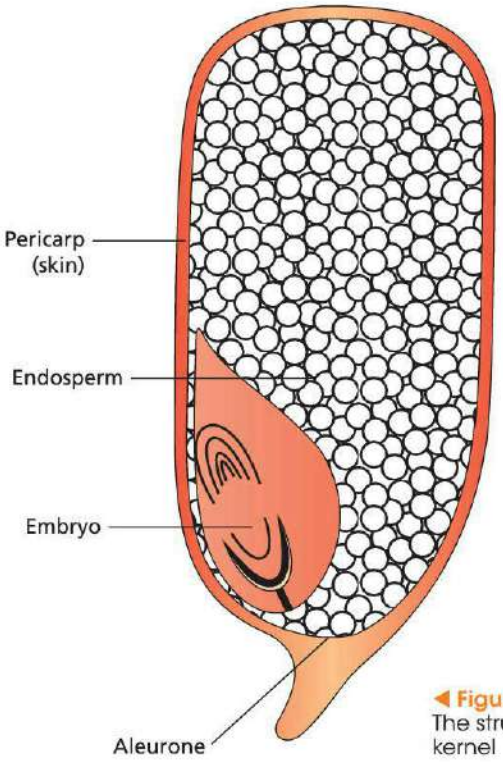
▲ **Figure 4.24**
Some examples of different corn varieties



◀ **Figure 4.25**
The structure of a corn kernel

Kernel colour is determined by the presence or absence of a pigment called anthocyanin in the aleurone layer. When the pigment is present the kernels are purple and referred to as 'coloured', and when it is absent the kernels appear yellow and are called 'colourless'.

Kernel shape may be described as smooth or wrinkled. The difference between the two is largely due to the ratio of sugar to starch content of the endosperm. An endosperm high in sugar dries out quickly, causing the kernels to collapse and become wrinkled. An endosperm high in starch will tend to retain water and cause a kernel to remain smooth.

In this investigation, you will examine these traits of corn kernels (colour and shape) to determine their inheritance patterns over three generations.

Aim

To predict the phenotypic ratios of F_2 offspring arising from a dihybrid cross in *Zea mays* (corn) and test the prediction against observed data

Materials

- Dihybrid maize model, boxed (produced by Carolina Biological Supply Company, sourced locally through Australian suppliers)
  Note: Alleles are assigned in the model. Ideally, these should be removed or obscured so that alleles are assigned during the investigation.
- Maize ear: segregation for dihybrid cross (Carolina Biological Supply Company)

Procedure

Part A: Predict the outcome of the dihybrid cross

A cross was set up between two purebred corn plants (P) to produce an F_1 generation. Representatives of the F_1 generation were subsequently self-pollinated to give the F_2 generation. A model of the two crosses is shown in the box.

1. Examine the model and determine the dominant and recessive phenotypes for the two independently assorting characteristics in corn: colour and shape. Copy Table A and enter your observations.
2. Assign alleles corresponding to each of the dominant and recessive phenotypes and enter them into Table A.
3. Copy and complete the first Punnett square (Table B) to show the genotype(s) of the F_1 generation kernels.
4. Copy and complete the second Punnett square (Table C) to show the predicted genotype(s) of the F_2 generation corn kernels.
5. Use the genotype in Table C to determine the corresponding phenotype of each possible zygote formed. List these possible phenotypes under Table C in your results section.
6. Use the phenotype data from your list to calculate the ratio of the predicted phenotypes of the corn kernels and record your results as Table D.

Part B: Gather experimental data and compare with predicted ratios

You are presented with the maize ear from the F_2 generation.

1. Identify the four different phenotypes with respect to kernel shape and kernel colour. Copy Table E and record the phenotypes in the top row.
2. Select and mark five columns of kernels with a pin (at the flat end of the cob). Count the number of kernels of each phenotype for each row and enter the data into Table E.
3. Calculate the total number of kernels for each phenotype and enter these into the second-last row of Table E.
4. Identify which of the phenotypes in Table E is represented by the smallest total number of individuals. Divide the total number of each phenotype by the smallest total number and round off to the nearest whole number. Enter these values into the final row to generate a ratio for the observed phenotypes.

Results

Copy and complete each of the tables, following the instructions given in the procedure.

Table A Corn kernel phenotypes and their corresponding alleles

Characteristic	Colour		Shape	
Phenotypes	Dominant phenotype	Recessive phenotype	Dominant phenotype	Recessive phenotype
Alleles				

Table B Punnett square representing the cross between two purebred P corn plants to give the F_1 generation

		Female gamete
Male gamete		

Table C Punnett square representing the cross between F_1 corn plants to give the F_2 generation

		Female gametes			
Male gametes					

Table D Ratio of expected phenotypes for the F_2 corn kernels

Phenotype				
Ratio				

Table E Observed data for F_2 generation corn

	Phenotype 1	Phenotype 2	Phenotype 3	Phenotype 4
Column 1 Number of kernels				
Column 2 Number of kernels				
Column 3 Number of kernels				
Column 4 Number of kernels				
Column 5 Number of kernels				
Totals				
Simplified ratio				

Analysis of results

1 How many different genotypes are represented in your Punnett square (Table C)?

2 How many different phenotypes are represented (Table D)?

3 Compare your expected and observed phenotypic ratios. Explain the discrepancy (if any) between the two ratios.

4 What could be done to improve the accuracy of the observed phenotypic ratio?

CORN DOMESTICATION

Learn about the history of corn domestication at the Max Planck Institute for Plant Breeding Research.

Discussion

1 Consider the coloured smooth corn kernels of the F_2 generation. What kind of experiment could be done to determine their genotypes?

2 Discuss how corn from the F_2 generation could be crossed to regenerate purebred lines of coloured smooth and colourless wrinkled corn.

See Chapter 7 to learn more about selective breeding.

QUESTION SET 4.3

Remembering

1 Define 'dihybrid cross'.

2 Describe 'independent assortment'.

3 Distinguish between multiple alleles and polygenes.

4 Explain why some phenotypes, such as height, can show continuous variation, yet others show discontinuous variation.

Understanding

5 Explain the origin of a 9:3:3:1 phenotypic ratio in a dihybrid cross.

Applying

6 Two purebred rabbits are mated: a doe with grey fur and black eyes is mated with a buck with white fur and red eyes. The litter contains only offspring with grey fur and black eyes. Assign the alleles and show the genotypes of the P and F_1 individuals. Draw a Punnett square to show a cross between individuals of the F_1 generation and predict the ratio of phenotypes with respect to fur and eye colour in the F_2 generation.

7 Feather colour in budgerigars involves two pigments, each under the control of a separate gene. One gene determines whether the first pigment is yellow or white. The other gene determines whether the second pigment is blue or white. If the yellow and blue pigments are both expressed, the feathers are green. If neither is expressed, the feathers are white. A purebred yellow female is mated with a purebred blue male. When their eggs hatch, the offspring are all green. Assign the alleles and draw a Punnett square to determine the phenotypic ratio of the feather colour in offspring resulting from mating a female and male among the generation of green birds.

8 The capacity to tolerate high salt concentration varies between different individuals of a population of salmonid fish. Plot a graph of salinity tolerance versus number of individuals in the fish population to show what you would expect if the trait is under the control of multiple genes.

9 Re-plot the graph from Question 8 to show what you would expect if the trait is under the control of one gene with two alleles: an allele for saline intolerance and another for high tolerance. Assume the alleles occur in equal proportions in the population and high tolerance is dominant to intolerance.

Sex-linked inheritance

In the process of fertilisation, male and female haploid sex cells fuse to produce a diploid zygote. In humans, normally all female gametes contain 22 autosomes and an X chromosome. But 50% of male gametes contain 22 autosomes and a Y chromosome and 50% contain 22 autosomes and an X chromosome. Thus, in humans there is a 50% chance that, in fertilisation, a sperm cell bearing a Y chromosome will fuse with an egg cell resulting in a male (XY) and a 50% chance that a sperm cell carrying an X chromosome will fuse with an egg cell resulting in a female (XX).

The X and Y chromosomes, or sex chromosomes, can be divided into homologous and differential regions. The homologous regions are those regions that the two chromosomes share in common. The differential regions are unique to each chromosome. In males, genes found in the differential regions are **hemizygous** in the sense that there is only a single copy of each of them.

Genes within the differential regions of the sex chromosomes show inheritance patterns that can be described as **sex-linked**. Sex-linked inheritance can be detected as phenotypes that segregate differently between males and females.

Scientific literacy: Evil gene would make punishment a tricky business

Are there evil genes or is it only people who can be evil? A recent story in *The Age* ('Deep Divide of "Evil Genes"') raised the question of whether criminals might evade responsibility for their crimes by blaming their genes.

The suggestion that there may be biological causes of crime is troubling in many ways. It reminds us of the horrors of the Nazis, and the evasion of responsibility seems like a slap in the face for victims of crime.

The very notion of a crime gene makes us reflect on the purposes of punishment. Should the courts try to give offenders what they deserve, or should they just protect the community from those with dangerous genetic profiles?

An example of a so-called 'evil gene' might be the low-activity MAOA gene. MAOA is a neurotransmitter in the brain and some research has suggested that those males who have low levels of the substance are particularly vulnerable to the effects of being maltreated when young.

Experience of childhood maltreatment has long been thought to be an influence on criminal conduct but it seems that being maltreated and having the genetic vulnerability is particularly likely to lead to bad behaviour.

But children don't get to choose their genetic profile nor whether they are maltreated. These are just things that happen to them and it is a matter of luck whether they receive the 'evil gene' and a matter of luck whether they are abused. Some are very unlucky on both counts.

So it's not a level playing field. Some people appear to have genetic and environmental misfortune that brings difficulties in complying with the criminal law.

This becomes problematic when punishing offenders. Once a person has been convicted of an offence, it is up to the judge to sentence them. But if a person has 'evil genes' – or, put another way, a genetic vulnerability – and is unlucky enough to have been maltreated, one might ask whether it is fair to give them the same punishment as an offender without these issues.

It is well recognised in the law that the characteristics of the offender are relevant to punishment. A mentally impaired young person from a severely dysfunctional background deserves less punishment than an unimpaired adult, even if they have committed the same type of crime.

It's just not fair to treat them the same because one has more difficulty in behaving well.

Similarly, it seems unfair to treat maltreated low-activity MAOA offenders the same as those who are more fortunate in their genetic profile and family circumstances.

But things are not so simple. Those with the 'evil gene' and difficult backgrounds may still be very dangerous.

This is how the purposes of punishment come into question. Should judges focus on giving offenders what they deserve or should they just try to prevent future crimes?

Perhaps judges should just lock them up until they won't cause any more trouble. Or even lock up those with 'evil genes' before they cause any trouble.

But that doesn't seem right. It seems to be a condition of a decent society that only the guilty are punished and that they not be punished in excess of their guilt. Criminals shouldn't get a worse punishment than they deserve and people who haven't committed a crime shouldn't get any punishment.

The proper consideration of 'evil genes' just draws attention to the complexity of the practice of punishment. It is easy to present it as a simple matter but it just isn't.

However, one thing seems clear. The ethical issues described here are likely to be forced on the courts by developments in science and there is no simple resolution in sight.

McCay, A. (2013) 'Evil gene would make punishment a tricky business', *The Age*, 22 April.

Questions

1. Describe what implications the function of the MAOA allele has for the interactions between genes and environment on criminal behaviour.
2. What kind of variation is implied by inheritance of the MAOA allele? Are you satisfied that this kind of variation best describes variation in human behaviour? Explain your reasoning.
3. Do you believe 'evil' is a valid phenotype? Outline two reasons in support of your answer.
4. Consider a situation where two first-time offenders were convicted of the same sort of crime. Would you argue that, if one of the offenders was shown to have an 'evil' genotype, the punishment should be more lenient, the same, or more severe as for the other convicted offender? Explain your reasoning.
5. Discuss at least two concerns with using a convicted criminal's genotype as a basis for determining their sentence.

X-linked recessive

In fruit flies, if a male with white eyes is crossed with a female with red eyes, all the F_1 fruit flies have red eyes, indicating red is dominant to white. If males and females of the F_1 generated are mated, $\frac{3}{4}$ of the F_2 fruit flies have red eyes and $\frac{1}{4}$ have white eyes. So far, this seems like a typical Mendelian ratio for a monohybrid cross but there is a twist. All of the white-eyed flies are male. Alternatively, if the initial cross is between a red-eyed male and white-eyed female, all the F_1 males have white eyes and all the F_1 females have red eyes. How can these observations be explained?

It makes sense if the gene for eye colour is carried on the X chromosome. In the first cross between the white-eyed male and the red-eyed female, the male is hemizygous for the white allele. The female is homozygous dominant with the red allele. In the F_1 generation, all the females are heterozygous for the red phenotype. The males, however, can only inherit one X chromosome, from their mother, and so are hemizygous with the allele for red eyes. In the F_2 generation, all the females have at least one allele for red eyes because they inherited one of their X chromosomes from their red-eyed father. On the other hand, males inherit only one X chromosome from their heterozygous mother: half get the red allele, half get the white allele.

In the alternative cross between a red-eyed male and a white-eyed female, all the F_1 females inherit an X chromosome with a red allele from their hemizygous father. All the F_1 males inherit their X chromosome with a white allele from their homozygous mother. Figure 4.26 summarises these crosses.

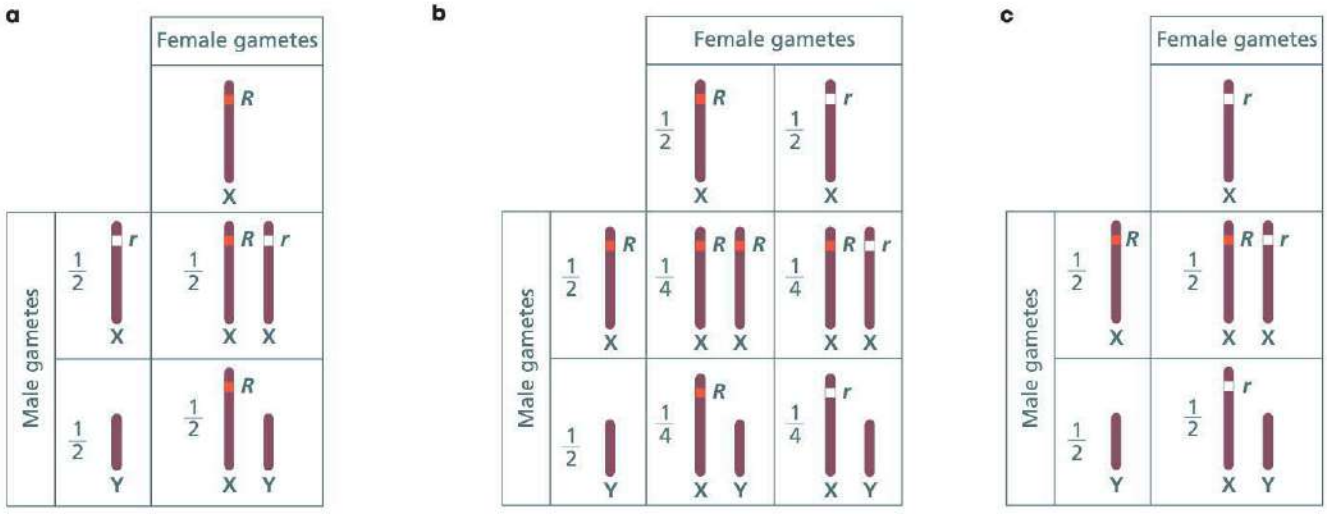


Figure 4.26 ▲ White eye colour in fruit flies is an X-linked recessive phenotype. The Punnet squares show a) the P generation and b) the F1 generation of a monohybrid cross between a white-eyed male and red-eyed female, and c) a monohybrid cross between a red-eyed male and white-eyed female.

When a recessive phenotype under investigation is determined by an allele on the X chromosome, it is said to be an **X-linked recessive** phenotype. Males who have the recessive allele on their X chromosome will always express the phenotype as they only have one X chromosome. Females will only express the phenotype when both X chromosomes have the affected allele. A heterozygous female will be a **carrier**.

To express the phenotype, males only need one copy of the affected allele, whereas females must have two. Consequently, males show X-linked recessive phenotypes much more often than females do. Red–green colour blindness and haemophilia are two recessive conditions in humans that are transmitted to offspring through **X-linked** inheritance.

In this type of inheritance, a male with the phenotype cannot pass on the trait to his sons, as they inherit his Y chromosome only. His daughters will get the affected X chromosome but they

will only show the phenotype if they inherit another affected X chromosome from their mother. As with autosomal recessive phenotype, some generations may not have any members showing the phenotype.

X-linked dominant

This type of inheritance is similar to sex-linked recessive inheritance except that heterozygous females will always show the phenotype and any individuals must have a parent with the phenotype. Males showing the phenotype will not pass the affected allele on to their sons (as they must inherit their father's Y chromosome) but they will pass it on to all their daughters, who will also show the phenotype. A heterozygous female is expected to pass on the allele to 50% of her offspring regardless of their sex.

Y-linked

If a trait is carried on the Y chromosome it is said to be **Y-linked**. The most conspicuous phenotype associated with genes of the Y chromosomes is male gender. 'Maleness' in humans is determined by the SRY gene carried on the Y chromosome. Other Y-linked genes are relevant to testis development and sperm production. By definition, inheritance of the Y chromosome is along the male line from father to sons.

See Chapter 3 to learn more about how the SRY gene achieves male development.

Alleles carried on the X and Y chromosomes show different inheritance patterns for males and females. X-linked recessive and Y-linked phenotypes are more common in males because they are hemizygous for the sex chromosomes.

QUESTION SET 4.4

Remembering

1 Define 'sex-linked', 'X-linked' and 'Y-linked' inheritance.

2 Define 'carrier'.

Understanding

3 Describe and explain the occurrence of phenotypes that are:

- a X-linked recessive.
- b X-linked dominant.
- c Y-linked.

Applying

4 Ichthyosis is an inherited condition characterised by scaly skin. The condition affects around 1 in 6000 males but female cases are almost unknown.

- a What might account for the differences in ichthyosis occurrence among males and females?
- b From which parent would an affected male have inherited the condition?
- c What is the probability the affected male would pass the responsible gene on to his sons? Explain your reasoning.

5 The novel Xg blood group is an X-linked phenotype that is observed in approximately equal proportions in males and females.

- a Explain how an X-linked phenotype could be observed in equal proportions in both sexes.
- b What is the probability that Xg sons are born to a heterozygous mother with Xg and a father without Xg? What is the probability that daughters of this mother and father show the Xg trait?
- c What is the probability that Xg sons are born to a mother without Xg and a father with Xg? What is the probability that these parents have Xg daughters?

CHAPTER SUMMARY

- The combination of alleles an organism has for a particular gene is its genotype. The two alleles may be identical (homozygous) or different (heterozygous).
- The interaction between alleles determines an organism's phenotype. Phenotypes may be described as dominant, recessive, codominant or partially dominant.
- In a monohybrid cross between two purebred (homozygous) parents:
  - the F_1 offspring are all heterozygous and show the dominant phenotype
  - in a subsequent cross between F_1 individuals, the F_2 offspring are predicted to show dominant and recessive phenotypes in the ratio of 3:1.
- In a dihybrid cross between two parents purebred for two independently assorting characteristics:
  - the F_1 offspring are heterozygous for both traits and show the combination of dominant phenotypes
  - in a subsequent cross between F_1 individuals, the F_2 offspring are predicted to show four combinations of phenotypes, dominant-dominant : dominant-recessive : recessive-dominant : recessive-recessive in the ratio of 9:3:3:1.
- Punnett squares are a convenient way to represent crosses and predict the resulting genotypes and phenotypes and their proportions.
- Observed phenotypic ratios for any cross may vary from those predicted by a Punnett square. This is due to:
  - random assortment of alleles on chromosomes during meiosis
  - the fertilisation of random gametes.
- Certain combinations of alleles sometimes lead to lethal phenotypes. Lethal phenotypes skew the phenotypic ratios of the surviving offspring.
- A test cross can be used to determine the genotype of an individual displaying a dominant phenotype.
- Discontinuous variation is displayed by phenotypes governed by a single gene. Continuous variation is shown for phenotypes governed by polygenic inheritance.
- Sex-linked inheritance is detectable by unequal phenotypic ratios between males and females.
  - X-linked recessive phenotypes are more common in males than in females.
  - X-linked dominant phenotypes show up in all affected females and males.
  - Y-linked phenotypes are exclusively male.

CHAPTER GLOSSARY

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

artificial selection the breeding of plants and animals to produce desirable traits in successive generations; also known as selective breeding

carrier usually used in reference to disease; a healthy, heterozygous organism carrying an allele for a recessive phenotype; the organism may transmit the recessive allele and resulting phenotype to its offspring or to others

codominant a state in which both alleles of a heterozygous individual are fully expressed in the phenotype

continuous variation a variation in a characteristic that shows a smooth range of different phenotypes

dihybrid cross a cross between two organisms that are heterozygous at two gene loci

dihybrid inheritance inheritance of two pairs of contrasting characteristics

discontinuous variation a variation in a characteristic that shows two or just a few clearly distinct phenotypes

dominant a phenotype that requires only one copy of its allele in an individual to be expressed

first filial generation (F_1) the first generation of offspring produced from a cross between two homozygous parents (P)

gene a unit of heredity that transmits information from one generation to the next; a segment of DNA that codes for polypeptide

genotype a specific combination of alleles for a particular gene locus belonging to an individual or cell

hemizygous a gene that occurs only as a single copy in a diploid organism or cell

heredity the study of inheritance; the genetic transmission of characteristics from one generation to another

heterozygous a genotype with two different alleles for a single gene locus

homozygous a genotype with two identical alleles for a single gene locus

homozygous lethal phenotype a phenotype that arises from a homozygous recessive genotype, leading to the premature death of an organism

incomplete dominance the state in which a heterozygous individual has a phenotype that is intermediate between those of the corresponding homozygous individuals

independent assortment when alleles of gene pairs redistribute independently into different combinations in gametes during meiosis

inheritance genetic acquisition of characteristics by offspring from their parents

linked genes – or their alleles – that are inherited together more frequently because they are located near each other on the same chromosome

monohybrid cross a cross between two organisms that are heterozygous at one gene locus

parental generation (P) two individual organisms that represent the start of a breeding experiment; their offspring are the F_1 generation

partial dominance see *incomplete dominance*

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

polygene a gene for which the alleles have a small additive effect on a phenotype; many polygenes together contribute to continuous variation in a phenotype

polygenic inheritance transmission between generations of characteristics that are controlled by polygenes

Punnett square a grid used to graphically predict the outcome of a cross or breeding experiment

purebreeding a line of organisms that always produce offspring with the same phenotype when crossed with each other

recessive a phenotype that requires two copies of its allele in an individual to be expressed

second filial generation (F_2) offspring of the F_1 generation; the second generation produced from a cross between two homozygous parents (P)

selective breeding a process by which humans domesticate animals or plants by purposely choosing individuals with the most desirable characteristics as parents for each successive generation of breeding

sex-linked a gene located on a sex chromosome

test cross a cross using an organism with a recessive phenotype to determine the unknown genotype of an organism with a dominant phenotype

trait a heritable characteristic; phenotype

X-linked related to a gene located on the X chromosome

X-linked recessive when a phenotype is determined by a recessive allele on the X chromosome

Y-linked related to a gene located on the Y chromosome

CHAPTER REVIEW QUESTIONS

Remembering

1 Describe the relationship between the following terms.
   - **a** Gene and allele
   - **b** Genotype and allele
   - **c** Genotype and phenotype

2 Match each item in the first column with a description in the second column. Each item can only be used once.

Hemizygous	Only one copy of the allele is required for the phenotype to be observed.
Heterozygous	Two different alleles are both fully expressed in the phenotype.
Homozygous	The phenotype is intermediate between each of those determined by two different alleles.
Recessive	Two copies of the same allele are present for a particular gene locus.
Dominant	There is only one copy of the gene in a diploid organism.
Codominant	Two different alleles are present for a particular gene locus.
Partially dominant	Two copies of the allele are required for the phenotype to be observed.

Understanding

3 Explain what 'purebreeding' means. Why was it important for Gregor Mendel to use purebred plants in his experiments?

4 Explain why siblings are not identical, even though they inherit half their chromosomes from each of the same parents.

5 Explain why the offspring of a tall pea plant and a short pea plant are not all of intermediate height.

6 Distinguish between the effects of random assortment of alleles and linked alleles on phenotype, and describe what accounts for these differences.

Applying

7 Two grey rats are mated. Half the offspring are grey, one-quarter are white and one-quarter are black.

- a Assign the alleles for coat colour.
- b Describe the genotypes of the parents and the offspring.
- c What kind of dominance is this?

8 There are four possible phenotypes for ABO blood groups in humans: A, B, AB and O. The most common of these, O, is actually the recessive phenotype. These phenotypes are determined by three alleles, of which I^A and I^B are codominant and i is recessive to both.

- a Write each possible genotype and the corresponding phenotype.
- b If a woman is heterozygous with blood type A and a man is heterozygous with blood type B, predict their children's possible blood type(s) and the probability of each. Support your conclusions with a Punnett square.

9 Consider the cross between the red-eyed male and white-eyed female fruit flies shown in Figure 4.27. Predict the proportion of red-eyed and white-eyed offspring and their gender resulting from a cross between an F_1 female and an F_1 male. Use a Punnett square to support your prediction.

10 In mice, coat colour is determined by an autosomal gene, and pink coat colour is dominant to brown. Dwarfism is caused by an X-linked recessive allele. If a brown female dwarf mouse mates with a purebreeding pink male of normal size, what will the phenotypic ratios in each gender be in the F_1 and F_2 generations?

11 In cherry tomatoes, a tall vine is dominant to dwarf vine, round-shaped fruit is dominant to pear-shaped fruit and red fruit is dominant to yellow fruit. If you crossed a purebreeding tall, round, red-fruited plant with a short, pear-shaped, yellow-fruited plant, what would you expect to be the appearance of the F_1 generation? Assuming that the genes controlling these three characteristics are inherited independently, what are the possible combinations of genes in the gametes of the F_1 generation?

Analysing

12 The snapdragon (*Antirrhinum majus*) can show a condition called 'aurea' in which the leaves appear a golden colour instead of green. A pair of aurea snapdragons with golden leaves was crossed and they produced 101 offspring, 67 with golden leaves and 34 with green leaves. Draw a Punnett square for the cross and use the data to explain how the ratio among F_2 offspring could have arisen.

13 A test cross of fruit flies with curly wings and red eyes produces offspring with red eyes, half of which have curly wings, and half straight wings. Identify the genotype of the original red-eyed fruit fly with curly wings and provide evidence to support your conclusion.

14 A male purebred fruit fly with the standard brown body is crossed with a female purebred with a yellow body. All the male offspring have yellow bodies, whereas all the female offspring have brown bodies.

- a Explain where the gene resides.
- b Predict the proportions of the phenotypes in the offspring produced by crossing the F_1 fruit flies.

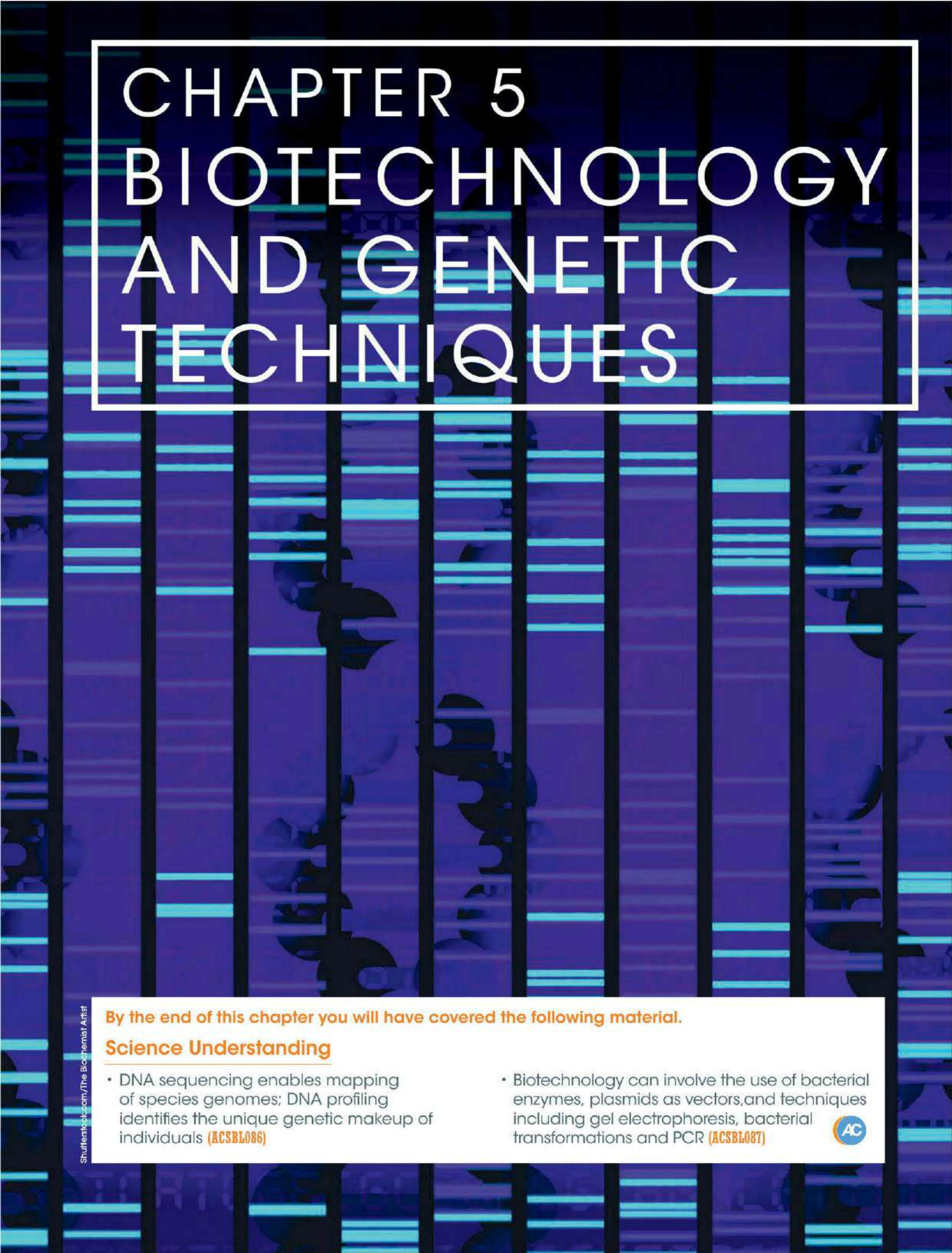
Evaluating

15 Discuss the benefits and limitations of studying Mendelian inheritance patterns in humans.

Creating

16 Would you consider most phenotypes to be monogenic or polygenic? Discuss the observations you would cite in support of your point of view.

CHAPTER 5 BIOTECHNOLOGY AND GENETIC TECHNIQUES



Shutterstock.com/The Biochemist Artist

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

Science Understanding

- DNA sequencing enables mapping of species genomes; DNA profiling identifies the unique genetic makeup of individuals (ACSBL086)
- Biotechnology can involve the use of bacterial enzymes, plasmids as vectors,and techniques including gel electrophoresis, bacterial transformations and PCR (ACSBL087)

Shutterstock.com/Aleksey Krylov

Figure 5.1 ▶ Domestication is one of the earliest examples of biotechnology.

Most of the farm animals we know today were domesticated between 10 000 and 4000 years ago. Early humans used the principle that offspring of individuals presenting good traits (e.g. larger size, faster growth, better milk production and improved fertility) were also likely to express those traits. Animals were selectively bred in order to improve, over time, the quality of farmers' herds. The same is true for rice, wheat and other crops. Present-day varieties bear little resemblance to their wild ancestors. Today, new scientific techniques enable us to create new domestic animals with enhanced phenotypes, by changing their genetic sequence. Along with this new technology comes the added responsibility to consider economic, social and ethical issues.

Biotechnology through the ages

The term **biotechnology** describes the use of living things to make new products or systems. While the term may be quite new, the concept is not. Traditional biotechnology has been with us ever since people began to grow crops and domesticate animals. Early Egyptian, Babylonian and Sumerian civilisations used micro-organisms to create bread, beer and wine. In recent times, increased knowledge of cell systems and molecular biology has revolutionised biotechnology, promising potential benefits for agriculture, the environment and medicine.

The gene revolution

Modern biotechnological techniques now enable scientists to manipulate the outcome of normal functioning genes to meet the needs of science and society in a more precise way than ever before. Because of this, much of modern biotechnology is called **genetic engineering**. This term simply means changing the genetic sequence of an organism through human use of modern biotechnology techniques. Such genetically engineered organisms are also called **genetically modified organisms (GMOs)** or **transgenic organisms**.

The term genetic engineering applies to a range of techniques and processes for investigating and modifying DNA, genes and **genomes** of species. It is possible for scientists to use genetic engineering to switch genes on or off, remove genes and introduce genes from one species into another. For example, a US company created a genetically modified salmon that grows twice as fast as wild salmon. This was achieved by introducing a gene from a separate fish species into the salmon genome.

Genetic engineering techniques

In genetic engineering, just as in the construction of buildings, tools are used for specific purposes. Biotechnology has its own set of specialised tools, which are mostly derived from other organisms. These include tools for synthesising, cutting and pasting DNA, along with tools and techniques for viewing and analysing DNA.

Cutting DNA

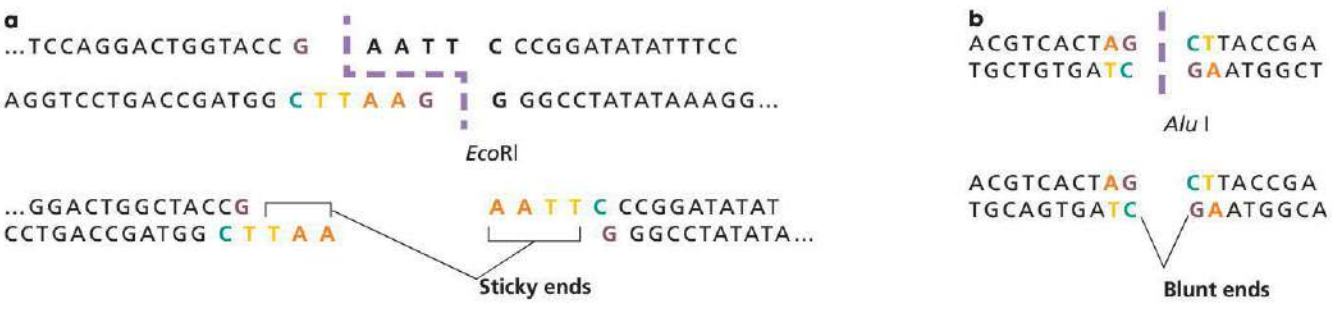
One of the essential requirements in genetic engineering is the ability to cut segments of DNA at known sequences. The cutting tools used are enzymes known as **restriction endonucleases** ('endo' = within, 'nuclease' = an enzyme that cleaves nucleic acids), or **restriction enzymes**. These are like 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**. Different restriction enzymes have different restriction sites, though some restriction enzymes do share restriction sites with other restriction enzymes. Most recognition sequences are palindromes of their complementary sequence.

Restriction enzymes occur naturally in bacteria, where they cleave (cut) 'foreign' DNA that enters from invading viruses, thus destroying any potential threat. In essence, they are the immune system of a bacterium. Restriction enzymes are named according to the bacterial strain from which they are derived. The first restriction enzyme was isolated from *Escherichia coli* RY13 strain and was thus named *Eco*RI. Table 5.1 identifies a number of common restriction enzymes and their source.

Table 5.1 Common restriction enzymes and their restriction sites

Enzyme	Bacterial source	Restriction site	After cutting
*Eco*RI	*Escherichia coli*	5′G↓AATTC3′ 3′CTTAA↑G5′	5′G AATTC3′ 3′CTTAA G5′
*Hind*III	*Haemophilus parainfluenzae*	5′A↓AGCTT3′ 3′TTCGA↑A5′	5′A AGCTT3′ 3′TTCGA A5′
*Alu*I	*Arthrobacter luteus*	5′AG↓CT3′ 3′TC↑GA5′	5′AG CT3′ 3′TC GA5′
*Bam*HI	*Bacillus amyloliquefaciens H*	5′G↓GATCC3′ 3′CCTAG↑G5′	5′G GATCC3′ 3′CCTAG G5′

To date, almost 4000 different restriction enzymes have been identified. Although each enzyme recognises a specific sequence of between four and eight nucleotide base pairs (bp) of the double-stranded DNA, multiple enzymes isolated from different organisms can recognise the same sequence. Restriction enzymes 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 5.2a), or **blunt ends** (Figure 5.2b), in which the cut has occurred at the same position in each strand of the DNA and there are no overlapping strands.



▼ **Figure 5.2**
a) Sticky ends produced by cutting DNA with the restriction enzyme *Eco*RI; b) Blunt ends produced by cutting DNA with the restriction enzyme *Alu*I

Recombining DNA

At times molecular biologists may want to combine two samples of DNA. **DNA ligase** is an enzyme that is 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′ hydroxyl end of one nucleotide with the 5′ phosphate end of another nucleotide.

To join two strands of DNA together using DNA ligase, the success rate is vastly improved by bringing them together somehow. When using restriction enzymes that 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. DNA ligase can then be used to recombine these two fragments, even if they are from two unrelated organisms. For example, *Eco*RI can be used to cut both human DNA and bacterial **plasmid** DNA, leaving sticky ends that are complementary and increase the chance of the correct two ends coming together (Figure 5.3).

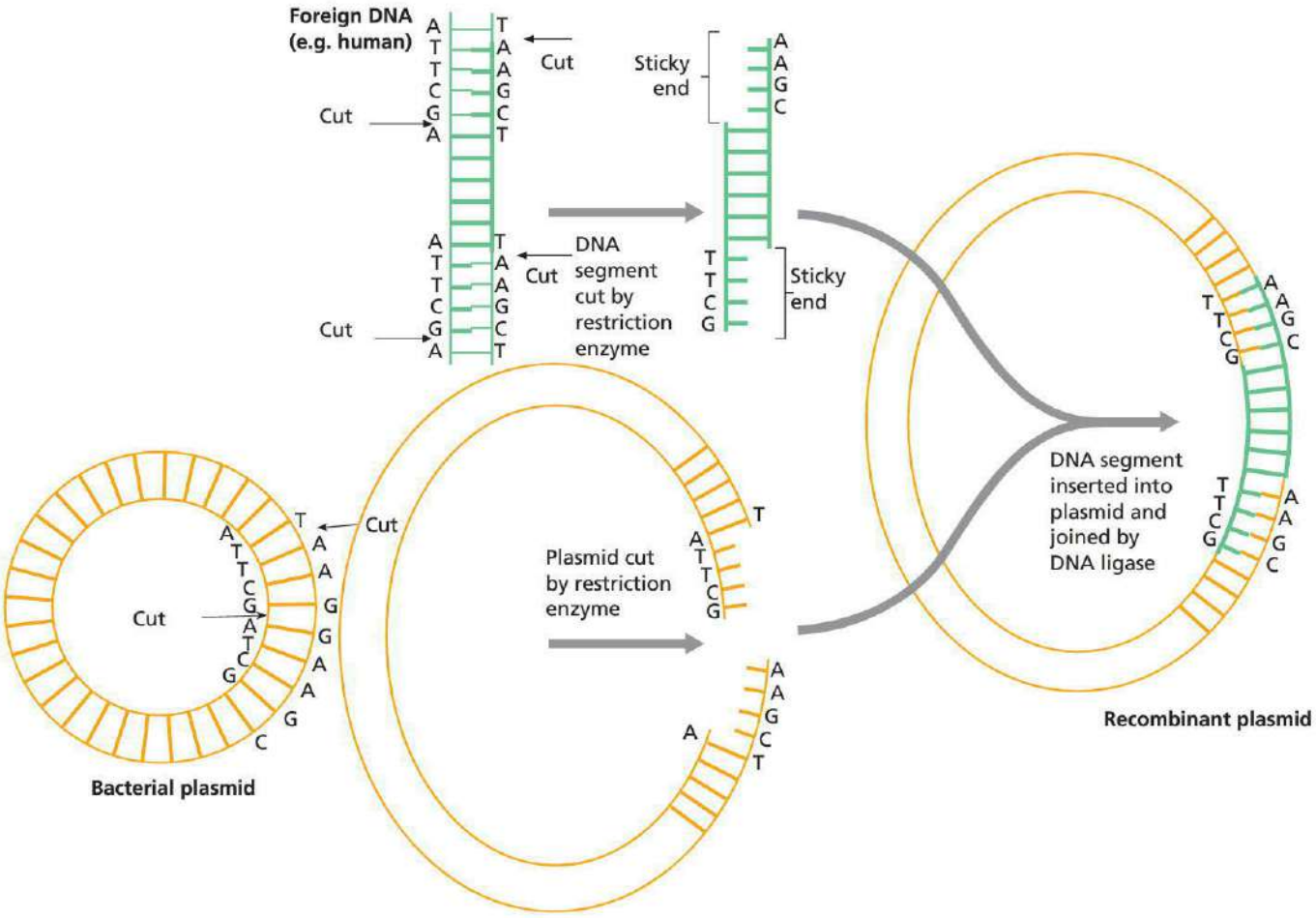


Figure 5.3 ▲ DNA ligases join DNA inserted from a foreign source that has complementary sticky ends.

Fragments with blunt ends can also be joined by DNA ligase, but this process is much less efficient. The technology that recombines DNA from different sources to modify the DNA sequence is called **recombinant DNA technology**.

Amplifying DNA: polymerase chain reaction

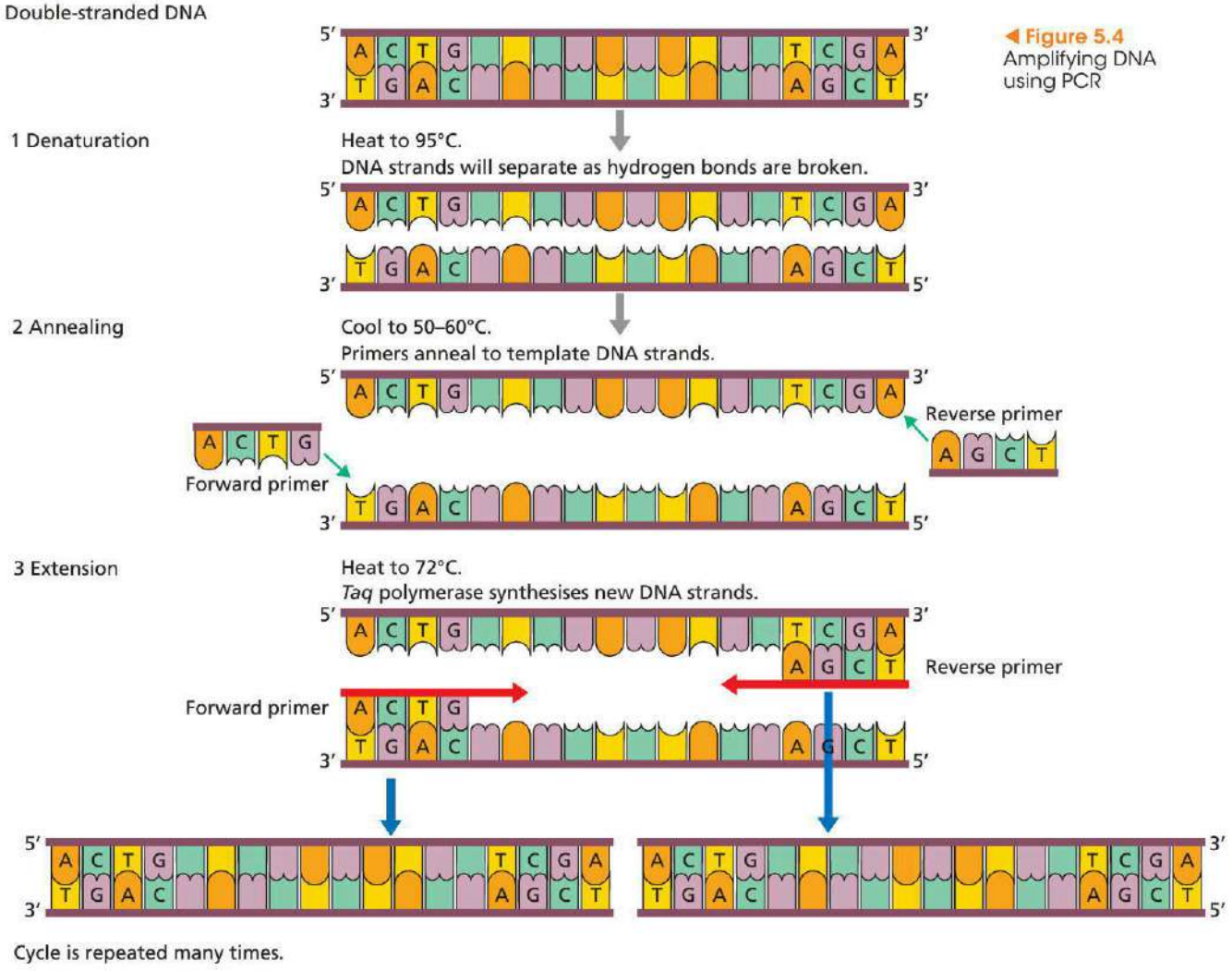
Each eukaryotic somatic cell has only two copies of a gene of interest, and prokaryotic cells have only one copy. This small amount of DNA poses a problem for scientists wishing to work with it. Similarly, only a small sample of DNA may be available for analysis, for example, at a crime scene or DNA samples obtained from bones. To increase the amount of DNA of a particular sequence the biotechnologist has another tool to work with: the **polymerase chain reaction (PCR)**.

PCR makes use of the enzyme **DNA polymerase**, which catalyses the formation of new DNA molecules in the nucleus from free nucleotides. PCR is used to amplify, or make many copies of, a specific sequence of DNA.

A number of components are required: the DNA to be copied (template), DNA polymerase, a buffer solution that contains salts and other chemicals that help the polymerase to function, a supply of the four nucleotides (i.e. A, T, C, G) from which to build the new DNA molecules, and two **primers**. The primers are short sequences (around 20 nucleotides) of single-stranded DNA, complementary to the nucleotide sequences at either end of the DNA section that is to be copied. These 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.

PCR has three steps (Figure 5.4).

See Chapter 1 for more information on the functions of DNA polymerase.



◀ **Figure 5.4** Amplifying DNA using PCR

1 Denaturation: The double-stranded DNA is heated to 95°C, breaking the hydrogen bonds between the bases, and thus causing the two strands to **denature**. This is sometimes called the melting stage.
2 **Annealing**: The temperature is reduced to 50–60°C, allowing the primers to anneal (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 DNA polymerase used in PCR. Starting from the primers, new DNA strands are synthesised using DNA polymerase and the available nucleotides. At the end of this phase there are two copies of the double-stranded DNA.

This cycle is repeated until sufficient quantities of the DNA are obtained to work with. Each cycle doubles the number of DNA strands; therefore, in just 20 cycles more than one million copies of target DNA will be produced.

PCR is a process that amplifies a specific DNA sequence for analysis. The sequence of the primers determines the DNA sequence to be amplified.

Figure 5.5 ▶ Thermal cyclers, in which the PCR is carried out as an automated process

Science Photo Library/Philippe Psaila

QUESTION SET 5.1

Remembering

1 Identify an example of the use of 'traditional' biotechnology.
2 Identify the new knowledge that has made genetic engineering possible.
3 List examples of new products derived via biotechnology.
4 Name the two types of restriction enzymes.
5 State the components of a PCR reaction.
6 Match the enzyme in the left column with an activity in the right column. Each item can only be used once.

DNA ligase	Replication of DNA
Restriction endonuclease	Joining of two fragments of DNA
DNA polymerase	Cutting of DNA at specific sites

Understanding

7 Outline the difference between gene technology and traditional biotechnology.

8 Outline the three steps of PCR.

Applying

9 If you start with five copies of a DNA region, how many copies will be produced if your sample goes through 10 cycles of PCR?

Visualising DNA

DNA molecules are far too small to see. One way to visualise them is to separate the fragments according to size, using gel electrophoresis. Alternatively, DNA fragments can be identified using a DNA probe, or the nucleotide sequence can be analysed using DNA sequencing.

Gel electrophoresis

Gel electrophoresis is a technique that separates fragments of DNA according to their size and charge. DNA has an overall negative charge due to the phosphate groups in its backbone. The technique of 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 created 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, and positive and negative electrodes are attached at each end of the gel. When the electric current runs, the 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 wiggle through the gel matrix faster than the larger strands, which take longer to migrate through the gel. This method therefore separates DNA strands based on their size.

DNA itself will not be visible in the gel. To view the separated DNA fragments, **ethidium bromide** or another fluorescent DNA-binding dye 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.

Science Photo Library/Simon Fraser

◀ **Figure 5.6**
A researcher injecting genetic material from coral into an agarose electrophoresis gel apparatus

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 **molecular size markers**. These are pieces of DNA of 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 5.7 shows four markers in the calibration lane: 1700 bp, 1000 bp, 500 bp and 200 bp, respectively.

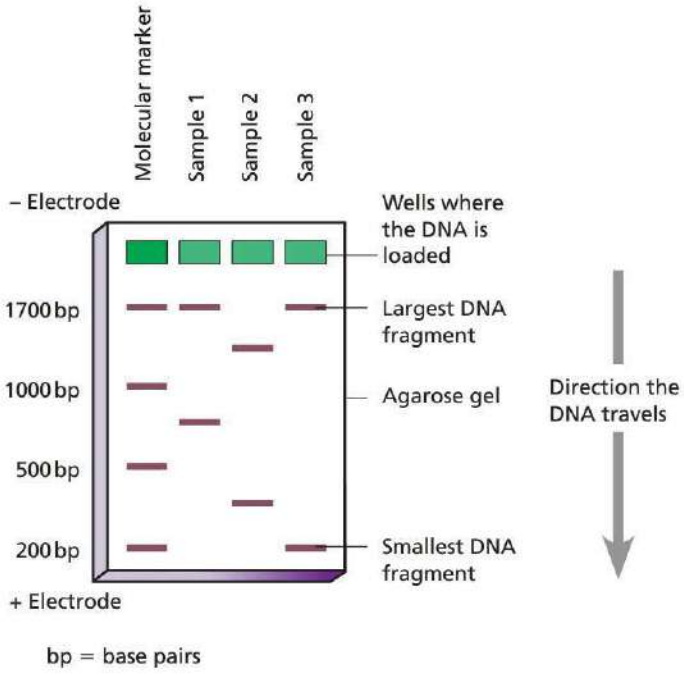


Figure 5.7 ▶ Molecular markers of known size are run alongside samples and allow estimation of the size of the DNA fragments migrating through the gel.

EXPERIMENT 5.1

GEL ELECTROPHORESIS ANALYSIS

The DNA from any living organism is chemically identical and behaves in the same way in agarose electrophoresis. This makes analysis and comparison relatively easy. Scientists are able to read agarose gels and map the restriction sites of a DNA molecule. To prepare a run, molten and cooled agarose gel is poured with a well comb in place; when the gel is set, the comb is removed to expose the wells for the loading of DNA fragments (Figure 5.8).

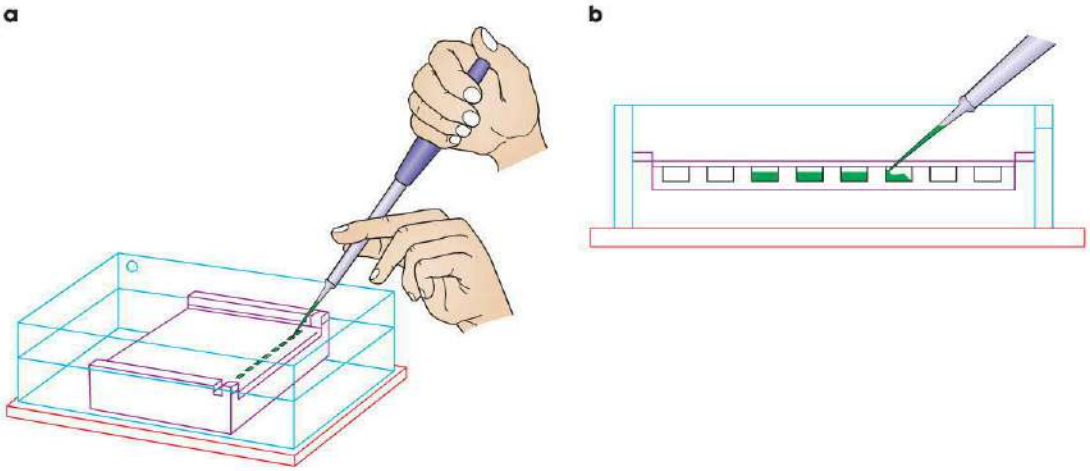


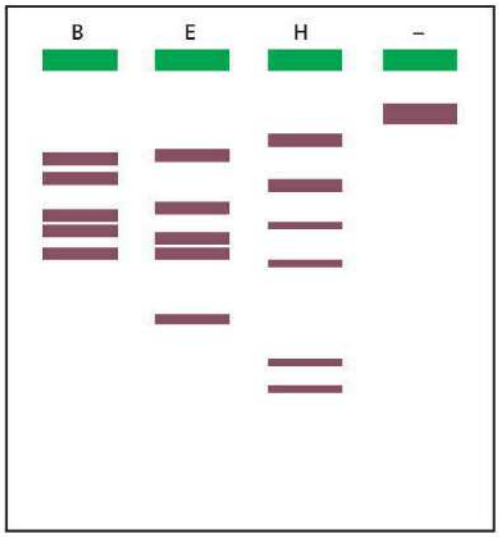
Figure 5.8 ◀ Loading samples of DNA into wells of an agarose gel showing a) the hand position and b) the samples being loaded.

DNA analysis is at the heart of recombinant DNA technology. In most cases when DNA is being cut for analysis, it is necessary to use a standard DNA fragment for comparison.

Bacteriophage λ-DNA is often used as a standard piece of DNA and can be found as either linear molecules or circular molecules. At each end of the linear molecule are single-stranded sequences of 12 nucleotides that are similar to the sticky ends produced by restriction enzymes.

In a standard test using λ-DNA, three restriction enzymes, *Hind*III, *Eco*RI, and *Bam*HI, have been used in a restriction digest. The following steps outline the procedure for obtaining the gel shown in Figure 5.9.

- Each of the restriction enzymes was placed in separate test tubes containing a buffer solution and incubated with λ-DNA for 20 minutes.
- A sample of DNA cut with *Bam*HI was placed into the first well (B) of the agarose gel.
- A sample of DNA cut with *Eco*RI was placed into the second well (E) of the agarose gel.
- A sample of DNA cut with *Hind*III was placed into the third well (H) of the agarose gel.
- A sample of DNA mixed with water was placed into the fourth well (–) of the agarose gel.
- After electrophoresis and exposure to ultraviolet light, the banding pattern presented in Figure 5.9 was obtained. Using the gel, it is possible to determine the approximate base-pair size of λ-DNA.



▲ **Figure 5.9**
The DNA fragments separate according to length.

Aim

To analyse the results of a restriction digest experiment using agarose gel electrophoresis

Material

Each student will require:

- ruler

Procedure

1. Measure the distance from the lower part of the well to the lower part of each band on the gel.
2. Copy Table 5.2 and enter each of your measurements under the appropriate 'distance' heading.
3. The base-pair sizes for fragments cut with *Hind*III have already been included.
4. Calculate (approximately only) the base-pair sizes of the fragments cut with *Eco*RI and *Bam*HI, and complete Table 5.2.

Results

Table 5.2

***Hind*III**		***Eco*RI**		***Bam*HI**	
Distance (mm)	**Size (bp)**	**Distance (mm)**	**Size (bp)**	**Distance (mm)**	**Size (bp)**
	23 130		21 226		16 841
	9416				
	6557				
	4361				
	2322				
	2027				

Discussion

1. From the bands observed in the *Hind*III well, what is the total size of the λ-DNA?
2. It is known that λ-DNA has a linear length of 48 502 base pairs. Are the actual numbers of base pairs equal to the numbers shown on the gel? If they do not match, how can you account for the differences in base-pair numbers?
3. Why was one of the wells in the gel filled with DNA that was mixed with water?

4 Why does the band produced by the DNA that has not been cut with a restriction enzyme appear to have stopped running on the gel first?

5 Describe in your own words how the process of gel electrophoresis works.

6 Explain the role that restriction enzymes play in the process of gel electrophoresis.

Conclusion

Write a summary to this activity.

Probing for genes

Huntington's disease (HD) is a genetic disorder of the central nervous system with the symptoms only appearing when an individual is an adult. Scientists can probe a person's genome for the *HD* allele and analyse it in family members to inform them of their chance of developing the disease. When searching for genes, another molecular tool is needed, one that can search for specific regions within the genome. This tool is called a **gene probe**.

A gene probe binds to target sequences in DNA. The probe is a specific single length of single-stranded DNA of between 20 and 40 nucleotides, or sometimes as large as 1000 nucleotides, that is complementary to a known sequence of DNA from a specific gene. The DNA being investigated is then heated to separate the two strands and expose their bases. The single-stranded probes will bind to any complementary sequences (Figure 5.10). In the case of HD the gene probe would be complementary to the allele responsible.

Gene probes have either a radioactive tag attached to them, which will show up when exposed to photographic film, or a fluorescent dye tag, which shows up when exposed to an ultraviolet light source. In the case of HD, this technique can be used to determine which family members have the allele and therefore will develop the disease.

Gene probes can be natural or nucleotide sequences synthesised in the laboratory. Gene probes have a variety of uses including finding a certain fragment of gene after a sample has been separated by gel electrophoresis, identifying the position of a gene on a chromosome and identifying an allele of a specific gene associated with a genetic disease.

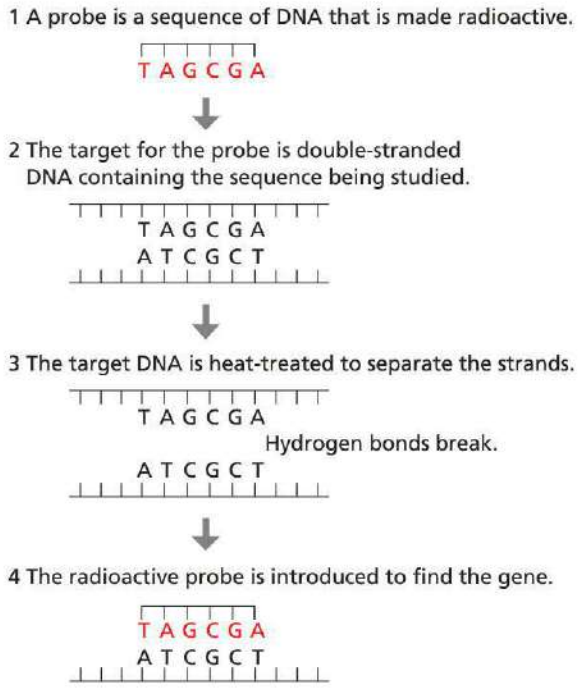
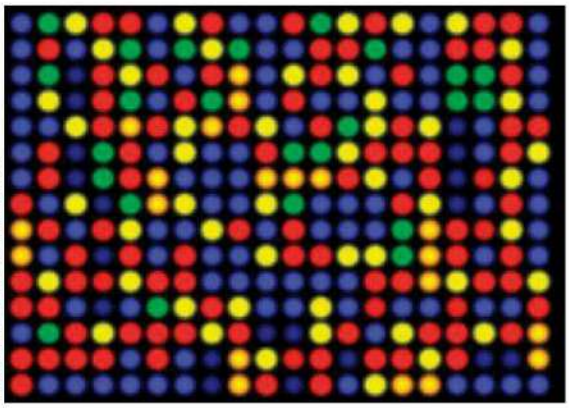


Figure 5.10 ▲ A probe is made up of 20–40 nucleotides complementary to its target sequence.

Figure 5.11 ▼ A DNA microarray indicating binding of cDNA to the DNA probes from one sample (red fluorescence), another sample (green) and both samples (yellow)



Science Photo Library/Alfred Pasieka

Gene probing uses a single-stranded DNA molecule complementary to a gene of interest to identify, isolate or position that gene on a chromosome.

Today, thousands of genes can be tested simultaneously using microarray technology. A microarray consists of thousands of DNA probes arrayed on a single microscope slide of glass or silicon chip (Figure 5.11). Each probe is designed to be complementary to a gene of interest in the target cell. The mRNA of the target cell is extracted, reverse transcribed into DNA (now called copy DNA, or cDNA) and labelled with a fluorescent marker. Fluorescently labelled DNA is then hybridised (allowed to bind) under stringent conditions to the probes cross-linked to the slide. A scanner measures the fluorescence for each DNA probe on the slide and from this information scientists can work out the activity of genes in the cell: the stronger the fluorescence, the more mRNA in the original sample and therefore the greater the activity of each of the genes.

Microarray technology can be used to detect genetic diseases. For example, genes that are usually turned off in normal cells may be turned on, leading to uncontrolled cell division and cancer. Conversely, genes that suppress the development of tumours may be turned off. Microarray technology offers a way of diagnosing the cancer at a molecular level.

DNA sequencing

Scientists often want to know the exact nucleotide base sequence of the DNA. Many mutations that cause genetic diseases are caused by single base substitutions or deletions, yet their effect can vary greatly. The process of **DNA sequencing**, that is, determining the exact nucleotide sequence of a gene, can help scientists identify individuals with deletion mutations, as in cystic fibrosis, or substitution mutations, as in sickle cell anaemia.

DNA sequencing can be done manually using gel electrophoresis or automatically using an automatic DNA sequencer that can sequence a large amount of DNA in a very short time. In this process, the four nucleotides are labelled with four different coloured fluorescent dyes. As electrophoresis proceeds, a laser scans across the bottom of the gel, detecting the different dyes and consequently the base sequence. A computer can then automatically analyse the information from the gel to read the base sequence. This technique is called the Sanger method.

A large number of faster and cheaper sequencing technologies are now available for use by biotechnologists. These methods are collectively called next generation sequencing and they use whole genomic DNA as a template, resulting in much greater sequencing efficiency. For example, one million DNA fragments of 700 bp can be sequenced in 24 hours, which is the equivalent of one full human genome every five days.

DNA sequencing can identify the exact nucleotide sequence of DNA fragments, which can be used to determine the genetic basis for particular phenotypes.

WOW

Comparative genomics between humans and fruit flies

Comparative genomics involves the comparison of different genomes. Recent studies have discovered that humans share 60% of their genes with fruit flies, *Drosophila melanogaster*. Two-thirds of the genes involved in human cancer are reflected in the fruit fly genome. The evolutionary links between species, or their genetic relatedness, can also be determined by comparing genomes. Humans and fruit flies diverged from each other about 990 million years ago, but we only diverged from chimpanzees about 5 million years ago.

QUESTION SET 5.2

Remembering

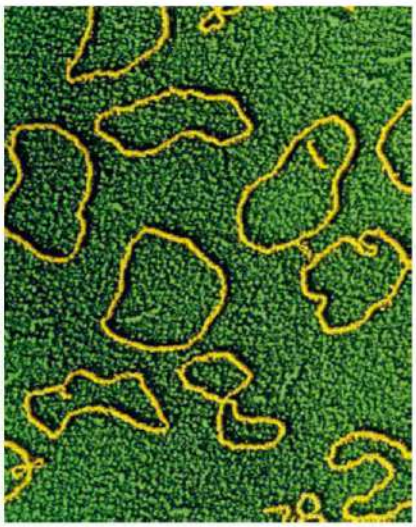
1 Recall the function of a gene probe. Identify one practical application.
2 State how DNA sequencing can help identify mutations.
3 Recall the steps involved in cloning by nuclear transfer compared to the Sanger method.

Understanding

4 Agarose gels can be made with different concentrations of agarose. If increasing the concentration of agarose results in tighter gel matrix, what would be the impact on migration speed?
5 Describe why gene probes are single stranded.

Genetic cloning: copying DNA using plasmids

An alternative to using PCR to generate a large number of copies of a DNA sequence is to insert it into bacteria. This process is called **gene cloning** and it has multiple advantages. It allows replication of larger segments of DNA and permits the analysis of any gene, and associated proteins, included in the DNA sequence in an environment where they are active.



Science Photo Library/Dr Gopal Murti

Figure 5.12 ▲ A transmission electron micrograph of bacterial plasmids from *Escherichia coli*

Plasmids are used to insert DNA into the bacteria. A plasmid is a circular piece of DNA that is found in bacteria and which reproduces independently of the bacterial chromosome (Figure 5.13). 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 5.14).

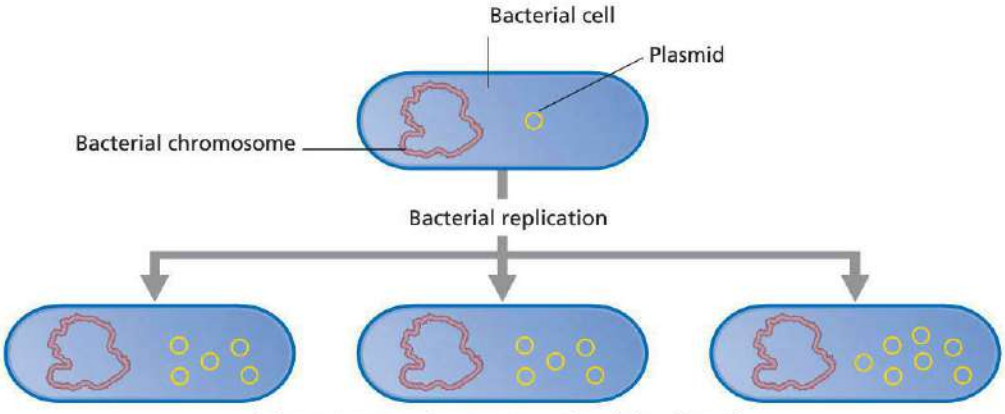
1. Plasmids are extracted from bacteria by rupturing the cell walls.
2. The same restriction enzyme is used to cut the plasmid DNA and the DNA of the gene to be inserted so that both pieces of DNA have complementary sticky ends.
3. DNA ligase binds the 'foreign' DNA fragment into the plasmid DNA. After 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.

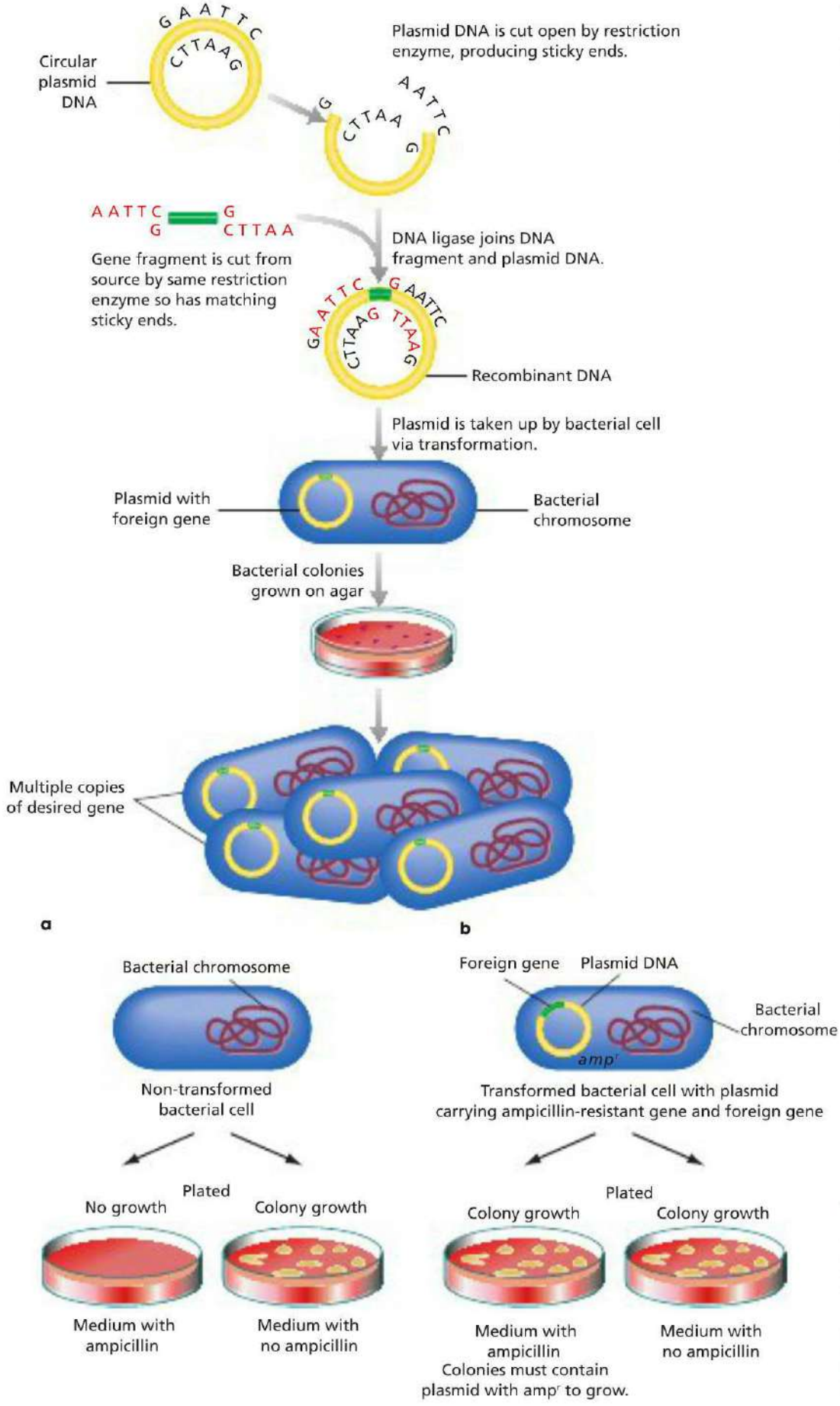
Gene cloning utilises bacterial plasmids to produce many copies of a gene.

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** (Figure 5.14). After transformation, the bacterial cells that contain recombinant plasmids have to be isolated from the majority of the colony, which has not taken up plasmids.

Plasmid DNA often contains genes for resistance to an antibiotic; for example, ampicillin. Bacteria that have been transformed with the plasmid are able to 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 as they are sensitive to the antibiotic ampicillin (Figure 5.15). This process is called antibiotic selection and is an important component of many biotechnology techniques.

Figure 5.13 ▶ Cloning a gene using recombinant plasmids





◀ **Figure 5.14**
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.

◀ **Figure 5.15**
Antibiotic selection of transformed bacteria. a) Non-transformed bacteria can't grow on media supplemented with ampicillin but grow well on normal media. b) Transformed bacteria can grow equally well on either media.

The bacteria with antibiotic resistance are then selected and grown in culture. To study the gene of interest, the plasmids can be isolated and analysed. 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. One example of this is the production of human growth hormone, which is used to treat people with a certain form of dwarfism. Prior to this technique, the hormone needed to treat these people was extracted from the pituitary glands of human corpses.

Transferring genes

On occasions, scientists may be interested in transforming organisms other than bacteria. For example, a sheep may be transformed with a gene for the blood clotting factor IX so that this protein is secreted in its milk. Factor IX can then be harvested from the milk and used to treat people with certain forms of haemophilia. Transferring genes is also the basis for gene therapy, which involves inserting functional copies of a gene into a patient with a genetic disease. The inserted gene will produce the missing protein, and thus alleviate the symptoms of the disease. For genes to be inserted into complex animals and plants, a method is needed to deliver the gene to the organism's cells. The gene needs to be able to function when it arrives in the host organism, whether it is a plant or animal. A number of different methods have been used to deliver genes. In most cases the gene is inserted into a **vector** that will carry the gene to the desired organism. In this context, a vector is a tool that can be used to transport DNA from one organism to another. Plasmids, viruses and liposomes can all act as vectors as they can transport small sections of DNA from one organism to another.

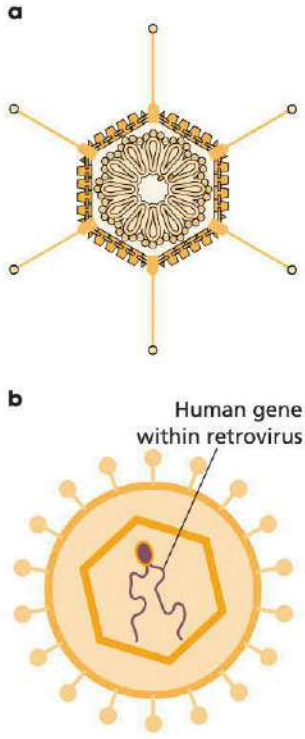


Figure 5.16 ▲
A vector can be:
a) an adenovirus,
b) a retrovirus or
c) a liposomes

Plasmid vectors

Purified recombinant plasmids can be inserted into a new organism directly. However, despite showing great potential, this method is not currently an efficient method of gene delivery as the plasmid DNA is not very stable in body cells in this form.

Viral vectors

Viruses infect target cells by injecting their nucleic acid into the host cell. By using recombinant DNA techniques, it is possible to insert desired genes into viral DNA or RNA, and use the virus to insert this new gene into the target cells. Viruses can accept large DNA inserts, making it relatively easy for them to accept foreign genes. As viruses are pathogens, it is also necessary to remove or disable the genes in the virus that cause disease symptoms. Two types of virus currently being used in this way are the adenovirus and the retrovirus (Figure 5.16). The main problem with using viruses as vectors is that human immune systems attack viruses and this may decrease their chance of survival within their new host. Furthermore, viral DNA insertion in the host genome can sometimes disrupt normal gene regulation and result in the development of cancer.

Liposome vectors

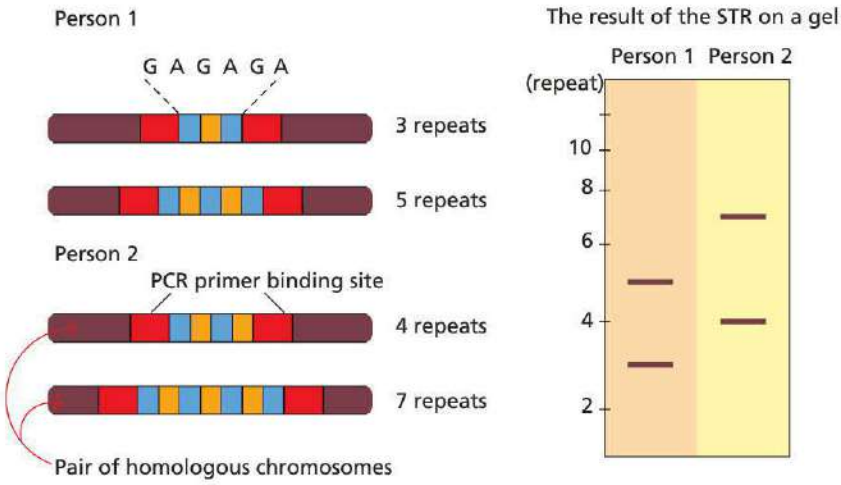
Liposomes are small spheres that are surrounded by a membrane composed of a phospholipid bilayer (Figure 5.16c). The phospholipid bilayer can fuse with other membranes, such as a cell's outer plasma membrane, thus delivering the contents of the liposome into the cell.

Liposomes can be made artificially and are designed to have molecules attached to their surface that can be recognised by specific types of cells in the host organism. Thus, when a liposome is inserted into a foreign organism, it can be targeted to reach specific cells, such as the kidney. A gene of interest can be inserted inside the liposome. This technique is used extensively to insert foreign DNA into cells cultured in Petri dishes.

DNA profiling

DNA profiling is a process used to compare the base sequence of two or more individuals to determine how similar or different they are. For example, your DNA will be more similar to your parents' DNA than to that of a next-door neighbour who is unrelated to you.

Short tandem repeats (STRs) and **variable nucleotide tandem repeats (VNTRs)** are sections of non-coding DNA that are repeated many times. STRs have a repeat sequence of two to five bases, while VNTRs have a repeat sequence of more than five bases. For example, the dinucleotide GA is repeated many times to form the STR, GAGAGAGA. The repeat is present in all members of the population, but the number of the repeats varies between individuals (Figure 5.17). Each individual usually has two alleles for each STR, one from each homologous chromosome. DNA profiling identifies people based on differences in the length of their DNA repeats for a large number of individual STRs. As every individual has their own unique number of repeats, this forms the basis of identification.



◀ **Figure 5.17**
STRs vary between individuals.

Constructing a DNA profile uses two techniques of the biotechnologist: PCR and gel electrophoresis. First, the DNA to be profiled needs to be isolated. This DNA can come from any somatic body cell that contains a nucleus, including blood or cheek cells. PCR is used to amplify the DNA of up to 13 different STR regions in the sample. The amplified DNA repeat fragments are then separated by gel electrophoresis, which sorts the fragments according to their lengths. Smaller fragments have fewer repeats and migrate further on the gel than do alleles with more repeats.

DNA profiling can be used to find relatedness with other individuals, identify criminals and conduct paternity testing.

WOW

Superb Fairy-wrens – not as faithful as you thought!

DNA profiling can be used to determine paternity. Through the use of microsatellite markers, a type of STR, researchers have established that approximately three in four Superb Fairy-wren (*Malurus cyaneus*) chicks are sired by a male other than their social fathers. This came as a surprise, because females have never been seen copulating with males other than their partners. It is believed that all copulations with other males take place under the cover of darkness either early in the morning or late in the evening.


Dreamstime/Ben Twist

▲ **Figure 5.18**
Paternity testing shows that Superb Fairy-wrens are actually promiscuous.

EXPERIMENT 5.2

BROOD PARASITISM AND FAMILY SIZE IN BLACK SWANS

Family size is highly variable in Black Swans and varies between one and seven. Interestingly, family size distribution seems to be bimodal with most families containing 1–3 cygnets or 5–7 cygnets. This has led many to speculate that the larger families are the result of brood parasitism, the result of a female laying her eggs in the nest of a second female and leaving this second female to raise her young. This process is quite common in ducks, but has not been investigated thus far in Black Swans.

Dreamstime/Hbfiala

Figure 5.19 ▲
A Black Swan family

One way to determine whether a female is the biological mother of her cygnet is to create a DNA profile for both the mother and the cygnet and determine if the cygnet shares half of the female's profile.

Aim

To determine, using DNA profiling, whether brood parasitism occurs in Black Swans and whether this explains the larger number of cygnets in some families

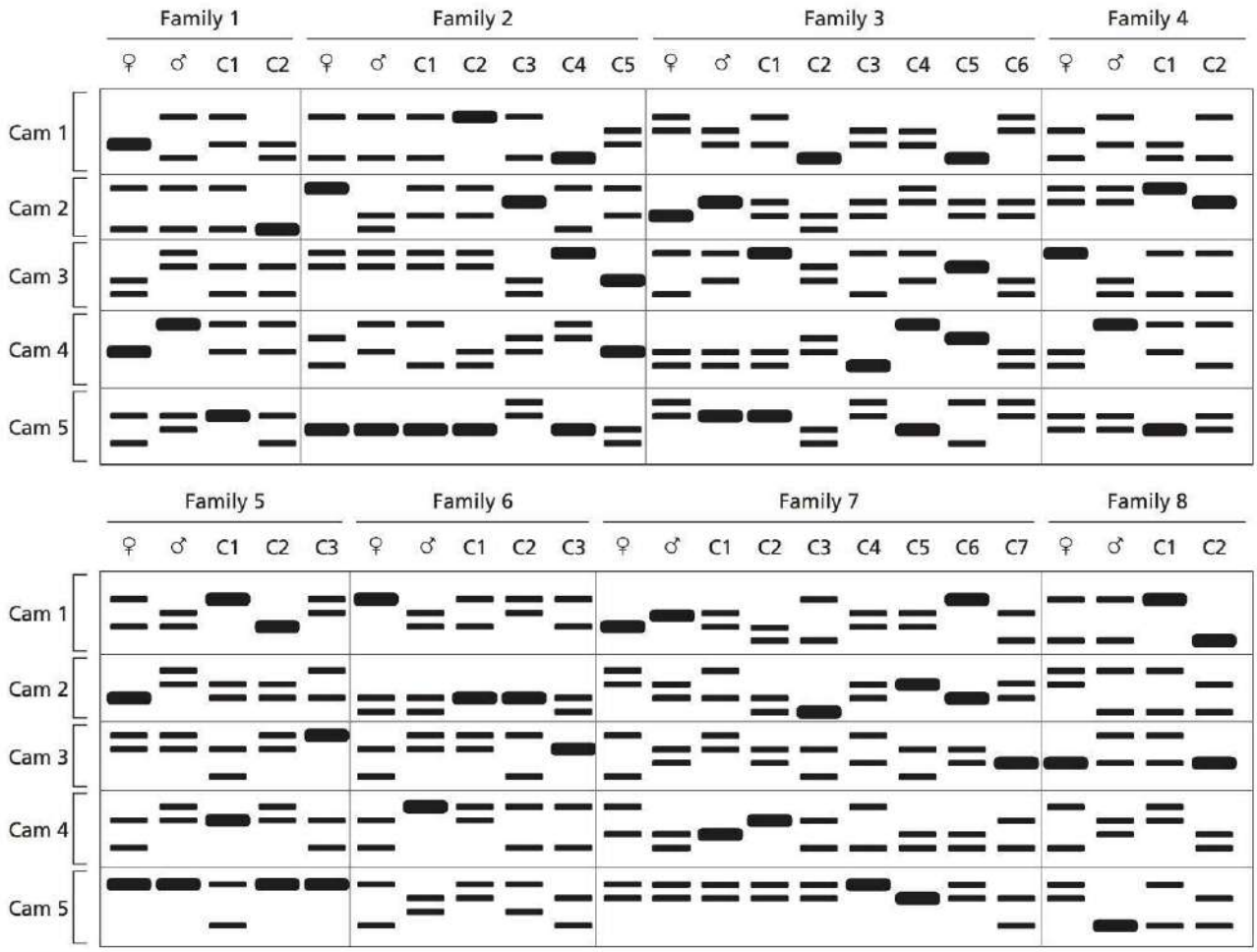
Material

Each student will require:

- ruler

Procedure

1. Consider the DNA profile in Figure 5.20. The necessary DNA was obtained by capturing swans, collecting a small blood sample from each, and extracting the DNA. Five STRs have been identified in Black Swans (*Cam*1, *Cam*2, *Cam*3, *Cam*4 and *Cam*5). Using PCR, these five regions were amplified in all adults and cygnets of eight families of swans. The PCR products were then separated using agarose gel electrophoresis. Figure 5.20 shows the resulting gel. Each individual has two alleles for each STR, but sometimes only one band is observed as the individual has two identical alleles.
2. Compare the profile of the mother of each family and the profile of each cygnet and determine if the female could have been the biological mother of the cygnet.
3. Copy Table 5.3 and record your results in the second column.
4. Calculate the proportion of parasitic cygnets in each family and include these in the third column.
5. Determine if there is any difference in proportion of parasitic cygnets between small and large families.



▲ Figure 5.20
DNA profiling for the eight black swan families that include a social mother (♀), social father (♂) and cygnets (C)

Results

Table 5.3

Family	Biological cygnets	Parasitic cygnets
1		
2		
3		
4		
5		
6		

Analysis of results

1 Using your results, identify any evidence of brood parasitism in Black Swans.

2 Calculate the maximum proportion of parasitic cygnets in this sample.

Discussion

1 Explain whether the results confirm the belief that large Black Swan families are due to brood parasitism.

2 Describe how you could determine whether a cygnet has been fathered by a male other than its social father.

QUESTION SET 5.3

Remembering

1 Define 'plasmid' and recall its use in biotechnology.
2 Define 'bacterial transformation'.
3 Recall the types of DNA sequence used for DNA profiling.

Understanding

4 Distinguish the three types of vectors.
5 When making a recombinant plasmid, clarify why it is important to cut the plasmid and the gene of interest with the same restriction enzyme.

Biotechnology applications for humans

Gene technology is revolutionising the diagnosis and treatment of diseases such as cancer in humans, prolonging and improving the quality of life for patients. Cancer is a group of diseases that is defined by an uncontrolled proliferation of cells. These cells also have the ability to metastasise, or spread, to other tissues. Cancerous cells arise when multiple mutations occur in key genes that control or regulate cell division.

Gene technology allows researchers to identify some of the genes disrupted in individual cancers and tumours. Microarray technology can be used to investigate which genes are disrupted in certain tumours, thus enabling determination of which drugs would be most effective in restoring gene activity to normal. Genetic testing also facilitates a more accurate diagnosis of the type of tumour a patient has and which treatments will be most effective. Gene therapies may also prove useful in the fight against cancer. Some therapies that are being researched involve injecting cancer cells with genes that give rise to toxic molecules when these genes are expressed, resulting in proteins that then kill the cancer cells. Another approach involves modifying the cells of the immune system of cancer patients so that they can recognise and destroy cancer cells. This process has been met with some success but is still very experimental.

The types of applications that scientists have developed that have potential use in medicine include:

- identifying alleles of genes responsible for serious medical conditions in an effort to speed up the discovery of a treatment for them
- screening for genetic diseases to determine if an individual or embryo is a carrier of a genetic disease
- developing transgenic organisms to produce large quantities of protein that can be used for commercial or therapeutic purposes
- gene therapy for humans with a genetic disease.

WOW

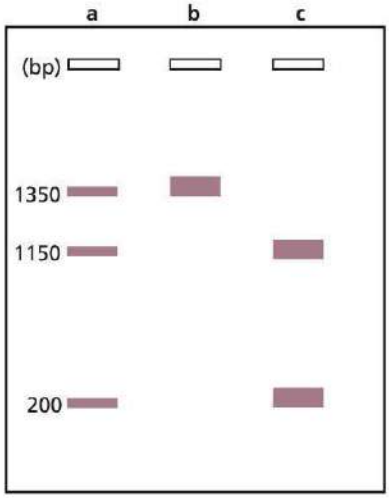
Pharmacogenomics

Patient response to chemotherapy is highly variable. Pharmacogenomics is an emerging area of biotechnology that examines the inherited variations in genes that determine our responses to drugs and explores the ways that these variations can be used to predict whether a patient will have a good response to a drug, a bad response to a drug, or no response at all.

Genetic testing

Genetic testing involves examining a person's genetic material. This can involve analysis of a person's DNA or chromosomes, or assessment of the products of genes or metabolites that result from the actions of proteins coded for by a gene. Genetic testing is most often done to determine if someone possesses an allele for a gene that is associated with a specific disease.

Various biotechnological techniques and processes are used to conduct genetic tests. All mutations causing diseases can be detected by direct DNA sequencing, but if the mutation changes a restriction site, it can be detected using a technique known as restriction fragment length polymorphism (RFLP) analysis. One example of such a mutation is the single base-pair change associated with sickle cell anaemia. Within the wild type sickle cell anaemia gene there are three *Mst*II restriction sites (CCTNAGG). The allele for sickle cell anaemia is due to a point mutation, which causes a change in a single amino acid in the beta-globin molecule of haemoglobin. This single base change also results in the loss of one of the *Mst*II restriction sites. Thus, restriction digestion of the PCR amplified region of the mutation-containing sickle cell anaemia gene locus in a normal patient will yield two fragments, but only one in an affected patient (Figure 5.21).



▲ **Figure 5.21**
The electrophoresis gel shows the patterns of: a) a carrier, b) a person affected with sickle cell anaemia and c) a normal individual.

Production of pharmaceuticals using gene cloning

Using genetic cloning, functional genes can be inserted in bacteria, allowing the production of larger amounts of proteins of interest. Individuals who are unable to produce a particular protein can be treated with injections of that protein. One example is the production of insulin. Some diabetic patients are unable to produce insulin and their blood sugar level cannot be controlled within precise limits. Insulin is now manufactured in large vats containing genetically altered *Escherichia coli* bacteria. People with insulin-dependent diabetes are able to purchase genetically engineered insulin at a low cost. Other examples include human haemoglobin and clotting factor VIII that are now extracted from transgenic animals.

Gene therapy

Gene therapy is a technique of delivering normal and fully functional genes to individuals to compensate for a disease-causing mutation (Figure 5.22). For gene therapy to be effective, the functional gene has to be located, isolated and cloned in sufficient quantities and a suitable vector has to be found. It would be impossible to replace every disease-causing allele in each body cell, so gene therapy needs to target the regions in the body that are affected by the disease (Figure 5.23). The inserted gene also needs to be able to persist and function normally within the target cells.

Gene therapy was once heralded as a cure-all for many conditions; however, scientists have encountered significant difficulties in overcoming problems associated with transferring genes safely and effectively into human cells.

A breakthrough came when scientists were trying to treat children who had a rare genetic disorder called severe combined immunodeficiency (SCID). SCID is caused by a gene that disrupts the functioning of both the B and T cells of the immune system. These children are extremely susceptible to infectious disease and must live in a completely sterile environment. Scientists were able to insert a functional copy of the affected gene into an adenovirus. This viral vector was then used to transform bone marrow cells harvested from the children. The bone marrow cells were then returned to the children, where they were now able to produce functional T and B cells. The children gained a normal immune system and have lived healthy lives for several years now.

See Chapter 12 for further information on SCID.

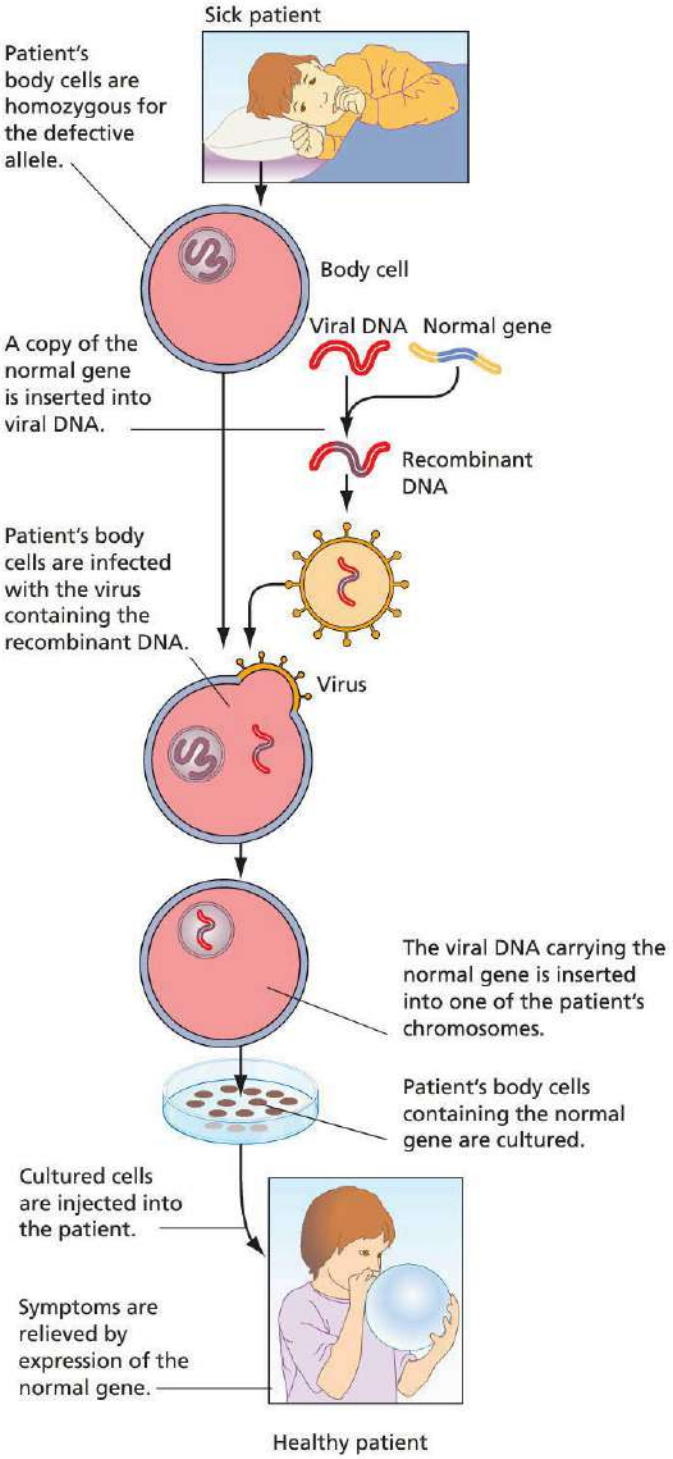


Figure 5.22 ▲
Experiments on gene therapy are continuing. New genes can be added to somatic cells. When they are returned to the body, the transgenic cells can produce the missing gene product.

A major difficulty with using gene therapy for treating many other conditions is delivering the therapeutic genes into tissues where they need to be expressed. For example, individuals with cystic fibrosis lack a functional *CFTR* gene, which is needed for the correct formation of protein that helps regulate the uptake of chloride ions into cells lining the lungs. Individuals with cystic fibrosis suffer from a build-up of mucus lining the lungs, which makes them very susceptible to infections. Clinical trials are currently underway to use liposomes to deliver functional copies of the *CFTR* gene to lung cells using an inhaler.

The applications of gene therapy described above are examples of **somatic cell gene therapy** as they alter the genotype of somatic cells; such changes are not heritable. For many conditions, a number of organs or cells of the body are affected. It is extremely difficult to insert new genes into every cell or organ affected. Somatic cell gene therapy does not solve the problem of people with a particular gene disorder passing on the affected allele to their offspring.

A possible solution would be to use gene therapy to transform eggs or sperm, so that any changes to their genotype will be passed on to successive generations and all cells of their offspring will contain the therapeutic gene. This is known as **germ-line gene therapy**. Naturally, this kind of therapy is extremely controversial as future generations will be affected if there are adverse effects of the therapy. Consequently, germ-line gene therapy is not an accepted medical practice.

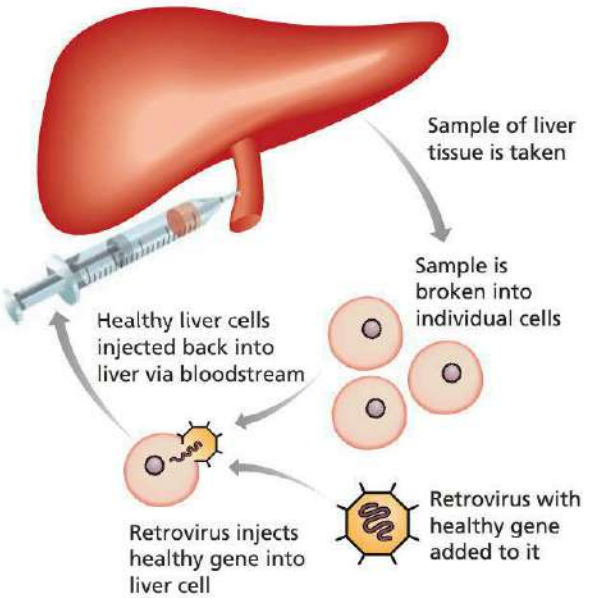


Figure 5.23 ▲
Gene therapy using liver tissue

QUESTION SET 5.4

Remembering

1 Recall what a somatic mutation is.
2 Define 'gene therapy'.

Understanding

3 Outline the different techniques used to identify genetic diseases.
4 Distinguish somatic cell from germ-line gene therapy.

Biotechnology applications for agriculture

Millions of people around the world are malnourished. Scientists have been trying to use their molecular tools and techniques to modify food crops, resulting in higher nutritional value and greater crop yields. Biotechnology has also been applied to reduce the impact of pests on crops, thus increasing the amount of food available in developing countries. The process used for most of these applications is transformation: taking a gene from one species and inserting it into another to obtain a desired characteristic.

Modifying plants for human nutritional benefits

Scientists are looking at ways to genetically engineer staple-diet plants as a way to supplement the human diet for essential nutrients that may be difficult to obtain from natural sources. Two essential nutrients currently being investigated are vitamin A and omega-3 oils (Case study, page 142).

More than one million people die each year from diseases caused by vitamin A deficiency, mainly in developing countries. With the use of biotechnology, golden rice has been engineered to contain beta-carotene, the nutrient required to form vitamin A. While 100 g of the original strain of golden rice only gives 5–8% of the recommended daily intake of vitamin A, newer strains can provide up to 60% for the same portion size.

Genes against pests

Stem rust is a disease of wheat in eastern Australia. It is treated by spraying plants with fungicides. However, pathogens that cause stem rust can develop resistance to fungicides, and new strains of the stem rust frequently appear. The CSIRO has developed a method of isolating the rust-resistant gene (*L6*) from a rust-resistant flax plant and then introducing it into a rust-susceptible plant. This genetically engineered plant grows resistant to rust. Other similar examples include ringspot virus-resistant papayas and yellow mosaic virus-resistant zucchini.

Cotton plants are susceptible to the caterpillar of the moth *Helicoverpa punctigera*. Normally, regular spraying of insecticide has been used to eradicate this pest. However, it has been discovered that the soil bacterium *Bacillus thuringienis* produces a range of proteins that are toxic to some insects. By inserting genes from this bacterium that are toxic to the *Heliothis* caterpillar into cotton plants, the plants are protected from damage. The use of genetically engineered cotton reduces the use of insecticides. Unfortunately, resistant strains have evolved in various insects.

Case study

Long-chain omega-3 oils in grains

Omega-3 oils, especially the long-chain fatty acid docosahexaenoic acid (DHA), are critical for brain and eye development in infants and are an essential part of a healthy adult diet. These healthy oils are not synthesised by humans and thus must be obtained through our diet. Land plants do not normally produce DHA. Only marine microalgae are known to possess the biochemical machinery to synthesise DHA, and fish contain large amounts of DHA because they feed on those algae. Fish are therefore the major dietary source of DHA, but fishing pressure is causing a worldwide decline of fish stocks.

In collaboration with Nuseed and the Grains Research and Development Corporation (GRDC), scientists from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) are working on a project aiming to solve this problem. The Canberra-based team is working towards the production of genetically modified canola that contains long-chain DHA levels similar to that of fish.

After years of research and development, in 2012 the CSIRO team created transgenic *Arabidopsis thaliana* (a commonly used laboratory model plant) by inserting genes (sourced from microalgae coding for DHA synthesis enzymes) in its genome. This was a breakthrough because these plants then produced seeds with levels of DHA similar to that found in fish oil. In 2013 the team reported similar results in *Camelina sativa* oilseed crop, which produced levels of DHA similar to commercially available fish oils.

Since then, the CSIRO team has successfully produced fish-oil-like levels of DHA in their canola crop in the laboratory and continue to develop this work with canola in mind, due to its adaptability to different growing regions and high oil content.

Field trials will begin in 2014, and canola containing long-chain DHA may be commercially available by 2018 provided that regulatory requirements and development milestones are met.

CSIRO

Figure 5.24 ▲
The CSIRO research team, left to right, front to back row: Dr Surinder Singh, Mr Lijun Tian; Dr Qing Liu, Dr Yoko Kennedy; Dr Srinivas Belide, Dr Pushkar Shrestha, Ms Anne Mackenzie, Dr James Petrie

Questions

1. Learn about omega-3 oils. Find alternative sources of these oils aside from fish. Where would vegetarians source these oils?
2. Think about alternatives. Would it not be simpler to eat algae rather than making crops that produce DHA? Why do you think this approach was not selected?
3. How would you feel about eating bread and cake enriched in DHA due to the use of genetically engineered wheat?
4. Why stop there? Can you imagine that genetically engineered crops will be developed to produce other essential nutrients or medications?

Herbicide-resistant crops

A major problem for farmers is the large number of weeds that grow throughout their crops. Spraying herbicides on crops to kill the weeds usually damages the crop as well. Herbicide-tolerant crops have been developed, which is proving to be an effective solution for farmers. These include canola, soybean and sugar beet.

Herbicide-resistant crops may be more productive and may be more environmentally sustainable but their use is questioned by many. It is too soon to be sure of the long-term effects on the environment and other organisms.

Genetically engineered animals

Many different genetically engineered animals have also been developed. These include salmon with increased growth rate, cows that produce milk similar to human breast milk, and enviropigs that release less phosphorus in their manure. As well as these examples of domestic animals,

laboratory animals including rats and mice have been engineered to contain alleles that make them model organisms for studying human disease and for discovering the functions of different genes.

Applications in biodiversity conservation

Many of the techniques we have discussed thus far have also been employed in efforts to preserve biodiversity and to help manage vulnerable populations. In small populations of animals and plants, there is a risk that closely related, and thus genetically similar, individuals will breed together. The resulting offspring will have an increased risk of deleterious recessive alleles becoming homozygous, causing genetic diseases. This is called inbreeding depression. Biotechnologists can use various techniques, including DNA profiling, to selectively breed individuals. This is very common in captive breeding programs of threatened species like the Mountain Pygmy Possum and the Orange-bellied Parrot.

Studies have been carried out to determine the genetic diversity of the gene pool of populations of elephants and cheetahs. This will help identify and preserve the genetic diversity that exists in their populations and thus improve their chances of long-term survival. DNA was extracted from material in their droppings. A major advantage of collecting faecal DNA is that it does not require capturing the animal. This avoids stress to the animal and danger to the researcher.

WOW

Inbred koalas

The koalas on Phillip Island in Victoria have become isolated from other populations. By studying their DNA and creating a genetic profile for each animal, researchers have found that the genetic diversity between the members of this population is very small and thus the population is inbred. With little to no variation between members of the population, they all become susceptible to the same disease, *Chlamydia*, which causes infertility and blindness.

Thinkstock/Adam Booth

▲ Figure 5.25
Koalas on Philip Island are inbred.

QUESTION SET 5.5

Remembering

1 Recall the advantages of golden rice over normal rice.
2 List three types of genetic engineering used in crops.

Understanding

3 Outline how biotechnology can diminish the risk of inbreeding depression in captive populations.

Emerging technologies

Biotechnologists are also investigating a number of new techniques and processes. These include cloning and **stem cell** therapy. In the future, these may be used on their own or in conjunction with other processes for benefits in both medicine and agriculture.

Cloning

Cloning is the process of making an identical copy of an original. In biology it is used in two contexts. First, cloning a gene involves using recombinant technology: a gene is extracted from one organism and then inserted into a bacterium, where it can be used and studied.

In contrast to genetic cloning, which involves making a copy of a gene, biological cloning involves cloning an entire organism; that is, reproductive cloning. Cloning can make it possible for cattle or sheep with desirable characteristics, such as high milk production or fine wool growth, to be produced more rapidly than through the normal cycles of reproduction and selection. It is achieved by embryo splitting or by nuclear transfer.

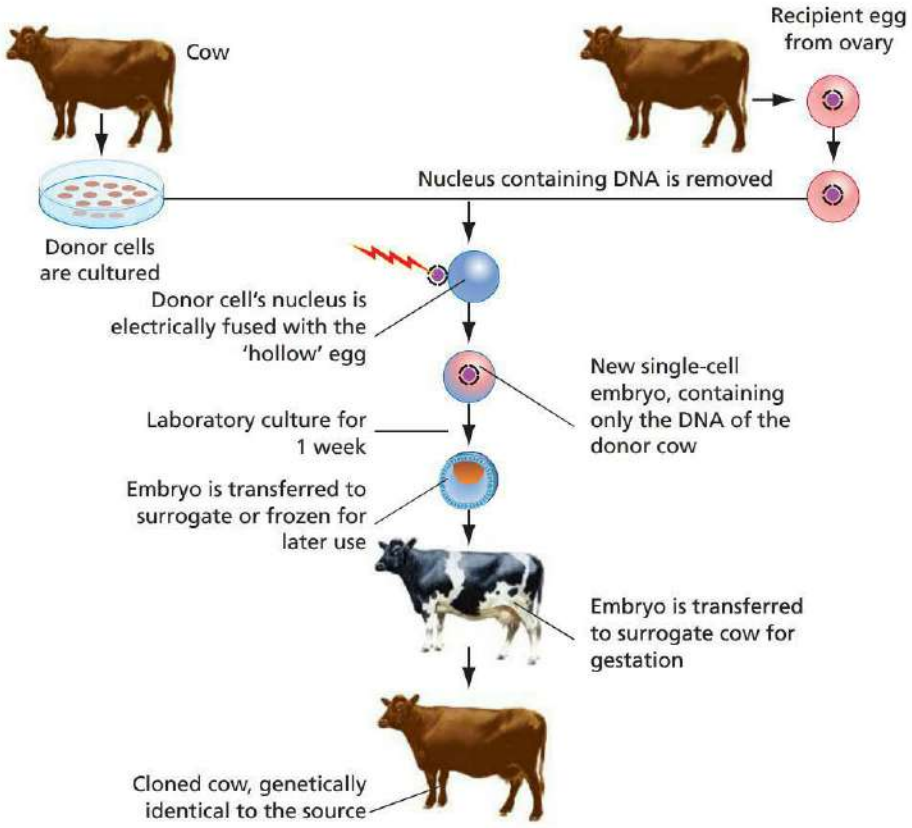
Embryo splitting

In embryo splitting, egg cells are removed from the donor female and fertilised *in vitro* (i.e. in tissue culture) by sperm from the male. After the zygote has divided, the coat around the two cells that promotes cell division is removed and the two cells are separated. Each cell is then given an artificial coating that promotes cell division. Embryos that have just begun to differentiate, called blastocysts, are implanted into surrogate mothers. The individuals are genetically identical; they are like identical twins but from different surrogate mothers.

Nuclear transfer

The process of cloning by nuclear transfer came to prominence when Dolly the sheep was cloned in 1996. Nuclear transfer involves removing mature donor somatic cells from a mature animal and a recipient egg cell from another mature animal of the same species (Figure 5.26). The donor cells are cultured in a nutrient medium before becoming inactive, and the nucleus of the recipient egg cell is removed.

Figure 5.26 ▶ Cloning a calf by nuclear transfer



The donor cell, with the intact nucleus, is fused with the 'hollow' egg by an electrical impulse. The new single-celled embryo is cultured for about a week, then cell division is activated and the developing blastocyst is surgically implanted in the surrogate mother. The offspring is genetically identical to the nucleus donor.

Cloning using nuclear transfer has not proceeded without some controversy. The success rate of live births is low and many of the offspring suffer from severe deformities. For these reasons alone, the scientific world is almost universally opposed to experimenting with reproductive cloning for humans.

WOW

Clone your pooch!

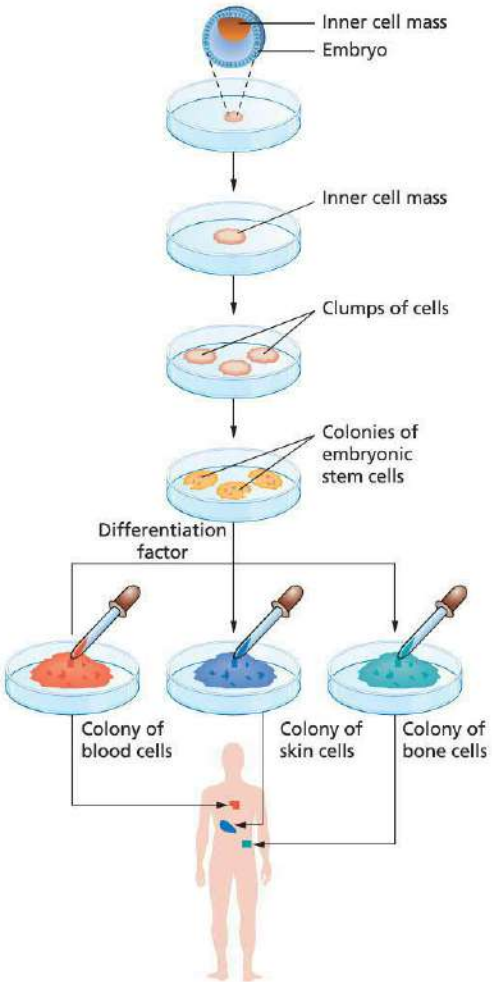
Pets are an important part of many families and thus when they die, the resulting sense of loss can be very strong. Have you ever hoped that your dog could live forever? Well there is a company in South Korea that can clone your dog if you can spare $100000. Although they cannot guarantee the personality of the dog, they promise to recreate a dog that is genetically identical to your original pet.

Stem cell research

Most of the cells of our body, for example blood, liver, brain and nerve cells, are specialised to perform particular functions. These specialised cells have become differentiated by a following a particular developmental pathway, and for them there is no turning back. On the other hand, **stem cells** are undifferentiated cells that have the potential to develop into many different kinds of cell. Unlike differentiated cells, stem cells also have the capacity to keep dividing and renewing themselves. These two characteristics make them ideal for cell-based therapies that aim to replace tissues that have degenerated or been damaged, such as in Parkinson's disease, diabetes, heart failure and spinal injuries. Stem cells may prove useful in the development of new methods for gene therapy, or for researching early stages in human development, and for understanding cell differentiation and function. Research into stem cells may also provide answers as to why cells become cancerous and how this may be prevented. When grown in culture, stem cells can be useful for testing drugs safely before they are trialled in humans, or for screening for the effects of potential toxins, such as pesticides, before they are used in the environment.

There are two main types of stem cell: embryonic and adult. **Embryonic stem cells** are derived from a four- to five-day-old embryo. They have the ability to form virtually any type of cell found in the human body, and are therefore said to be **pluripotent,** which makes them particularly attractive for cell-based therapies. Embryos are obtained from parents who have completed infertility treatment and have consented to donate excess embryos to research. However, to obtain the stem cells requires the destruction of an embryo, which raises significant ethical issues, and governments have had to ensure that there are strict regulations for controlling the use of this technology. Embryonic stem cells grown in culture (Figure 5.27) also have a tendency to form tumours called teratomas, which may limit their usefulness.

Adult stem cells are undifferentiated cells found among differentiated cells in a tissue or organ after birth. They are **multipotent** and give rise to a more limited range of cells types, which may limit their usefulness in cell-based therapies. However, they do not form teratomas and therefore may be safer to use. Adult stem cells also have the advantage of avoiding the ethical issues associated with the use of embryonic cells, and tissues generated from these cells can circumvent problems with transplant rejection if they are derived from the person who is to receive the stem cell therapy.



▲ **Figure 5.27**
Embryonic stem cells from humans can be cultured and made to differentiate into any type of human tissue.

Under specific circumstances, differentiated cells can also be made to divide again by artificially inducing the expression of specific genes. These are called induced pluripotent stem cells and can also be used as a source of stem cell. Much research is being undertaken to try to understand how to make and use induced pluripotent stem cells.

There are pros and cons for the use of both types of stem cells and research is continuing with both types. Other research is also looking into obtaining stem cells from the umbilical cord and placenta.

QUESTION SET 5.6

Remembering

1 Define the following terms.
   a Cloning
   b Stem cell

Understanding

2 Predict how similar genetically your clone would be to yourself.
3 Summarise the differences between embryonic stem cells and adult stem cells.

Ethical issues associated with biotechnology

The ability to manipulate and modify DNA carries responsibility. The implications of gene technology and the issues associated with the application of gene technology have to be considered in any decisions about what should or should not be done.

When considering the implications of gene technology the following questions can be helpful.

- Is the effect on the organism temporary or permanent? If permanent, is the change heritable?
- Is the structure or functioning of an organism affected?
- Is the health or survival of non-target species affected either directly or indirectly?
- Is there a change in the selective advantage of an organism as a result of its modification?

GMOs

GMOs such as crops are theoretically very appealing: farmers get a better yield, spend less on pesticides and herbicides, and don't pour dangerous chemicals into our environment. Yet how is the modified crop going to affect the ecosystem? This question will probably not be answered for 10 or more years after a modified crop's first use in agriculture. Studies in India have demonstrated the evolution of toxin resistance in aphids and mealy bugs resulting in the inefficacy of genetically modified corn.

What about the spread of modified genes? This depends on how the plant is pollinated. Plants such as cotton are usually wind pollinated. This could lead to the spread of modified plants to other nearby crops belonging to farmers who may not want to use this type of crop because of the unknown long-term effects or its poor acceptance among some consumers. The effect of GMOs on non-target species is also controversial. Some researchers claimed that the pollen from pest-resistant corn could threaten the Monarch Butterfly, but these claims have now been disproved.

Some other concerns raised by consumers relate to the increasing allergenic effect of combining genes from two organisms. If a gene that produces a protein from one plant is introduced into a second plant to induce a desired trait, there is a chance that the introduced transgene might cause an allergic reaction in some people.

The application of biotechnology is fraught with controversy: there are arguments for and against, and the merits of each application have to be evaluated from a number of perspectives. Scientific, industrial, commercial and governmental interests have to be examined along with

the views of society. Gene technology is costly. Who will benefit? We have powerful tools available to us, capable of changing the course of life, and their use has to be debated. The common arguments for and against gene technology are presented in Tables 5.4, 5.5 and 5.6.

Table 5.4 Arguments for and against biotechnology

For	Against
Biotechnology is natural; genetic engineering has existed for years; for example, farmers breed specific cattle to achieve the desired traits. Biotechnology is simply an extension of this process.	Biotechnology is not natural; selective breeding only involves individuals from the same species, yet biotechnology is transferring genes across species, which rarely happens naturally.

Modifying plants and animals and releasing them into the environment raises the issue of their effect on the relationships of organisms in ecosystems. Table 5.5 presents some of the arguments for and against.

Table 5.5 Arguments for and against the use of biotechnology with respect to effects on the environment

Possible positive effects on the environment	Possible negative effects on the environment
Herbicide-resistant crops are resistant to a herbicide that is not very toxic to the environment. This enables the farmers to use more of this herbicide rather than use a more toxic herbicide, which may be damaging to the environment.	Herbicide-resistant crops may encourage farmers to use more herbicide on their crops, which could potentially be more damaging for the environment.
Researchers, so far, have found that there is little transfer of genes between species.	There may be gene transfer between closely related species; weeds may become resistant to the herbicide.
	We may not know what gene transfers may have occurred.
	We may not know what transgenic organism may have escaped into the environment.

Proteins are being produced for use by humans using other animals or bacteria, and crops are produced that have a higher yield. Fruit such as bananas and tomatoes are being modified so that they don't bruise on the way to market or ripen too quickly. With these advancements come another set of issues for people who are going to eat the GMOs (Table 5.6).

Table 5.6 Arguments for and against genetically engineering foods with respect to public health

Arguments for	Arguments against
Biotechnology can vastly improve the health, nutritional value and growth capacity of agricultural species, and therefore greatly help to combat a global food crisis and benefit public health.	Selective breeding has provided us with crop improvements in the past and can be a source of steady improvement in crop quality.
There are strict guidelines that aim to ensure all genetically engineered food is as safe as non-genetically engineered food. The genetic code is common in all living species.	The long-term effects of genetic modification of crops are essentially unknown.

With the increased use of biotechnology, governments of the world have an obligation to keep the public informed about issues that are important to them. Many issues have emerged from the use of genetic engineering. Advisory committees have been set up worldwide for the purpose of alerting the authorities to any risk factors and to ensure guidelines are consistent worldwide.

Scientific literacy: Superweed outbreak triggers arms race

Hardy superweeds immune to the Farm Belt's most effective weedkiller are invading fields, prompting a counterattack from agribusiness that could leave farmers using greater amounts of harsh old-line herbicides.

The flagging weedkiller is Roundup. Its developer, Monsanto Co., also sells seeds for corn, soybean and cotton plants unaffected by the chemical, enabling farmers to spray it on freely without fear of harming their crops. Farmers now do so en masse, using 'Roundup Ready' crop varieties for 90% of the soybeans and 80% of the corn grown across the US.

The rise of Roundup, more than a decade ago, sent older herbicides that damage both weeds and crops into deep eclipse. But now, as nasty invaders with names like pigweed, horseweed and Johnsongrass develop immunity to the mighty Roundup, chemical companies are dusting off the potent herbicides of old for an attack on the new superweeds.

And big chemical companies – taking a page from Monsanto's book – are engineering crop varieties that will enable farmers to spray on the tough old weedkillers freely, instead of having to apply them surgically in order to spare crops.

Kilman, S. (2010) 'Superweed Outbreak Triggers Arms Race', *The Wall Street Journal*, 4 June. Reprinted with permission of Wall Street Journal © 2010. Dow Jones & Company, Inc. All Rights Reserved Worldwide.

Questions

1. Glyphosphate is the active ingredient of Roundup. Research and explain why Monsanto initially chose to engineer crops that are resistant to glyphosphate rather than to some other chemical.
2. Evaluate the return to older, more powerful herbicides as a long-term solution to this problem.
3. Propose how pigweed may have acquired glyphosate resistance.
4. Given the information provided above, discuss the benefits of herbicide-resistant crops to farmers and how this balances with the benefits to the company that produces them.

In Australia in June 2001, the Office of the Gene Technology Regulator came into effect as a result of the *Gene Technology Act 2000*. The purpose of this regulator is to ensure that no GMOs pose a risk to the environment and that all laboratory work is contained.

Issues for biotechnology in medicine

Biotechnology is a rapidly growing area with advances in medicine constantly being made. Techniques are being improved and developed and new applications are discovered. However, many of the applications are not fully commercialised or being used in medicine. Gene therapy is still in the early stages of development, whereas gene cloning has been used widely for a number of years now. The potential applications in medicine are enormous, although the practicalities and ethical issues surrounding biotechnology will determine whether these are realised.

Genetic tests for all

Life insurance companies could use genetic tests to determine the risk of insuring people. Eventually, these companies may be able to estimate the life span of a policy holder. To forecast the possibility of a policy holder having a heart disease or cancer will mean higher premiums for insurance. Under existing insurance company practices, results of genetic tests can be made available to the insurer for the purposes of classifying a risk.

Who has a right to know?

In situations where genetic screening has revealed that a person is a carrier for a defective heritable gene, such as for cystic fibrosis, thalassaemia, Duchenne muscular dystrophy or haemophilia, who in the family has a right to know?

Patenting genes

The cost of research by companies to discover biological pathways and develop new drugs is immense and, to recoup those costs, patents are usually sought that give the patent holder exclusive rights to specific therapeutic treatments or other applications resulting from their

discovery. An alternative to patents is for laboratories to keep research secret. Scientific research relies heavily on communication and, to a certain extent, cooperation with other laboratories and so secrecy in research could bring about a stagnation of ideas.

In the past, a company that is researching a particular gene could apply for patents covering all future research and development and all possible gene mutations. This stifled other research and maintained a monopoly on specific treatments and, in July 2013, a US court ruled that genes cannot be patented, overturning thousands of US patents on genes.

Animal ethics

One important aspect of the debate about GMOs that must be contemplated is the issue of animal welfare. Genetic engineering of animals for our needs, as well as the use of laboratory animals in research, must be carefully considered. Any research conducted on animals is governed by strong ethical principles and is reviewed and overseen by institutional ethics committees comprising scientists, veterinarians, members of animal welfare organisations and members of the public. These committees have the power to stop or modify studies involving animals if welfare issues are not adequately addressed.

QUESTION SET 5.7

Remembering

1 Recall the function of the Gene Technology Regulator.
2 List some of the fears associated with genetically engineered foods.

CHAPTER SUMMARY

- A growing understanding of molecular biology has allowed us to exploit cellular tools for use in biotechnology, fuelling the 'gene revolution'.
- Restriction enzymes are enzymes isolated from bacteria that cut DNA at specific sites known as restriction sites. These sites are four to eight nucleotides long. Cutting can result in the formation of either sticky ends or blunt ends depending on the enzyme.
- DNA ligase is an enzyme used to join two DNA molecules with complementary sticky ends or with blunt ends.
- PCR is a process through which a specific DNA sequence can be amplified for analysis.
- Using gel electrophoresis, it is possible to separate DNA fragments according to size and to visualise them by using a DNA-binding dye that fluoresces under UV light.
- Gene probing uses a single-stranded DNA molecule complementary to a gene of interest to identify, isolate or position that gene on a chromosome.
- DNA sequencing can allow the determination of the exact nucleotide sequence of DNA fragments, which, among other uses, can help to identify genetic mutations that lead to disease.
- Gene cloning is a process through which a large number of copies of a gene of interest can be made in bacteria by incorporating the gene in a plasmid.
- Genes can be transferred from one organism to another using different vectors including plasmids, viruses or liposomes.
- DNA profiling is a technique that can be used to determine an individual's genetic profile. This profile can be used to find relatedness with other individuals, identify criminals and conduct paternity testing.
- Biotechnology has many uses in medicine. They include:
  - identifying mutations that can cause disease
  - screening the genome of individuals for mutations that are known to cause disease

  - creating genetically engineered organisms that can produce large quantities of proteins that have therapeutic use in humans
  - allowing gene therapy to treat human disease.
- Biotechnology has been used extensively in agriculture to create genetically engineered crops that produce greater yields, are protected from pests and diseases, are resistant to herbicides and/or produce desirable nutrients.
- Biotechnology can provide very important information for the conservation of species by showing the genetic profile of individuals, allowing breeders to choose the least-closely related individuals for breeding purposes, and hence help to maintain genetic diversity.
- Cloning can be accomplished using either embryo splitting or nuclear transfer and results in the production of an individual genetically identical to the DNA donor.
- Stem cell research encompasses a number of techniques that use stem cells: cells that can differentiate into various tissues. These cells include embryonic stem cells, adult stem cells and induced pluripotent stem cells.
- The use of GMOs is controversial and, prior to their widespread use, potential impacts on the environment, their potential toxicity and allergenic potential need to be investigated.
- Ethical considerations are an important aspect of the debate over the use of genetically modified organisms, particularly the consideration of animal welfare.

CHAPTER GLOSSARY

agarose gel gel matrix used for electrophoresis

annealing in PCR, a process of joining separate strands of DNA together as a result of hydrogen bonds pairing; occurs when the temperature is lowered

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

denature in PCR, to change the molecular structure of a protein or DNA by applying high temperature; in DNA, the hydrogen bonds break and the two strands separate

DNA ligase an enzyme used to catalyse the formation of a bond between two pieces of DNA

DNA polymerase an enzyme capable of making exact copies of fragments of DNA

DNA profiling a process that is able to identify natural variations that exist within an individual's genome, by using the polymerase chain reaction and gel electrophoresis

DNA sequencing a process of establishing the nucleotide sequence of a piece of DNA

embryonic stem cell a pluripotent stem cell derived from an embryo

ethidium bromide a chemical that binds to double-stranded DNA and fluoresces pink when exposed to ultraviolet light; used to locate DNA in an agarose gel following electrophoresis

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

gene probe a specific short length of single-stranded DNA molecule that can bind specifically to a gene of interest

gene therapy a method of delivering normal and fully functioning genes to individuals who have a mutated defective version of the particular genes

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 organisms (GMOs) see *transgenic organisms*

genome all of the genetic material contained in an organism or a cell; includes the chromosomes within the nucleus and the DNA in mitochondria and chloroplasts

germ-line gene therapy replacement of a faulty gene within a germ-line cell

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

multipotent a stem cell that is able to differentiate into a limited number of cell types

plasmid a small circular piece of DNA, found in bacteria, which is able to replicate independently of the cell's chromosomes; plasmids carry antibiotic resistance markers

pluripotent a stem cell with wide regenerative capacity, able to differentiate into very many different cell types

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

primer a single-stranded DNA molecule that acts as the start of the amplification process

recombinant DNA technology transferring a gene from a cell of a member of one species to the cell of a different species

recombinant plasmid a plasmid with foreign DNA inserted into it

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 specific nucleotide sequence (usually 4–8 bp) that is recognised as a cleaving site for a restriction enzyme

short tandem repeats (STRs) a short non-coding region of DNA that is repeated many times in the genome of an organism; it is highly variable between individuals and can be used in DNA profiling; STRs have a repeat sequence of two to five bases

somatic cell gene therapy gene therapy for a body cell

stem cell an unspecialised cell with the potential to differentiate into many different kinds of cells

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 DNA is taken from one organism and inserted into another organism using a plasmid

transgenic organism an organism that has been modified by incorporating into its genome a piece of foreign DNA

variable nucleotide tandem repeats (VNTRs) a short non-coding region of DNA that is repeated many times in the genome of an organism; it is highly variable between individuals and can be used in DNA profiling; VNTRs have a repeat sequence of more than five bases

vector a living organism that transmits pathogens from one host to another; a vehicle used to transfer DNA sequences from one organism to another

CHAPTER REVIEW QUESTIONS

Remembering

1 Match each item in the first column with a description in the second column. Each item can only be used once.

DNA ligase	Small circular self-replicating DNA molecule
Vector	Sorts DNA molecules based on size and charge
Primer	Joins two single-stranded sections of DNA together
Blunt ends	Specific site at which restriction enzymes cut DNA
Plasmid	Vehicle to introduce DNA into a host cell
Restriction site	An enzyme that catalyses the synthesis of DNA
Gel electrophoresis	Results from cleavage by a restriction enzyme in the middle of the recognition sequence
DNA polymerase	Synthetic short, single-stranded DNA molecule

2 Recall the two most common virus vectors.

3 Outline how bacteria transformed with a plasmid can be discriminated from bacteria that have not taken the plasmid.

4 State why the temperature is lowered to 50–60°C during the annealing phase of PCR.

Understanding

5 Predict whether the following cuts made by restriction enzymes will produce sticky or blunt ends. The arrows show where the cuts occur in the double-stranded DNA.

a TCCGCGGA
AGGCGCCT

b AGGGCCT
TCCCGGA

c GCGGCCGC
CGCCGGCG

6 Summarise why radioactive or fluorescent tags are added to gene probes.

7 Summarise the processes involved in using gene therapy to treat a child who has severe combined immunodeficiency.

8 Outline the major disadvantages of using viruses as vectors to transfer genes from one organism to another.

Applying

9 Predict the minimum band sharing percentage in the DNA profiles of a mother and her baby.

10 Look carefully at the gel in Figure 5.28. Based on the figure, match the size of fragments in lanes 1, 2, 3 and 4 to the sets of measurements presented below.

- a 200, 250 and 900 bp
- b 150, 400 and 600 bp
- c 50, 450 and 650 bp
- d 100, 100 and 450 bp

11 Predict whether digestion of the human genome with *Alu*I or *Eco*RI would result in the larger number of fragments.

12 The section of DNA in Figure 5.29 shows a sequence of 120 bases in one strand of DNA. Refer to Table 5.1 on page 123 for restriction sites.

- a How many *Bam*HI and *Alu*I restriction sites are there in the sequence?
- b If the sequence is cut by *Bam*HI, how many fragments of DNA would be produced?

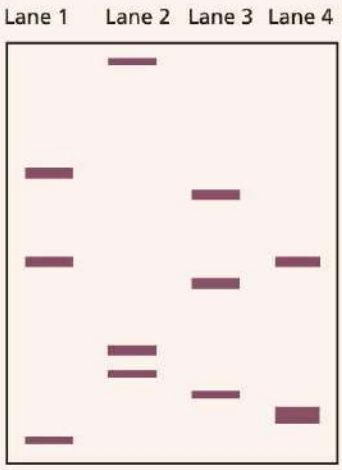


Figure 5.28 ▲
Gel electrophoresis

```
ATATGTGT GGATCCGT CTTAGGTT ATCGAATT CTAGAGCT
ATGGCCTA TTAGCTTC CTGGATCC AACCTGTA TAGAGCTA
CTCGTCAG CTATTGCT ACGGGATC CTAGCTGA TTGGATTC
```

Figure 5.29 ▲
DNA sequence

- c If the sequence was cut by *Alu*I, how many fragments of DNA would be produced?
- d If the sequence was cut by both *Bam*HI and *Alu*I, 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 *Bam*HI to get the number of fragments stated in part b?

13 Looking at the profiles of the black swan family 5 in Figure 5.20 on page 137, determine whether the male is the biological father of all cygnets.

14 Demonstrate the use of stem cells in human medicine.

Analysing

15 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 from occurring.

16 Identify some of the instances when only a small sample of DNA may be available.

Evaluating

17 Debate the use of genetically engineered animals from an animal welfare and ethics point of view and weigh this against the benefits to public health of having safe and effective medicines to treat many of our ailments.

18 From a consumer's point of view, identify some of the issues associated with the production of foods from genetically engineered crops such as canola.

Creating

19 Based on your knowledge acquired in this chapter, design a way to test for a mutation resulting in the deletion of a region of 100 nucleotides that does not contain any restriction sites.

20 Explain how you would use DNA profiling to design a breeding program that minimises inbreeding of an endangered species.

Reflecting

21 Discuss whether you would buy genetically engineered food, weighing up the arguments for and against and justifying your decision.

22 Put forward two arguments for and two arguments against testing for genetic diseases.

CHAPTER 6
EVIDENCE OF CHANGE

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

Science Understanding

- Life has existed on Earth for approximately 3.5 billion years and has changed and diversified over time (ACSBL088)
- Comparative genomics provides evidence for the theory of evolution (ACSBL089)

Figure 6.1 ▶ An artist's rendering of *Anomalocaris*, an extinct sea-dwelling organism fossilised in the Burgess Shale

One day in 1886, a construction worker in the Canadian Rockies stumbled upon what is now regarded as one of the world's most significant **fossil** sites – the Burgess Shale. Unknown fossils of seemingly headless shrimp with odd appendages and other wondrous creatures baffled observers. Charles Walcott from the Smithsonian Institute visited the site and collected more than 60 000 important fossils, giving us a window to life in the past.

We can find out about the past by looking at the traces left in the earth. This has helped us understand the processes that establish change in a group of organisms, and we now know that, with enough time and enough variation, a group of organisms can change beyond recognition – or die out.

Evolution's revolution

The diversity of life on Earth today is astonishing. It is the result of **evolution**, the scientific explanation for the mechanisms that drive species to change over time. Understanding how evolution works, and how evolutionary theory has developed, can be complex. The contemporary view of evolution, the 'modern evolutionary synthesis', has come from more than 150 years of research and observations. Research in the fields of genetics and earth sciences, as well as countless newly discovered species of living and fossil organisms, all provide evidence that builds on early ideas. Emerging technologies can help with identifying new discoveries and can also be used to identify mechanisms that drive evolution, and patterns within the process itself. But understanding the scale of time involved can be difficult to comprehend; in order to understand the history of life on Earth, we first need to understand the history of Earth itself.

The life and times of Earth (so far)

Human lifetimes are, in the grand scheme of things, short. Our lives are determined by hours, days, years and decades. This framework works for our own lives, but when we try to understand how old Earth is, or how long it takes for species to diversify, our terms of reference quickly become meaningless. We need something bigger than our own lifespan to measure the time scales of these changes. Instead of understanding the planet's history in years, 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 geologic time and are expressed in millions of years ago (**mya**).

Earth has changed significantly over billions of years; far from being stable, it is changing right now as you read, and will continue to change. Our dynamic planet can only harbour equally dynamic inhabitants; any organism's failure to respond and adapt to Earth's changes results in its extinction. Life has flourished and dwindled throughout various stages of Earth's history, responding to the changes by either adapting or dying out.

It is possible to track a number of physical and climatic changes that have occurred during the history of Earth. Some of these changes in the past were rapid and dramatic, causing major changes to sea levels and vegetation patterns; others occurred more slowly over time. In turn, these affected animal populations. Key events that have occurred so far in Earth's timeline are summarised in Table 6.1.

Table 6.1 Geologic timeline and key events

Eras and eons	Periods (and epochs)	mya	Continental associations	Animals and plants
Precambrian eon		4560–570		First archaea First bacteria First eukaryotes First multicellular organisms
Palaeozoic	Cambrian	570–510	Landmasses aggregate at equatorial zone Australia part of Gondwana North America and Greenland part of Laurentia Europe part of Baltica	First invertebrates Arthropods, including trilobites, brachiopods dominant
	Ordovician	510–439	Northern landmasses form supercontinent Laurasia	Diverse marine communities, reef-forming organisms Brachiopods and cephalopods Jawless fish
	Silurian	439–408		First land plants and arthropods Jawed fish
	Devonian	408–362	Gondwana moves south	First trees Land plants and fish spread First land vertebrates (tetrapods) descend from lobe-finned fish
	Carboniferous	362–290		Ferns dominant Swamp forests Insects dominate as the first winged animals First reptiles and amphibious tetrapods abundant
	Permian	290–245	Laurasia and Gondwana unite to form Pangaea	Reptiles dominant, rise of reptilian ancestors of mammals
Mesozoic	Triassic	245–208		Catastrophic mass extinction eliminates most life. Surviving organisms start to diversify, including dinosaurs and marine reptiles First mammals
	Jurassic	208–146	180 mya: Pangaea begins to break up 160 mya: Africa breaks from Gondwana	Dinosaurs dominant Cycads and conifers Flying reptiles (pterosaurs) *Archeopteryx* (first dinosaur–bird fossil) dies and fossilises in Bavaria

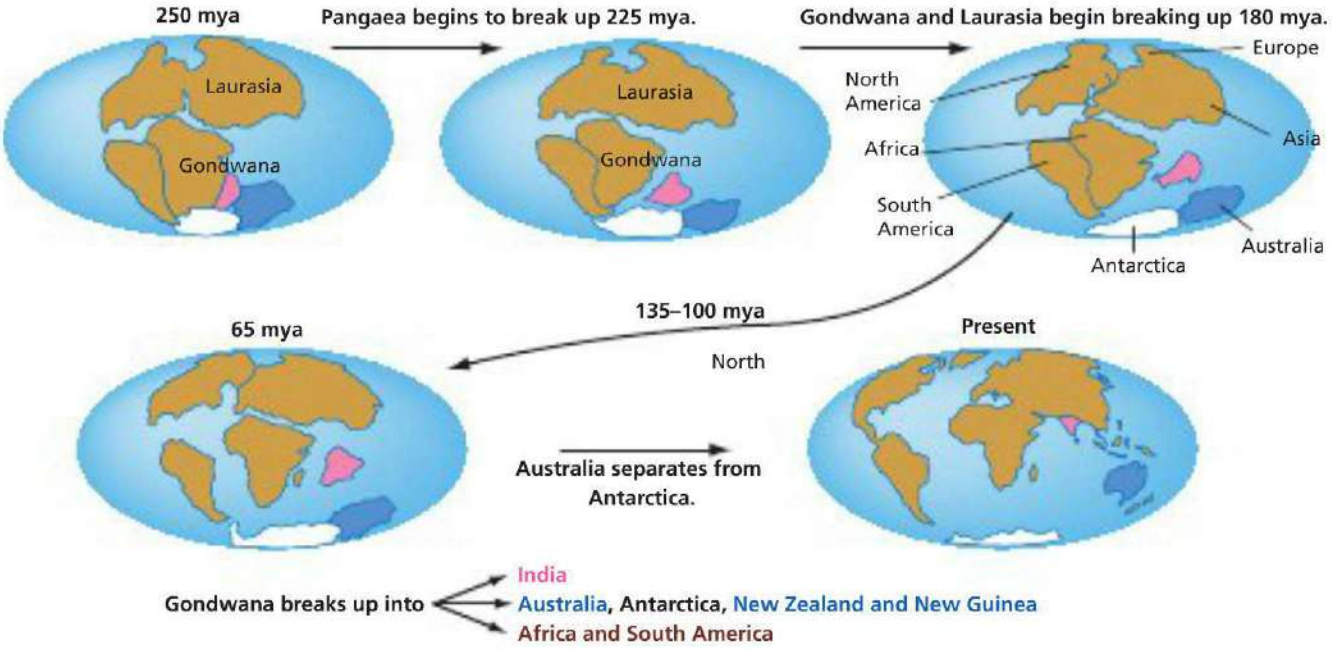
(continued)

Table 6.1 **CONT.**

Eras and eons	Periods (and epochs)	mya	Continental associations	Animals and plants
	Cretaceous	146–65	120 mya: India breaks from Gondwana, moves north Gondwana breaks from Laurasia and drifts south Gondwana breaks up Late Cretaceous: Australia and Antarctica still attached	First flowering plants Arrival of marsupials in Australia via Antarctica Dinosaurs populate huge rift valley between southern Australia and Antarctica Cool temperate forest of podocarps, celery pines, proteas Southern beech (*Nothofagus*) established
Cenozoic	**Paleogene** - Palaeocene	65–54		Dinosaurs now extinct Flowering plants, birds and mammals radiate into newly vacant niches left by dinosaurs
	- Eocene	54–40	50 mya: Australia begins to break from Antarctica and drifts north Inland seas form as eastern highlands lift Antarctic ice cap begins to form	
	- Oligocene	40–23	30 mya: separation of Australia and Antarctica complete	First *Eucalyptus* species
	Neogene - Miocene	23–5	Slow drying of southern parts of the Australian continent	Rainforests contract to the equator First *Acacia* species Large marsupials are well established
	- Pliocene	5–2.6		Australia close enough to Asia to allow exchange of plants and animals (e.g. bats, rodents)
	Quaternary - Pleistocene	2.6		Major ice ages First humans arrive and increase in range
	- Recent (Holocene)	(10000 years)		8000 years before present: formation of Great Barrier Reef begins

The changing face of the planet

The major plates of Earth's crust float on the fluid mantle that lies over the core of Earth, resulting in a process called **continental drift**. These plates are in constant movement, tearing apart or colliding and causing uplift of Earth's crust. (For example, the plate on which Australia sits is moving north at about 5–7 cm each year.) This movement is known as plate tectonics and may result in earthquakes, volcanic activity, or a combination of the two. If an earthquake happens under the sea, a tsunami can occur. Colliding plates can also create major mountain ranges where sections of continents crush together, forcing soft rocks upwards. The Himalayas are an example of this, where the upwardly thrust rocks were once sea-floor sediments.



▲ **Figure 6.2**
The breaking up of the supercontinent Pangaea to form the present-day continents

Geological (Figure 6.2) and **fossil** evidence (Figure 6.3) show that 200 mya a single supercontinent known as Pangaea existed. Over a period of about 20 million years, Pangaea broke up into two landmasses: the northern continent Laurasia (which would later give rise to North America, Asia and Europe) and the southern continent Gondwana (which would eventually become Antarctica, Australia, New Zealand, India, Africa and South America). Gondwana then broke up over the following 100 million years. Africa and India drifted north, and South America, Antarctica and Australia initially stayed together. By the Eocene epoch (45 mya) Australia and Antarctica were all that remained of the once great southern landmass of Gondwana. Finally, Australia broke free and began its trip northward: a journey we are still on.

◀ **Figure 6.3**
Distribution of fossil evidence for former Gondwana land mass

Evidence for continental drift is supported by both the geological and the fossil record.

Geological and biological evidence has helped construct maps of continental movement. Another line of evidence comes from observing changes in Earth's magnetic field; over millions of years, our magnetic field has changed frequently, alternating between 'normal polarity' such as we see today and 'reversed polarity', where the magnetic poles were reversed. Also, some iron-rich minerals found in rocks originating from lava flows are magnetised, serving as 'frozen compasses', effectively recording their orientation relative to the position of the magnetic poles when the rocks first formed or solidified.

Temperature, climate and sea levels

Over the course of its history, Earth's climate has oscillated between hot, humid periods and cold, dry periods. Evidence for this is found in ice cores drilled in Greenland and Antarctica. Some ice cores are several kilometres long and contain a record of climate change dating back 100 000 years.

For much of its history, Earth was much warmer than it is today and the temperature gradation from the equator to the poles was not as wide. Evidence suggests that past fluctuations have been dramatic.

Towards the end of the Precambrian era (570 mya), during the Carboniferous and Permian periods (245–362 mya), and during the Oligocene epoch (23 mya), snow, glaciers and sheets of ice covered much of Earth. Between these cold, dry periods there were long periods, millions of years, of warmer temperatures when the ice melted, the sea level was higher (Figure 6.4), humidity was generally higher and vegetation was generally more tropical. Such climate variations inevitably affect life in some way or another, and it is possible to track some of these changes through the fossil record, especially fossil plants.

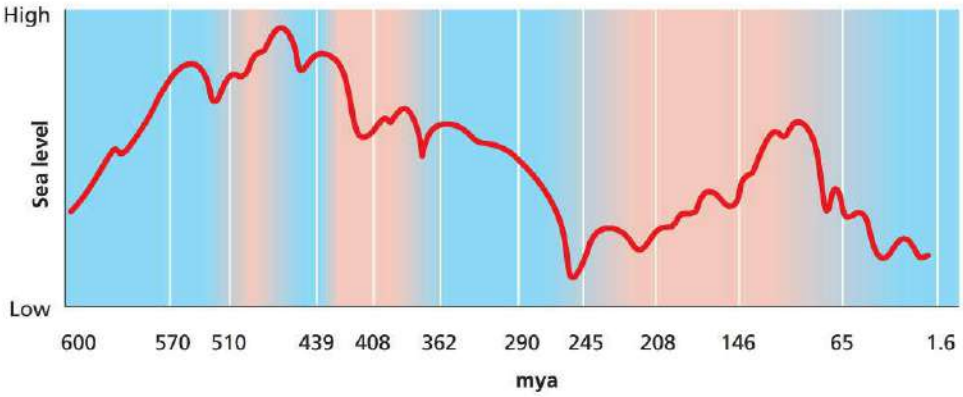


Figure 6.4 ▶ Sea levels have changed repeatedly with the changing temperature of Earth. Fossils record some mass extinctions, where dramatic variations in climate and sea levels appear to have influenced the severity of the extinction events.

THE GEOLOGIC TIME SCALE

Explore the geologic time scale and the animals, plants and environments of Victoria over the last 600 million years.

Oxygen levels

A critical environmental factor for life is the composition of the atmosphere: it affects all living things worldwide, including anaerobes. The first atmosphere of Earth most likely had very little oxygen. Evidence from ice cores shows that the oxygen concentration began to increase about 2.5 billion years ago. By 1.5 billion years ago the level was about 1% of the present level, making up about 0.2% of the atmosphere. By 600 mya it had risen to 5% of the present level, making up about 1% of the atmosphere. This increase in atmospheric oxygen came from large numbers of cyanobacteria or 'blue-green algae' in the oceans. These algae are able to use water as a resource in photosynthesis, converting carbon dioxide to organic compounds and producing oxygen as a waste product. A few descendants of Earth's original 'atmosphere engineers' are very much alive today and continue to form stromatolites in places such as Hamelin Pool in Western Australia, just as they did hundreds of millions of years ago.

QUESTION SET 6.1

Remembering

1 Briefly describe 'Pangaea', 'Laurasia' and 'Gondwana'. Identify how each relates to the others.

2 Identify the type of evidence that would support the idea that Antarctica and Australia were once connected.

Understanding

3 Suggest what process might explain how fossils that died on the sea floor could be found high in the Himalayan mountains.

4 Recount how the level of oxygen in the atmosphere has changed since Earth was formed. How has this change affected the type of organisms – in terms of cell type and cell size – that were able to survive as time progressed?

5 Consider the effect on large land herbivore populations of warmer, wetter global conditions in contrast with cooler, drier conditions.

6 Refer to Table 6.1 to complete the following tasks.

- a Identify the era in which life first appeared.
- b List all periods in which dinosaurs existed.
- c Determine whether *Eucalyptus* species would be expected in Africa. Explain your reasoning.

Evidence for evolution: the fossil record

What are fossils? Put simply, 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 has 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* (Figure 6.5), fossils can also include footprints, burrows and even preserved waste products such as coprolites: fossilised faeces.

So what can the fossil record tell us? Much of our knowledge of the changes that have occurred in living things over time is derived from fossils. Only a very small percentage of organisms leave fossilised remains. Many fossils are destroyed by natural processes such as weathering and erosion.

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▲ **Figure 6.5** An immaculately preserved fossil of the extinct fish *Ceratoichthys*: a rare example of a complete fossilised skeleton

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 toward organisms that lend themselves more easily to the fossilisation process. To become a fossil, organic matter needs to be deposited and covered in sediments in an environment that lacks oxygen. Plant and animal remains can be preserved if they are covered in waterborne mud, sand or clay, depriving the remains of oxygen, as can happen in the beds of lakes and rivers or in calcium-rich sea beds. 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.

WOW

Ancient platypus found in New South Wales

The jaw bone of an ancient relative of the platypus was discovered at Lightning Ridge in New South Wales. At more than 100 million years old, this is one of Australia's most ancient mammal fossils. It is a small jaw with three teeth beautifully preserved in translucent opal, so that tiny details of the root and nerve canals can all be seen.

Fossils can form when organisms are covered with sedimentary material, such as mud, silt or sand, generally carried by rivers and streams and deposited. These materials are consolidated to form sedimentary rock. This overlying sedimentary material protects organic matter from scavengers and also slows its decay long enough for it to fossilise. The resulting fossils are generally only the 'hard parts' of organisms (such as bones and teeth that are slow to decay) but rarely they can include more delicate tissue such as feathers. Fossils of this type are not found in volcanic rocks because molten lava solidifies at about 1000°C, which is hot enough to burn any organic material; however, they can be found in sedimentary layers of eroded volcanic ash. Metamorphic rock does not usually bear fossils, as the pressure and heat of metamorphism generally (although not always) destroys any trace of fossil.

Thin tissue, such as leaves and muscle, is sometimes preserved as films or impressions left in the rock. Fossils are also formed when soft material, such as volcanic ash, fills an impression, or when minerals later form in a pocket in sedimentary rock left by a decomposed organism, which can result in fossils composed of opal. A 3-million-year-old set of footprints from a family of early humans, including children, is preserved in this way in the Afar triangle region of Africa. Dinosaur footprints can also be found in sandstone and mudstone.

There are several other ways a fossil can form. They can be formed as a result of freezing and subsequent dehydration. Plants are also quite commonly fossilised. The original plant material may be partly dissolved and some tissue replaced with dissolved salts, which 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. As a consequence, fossilisation can tell us a great deal about past life and how it differs from what we see in the world today. But in order for this to make any sense, we need to calculate the age of fossils. This can be done using dating techniques.

Evidence for evolution comes from five lines of evidence: palaeontology, biogeography, developmental biology, morphology and genetics. Developments in comparative genomics, comparative biochemistry and bioinformatics identify further evidence for evolutionary relationships.

Figure 6.6 ▲ A reconstructed model of the bird-like dinosaur *Archaeopteryx*, an example of a transitional form between feathered dinosaurs and modern birds

Transitional forms and the pace of evolution

Close examination of the fossil record reveals interesting occurrences when looking for evidence for evolution. The fossil record reveals many examples of intermediate states between an organism's ancestral form and that of its descendants, such as the famous bird/dinosaur transitional form *Archaeopteryx* (Figure 6.6). These intermediate states are called transitional forms. Transitional forms give us copious evidence for evolution, documenting change over time on a broad scale. Transitions between species are harder to identify due to the bias of the fossil record.

There is always bias in the fossil record. Given the specific requirements for fossilisation and thus the nature of the remains that can be fossilised, there will always be chapters missing from the story. Even with this bias, it is possible to observe a gradual change over time of organisms as their shape or size transitions to different forms in some cases. In other cases, no such gradual transition is evident, the changes seem sudden and inexplicable. The change appears as a burst of evolutionary speed. The burst of evolution suggested by a gap in the fossil record may be explained by aspects of two theories of evolutionary patterns: **gradualism** and **punctuated equilibrium**.

Gradualism

The concept of gradualism assumes that evolution occurs as a steady, slow divergence of lineages (ancestral tree branches) at an even pace. Gradualism states that apparently sudden bursts of evolution implied by the fossil record are not a real indication of an evolutionary history, but an illusion of the fossil record. Evolution only appears as a burst because of the absence of sediments containing fossils that document this transition, or perhaps a change in conditions that made fossilisation impossible. Even if a small section of potentially fossil-bearing sediments were absent, this may account for fossils missing from millions of years in the fossil record. Were this section of strata still present, the fossils within it would show a divergence pattern that is slow, even and steady,
in other words, gradual.

Punctuated equilibrium

In contrast to gradualism, the theory of punctuated equilibrium states that the apparent burst of evolution is not an illusion, but real. It states that species remain fairly stable for long periods of time but may swiftly change to a new species; for example, in response to rapid changes in the organism's environment. Like gradualism, punctuated equilibrium accepts the existence of transitional forms between species, but for such brief periods that they were not preserved as fossils. Punctuated equilibrium is thought to be a successive process of stasis followed by a period of rapid change of a subset of the population. This theory does not imply that the natural selection theory is incorrect. Examples of gradual and punctuated bursts of change are both seen in the fossil record.

PUNCTUATED EQUILIBRIUM

Find out more about punctuated equilibrium.

ACTIVITY 6.1

FOSSILS

Evidence of evolution comes from studying organisms living today, but further evidence can be obtained by studying the animals and plants of the past as seen in the fossil record. Fossilisation is a rare occurrence and requires precisely the right conditions to occur. But how do fossils form and how much information can they reveal to us about organisms that lived in the past?

Aim

To investigate how fossils are formed and what they can reveal about organisms that lived in the past

You will need

- four fossil samples (e.g. fossilised coral, fossil footprint, trilobite, ammonite, shark's tooth, leaf fossil)
- reference material with information on fossils
- hand lens (one per group)

What to do

1 For each of the fossils that you have been given, complete as many observations as possible and record them. Your observations should include the following.
   - Sketch of fossil
   - Name of organism
   - Phylum or classification
   - Location or habitat where fossil was found
   - Type of rock in which fossil was found

2 Examine the individual fossil specimens carefully and attempt to classify them into the phylum (or class or order if possible) to which they belong.

3 Examine the rock surrounding the fossil specimen using a hand lens and try to identify the type of material it is. Check to see if the information provided with the fossils gives you any insight into what the material might be.

4 Sketch the fossil specimen and note which parts have been preserved and which have not.

What did you discover?

1 Identify and explain how each of the fossils has been preserved. Compare the material the fossil is made of with the original living tissue. Explain how the two are different and how the composition of the fossil may have come about.

2 Compare the fossilised specimens to similar species that exist today and identify which parts have been preserved and which parts have disappeared. Explain why this would be the case.

3 Describe how much information scientists can gain about an animal from a single fossilised tooth. Investigate this topic on the Internet, using the example of *Carcharodon megalodon* (an extinct shark) as the focus of your research. Find out why this particular example of animal reconstruction has a controversial history.

QUESTION SET 6.2

Remembering

1 Recount the steps involved in the process of fossilisation.

2 Identify two ideal types of environment or scenario in which fossilisation could likely occur.

Understanding

3 Most of our knowledge of the evolution of sharks is based on the remains of fossilised shark teeth. Suggest why other fossilised body parts of sharks haven't been found in abundance.

4 Given we have more than just fossils available in the case of sharks, suggest alternative ways to determine how closely related different present-day animals may be to each other.

5 Palaeontologists have found tracing the evolution of sea jellies ('jellyfish') to be very challenging. With your knowledge of fossils and the process of fossilisation, suggest why this may be the case.

Fossil dating methods

In order to make sense of the fossil record and to examine it for evidence of evolution, we first need to understand same basic information about the fossils and their geological settings. How old are fossils, which organisms arose first and which organisms lived together? These questions can only be answered if we are able to accurately determine the age of the evidence. A combination of comparative and absolute dating techniques is used to estimate the ages of sedimentary rocks and fossils within them.

Dating techniques that work comparatively

Comparative dating (also called relative 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.

Sedimentary rock, as you would expect, is composed of sediment: weathered material from Earth's surface, such as gravels, silts, sands and muds that have been transported by water and deposited in river beds, flood plains and sea floors. Sediment transport and deposition is an ongoing process; it has been continuously occurring on Earth for billions of years and can still be observed today. Over time, these deposited sediments form defined layers that consolidate into sequences of sedimentary rock. These sequenced layers are called strata, and a section showing successive layers of sedimentary deposition is called stratification. Strata are deposited in a time sequence, with the oldest on the bottom and the youngest on the top. Assuming natural processes like tectonic movement haven't twisted or inverted the layers, palaeontologists can then assign relative ages to fossils based on the strata in which they are found. While this technique can't give an age in years, for the sequence of the strata to be estimated relative to each other.

THE LAW OF SUPERPOSITION

Read about the law of superposition and watch the animation that explains it.

Dating techniques that work 'absolutely'

Absolute dating (or chronometric dating) is a technique that assigns a numerical age in years to a fossil or rock. There are three main types of absolute dating: radiodating, electron spin resonance and luminiscence.

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. 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 the presence of different radioactive isotopes present, an age in years can be estimated for the sample.

Radiometric dating

Some 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 natural isotopes: carbon-12, carbon-13 and carbon-14. Carbon-12 (^{12}C) has 6 protons and 6 neutrons in each nucleus, and carbon-14 (^{14}C) has 6 protons and 8 neutrons. Some isotopes have an unstable nucleus that emits energy in the form of radioactivity (alpha, beta or gamma rays) at a measurable rate. The half-life of an isotope is the time taken for half of the radioactive atoms in an initial sample to decay.

Carbon-14 is a radioactive isotope that breaks down at a known rate to produce nitrogen-14 (^{14}N) (Figure 6.7). This measurable rate of decay is the basis of carbon dating; if it is assumed that the ratio of carbon-14 to carbon-12 in the atmosphere is fixed, it is possible to measure the amount of carbon-14 in an organism at the time of its death and the amount that is present in the atmosphere now.

Using the half-life of carbon-14 we can determine the age of the sample: in other words, the time taken for the original amount to decay to the present amount. However, data from tree rings shows that the amount of carbon-14 in the atmosphere can change with time. For this reason, the time calculated from carbon dating is expressed with a degree of accuracy (usually as plus or minus *x* years), and has to be corrected into calendar years.

The older the object, the greater the margin of error. Carbon dating is thought to be accurate for samples up to about 12 000 years old. After this time it is difficult to measure the level of carbon-14 accurately and other radioisotopes, such as potassium-40 (which decays into argon), are used (Table 6.2).

Carbon-14 dating is generally not applied to fossils for two main reasons: in most cases fossils have been mineralised and the organic (carbon-containing) tissue has been chemically altered or replaced, and the process of fossilisation generally takes longer to occur than the maximum age of accuracy for carbon-14.

Figure 6.7 ▶
Graph of the half-life of carbon

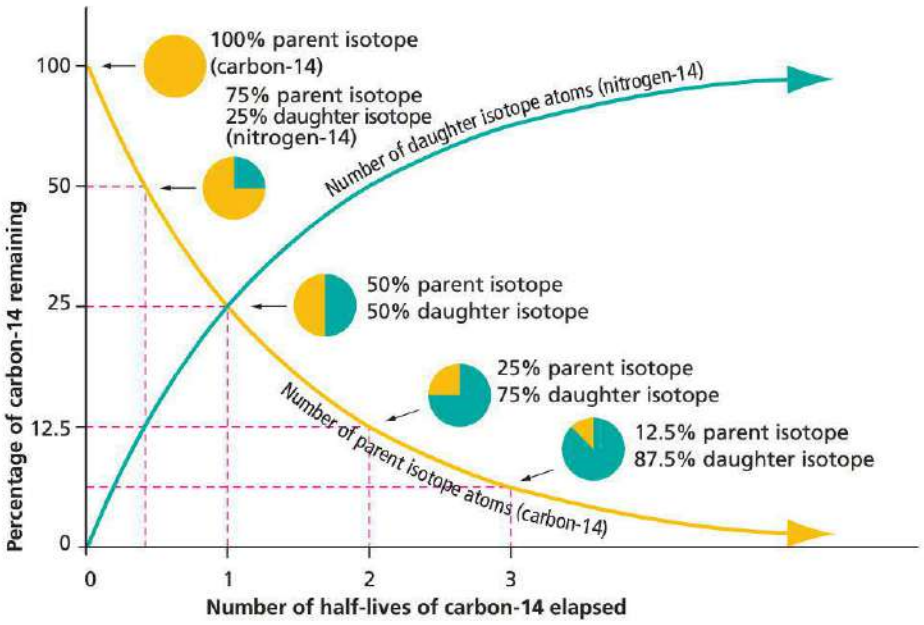


Table 6.2 Half-life and product of decay of some elements used in radiometric dating

Element	Product	Half-life (years)
potassium-40 (^{40}K)	argon-40 (^{40}Ar)	1.25 billion
thorium-232 (^{232}Th)	lead-208 (^{208}Pb)	14 billion
carbon-14 (^{14}C)	nitrogen-14 (^{14}N)	5730
rubidium-87 (^{87}Ru)	strontium-87 (^{87}Sr)	48 billion

Electron spin resonance

Electron spin resonance is a relatively new absolute dating technique that measures the properties of electrons in the crystals of minerals. Some common minerals 'collect' electrons in their crystal lattice over time at a predictable rate, either from radioactive sources in the surrounding rocks or by absorbing cosmic rays. The electrons become fixed within these crystalline lattices and the trapped electrons are mildly magnetic. This magnetism of the trapped electrons can be measured to give an electron spin resonance reading. As the amount of radiation increases with the time, the accumulated amount of radiation can be divided by the background dose rate to determine the age of the sample.

Luminescence techniques

Thermoluminescence and optically stimulated luminescence are two other forms of absolute dating techniques that are used in Australia, and both measure characteristics of minerals within sedimentary rock. The basis of thermoluminescence is the measurable light that is emitted from a mineral when it is heated. In contrast, optically stimulated luminescence is the light that is emitted from a mineral when it is exposed to visible light. Both techniques are useful for dating minerals and the occurrence of mineralisation, but cannot generally provide an estimate for the absolute age of a sedimentary rock.

Fossil evidence can be dated using comparative dating that relates to rock strata. Absolute dating techniques such as radiodating, electron spin resonance and luminescence are also used.

QUESTION SET 6.3

Applying

1 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:
   a the way in which the rocks were formed
   b the age of the fossil at location Y.

2 Stone tools have been found with camp-fire charcoal. Explain how the technique of carbon dating could be used to determine the time at which the tools were made.

3 Explain the basis of the technique of electron spin resonance.

Analysing

4 Identify a limitation of luminescence in dating sedimentary rock.

Making sense of the evidence: evolution and its patterns

So far we have looked at the evidence for evolution, and given it context in terms of timing, as well as geology and climate. However, our understanding of the mechanisms that explain what we see around us today has itself undergone many historic changes to reach this point: the concept of evolution has itself evolved. Our understanding of evolution now shows that additional evidence – patterns observed in the world today – gives even greater explanatory power to the process of evolution.

UNDERSTANDING EVOLUTION

This website contains an explanation as to how research in evolutionary biology is performed, and how ideas in this area have changed over time.

How did we get to here? A brief history of evolutionary thought

In the late 1600s, Western civilisations believed in the idea of 'natural theology' – that every 'kind' of organism has essential, unalterable characteristics. As biological studies blossomed, naturalists began noticing variability in species, and the discovery of the remains of animals unlike anything seen before introduced the idea of extinction, which challenged natural theology: where did these giant animals come from, and where did they go?

Questions like this prompted naturalist Jean-Baptiste Lamarck to devise the 'transmutation of species by spontaneous generation'. Lamarck suggested that organisms pass on to their offspring characteristics that they acquire during their lifetimes; that is, individual efforts during the lifetimes of organisms were the mechanism that drives adaptation. Although now discredited, this was the first, albeit flawed, theory that embraced evolution.

In the 1850s, two naturalists named Alfred Russel Wallace and Charles Darwin were studying and collecting forms of life in different parts of the world. By coincidence, they both arrived at the same idea about how species 'came to be'. To refute Lamarck's theory, Darwin proposed a new theory of evolution and 'called this principle, by which each slight variation, if useful, is preserved, by the term Natural Selection'. Originally published in 1859 as *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*, Darwin's work is now commonly known as *The Origin of Species*. The basis of Darwin's theory of evolution was that individuals within a population showed a range of variation in their characteristics. Those with characteristics, or traits, most suited to their

environment would have an advantage over other individuals, making them more likely to pass these alleles (which encode these favourable traits) to the next generation. In each generation, favourable alleles become more common and the population gradually changes to become better suited to its environment. Darwin provided evidence for descent with modification (branching evolution) based on patterns in variation of domesticated and wild species, and patterns of species distributions in time and space. This was a new approach to understanding evolution. Much of the previous work had viewed relationships between organisms to be 'ladder-like' – that life can be organised in a hierarchy of lower to higher organisms. Darwin proposed a more 'tree-like' scenario, where life's lineages can be mapped on a branching diagram. In this analogy, the forks in the branch mark points at which new species arise – evolutionary events that occurred when populations became so different from other populations of the same species that they could no longer interbreed. This important concept is the basis of **phylogeny**. Phylogeny seeks to reconstruct the evolutionary history of any given group of organisms, studying the patterns of relationships among them.

You can revise the concepts of inheritance in Chapter 4 of this book.

Evolution is gradual: small genetic changes driven by natural selection accumulate over long periods that are consistent with known genetic mechanisms. Species discontinuity arises gradually through geographical isolation and extinction.

Evidence for evolution: biogeography

Biogeography is the study of the distribution of organisms and ecosystems across the world and through geologic time. The fauna and flora of Australia owe their uniqueness to the relatively recent isolation of the landmass. However, Australia and other landmasses in the southern hemisphere share many plant and animal groups.

Many genera of Indian plants are similar to those of the monsoonal environments of northern Australia. Some Malaysian rainforest genera occur in the rainforests of tropical eastern Australia. Southern beech trees, *Nothofagus*, are found as both living and fossil specimens in mainland Australia, Tasmania, Papua New Guinea, New Caledonia, New Zealand, Antarctica and South America (Figure 6.8). The mountains and dry valleys of

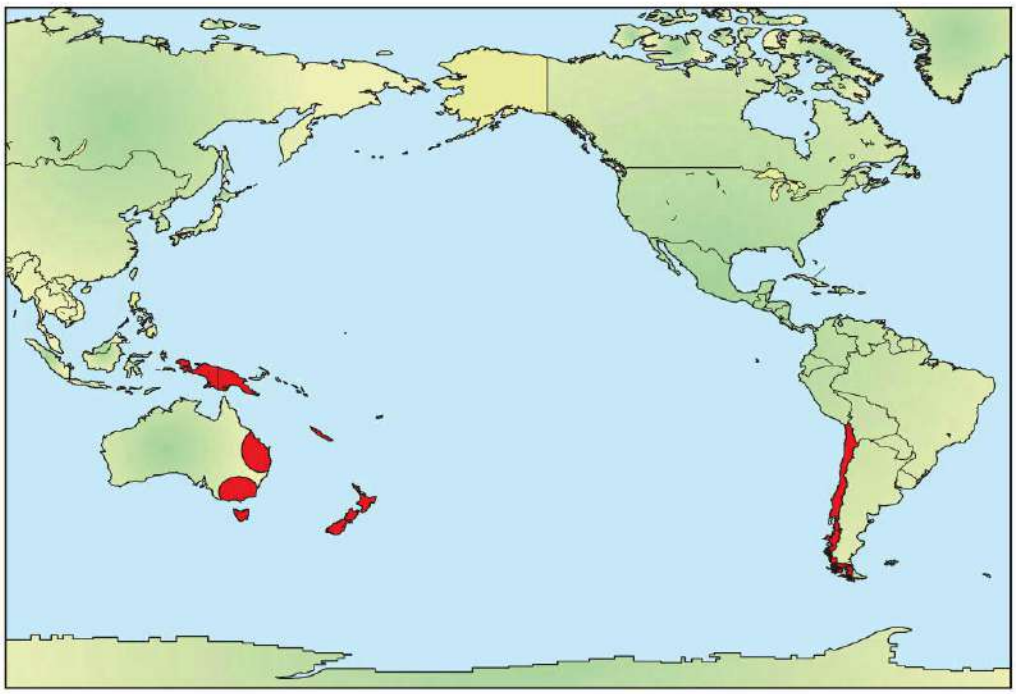


Figure 6.8 ▶ The red shaded areas show where Southern beech trees, *Nothofagus*, are found as both living and fossil specimens in Australia, Papua New Guinea, New Caledonia, New Zealand and South America.

Antarctica have fossils of *Glossopteris* seed ferns (embedded in rocks and coal seams) that are the same as those found in coal deposits of India, South America, South Africa and Australia. These were all laid down in ancient forests prior to and including the Permian period of the Palaeozoic era, that is, up to 245 mya. The far-flung distribution of these groups provides evidence that Gondwana once existed.

Wallace drew the line

Alfred Russel Wallace (1823–1913) is known as the 'father of biogeography'. He was a self-taught naturalist, a professional collector of flora and fauna, and an important intellectual of the 19th century. Wallace's travels took him to the Amazon for 4 years and, later, Malaysia and Indonesia for 8 years. During his time in Southeast Asia, he collected more than 126 000 specimens. In Indonesia, he was struck by the stark difference in the bird families he encountered between the islands of Bali to the west and Lombok to the east, a distance of 25 km. On Bali, the birds were more closely related to those of the larger islands of Java, Sumatra and mainland Malaysia, the 'Asian' fauna. Those on Lombok were related to the 'Australian' fauna of Papua New Guinea and Australia.

Birds were only one example of the faunal differences Wallace found. Further observations of some species of fish and large mammals indicated a distinct break between two regions. The line between them became known as Wallace's line (Figure 6.9), but the reason behind this dividing line remained mysterious for many years. The mystery of 'the line' was later solved with the theory of plate tectonics, as the line approximates the collision zone between the Australian and Asian plates.

▼ **Figure 6.9**
Wallace's line shows the demarcation of Asian fauna from Australian fauna.

ALFRED RUSSEL WALLACE

This website contains a look at the life and work of Alfred Russel Wallace, and the legacy of his contributions to evolutionary biology.

WOW

Death and collecting in the Amazon

At the age of 25, Wallace embarked on a collecting journey to South America. His younger brother Herbert joined him for a time, but became injured. After 4 years, having collected thousands of specimens and found hundreds of new species, Wallace emerged from the Amazonian jungle. Herbert had died of yellow fever. On the journey home, Wallace's ship caught fire and most of his work was lost at sea. He managed to retrieve only a few notebooks and sketches.

Divergent evolution

Divergence is a pattern of evolution where differences between groups of organisms accumulate to a critical point that leads to **speciation**, the development of a new species. This pattern 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. A group of organisms that has a recent **common ancestor** may evolve different **adaptations** in response to a range of environmental pressures.

As members of the population develop adaptations driven by mutations over successive generations, they may diverge enough to become new species. The process is referred to as **adaptive radiation**.

For example, koalas (tree-dwelling herbivores), Tasmanian devils (ground-dwelling carnivores) and marsupial moles (dune-burrowing insectivores) are related because they have a common marsupial ancestor (Figure 6.10). However, they show quite different feeding structures that adapt them to different diets. These animals are an example of adaptive radiation. Also, because they have evolved into separate species, they are an example of **divergent evolution**.

▲ **Figure 6.10** a) Koalas, b) Tasmanian devils and c) marsupial moles evolved from a common ancestor that probably lived during the Eocene epoch. They are examples of the divergent evolution of marsupials.

Adaptive radiation

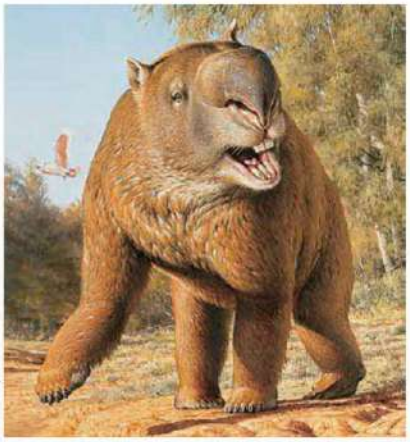
You can learn more about the mechanisms of evolution and speciation in Chapter 7.

Adaptive radiation is a pattern of divergent evolution where organisms rapidly diversify into numerous new forms, particularly 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 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 is therefore an example of adaptive radiation.

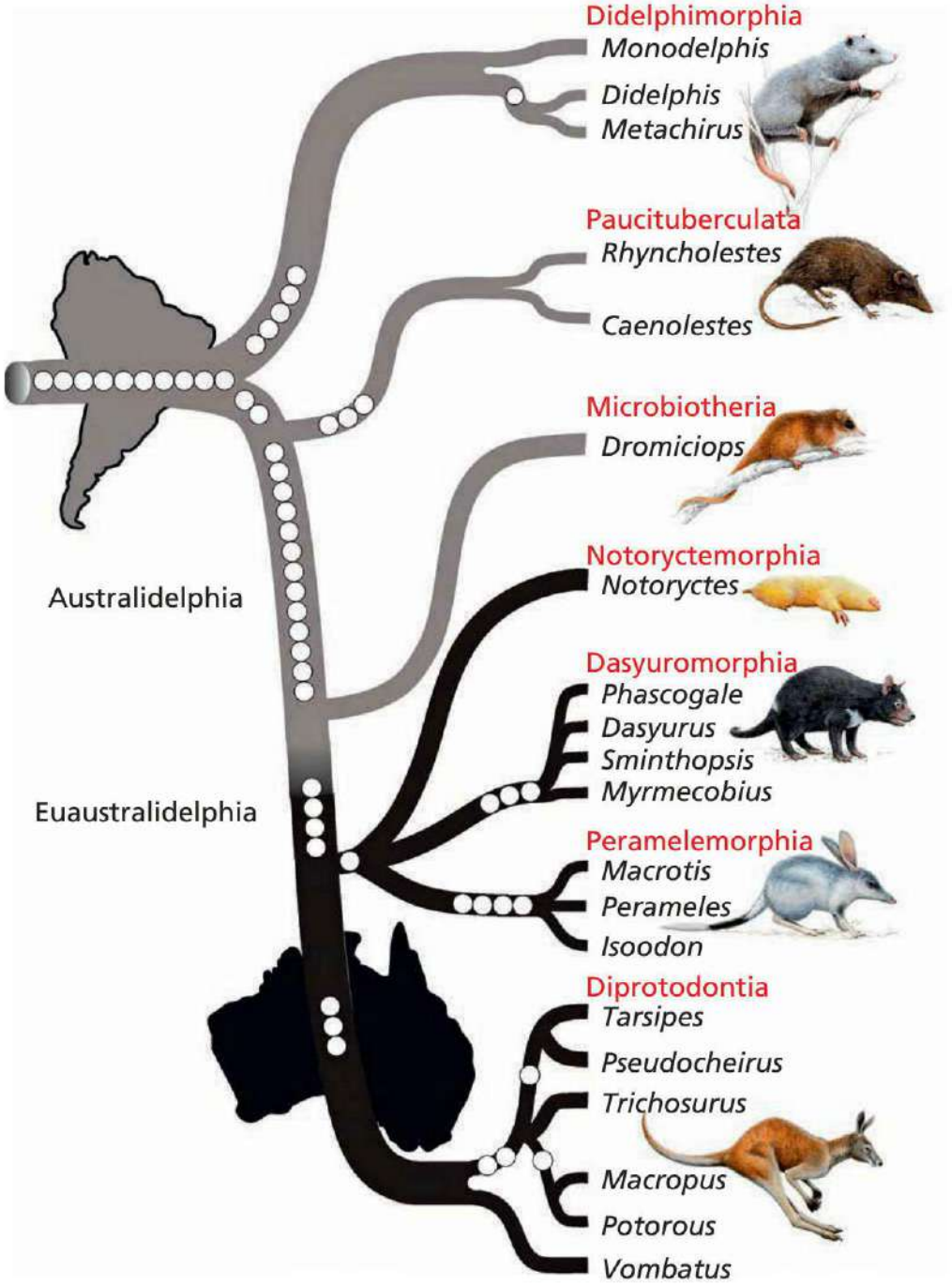
Slowly, the tropical centre of Australia began to dry out during the Pliocene epoch (approximately 5 mya). This brought an end to the tropical habitat, which gave way to broad grasslands. Large browsing mammals called diprotodontids (Figure 6.11) and a variety of possums could not survive with the reduction 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.



Courtesy of Australia Post

◀ **Figure 6.11**
The giant *Diprotodon optatum* was a type of megafauna that browsed on leaves.



◀ **Figure 6.12**
Adaptive radiation of marsupials began in South America, which was then joined to modern Australia in the supercontinent Gondwana. Most surviving marsupials are now restricted to the Australian continent.

Convergent evolution

Convergent evolution is a pattern that occurs when unrelated organisms evolve similar adaptations in response to their environment. An example of convergent evolution is provided by anteaters. Many animals eat ants and 'white ants' or termites, and have developed similar structures even though they are not closely related.

Modern anteaters include echidnas, which are monotremes; numbats, which are marsupials; and aardvarks and pangolins, which are placentals (Figure 6.13). All of these species have an elongated snout that functions as a smelling and digging device, a long, extendible tongue that can extract ants from crevices, and powerful claws that are used for digging up ant and termite nests.

The different species of ant-eating mammals have a common ancestor, but not a recent one; they belong to different orders. They have developed ant-eating habits indepently and coincidentally, rather than it being a legacy from their common ancestor. The first mammal-like animal probably emerged in the Triassic period, around 208 mya.

The results of convergent evolution often show up as **analogous structures**: adaptations of very different types of structures that solve a problem in a similar way.

Figure 6.13 ▼
Ant-eating mammals including: a) echidnas (monotremes), b) numbats (marsupials) and c) pangolins (placentals) show convergent evolution with ant-eating structures.

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QUESTION SET 6.4

Understanding

1 Define 'divergent evolution' and give an example of how it has affected the evolution of species.
2 Define 'convergent evolution' and give an example of how it has affected the evolution of species.

Analysing

3 Explain the significance of *Glossopteris* plant fossils in understanding the biogeography of Gondwana.
4 Explain the importance of using living and fossil forms in constructing phylogenies.

Evidence for evolution: comparative anatomy

While we now know that all life shares a common genetic code, and relies on similar physiological and biochemical processes, early evolutionary theorists could only use evidence that they could observe at the time. Even to the casual observer, it's evident that different species can appear vastly different, seeming to bear very few similarities to each other, while others appear so similar that their shared common ancestor must have existed relatively recently. Closer examination of the physical characteristics of species, at both the embryonic and adult stages, can reveal further evidence for evolution.

Case study

Dr Erich Fitzgerald and the evolution of baleen whales

Dr Erich Fitzgerald is Senior Curator of Vertebrate Palaeontology at Museum Victoria. Erich researches the evolutionary history of marine mammals. To undertake his research, he uses a combination of field work and interpretation of fossils and animal remains that are housed in museums around the world. Erich seeks to answer questions on what drives the evolution and extinction of marine mammals.

His research would not be possible without advances in information technology, such as computational phylogenetic analysis. 'To get to the bottom of the evolutionary history of organisms, you need to place them in an evolutionary context. To do that, we subject fossils and living species to phylogenetic analysis, computationally estimating the evolutionary "tree" based on large data sets of characteristics across large numbers of taxa. We then use different programs to interrogate that tree for other patterns to test our hypothesis.'

'Using computers as a way of capturing data, imagery, 3D and CT scanners and communication between researchers internationally has been a huge benefit to palaeontology; it means we are forced to access a wider range of data in order to get an accurate evolutionary picture of what we are looking at. Computers can deal with large data sets of measurements and characteristics and identify patterns that could easily be otherwise overlooked; as such, computers are as important to palaeontology as a hammer and chisel.'

Erich's research has unveiled unexpected results. 'For a long time there was a gap in our knowledge of the relationships between the toothed ancestors of modern whales – Archaeocetes – and living baleen whales. How on earth does such a specialised feeding structure like baleen evolve? For some time there was the idea that Archaeocetes probably closed their jaw and used their teeth like a sieve, like some seals do today.'

Newspix/Paul Trezise

▲ **Figure 6.14** Erich Fitzgerald and the fossil skull of the ancestral toothed whale *Janjucetus hunderi*

Extensive data sets, including measurements from the skulls, teeth and other bones of fossil and contemporary animals, allow for the use of computational phylogenetics, showing some surprising results. 'Our research points to a complex story of "false starts" and "experiments" in evolution. It shows whales didn't have an intermediate stage using both teeth and baleen; the evidence suggests the transition between teeth and baleen happened a different way. The question is now how did this happen; that's what I'm try to solve. Understanding the evolution of organisms is vital. In order to gain any understanding of the dynamics of biodiversity, you have to understand how it has occurred over the time scales over which it has evolved, and those time scales are only accessible to palaeontologists.'

Questions

1. Account for how our view of current biodiversity is biased if we ignore evolutionary history and the fossil record.
2. Thinking of baleen whales and their diet, assess and discuss whether the evolutionary 'false starts' and 'experiments' that the fossil record shows are examples of divergent evolution.
3. Prior to the advent of computer-assisted phylogenetic analysis, estimating evolutionary relationships of vertebrates was based largely on bone and tooth morphology, or shape. Phylogenetic analysis now incorporates other elements to develop phylogenetic trees. Explain how computational technology has improved the identification of possible relationships of fossil and living animals more efficiently and rigorously than had been previously possible.

Embryology

Comparative anatomy 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 have, at some stage of their development, a dorsal notochord (a solid tissue 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 6.15a). Sea squirt larvae, however, have the requisite characteristics, including a notochord (Figure 6.15b). Vertebrates have lost the notochord and it is replaced with vertebrae.

The similarities observed between embryos of fish, humans and many other organisms are suggestive of a shared ancestor from which all these species have evolved. No other theory can adequately explain why the same structures occur in all chordate embryos, whose adult forms are so diverse.

Figure 6.15 ▼
a) Adult sea squirts show few characteristics of chordates. b) The free-swimming larva of the sea squirt shows the characteristic features of chordates, revealing its evolutionary affinity with chordates.

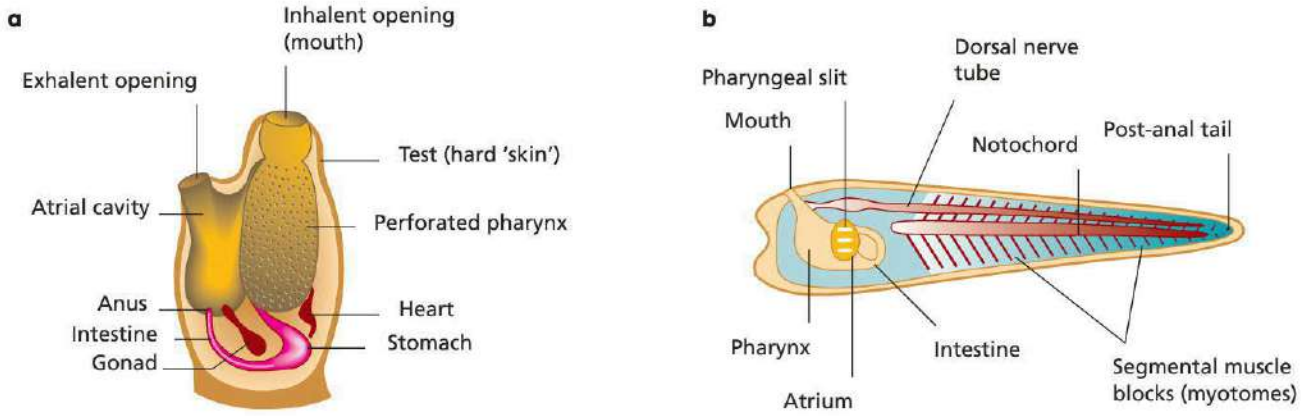
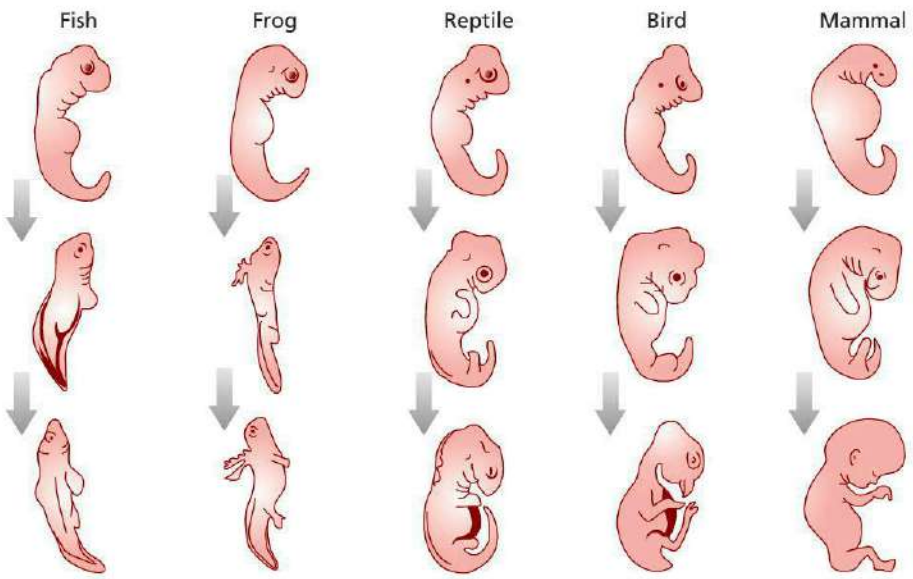


Figure 6.16 ▶
Similarities between chordate embryos suggest a common ancestor.



Scientific literacy: 'Terror bird' was scary-looking vegetarian

Giant prehistoric 'terror birds' looked so fierce that many palaeontologists assumed they were terrifying predators, but new research finds that the would-be carnivores were probably herbivores.

The terror bird, aka *Gastornis*, grew to nearly one-and-a-half metres tall. It lived between 55 and 40 mya in what is now Europe and possessed a huge, sharp beak.

'The terror bird was thought to have used its huge beak to grab and break the neck of its prey, which is supported by biomechanical modelling of its bite force,' says Thomas Tütken from the University of Bonn, who led the research.

'It lived after the dinosaurs became extinct and at a time when mammals were at an early stage of evolution and relatively small; thus, the terror bird was thought to have been a top predator at that time on land.'

Wrong, according to the latest findings, presented by Tütken and his team at the Goldschmidt conference in Florence this week.

An early clue came by way of footprints likely left behind by an American cousin of *Gastornis*. The footprints do not show imprints of sharp claws, which would have been expected as tools to grapple prey. Today's raptors, for example, sport such sharp claws.

Another clue is more obvious — the bird's hefty size and build. Can you imagine Sesame Street's Big Bird (with a big beak) running swiftly after prey? All of that bulk would not make for a very swift hunter. Some researchers theorised that terror birds ambushed prey, but even that seems pretty far-fetched.

To further explore the possibilities, Tütken and colleagues took a geochemical approach. They analysed the fossilised bones of the birds, focusing on calcium isotope composition. Isotopes are atoms of the same element with different numbers of neutrons.

In prior experiments, the scientists determined that the calcium isotopic composition becomes 'lighter' as it passes through the food chain. They tested the method first with herbivorous and carnivorous dinosaurs — including top predator *T. rex* — as well as mammals living today. For this latest study, they applied the method to terror bird bones housed at the Geiseltal collection at Martin Luther University in Halle.

They discovered that the calcium isotope compositions of terror bird bones are similar to those of herbivorous mammals and dinosaurs, and not to carnivorous ones.

'Tooth enamel preserves original geochemical signatures much better than bone, but since *Gastornis* didn't have any teeth, we've had to work with their bones to do our calcium isotope assay,' Tütken explains.

As for many scientific puzzles, the case isn't completely closed just yet.

'Because calcium is a major proportion of bone — around 40 per cent by weight — its composition is unlikely to have been affected much by fossilisation,' he says.

'However, we want to be absolutely confident in our findings by analysing known herbivores and carnivores using fossilised bone from the same site and the same time period. This will give us an appropriate reference frame for the terror bird values.'

Even if the food was just plant based, it had to have been large and tough, given the impressive beaks the birds evolved.

Viegas, J. (2013) '"Terror bird" was scary-looking vegetarian', *Discovery News* online, 29 August.

Questions

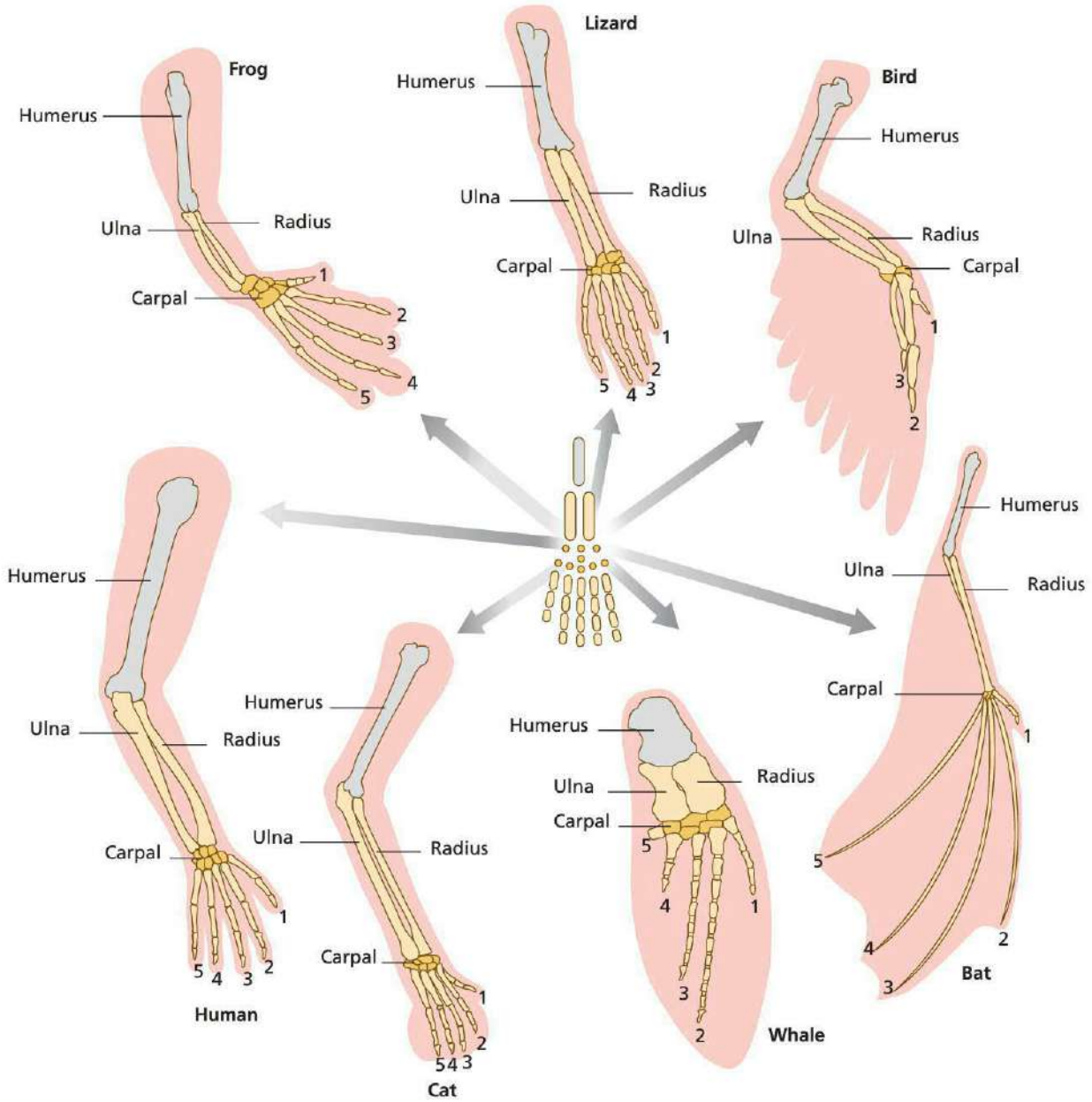
1. Suggest why it had been assumed that *Gastornis* was a predator, and what evidence may have pointed away from this before the so-called 'geochemical approach'.
2. Outline the reason for Tütken's caution of applying this geochemical analysis on fossil birds.
3. Suggest an alternative reason for the apparent absence of raptor-like toe claws on *Gastornis*.
4. Identify the possible food plant sources of *Gastornis*, assuming further evidence supports the theory of it not being carnivorous.

Homologous structures

Homologous structures are common physiological 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 in relation to the habitat that they occupy, and may function in defence, temperature maintenance or camouflage. The different types of scales are examples of homologous structures.

The example of homologous structures in lizards is one that has a relatively recent evolutionary history, but some homologous structures have evolved from a much more distant common ancestor and may have 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 the same basic structure: the pentadactyl limb, a hand or foot with five fingers or toes. However, in each species the limb has been modified to suit a variety of different ways of life, demonstrated by the different bone lengths and coverings of the limbs (Figure 6.17).

Figure 6.17 ▼ 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 act as defensive spines or fleshy water stores (Figure 6.18).

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 basic arrangement.

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◀ **Figure 6.18**
Homologous structures derived from leaves. a) The spines of a cactus and b) the bracts of *Heliconia* are derived from the same basic structure but now have different forms. In this case, the spines serve different functions; they are homologous structures. In other examples, homologuous structures can share functions, but different environments can influence how these functions are necessarily performed.

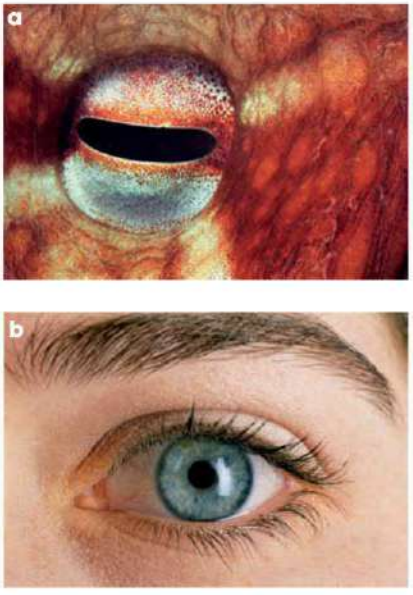
Vestigial homologous structures

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: from skeletal structures on vertebrates, soft-tissue such as organs or even 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 point to shared ancestry. Among humans, we need look no further than our vermiform appendix, a small pouch-like structure on the colon that appears to be the shrunken remains of the cecum, a far more extensive structure found in the digestive tract of other more predominantly herbivorous primates.

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 6.19). However, there is one telling 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 lacks one. 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 evolution.



Alamy/All Canada Photos

Photos.com/Jupiterimages

▲ **Figure 6.19**
a) Octopus eyes and b) human eyes are the solution to the same problem with similar adaptations.

EXPERIMENT 6.1

HOMOLOGOUS STRUCTURES

Charles Darwin noted that many animals shared similarities in body structure. He argued that this seemed to suggest that the structures had developed from a common ancestral form. Are the similarities in structures as obvious as he suggested?

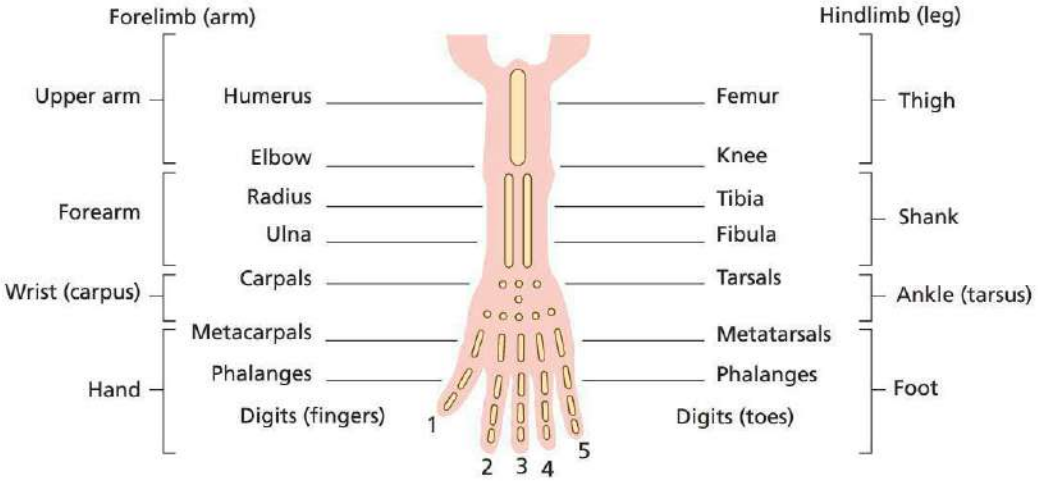
Aim

To investigate homologous structures in the pentadactyl limb of various vertebrates

Materials

- four examples of vertebrate pentadactyl limb. These could be actual skeletons, models, photographs or illustrations of the limbs (e.g. frog, bird, dolphin, dog, cat)

Procedure



▲ Figure 6.20 A generalised pentadactyl limb

For each of the samples that you have been given, complete as many observations as possible and note them in your results.

1. Examine the forelimbs and hind limbs of each specimen carefully and draw a quick sketch in your results. Make a table to record the number of bones that make up each individual digit on the forelimbs and another table for the hindlimb. Include the hand/foot area, wrist/ankle area, forearm/shin area, and the upper arm/thigh area.
2. Describe any other differences that you may have observed in each specimen when it is compared to the generalised diagram of a pentadactyl limb (Figure 6.20).

Results

Your results should include:

- Name of organism
- Sketch of forelimb
- Sketch of hindlimb
- Summary table of counts
- Descriptions of differences

Discussion

1. Analyse how the number of bones in each area of your specimens compares to the generalised pentadactyl limb.
2. Other than bone numbers, identify and explain what other differences you find in the limb structures.
3. Suggest and explain reasons for the differences noted for each particular animal.

4 Suggest what advantage these differences might offer to the species concerned.

5 Identify the basic similarities in the different limbs and explain how these can be found in so many different species that may occupy a variety of different habitats.

Taking it further

Use the Internet to examine the limb structure of other animals to see how they compare to the ones you've examined in this activity. Does it make a difference how closely animals are related to each other in terms of their similarities?

Conclusion

Write a conclusion that summarises your findings.

Molecular evidence for evolution: history is written in our genes

Different organisms share **molecular homologies** as well as structural ones. DNA itself is a very simple example of a molecular homology that links all life on Earth. Both DNA and RNA possess a four-base code that provides the basis for all life; however, the homologies are more complex, and profound, at a genetic level.

Protein conservation

Proteins, and the alleles that encode them, are subject to the same process of evolution by natural selection as the larger traits that individuals possess. A protein that is well suited to its function will be preserved, or **conserved**, while other traits around it may evolve. Two distantly related species may share very similar protein sequences for a protein whose function is much the same in those species, such as the histone proteins.

Mutations that arise over time may alter a protein's function, usually making it less suited to its function. If a point mutation results in the loss of an amino acid that is essential for the protein's function, the mutation may not be preserved. Protein sequences can be compared across species and conserved amino acids can be identified. This is another line of enquiry for identifying the evolutionary relationships between different species.

See Chapters 1 and 2 for more on allele expression, and histone proteins.

Occasionally, mutations may arise that change an encoded amino acid to one with a very similar charge and shape. Thus, the amino acid at that site is still essentially conserved, as the substituted amino acid will allow the protein to have the same function.

Genetic comparison

In the absence of external influences such as ionising radiation and chemical mutagens, a baseline rate of mutation occurs naturally to DNA. If mutations change the structure or function of the proteins that are encoded, they will change the way those proteins are passed to the next generation, making them either more or less common in subsequent generations. In many cases, mutations may arise in non-coding regions or may change a codon to one that encodes the same amino acid as before, resulting in a neutral mutation. The frequency of neutral mutations is fairly constant within a species and is called the **mutation rate**. When comparing the genomes of two species, the mutation rate can be used as a molecular clock to estimate at what point in time those species diverged from a common ancestor. For humans, the mutation rate is estimated to be approximately 10^{-8} per site (i.e. nucleotide) per generation.

Comparative genomics: a deluge of data

The comparison of genome sequences of different species or individuals gives a very broad picture of DNA sequence conservation and mutation frequencies, making it possible to trace evolutionary processes responsible for the divergence of two genomes. **Comparative genomics**, however, produces huge amounts of data that must be stored and analysed in a logical and meaningful way.

The scale of computational framework for this volume of biological analysis is huge. Only very recently has it become possible to undertake these analyses, due to advancements in computer science, engineering and mathematics via **bioinformatics**. Bioinformatics is the digital storage, retrieval, organisation and analysis of biological (in this case, genomic) data. Bioinformatics has dramatically increased the size, accuracy and scope of data sets, such as those needed for comparative genomics.

Bioinformatics has provided significant advances in our knowledge of the entire genomes of organisms, and in turn this has revealed yet more evidence of evolution. When contrasting the base-pair composition of genes of seemingly unrelated organisms that code for comparable structures (say, the code that provides instructions on how to build an eye), the composition of each gene is remarkably similar. For example, the genes that code for 'building eyes' on vertebrates such as humans, called *PAX6*, are more than 78% similar in their protein arrangement to those responsible for building the eyes of octopus. The similarities in sequence, function and abundance of these genes across organisms across a broad spectrum of phyla are yet another example of homology, in this case at a molecular level. This is an example of how the identification of molecular homologies via comparative genomics can reveal the shared common ancestry of diverse species (Figure 6.21).

You can revise the concepts of genetic variation and genomes in Chapter 3.

Molecular homologies such as these also have application in building branching phylogenetic trees in the technique of **molecular phylogeny**. In the example of the eye-building *PAX* gene, it is possible to conclude that as descendent lineages evolved, the gene was modified in a variety of ways in different lineages, giving rise to a diversity of eye-building genes seen in modern animals.

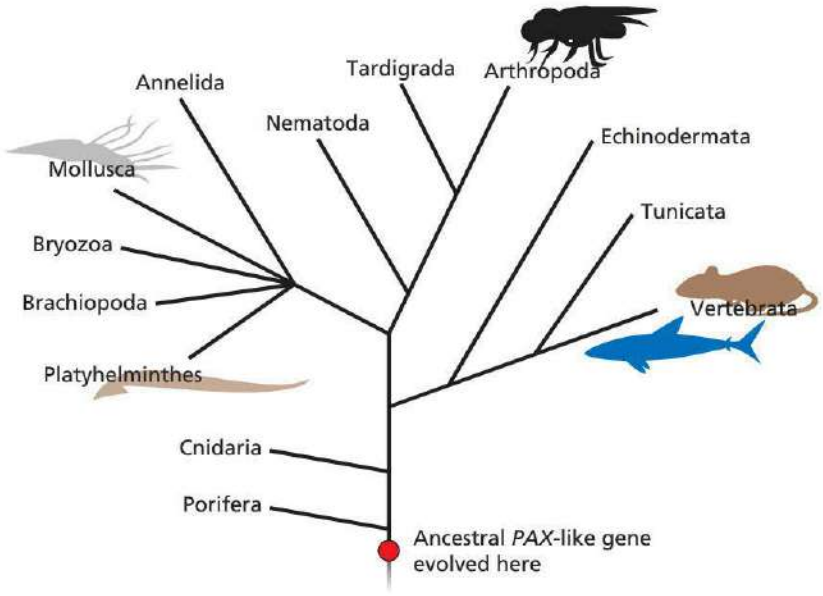


Figure 6.21 ▶ Comparative genomics has found shared 'eye-building' genes across all animals with eyes. From this, we can map a phylogenetic tree.

QUESTION SET 6.5

Understanding

1 Adaptations may be behavioural, physiological or structural.
   a Explain what is meant by each of these terms.
   b Give an example of each type of adaptation and name an organism with this type of adaptation.
2 Some species are phenotypically similar. Explain if this means that they have a recent common ancestor.

Analysing

3 Explain why the spikes of a bearded dragon lizard and the hard plates of a tortoise shell are homologous structures.
4 Explain why seemingly unrelated organisms could have a high percentage of very similar genes.
5 Explain the advantage of mass data storage (provided by technological advances in the use of bioinformatics) when examining the relationships of seemingly unrelated taxa.

CHAPTER SUMMARY

- Earth and its inhabitants have changed enormously over 3.5 billion years. Some changes are rapid and can be witnessed in a lifetime.
- The position of landmasses are all in constant change. Geological and fossil evidence tell us that 200 mya a single supercontinent – Pangaea – existed, which would later separate into smaller landmasses.
- Contemporary evidence for evolution comes from five main lines of evidence: palaeontology, biogeography, developmental biology, morphology and genetics.
- Comparative dating is used to determine the relative age of a rock or fossil. Absolute (or chronometric) dating assigns a numerical age in years to a fossil or rock.
- Biogeography is the study of the distribution of organisms and ecosystems across the world and through geologic time.
- Comparative anatomy is used to establish evolutionary relationships on the basis of structural similarities and differences, including the comparative study of embryos.
- Different organisms share molecular homologies as well as structural ones. Examination of the genes of different organisms indicates that all modern life descended from a single population of organisms.
- Comparative genomics provides evidence for the theory of evolution and helps us map the degree of species relatedness.
- Darwin's theory of evolution by natural selection refuted Lamarck's theory of transmutation of species by spontaneous generation.

CHAPTER GLOSSARY

absolute dating the process of determining the age of rocks and their contained fossils in years on the basis of the physical or chemical properties of materials in the rock

adaptation a developed characteristic that enhances an organism's survival in its natural environment

adaptive radiation a process where a lineage of organisms rapidly diversifies into many different forms and taxa with different adaptations; it can be triggered by many factors, such as changes to available resources, or other new challenges or opportunities; this is a type of divergent evolution

analogous structures features of organisms that have the same function but not the same structure

biogeograpy the study of the distribution of living things over a geographical area through geologic time

bioinformatics the science of managing and analysing biological data using advanced computing techniques; it is especially important in genomics research because of the large amount of complex data this research generates

common ancestor a species from which other species have evolved

comparative dating the process of determining the age of rocks and their contained fossils relative to each other, allowing an estimation of 'oldest to youngest' without assigning an actual age in years

comparative genomics the process of contrasting the entire hereditary information of organisms as encoded in their DNA

conserved (sequences) DNA or protein sequences that are preserved across species

continental drift the relative movement of Earth's continental landmasses that appear to drift or 'float' over Earth's mantle

convergent evolution a process whereby unrelated organisms evolve similar adaptations in response to their environments

divergent evolution when related species evolve new traits over time, away from the common ancestor, to give rise to new species

eon a division of geologic time that can be divided into periods, epochs and ages

epoch a division of geologic time that is shorter than a period and is marked by one or more significant events

era a division of geologic time comprising periods and epochs

evolution the process of gradual change in the gene pool of a population of organisms that results in new species

fossil preserved remains or traces of an organism

gradualism a theoretical model of the pace of evolution occurring as a steady, slow divergence of lineages at an even speed, irrespective of gaps in the fossil record

homologous structures features of organisms that have the same general structure but different functions

isotope atoms of an element that have the same number of protons but different numbers of neutrons, and therefore different relative atomic masses

molecular homology the identification of shared biomolecular elements – generally genes – used to

test the relationships between organisms, which can demonstrate common ancestry

molecular phylogeny the study of evolutionary relationships using comparative genomics

mutation rate the number of changes per gene copy in a population over a period of time

mya millions of years ago, sometimes expressed as millions of years before present (myBP), or simply millions of years (my); for example, a fossil dated as being 5 million years old lived 5 mya

niche an organism's habitat; or way of life or function of an organism in its environment

period a division of geologic time; periods and epochs together make up eras

phylogeny evolutionary relationships that exist between species, often expressed as a tree-like diagram

punctuated equilibrium a theoretical evolutionary model of an organism's change occurring rapidly and in relatively brief events between longer periods of stasis (or equilibrium) without record in the fossil record

speciation the evolution of one or more new species from an ancestral species

vestigial structures structures found in organisms that have lost most, if not all, of their 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

CHAPTER REVIEW QUESTIONS

Remembering

1 List the categories of evidence for evolution.
2 Recall Lamarck's theory of evolution.
3 Define the following terms.
   a Gradualism
   b Punctuated equilibrium
   c Biogeography
   d Vestigial structures
   e Homologous structures
   f Comparative genomics
4 The fossil record is a vital stream of evidence in the modern evolutionary synthesis, but it is patchy and incomplete. Recall the reasons for this patchy record.

Understanding

5 The Thylacine (marsupial) and the American grey wolf (placental) evolved independently of each other in remote biogeographic locations, but both animals had a similar appearance and occupied similar ecological niches. The similarities between the two organisms are most likely a result of which evolutionary pattern?
6 Describe the fundamental difference between Darwinian evolution and its precursor theories in terms of how descent should be viewed.
7 Both birds and bats have wings, while mice and crocodiles don't. Explain if this means that birds and bats are more closely related to one another than to mice and crocodiles.
8 Forty per cent of the world's species of fruit fly are found on the islands of the Hawaiian archipelago.
   a Propose why the Hawaiian archipelago might provide a suitable habitat for so many different species of fruit flies.
   b Explain how adaptive radiation may have been involved in the evolution of Hawaiian fruit flies.
   c Describe three ways that ancestral fruit fly genes may have been transported from one island to another.
9 The sugar glider and the flying squirrel have a similar appearance. Both have a flap of skin between the forelimbs and hind limbs that enables them to glide from branch to branch. The flying squirrel is a placental mammal found in the Northern Hemisphere and the sugar glider is a marsupial found in Australia.
   a Name the process that has resulted in these species having similar features.
   b Name and describe the evolutionary pattern that accounts for the similarity of these two species.
   c Suggest how these two animals – one a placental and one a marsupial – are different in other ways.

10 Mimicry is a common phenomenon in natural systems. The mimic seeks to take on the appearance of another organism. The organism being mimicked, called the model, is either 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** Describe the type of evolution is involved in mimicry.
- **c** Explain what would you expect the ratio of models to mimics to be in natural systems.
- **d** Describe how the disappearance of the model might affect the mimic.

Applying

11 Species are defined as a group of organisms that can interbreed and produce fertile offspring. Subspecies are defined as distinct populations of a species that can interbreed and produce fertile offspring with other members of the species, but due to isolating factors, they don't interbreed in nature. Many subspecies exist today among all organisms. Identify and explain the evolutionary pattern that is occurring in this example.

12 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.

Analysing

13 New Zealand has no large native land mammals, but has been home to some highly specialised bird species. Many of these birds have lost the ability to fly, and in the case of the five species of Kiwi, have developed some distinctive features. Mammal-like characteristics such as a keen sense of smell, bone marrow (which makes bones heavy and unsuitable for flight) and a pair of functional ovaries in females (most birds have only one functional ovary) are highly unusual for birds.

- **a** Research the five species of Kiwi and explain how they show examples of:
  - **i** divergent evolution.
  - **ii** convergent evolution.
  - **iii** adaptive radiation.
  - **iv** analogous structures.
- **b** What would molecular homology studies illustrate about the relationship of the five species?

14 There is a variety of types of tortoise on the Galapagos Islands. One type has a domed shell and a short neck, and is found on islands with high moisture content. The other has 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.

Evaluating

15 The Hoatzin (*Opisthocomus hoazin*) is a remarkable bird from South America. It has only one known fossil ancestor, a 10-million-year-old skull fragment found in Colombia. The age of the fossil demonstrates 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 is unique, perhaps because of its extensive history of geographic isolation, and has its own suborder. Chicks of the Hoatzin show a characteristic shown 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 the above description, identify whether the lines of evidence for evolution described are from the disciplines of:

- **a** palaeontology, via the fossil record.
- **b** biogeography.
- **c** developmental biology.
- **d** morphology.
- **e** genetics.

Creating

16 The 1861 discovery of the Jurassic-age fossil skeleton of the feathered dinosaurian bird ancestor *Archaeopteryx* from Germany was a key moment in the development of Darwinian theory. The discovery of the pigeon-sized animal was brought to the attention of Charles Darwin, who commented that 'hardly any recent discovery shows more forcibly than this how little we as yet know of the former inhabitants of the world'.

The skeleton of *Archaeopteryx* clearly shows that it had claws on its forelimbs, well-developed feathers on its wings (allowing for weak gliding flight), teeth and a long bony tail.

a Define which of these characteristics point to a relationship to birds on the basis of:

i embryology.

ii homologous structures.

iii analogous structures.

b Explain how the relationship of *Archaeopteryx* to dinosaurs and birds has limitations based on molecular homology and comparative genomics.

c Predict how the potential diet (as influenced by the climate, continental associations, and other animals and plants) would have affected the *Archaeopteryx* in terms of its:

i teeth.

ii size.

iii locomotory adaptations.

CHAPTER 7 NATURAL SELECTION AND SPECIATION

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

Science Understanding

- Natural selection occurs when selection pressures in the environment confer a selective advantage on a specific phenotype to enhance its survival and reproduction; this results in changes in allele frequency in the gene pool of a population (ACSBL090)
- In addition to environmental selection pressures, mutation, gene flow and genetic drift can contribute to changes in allele frequency in a population gene pool and results in micro-evolutionary change (ACSBL091)
- Mutation is the ultimate source of genetic variation as it introduces new alleles into a population (ACSBL092)
- Speciation and macro-evolutionary changes result from an accumulation of micro-evolutionary changes over time (ACSBL093)
- Differing selection pressures between geographically isolated populations may lead to allopatric speciation (ACSBL094)
- Populations with reduced genetic diversity face increased risk of extinction (ACSBL095)

Alamy/Neil Hardwick

Alamy/Frank Hecker

Figure 7.1 ▲ The peppered moth, *Biston betularia*, has a) a white speckled *typica* form and b) a dark *carbonaria* form.

The peppered moth, *Biston betularia,* is widespread in Britain. Historically, the standard moth form, *typica*, was white, liberally speckled with black. 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 of Britain, such as Manchester, were black (form *carbonaria*). A well-known lepidopterist, J. W. Tutt, proposed an evolutionary link between the Industrial Revolution and the moth population. Dark pigmentation was part of the natural, inheritable variation of the *B. betularia* population, but very rare. Blackening of tree trunks by soot presented a new environmental pressure for the moth population. The dark coloured moths were better able to evade bird predation than the common white speckled form. Over time, black moths came to dominate the population.

Since 1950, when clean air legislation was passed, the situation has reversed; once again dark coloured moths are suffering greater predation on the naturally white tree trunks, and their presence in the population is less common. Both dark and white forms continue to exist in the population.

Variation in populations

EVOLUTION OF THE PEPPERED MOTH

Explore the evolutionary story of the peppered moth.

Like the peppered moth example, individuals in any **population** express a range of different phenotypes. This is because members of a population have variation in genotypes that causes variation in their phenotypes. This genetic variation is **inheritable**; 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 case of the peppered moth, a **mutation** in genotype produced a dark-coloured form in this population. This dark phenotype conferred a survival advantage in the changed environment. On the other hand, the genotypic variation may also give a disadvantage or have no effect at all. Either way, genetic mutation introduces new alleles and, therefore, new variation into populations.

Variations in populations can be very small, but they are the basis of evolution.

Gene pools

Genes are the means of transmitting phenotypes from one generation to another. Many genes can exist in different forms as alleles, and the characteristics of individuals are determined by the alleles they inherit. It is this variation in alleles carried by different individuals that leads to most of the variation in a population. The total collection of alleles within a population is referred to as a **gene pool** (see Figure 7.3). In biological terms, a population is a group of individuals of the same species that live in the same geographic area and readily interbreed to produce fertile offspring, so that they belong to the same gene pool.

Genetic mutations introduce new alleles into populations. These act as the main source of variation. The sum total of all alleles present in a population is called the gene pool.

The range of variation possible in a population is restricted by the alleles available in its gene pool. For example, bearded dragons do not carry genes for wings or hard-shelled eggs or the enzymes required to synthesise chlorophyll or to digest cellulose. All bearded dragons do, however, carry genes for a tail, rudimentary teeth, scales and four legs (Figure 7.2). The many genes that have only one possible allele in a gene pool, and so do not contribute to any variation, are said to be 'fixed' in the population. Scientists believe that approximately 80–85% of our genes are fixed in this way. These genes do not make a significant contribution to evolution since there is no variety to draw on. It is the other 15–20% that can be drawn upon during evolutionary change.

Alamy/Juniors Bildarchiv GmbH

▲ **Figure 7.2**
Bearded dragons carry genes for a tail, scales and rudimentary teeth, but do not have genes for wings.

Allele frequencies

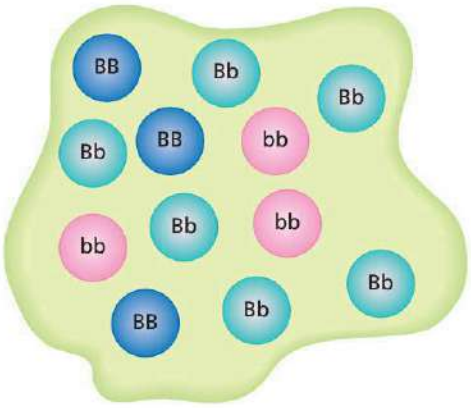
For variation to occur in phenotypes, more than one allele of a gene must exist. Phenotypes that vary due to genetic differences are termed genetic polymorphisms (*poly* meaning 'multiple'; *morph* meaning 'form'). The frequency of polymorphic alleles is not usually constant and can be affected by:

- mutation of an allele
- immigration of individuals; that is, movement into the population
- emigration of individuals; that is, movement out of the population
- the reproduction rate of various individuals in the population; that is, the number of offspring born per year to an individual.

Other factors that can change an allele frequency are:

- **genetic drift**
- the **bottleneck effect**
- the **founder effect**.

These factors are discussed in more detail below.



▲ **Figure 7.3**
The sum total of all alleles found in a population in called the gene pool.

The basis of evolutionary theory is that favourable traits become more common in each successive generation. Those members of a population that survive and reproduce in their habitat carry the traits most suitable for their circumstances, and so, over time, the population becomes more suited, or better adapted, to its habitat. But what happens when the habitat changes? In most cases where there is genetic variation in the population, some members will survive changes and pass on their genes. For example, some members of a locust population may be resistant to local pesticides. Those members would survive seasonal crop spraying and pass on their genes. If no members in the locust population possessed a genotype that resisted pesticides, the local locust population would not survive.

Such variation in the gene pool is essential. So, where do new alleles come from? The answer is that they generally come from old alleles through mutation. Mutations are rare and mostly produce harmful effects. In a large population they are barely noticeable. But despite this they are essential to evolution because they are the ultimate source of variation within populations.

Many mutations produce recessive alleles that can be masked by the effects of the original allele, which remains the dominant allele. We each may carry several hundred mutations, most of which will never be noticed, particularly as most of us will 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 population. Before the Industrial Revolution, the dominant *carbonaria* forms were extremely rare, and appear to have been maintained mainly through the occurrence of spontaneous mutations. However, during the Industrial Revolution recessive alleles coding for the white (*typica*) trait were able to survive in the population at a low level. Only the extremely rare homozygous individuals experienced the selective pressure of increased predation. In this case, the population responded quickly to a changing environment. The evolution of sexual reproduction, with the random mixing and assortment of traits from one generation to the next through meiosis, has been very important in producing populations with variation.

See Chapter 3 for detail on the possible causes and effects of mutations on alleles.

The gene pool of a population is subject to many external influences. Gene pools are shaped by the movement of individuals and by environmental events that can sometimes rapidly and considerably change the composition of populations.

Mutations provide the raw material for evolution since they introduce new alleles into a population. Evolution is any change in the gene pool over time.

Migration and gene flow

Populations, in a biological sense, 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** may occur if the migrants breed. For example, immigrants may add new alleles to the gene pool and emigrants may completely remove some alleles or significantly change the frequency of others.

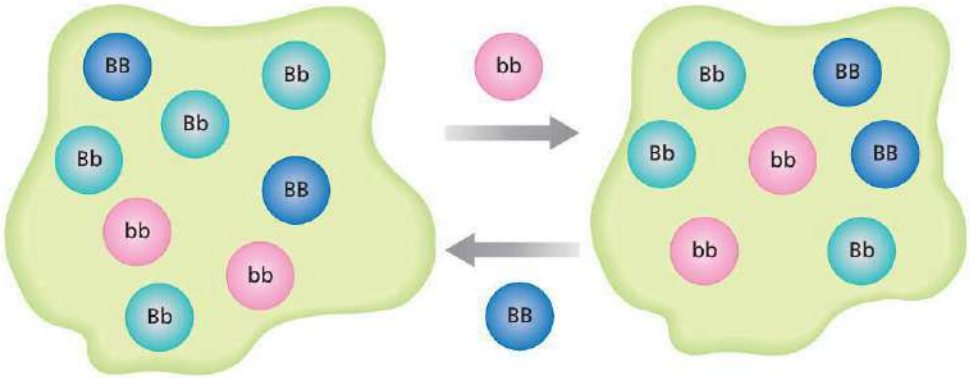


Figure 7.4 ▶ Gene flow is the transfer of alleles that results from emigration and immigration of individuals between populations.

Humans are polymorphic for a range of blood groups, including the ABO blood group. Indigenous Australians have some alleles that are present at frequencies different from other populations in the world. They have largely been isolated for 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 possess the I^B allele of the ABO blood group that results in either the B or AB type blood. The I^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 I^B allele is increasing within the Indigenous Australian population due to migration of people from Asia and Europe into Australia and the genetic flow between these populations.

See Chapter 4 for more on the inheritance of the ABO blood group.

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 respective parents passed on to us was a matter of chance. In large populations this randomness in inheritance of alleles is not noticeable overall. 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. Alleles may be easy to lose, but they are virtually impossible to replace.

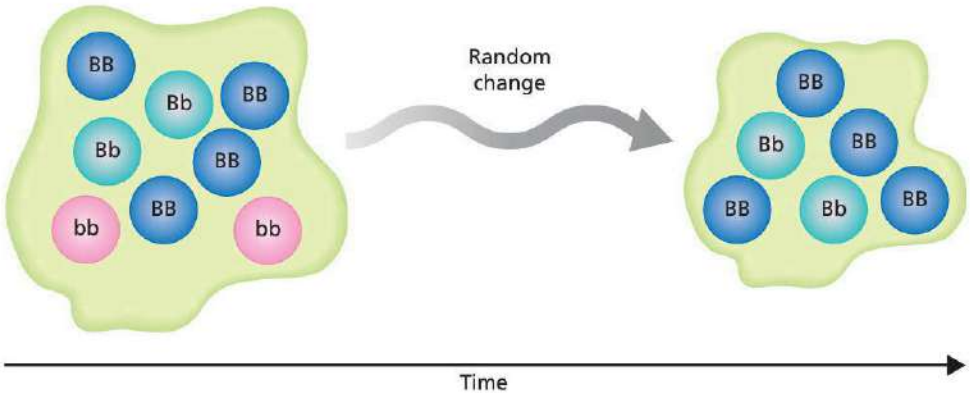


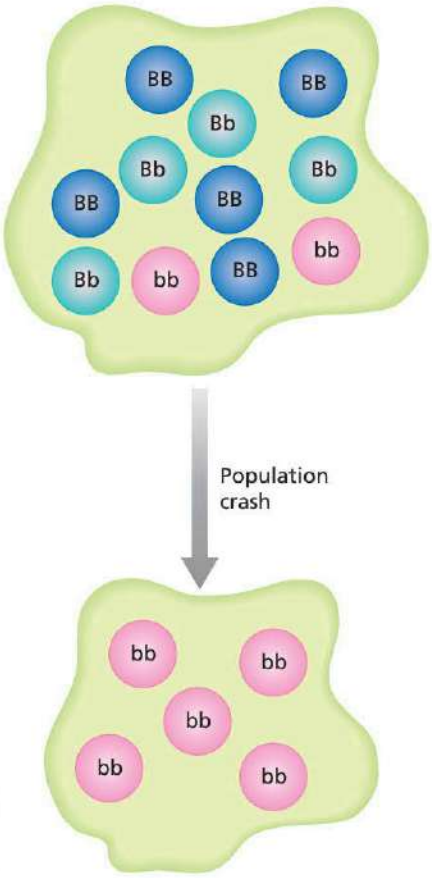
Figure 7.5 ▶ Chance events can cause the allele frequency in a population to change. This is known as genetic drift.

Genetic drift can occur in a small population or when a large population is suddenly reduced due to 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.

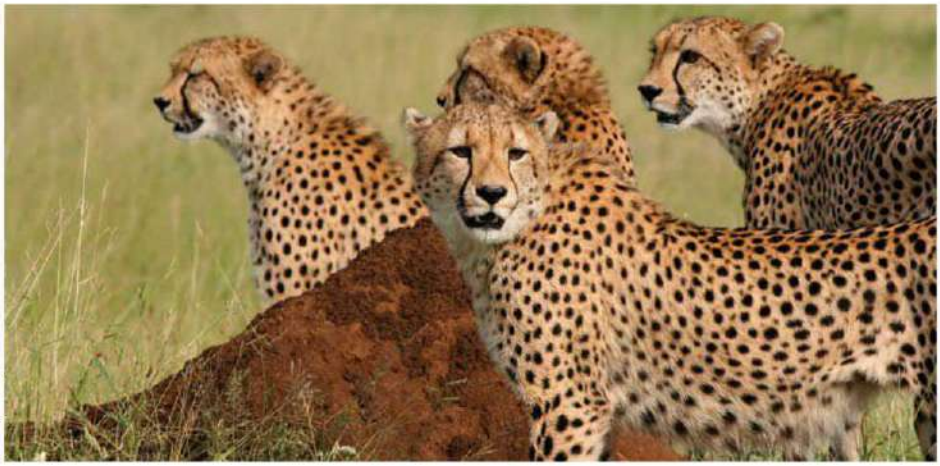
Bottleneck effect

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. If some portion of the population survives the catastrophe, the original population 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.

Cheetahs are an endangered species that have survived a drastic genetic bottleneck. Facing a declining population, the surviving 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. There are only around 10 000 cheetahs left in the world today.



▲ **Figure 7.6**
A catastrophic decrease in population size can result in a loss of some alleles from the gene pool. This is the bottleneck effect. Deleterious genes can be preserved by chance.

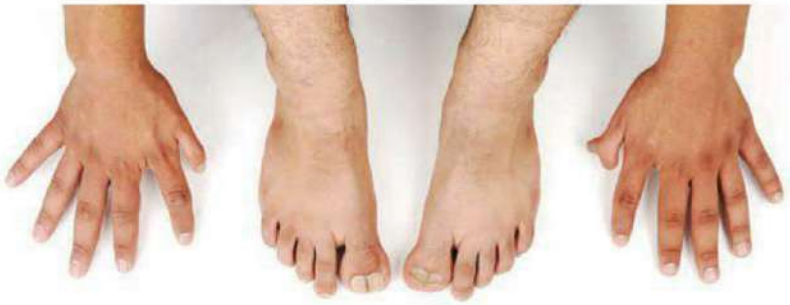


iStockphoto/DaleBHalbur

▲ **Figure 7.7**
Cheetahs survived a severe bottleneck that increased the frequency of some mutated alleles.

The 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. This means that the isolated population has less genetic diversity than the original population and deleterious recessive alleles may have a higher chance of coming together than they did in the original population.



Shutterstock.com/Zurijeta

▲ **Figure 7.8**
Examples of polydactyly, one of the symptoms of Ellis–van Creveld syndrome

This effect has been observed in human populations when small groups of particular religious or ethnic backgrounds have settled somewhere new and mixed very little with other settlers. Around 200 people originally settled the Amish community of North America and at least one of the settlers harboured a recessive allele for Ellis–van Creveld syndrome. This syndrome, symptoms of which include dwarfism, polydactyly (extra toes or fingers) and sometimes a hole in the heart, has been common among Amish people of this region ever since.

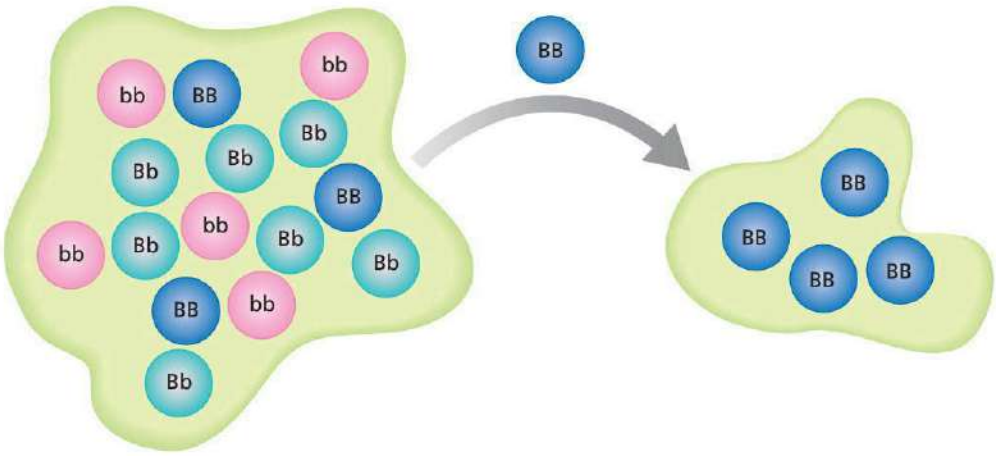


Figure 7.9 ▶ The founder effect occurs when a few individuals carry alleles to a new, isolated area and a new population is formed with different allele frequencies to the original population. This is also a type of gene flow.

Genetic drift, the founder effect and bottlenecks can lead to a change in the gene pool of small populations.

QUESTION SET 7.1

Remembering

1 Define the following terms.
   - a Founder effect
   - b Genetic drift

2 Recall the relationship between genotype and phenotype.

Understanding

3 Distinguish between a gene and an allele.

4 Outline why variations have to be inheritable for them to be relevant to evolutionary change.

5 Describe the mechanisms that can lead to changes in the gene pool of a population.

6 Outline how gene flow can affect allele frequency.

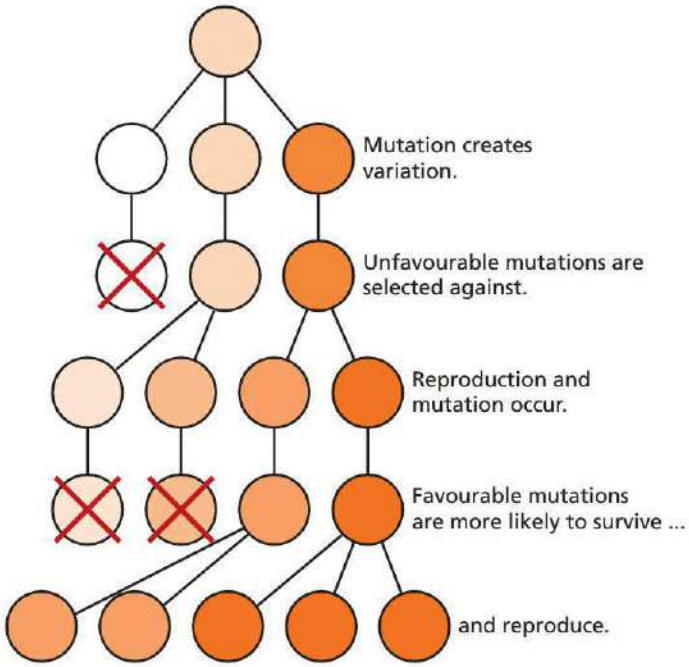
7 Mutations are rare and, in large populations, barely noticeable. Describe how mutations are still essential to evolutionary change.

Analysing

8 Construct a diagram that illustrates two examples of how recessive traits that are deleterious can survive in a population.

Natural selection leading to adaptive evolution

In 1868 two publications were released simultaneously through the Royal Society in London. These papers were by Charles Darwin and Alfred Russel Wallace. They outlined their ideas on the evolution of life, what they referred to as **descent with modification**. This term highlights the important idea that all life that exists today has descended from shared ancestors. Their proposed mechanism by which this happened was the process of **natural selection**, which has shaped nearly every feature of living things found in the world today. In this way, favourable traits are selected for and inherited, and become more common in subsequent generations.



◀ **Figure 7.10**
A diagrammatic representation of natural selection. In this example, the darker alleles confer an advantage over lighter alleles.

The principal of natural selection leading to evolutionary change rests on a few propositions.

1. **Individuals differ from one another; that is, individuals within populations show variation.**
2. **Many of these variations are caused by mutations in alleles and are inheritable.**
3. **In general, more offspring are born than can survive to maturity and reproduce. Because of this, there is a struggle for existence and only some organisms can reproduce.**
4. **Some individuals have traits that make them more suited to their environment than others, making them better able to reproduce and pass on their alleles to the next generation.**

Natural selection acts on individuals to produce changes in whole populations over time. Natural selection acts on the phenotypes of individuals, so that some survive and reproduce while others do not. The capacity of an individual to survive and reproduce is sometimes referred to as its **fitness**. Natural selection is the only selection mechanism that can lead to **adaptive evolution**. This means that it is the only mechanism that leads to new populations, and the mechanism that produces species that are better adapted to their environment.

The driving force for adaptive evolutionary change is natural selection.

▼ **Figure 7.11**
The greyhound is an ancient European dog breed dating from around 5000 years ago. Its appearance is very different to the original domesticated dog, which probably looked more like a cross between a Siberian husky and a large Alsatian.

Artificial selection: animal and plant breeding


iStockphoto/Xseon

Both Darwin and Wallace understood the importance of inheritable variation for any sort of selection to work, but their understanding was very limited at that time. If all individuals within a population were identical then selection would have no effect.

Darwin drew long comparisons with breeding programs for domestic animals, including dogs and pigeons. The processes for breeding different strains of dogs, or different varieties of pigeons

(Darwin was an avid pigeon fancier) were quite well understood. Parental stock with certain desirable traits are 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**, and relies upon human intervention to determine which traits are selected for.

Selection pressures

Darwin believed that the rapid changes produced by selectively breeding dogs, or pigeons, could also occur naturally in the wild. Naturally occurring **selection pressures** would act on traits in the population, resulting in some traits becoming more common as others became less so. Selection pressures are factors that influence the survival of an individual, a population or a species. Some examples are:

- competition between species for food and territories
- predator–prey relationships
- competition within species for food or water
- competition within species for territories or nesting places
- sexual selection; that is, selection of traits that successfully attract mates.

Darwin and Malthus

In 1798, Thomas Malthus, an economist, published his *Essay on the Principles of Population*. In this essay he suggested that the human population had a natural tendency to increase at a greater rate than food supplies and other resources, and this caused much of human suffering: disease, famine and war. Human populations therefore faced a constant struggle to survive. There has been much discussion about the extent to which Darwin was influenced by this essay. We know that he read a later edition of Malthus' essay and he also adopted the term 'struggle' in his writings. While Malthus was mostly concerned with showing that human populations were destined to struggle in this way, Darwin's real focus was the struggle that occurred between individuals in the same population or species, to survive and reproduce, leading to evolutionary change.

Sexual selection

Sexual selection is a process linked to mating behaviour in animals. It describes a form of selection where individuals with certain inherited characteristics or behaviours are more likely than others to obtain mates and pass on their genes. Sexual selection can produce quite spectacular effects, such as the enormous antlers of a moose, or the long, showy tail of a male peacock.

Sexual selection occurs when individual animals with certain inherited characteristics are more successful than other individuals in finding mates.

Special characteristics such as the large tails of peacocks and lyre birds or antlers of moose are actually quite costly to the animal that is carrying them, and do not directly give them any extra survival advantage. In many cases, these attributes can be a threat to their survival. Loud and elaborate courtship displays attract predators as well as mates and growing new antlers every year costs energy. So, what is the evolutionary advantage? One theory suggests that the females are selecting for a very obvious characteristic that correlates with other beneficial alleles. There have been some experiments carried out that suggest that this might be the case.

Sexual selection can also produce a phenomenon called **sexual dimorphism** . This term refers to situations where males and females have different appearances or size.

Some examples of sexual selection can be surprising. For example, the Soay (*Ovis aries*) is a primitive breed of sheep that live on the rocky islands off the coast of Scotland. They are well known for their agility on cliffs and for the large horns on many males. Large horn size appears to be a sexually selected characteristic and provides males with a significant advantage in securing mates. Variation in this trait appears to be controlled by a single gene. One allele (Ho^+) is linked to large horns and the other allele (Ho^P) with smaller horns.

Biologists have often hypothesised that sexual selection helps females somehow choose males who posses genes that confer a high level of fitness. But in the case of the Soay they found that males with large horns actually have less fitness overall. Rams with small horns had a better chance of surviving the harsh winters and rams that were heterozygous, carrying one of each allele, were most successful overall in terms of survival and reproduction. This ensured that the Ho^P allele survived in the population even though it rendered the rams less sexually fit.

Alamy/David McGill

▲ **Figure 7.12**
Soay rams, showing their large horns

WOW

The multimedia display of the lyrebird

The male lyrebird may not have the most spectacular tail in the world, so you might think it would not be attractive to females. But when you consider the bird's song display, it is a different story. The lyrebird is one of the most accomplished mimics in the animal kingdom. Males sing complex songs mimicking animal and bird sounds and even mechanical sounds, such as chainsaws. The males with the greater repertoire achieve better reproductive success.

Getty Images/Craig Dingle

▲ **Figure 7.13**
The male lyrebird displaying its tail

SONGS OF THE LYREBIRD

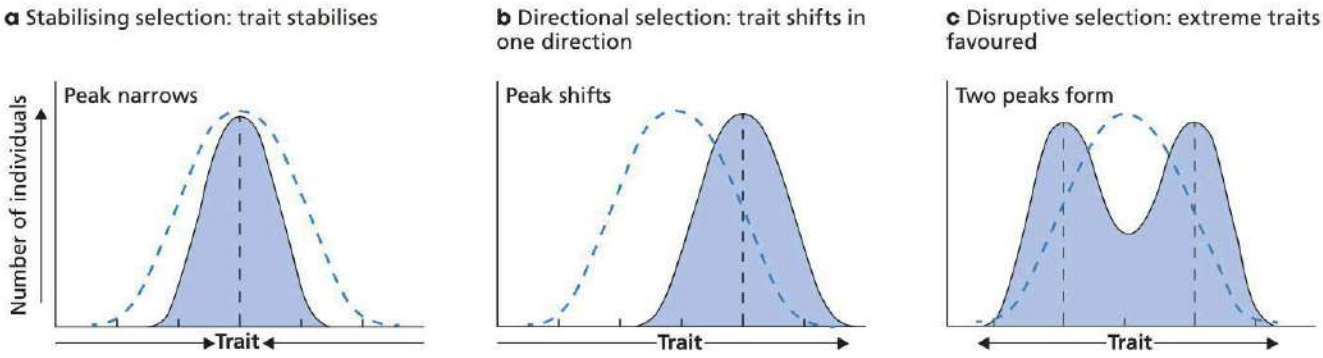
Explore with David Attenborough the repertoire of one bird.

Natural selection can be stabilising, directional or disruptive

Natural selection is most obvious when it is leading to changes in the gene pool of a population, causing some observable change in phenotype. The population may be gradually changing colour or becoming larger or smaller due to selective pressures in a changing environment. As long as the environment of an organism is not changing, then the selective pressures will act against deleterious alleles that cause a departure from the optimal phenotype. This is referred to as **stabilising selection** (shown in Figure 7.14).

Directional selection leads to a change in a trait over time. Changes in environment lead to selective pressures favouring organisms with new or more extreme traits.

A third mode of selection, called **disruptive selection**, operates in favour of extremes. For example, a drought may kill off a local species of shrub that produces medium-sized seeds. A species of seed-eating bird may experience disruptive selection in this situation, when there are only large seeds (or only small seeds) available to eat. Birds with intermediate-sized bills would not be as well adapted for eating either the large or the small seeds and would be selected against.



▲ Figure 7.14
Selection can change the distribution of phenotypes (and therefore genotypes) in three different ways. The original distribution of traits is shown with dotted lines in each graph.

QUESTION SET 7.2

Remembering

1 Outline the meaning of the following and give an example of each.
   a Natural selection
   b Sexual selection

Understanding

2 Describe how natural selection contributes to evolutionary change.
3 Identify the role of variation in evolutionary change.

See Chapter 4 for more on Mendel's contribution to our understanding of inheritance.

Putting it all together: the principles of evolution

The idea of adaptive evolution through natural selection is one of the most important 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 inheritable. Subsequent understanding of the inheritance of traits, initially through the work of Mendel, fitted perfectly with their theories to produce a combined theory referred to as the **modern synthesis**. The modern synthesis of evolutionary theory, sometimes referred to as neo-Darwinism, is one of the greatest refinements of a major theory to occur in biology.

TREE OF LIFE

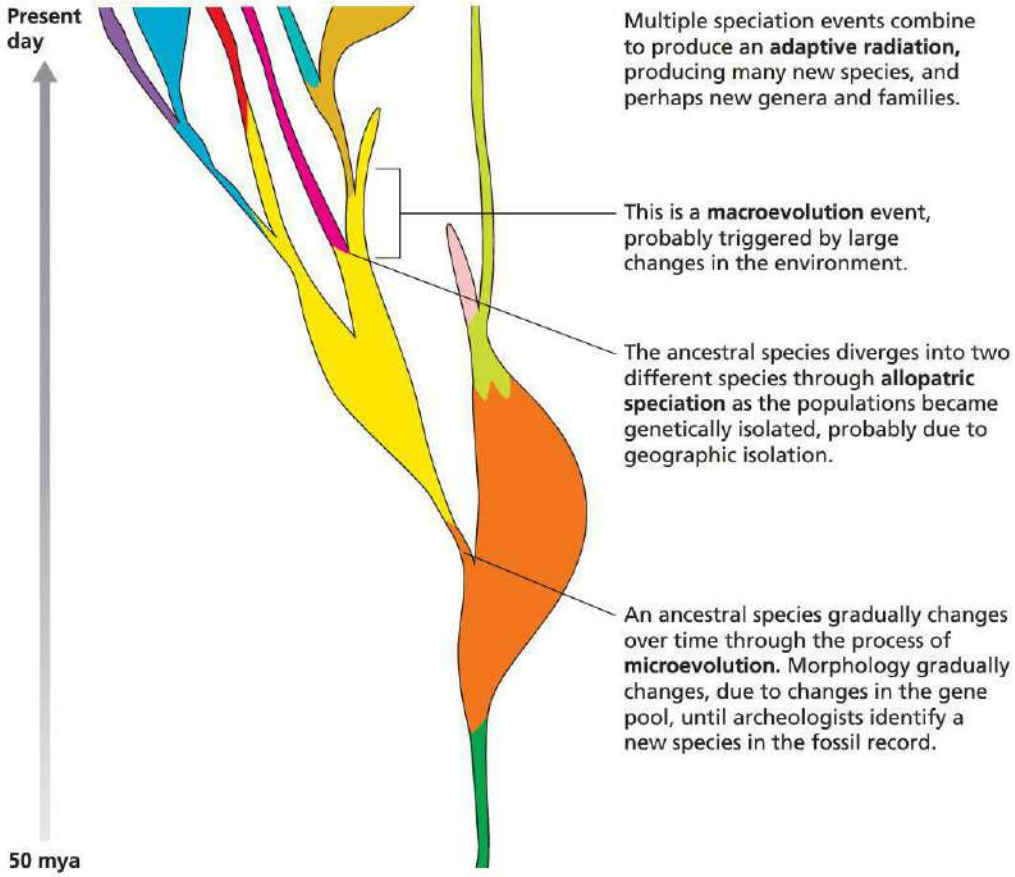
The Tree of Life project is a collaborative effort of biologists from around the world. It provides up-to-date information about biodiversity, the characteristics of different organisms and their evolutionary history (phylogeny).

Microevolution

The significant outcome of natural selection pressure is a change in the frequency of various alleles within a population, a process called **microevolution**. Microevolution refers to any change in the gene pool of a population. The idea of microevolution puts the spotlight of evolutionary theory firmly on the genetic makeup of populations. We now see a population as a large pool of alleles that can change over time for a variety of reasons. Regardless of how this change is occurring, if the gene pool is changing over time then evolution is occurring.

Macroevolution

Major evolutionary changes above the species level are sometimes referred to as **macroevolution**. **Adaptive radiation** occurs when a single species eventually gives rise to a whole new group of organisms comprising many new species. These species have adaptations that allow them to exploit new ecological roles or **niches**. Major radiations appear to be relatively rare and often seem to depend on chance events. These often correlate with major changes in environmental conditions or catastrophic geological events causing **mass extinctions**. A mass extinction event 250 million years ago (mya) during the Permian period saw the extinction of 96% of all marine species and 76% of terrestrial vertebrates. With the loss of so many species Earth now contained many new and under-utilised ecological opportunities (niches). New speciation events occurred quickly. One of these saw the evolution of many new species from a single ancestral dinosaur over just a few million years. This example is one of several that demonstrate how the evolutionary trajectory of life on Earth is greatly affected by chance events.



◀ Figure 7.15
The evolution of diversity. A hypothetical phylogenetic tree showing the evolution of increasing diversity in a group of animals. Colour changes show changes in species identified in the fossil record. New species arise within single lineages as well as when species diverge. Each lineage may contain up to five different species over 50 million years. The thickness of each line represents population size.

Microevolution is any change in the gene pool of a population over time.
Macroevolution is any change in groups of organisms above the species level.

The human story: are humans still evolving?

Evolution is an ongoing process and humans are still evolving, just like all other forms of life. The rise of the human family tree over the last 4 million years is an example of a recent adaptive radiation. We currently represent the only remaining species within a whole genus (*Homo*), within the Family *Hominidae* (the Great Apes, including humans, plus all ancestors). Most **phylogenetic trees** are much fuller and more diverse than this. They have multiple species within each genus and certainly within each family. Within the hominids, modern and

1000 GENOMES PROJECT

The 1000 Genomes Project aims to sequence the genomes of about 2500 unidentified people from about 25 populations around the world, using next-generation sequencing technologies. The results of the study will be freely and publicly accessible to researchers worldwide.

ancestral humans are classified as hominins and they branch away from the other apes. Fossil discoveries over the last 30–40 years have revealed that until quite recently the human family tree also had multiple branches, indicating an adaptive radiation of hominins that occurred starting around 4 mya.

Up until 600 000 years ago there were many different species and several different genera of hominids living throughout Africa, Europe and Asia. This adaptive radiation appears to have been the result of climatic and geological changes in East Africa that led to the evolution of multiple species of *Australopithecus* and, later, the genus *Homo*. Various species within the *Homo* genus appear to have migrated and adapted to a wide range of different environments, leading to their expansion across the globe.

Population genetics is the study of allele frequencies in populations and how they change over time in response to various evolutionary processes. This area of study is also applied to the human population, which appears to be undergoing a major transition at the moment. Historically there were many isolated small communities with distinct gene pools. Now, increased gene flow is leading to greater heterozygosity, which could have effects on health and survival.

Medical and genetic technologies interfere with the human evolutionary changes that might take place if we lacked these advances. Despite these technological advantages, several recently published research studies have found evidence of continued human evolution. University of Chicago scientists discovered that hundreds of our genes have undergone natural selection over the past 10 000 years. Genes for skin pigmentation, breaking down alcohol, and lactose tolerance are among the relatively recent changes. Findings from the University of Wisconsin reveal shrinking brain size and the University of Quebec discovered an island population with a genetic trend for early reproductive age.

HUMANS ARE BORN TO THROW

Discover the features that have evolved in humans that enable us to throw so well.

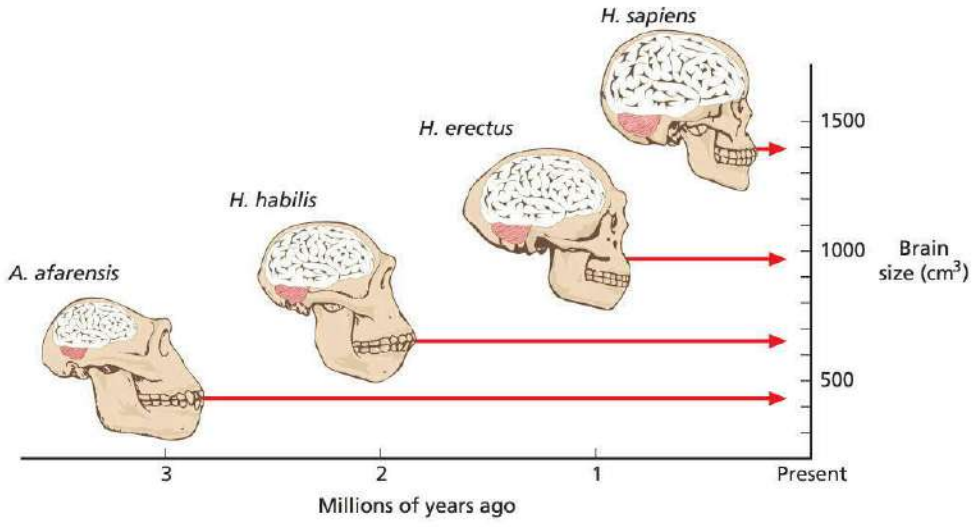


Figure 7.16 ▶ Humans, *Homo sapiens*, have the largest brain size of all hominin ancestors.

WOW

Born to throw

The ability to throw well seems to be a very human adaptation that sets us apart from all other apes. This evolutionary change may have occurred with the first evolution of the *Homo* genus up to 2 mya. Scientists at Harvard University have investigated the physiological changes that led to this adaptive advantage for our ancestors as they developed sophisticated hunting techniques.

Scientific literacy: Why humans continue to evolve despite the many benefits of hygiene and modern medicine

Within a decade of the publication of *On the Origin of Species*, the misconception developed that modern hygiene and medicine have caused natural selection to stop working on human populations. This was fuelled by another misconception: that selection operates only through differences in survival. We now know that natural selection on traits occurs whenever there is variation among individuals in fitness and in traits and when the variation in traits is correlated with the variation in fitness. A response to selection will then follow if some portion of the variation in the traits is heritable. A good proxy for fitness is lifetime reproductive success (LRS) or number of children per parent per lifetime. LRS has both a survival component – one must survive to reproduce – and a reproductive component. Good hygiene and medical care that reduce prenatal, infant and child mortality rates reduce the variation among individuals in the survival component but that does not eliminate natural selection, as substantial variation among individuals in the reproductive component remains. For example, consider an extreme case in which medical and public health measures were so good that everyone who was born survived to age 80. This would not eliminate natural selection, as individuals would still differ in their LRS and that variation would drive natural selection. The potential for natural selection only vanishes when all individuals have exactly the same reproductive success or when no trait is correlated with the variation in reproductive success that still exists. These states are unlikely ever to occur in any population.

The effect of culture on biology raises interesting issues. Birth control, assisted reproductive technology and the increased prevalence of late marriage and divorce complicate the evolutionary genetics of reproduction. These factors can be dealt with by regarding them as part of a changing environment that is changing selection intensities. A more fundamental solution awaits the development of methods of analysing gene–culture co-evolution that can be applied to large, longitudinal human data sets.

Stearns, S. C., Byars, S. G., Govindaraju, D. R. & Ewbank, D. (2010) 'Measuring selection in contemporary human populations', *Nature Reviews Genetics, 11* (September), pp. 611–22 doi:10.1038/nrg2831.

Questions

1 Summarise this article as four dot points.

2 Interpret the meaning of the statement, 'We know now that natural selection on traits occurs whenever there is variation among individuals in fitness and in traits and when the variation in traits is correlated with the variation in fitness.'

3 Propose how natural selection could still operate despite medical and public health measures extending life expectancy beyond 80 years old.

4 Discuss whether or not the effects of human culture can be incorporated into evolutionary theory.

5 List the arguments to support or refute the statement that 'modern hygiene and medicine have caused natural selection to stop working on human populations'.

QUESTION SET 7.3

Remembering

1 Define the following terms.
   a Microevolution
   b Macroevolution

2 Describe one piece of evidence that supports the idea that humans are still evolving.

3 In what way is the modern synthesis of the theory of evolution different to that proposed by Darwin and Wallace?

Applying

4 Construct a table summarising the different processes that can contribute to microevolution.

The diversity of species

The Galapagos Islands lie 1000 km west of Ecuador (South America) in the Pacific Ocean. When Darwin visited them in 1835 during his famous voyage on the 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 animals on the Galapagos Islands is the giant tortoise (*Chelonoidis nigra*), whose ancestral species, the Chaco tortoise, still exists on the mainland today. Darwin wondered how they had got to the islands, and how they had changed into a new 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.

Dreamstime/Rico Leffanta

Nature Picture Library/Gabriel Rojo

Figure 7.17 ▲ a) The famous Galapagos tortoise. Nothing like it exists anywhere else in the world, although it is similar to b) the much smaller Chaco tortoise, *Geochelone chilensis*, found in South America.

See Chapter 6 for more on the fossil record.

Before Darwin's theory of evolution by natural selection, there was a general belief that species were **immutable**, or unchanging; that each species had been put on Earth in its current form and could not change over time. Indeed, our current understanding of evolution tells us that sometimes natural selection produces very little change.

In general, though, the fossil record shows that not only do species change, but that these changes can be dramatic. It also shows that a single species can diverge to produce several new species. The ancestral type of tortoise that still exists on the South American mainland had somehow split to create a new species of giant tortoise on the Galapagos Islands. How this occurred was a key aspect of Darwin's theory. Darwin wondered how species arose only to disappear and be replaced by new animals and plants. We call this **speciation**.

Scientists have hypothesised that there may be more than be 8 million different species on Earth, but this is difficult to estimate accurately because only around 1.2 million species have been identified and classified so far. How new species have evolved in such large numbers is a key part of the theory of evolution.

There are three broad processes that work together in the evolution of this great diversity.

- Natural selection favours phenotypes that make the population better adapted to its environment. Populations change over time as their gene pools accumulate small changes in response to natural selection. This is called microevolution.
- Eventually a population accumulates so many changes that a new species can be identified. This process can lead to speciation, the multiplication of species.
- Sometimes a rapid series of speciation events leads to the development of a whole collection of new species, or even genera, families, or higher classification groups. This is referred to as macroevolution.

Evolution is also marked by another powerful process: extinction. Most species that have ever evolved are now extinct. The broad sweep of evolution is exactly how Darwin imagined it: the constant appearance and disappearance of new species over vast periods of time.

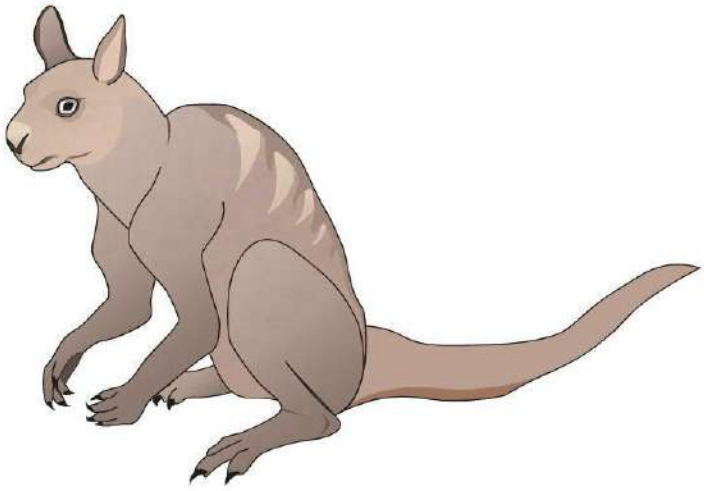
A key idea required to make sense of these processes is the concept of what defines a species, and how a species can be identified in the present time and in the fossil record.

The biological species concept

See Nelson Biology Units 1 & 2 for the Australian Curriculum, Chapters 1 and 2, for more on the definition of species.

Species can be identified 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 model 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 interbreed within itself. In this sense a species is represented by a totally isolated gene pool.

Sometimes, the only evidence that a species existed is in the fossil record. When dealing with fossils only, the **morphological species concept** can be applied. This concept identifies different species based on their physical and physiological characteristics but is limited to what can be observed in the fossil record. For example, red and grey kangaroos are two of Australia's most recognised marsupials. Kangaroos are quite well represented in the fossil record. Twenty-five mya the ancestors of modern kangaroos lived in rainforests and fed on fruit. Kangaroos of today are connected to these distant ancestors through an unbroken line of descent.



▲ **Figure 7.18**
Ancestral kangaroos 25 mya would have looked quite different to modern kangaroos but are connected with them through an unbroken line of descent.

The biological species model defines a species as a reproductively isolated group of organisms. These can be identified through consistent differences in morphological and physiological traits as well as genetic differences.

Mechanisms of speciation

Speciation occurs when a single population becomes two separate populations that are unable to interbreed due to changes that produce physical, biological or behavioural barriers. This separation, termed reproductive isolation, results in the gene pool of the original species being divided. Selection pressures act on the separated populations to cause microevolution, which can begin to change them in different ways. Over time their allele frequencies may become so different that the individuals are no longer able to interbreed even if they are reunited, and we come to regard them as two distinct species.

For example, small species such as frogs can cover long distances if enough time is available. Thus, during a period of hundreds of thousands of years, frogs can 'pond hop' hundreds of kilometres, which means that they can colonise new habitats and exploit new breeding sites. It seems that Victorian frogs colonised Tasmania in this way during the succession of recent ice ages. They did not evolve into new species until the subpopulations became isolated, in this case by the rising sea waters of an interglacial period.

KANGAROO FOSSIL?

A fossil recently discovered in north-western Queensland sheds light on the evolution of the kangaroo.

Reproductive isolating mechanisms

Isolating mechanisms separate two groups and prevent them from producing fertile, viable offspring – that is, offspring that survive and can themselves reproduce (Figure 7.14). These mechanisms can operate before reproduction has occurred or after reproduction. Genetic isolation (where populations become so genetically different that they can no longer interbreed) can occur before or after physical isolation. In either case, once isolation has occurred, the two groups can acquire different phenotypes, as natural selection works on the members of the two groups so they become adapted to their new, different environments.

Auscape/Densey Clyne

ANT Photo Library

Figure 7.19 ▶
Two Victorian frogs: a) *Geocrina victoriana* and b) *Pseudophryne semimarmorata* breed in the same habitat at the same time but are prevented from interbreeding by alternating their calls. If they do mate, their tadpoles do not develop.

Pre-reproductive isolating mechanisms

Some isolating mechanisms prevent organisms from being able to interact to reproduce. **Pre-reproductive isolating mechanisms** include the following.

- Geographic mechanisms: individuals are separated by geographic features, such as seas, mountains, distance or habitat.
- Temporal (time) mechanisms: individuals breed during different seasons of the year or times of the day.
- Behavioural mechanisms: individuals have different courtship patterns.
- Morphological mechanisms: individuals have different reproductive structures – that is, genitalia of different size, shape or location – so that mating is physically impossible.

The effectiveness of a geographic barrier as an isolating mechanism depends on the size and mobility of the individuals concerned. For example, small organisms may be easily transported across ocean barriers by being carried by other animals. Parts of plants, such as seeds and stems, can float; small rodents can cling to floating vegetation carried by tides; and winds may carry insects over bodies of water.

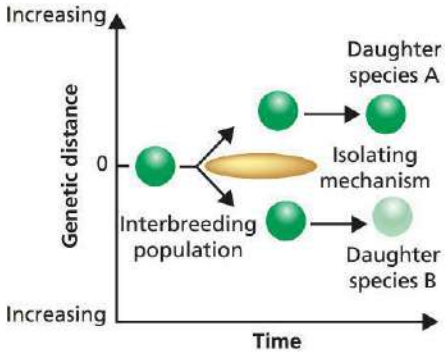


Figure 7.20 ▲
An isolating mechanism can prevent two subgroups of a species from breeding, until they are so genetically diverse that they form two new species. After a period of time they are no longer able to interbreed, even if the populations come back together.

Insects, in particular, can have very precise timing systems that determine when mating occurs. Periodical cicadas have one of the longest insect life cycles known. In North America there are several species of periodical cicadas (genus *Magicicada*). Recent studies have focused on several species; some that hatch out every 17 years and others that hatch every 13 years. It is possible that the unusually lengthy life cycle acts to prevent different populations interbreeding and producing **hybrid** offspring.

Another example of a pre-reproductive isolating mechanism can be seen in frogs. The mating calls of frogs may sound very similar to us but to other frogs they sound vastly different. Frogs usually reproduce only with members of their own species so their call acts as a pre-reproductive isolating mechanism. In many cases, frogs have undergone speciation because their mating calls ensure that they mate only with their own species.

iStockphoto/traveler1116

Figure 7.21 ▶
Magicicada, a periodical cicada endemic to the Northern United States

Post-reproductive isolating mechanisms

If a frog does accidentally mate with a frog from another species, they will not produce fertile, viable offspring because the parents' chromosomes cannot line up successfully during meiosis, and no zygotes are formed (see Figure 7.19).

Methods such as this are called **post-reproductive isolating mechanisms.** They do not prevent mating from occurring but they do prevent young from being produced. These genetic post-reproductive isolating mechanisms include the following.

- Gamete mortality: the gametes do not survive.
- Zygote mortality: the zygote forms but does not survive.
- Hybrid sterility: adult offspring are formed but are infertile because they are unable to produce viable gametes, usually as a result of a different number or structure of chromosomes from each species.

In general, hybrid sterility acts as a post-reproductive isolating mechanism in animals but not in plants. Many plants can interbreed; for example, polyploidy, or multiple sets of chromosomes, is common in eucalypts. Species of coffee plants with 22, 44, 66 and 88 chromosomes are known; this suggests an ancestral plant with a haploid number of 11 and a diploid number of 22.

The key to the formation of new species involves reproductive isolation combined with selection pressures, leading to a disruption of the flow of genes.

Allopatric speciation

In **allopatric speciation** (from the ancient Greek 'allos' = other and 'patra' = homeland) gene flow is disrupted as populations become physically separated through geographical isolation. The populations diverge. This may be because of different selection pressures on the two populations, or it may be due to other random processes such as genetic drift (see page 185). The isolation may happen on a very small scale such as when a river or stream changes course and divides a population of small animals that can't cross it. On a somewhat larger scale deserts may expand, cutting off populations that cannot live under desert conditions. Allopatric speciation is the most common form of speciation and species can, in terms of evolutionary time scales, be easily and rapidly separated by:

- water, for terrestrial organisms
- land, for aquatic organisms
- mountains
- continental drift
- rising sea levels
- climate change.

Islands are home to many examples of allopatric speciation. On the Galapagos Islands Darwin noticed a flightless cormorant. This species most likely originated from a small population of ancestral flying species that reached the islands from the South American mainland. The two populations would have been physically isolated by the 1000 km of ocean between the islands and the South American mainland. There would have been no gene flow between the two populations. The islands were totally free from predators. Reduced predation changed the selective pressures acting on this cormorant population. There were still selection pressures for efficient movement underwater but there was less pressure for efficient flight. This led to a reduction in the size of the wings in the cormorant population, to a morphology that was well suited to movement under water but which no longer allowed flight. This led to allopatric speciation.

The more recent arrival of feral dogs and cats to the islands has once again led to a change in selection pressures on this animal. This has led to dramatic reductions in the cormorant population, which is now less well adapted to the new predation pressures because it cannot fly. It is now recognised as an endangered species. Phylogenetic studies have only recently identified which mainland species (all flighted) the Galapagos cormorant is most closely related to (Figure 7.22).

Most speciation events seem to occur as a result of populations becoming physically separated through geographical isolation, leading to the disruption of the gene flow. This process is called allopatric speciation.

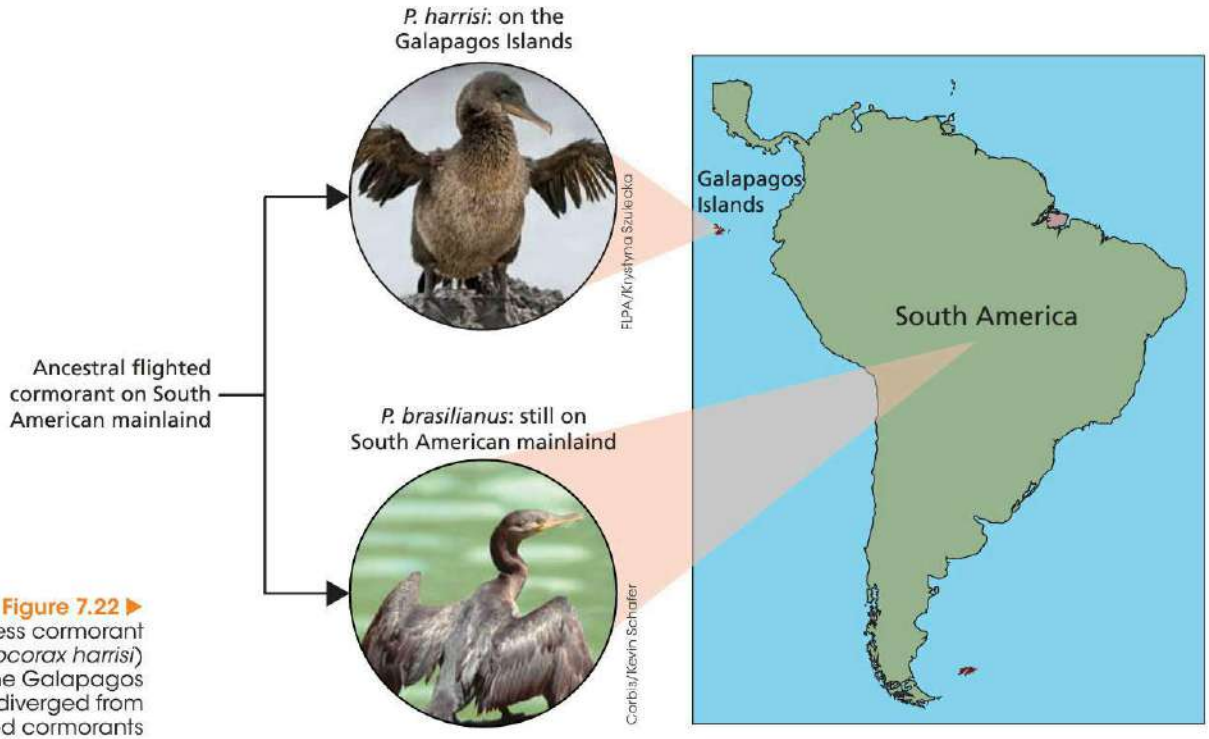


Figure 7.22 ▶ The flightless cormorant (*Phalacrocorax harrisi*) of the Galapagos Islands diverged from flighted cormorants on the mainland through allopatric speciation. *P. harrisis* is most closely related to cormorants such as the Neotropic Cormorant (*P. brasilianus*), which is widespread throughout tropical regions of South and North America.

Sympatric speciation

Allopatric speciation seems to be the main mechanism producing new species throughout evolutionary history. But sometimes species diverge without any obvious physical or geographical isolation. **Sympatric speciation** refers to the evolution of two or more new species from a single population within the same place. How could new species arise without physical separation? It might be that groups within a single population feed on different things, or choose mates based on different characteristics. They may also choose to mate at different times. The genetic separation may occur due to the various pre-zygotic and post-zygotic processes introduced in an earlier section. There are not as many clear examples of this type of speciation but a few are quite striking, as in the case of *Magicicada* (Figure 7.21).

Sympatric speciation refers to the evolution of two or more new species from a single population within the same place.

QUESTION SET 7.4

Remembering

1 Describe the different types of selection that can lead to speciation.
2 Describe the mechanisms that can lead to the isolation of populations.
3 Explain how isolation of populations can lead to microevolution changes.
4 Explain 'allopatric speciation' and provide an example.
5 What factors can act as geographic barriers?

Understanding

6 What effects do isolating mechanisms have on a population?

Extinction of species

The fossil record shows that nearly all species that ever lived are now extinct. In most cases they represented the end of an evolutionary lineage and left no descendants. Although extinction occurs quite regularly there have been periods when the rate of extinction has been very high. These are referred to as mass extinctions.

Five mass extinctions are documented in the fossil record within the last 500 million years, and there may have been many more before that. The Cretaceous mass extinction, 65 mya, is the best known and has received a lot of attention. It saw the demise of the dinosaurs, which had dominated the land for the previous 180 million years.

The most dramatic mass extinction event, often called the 'Great Dying' appears to have occurred at the end of the Permian Period 250 mya. This appears to coincide with one of the most extensive periods of volcanic activity Earth has ever seen. In fact some scientists believe that this event went close to wiping life out completely. However, one of the few survivors of this catastrophe was the ancestor of the dinosaurs, one of the most successful vertebrate groups ever to have evolved.

See Chapter 6 for more on mass extinctions and the fossil record.

The sixth mass extinction

Most biologists agree that Earth is facing a loss of species at a rate that rivals many of the previous mass extinctions. They refer to this as the sixth mass extinction event. The cause of this extinction event appears to be our own species, *Homo sapiens*, and the event continues today with modern agriculture and development practices. The first phase of this extinction event began around 50 000 years ago, when modern humans first spread out of Africa across Asia, Europe and Australia. These modern humans brought significant technology and skills with them; they were very effective hunters and many animal species faced predatory pressures that they had not faced before. But modern humans also brought another powerful technology with them wherever they went: fire. The extinction of several species of **megafauna** coincided with the first arrival of humans and a different fire regime in Australia, but the exact reasons for their disappearance are an issue of ongoing research.

▼ **Figure 7.23**
Dromornis (*Dromornis stirtoni*), believed to be the heaviest bird to occupy Earth, lived in Australia from the late Miocene (6 mya) to the early Pliocene (1.8 mya).

Getty Images/SPL Creative

Preventing extinction by preserving genetic diversity

Populations with reduced diversity face increased risk of extinction, so efforts of conservation are usually focused on maintaining genetic diversity. When large-scale extinctions occur not all species are lost, and some seem to be at more risk than others. Rapid extinction events can lead to the loss of larger organisms rather than smaller ones. Large populations can be more resilient than small populations, probably because the population has a more diverse gene pool. That is, it holds a greater reserve of different alleles to draw on as the pressures from natural selection change.

Populations with reduced diversity face an increased risk of extinction.

ACTIVITY 7.1

SPECIATION AND CONSERVATION: THE EASTERN BARRED BANDICOOT

Corbis/Steve Kaufman

▲ **Figure 7.24**
The Eastern barred bandicoot (*Perameles gunnii*)

The Eastern barred bandicoot (*P. gunnii*) belongs to the marsupial family *Peramelidae*. It is small (body about 300 mm, tail 200 mm), grey-brown in colour, with four pale stripes or 'bars' on its hindquarters. It has three claws on the front feet, which it uses for digging, while the back feet are long, similar to those of a kangaroo.

Populations of the Eastern barred bandicoot were once common over a wide area of south-western Victoria. Numbers were reduced dramatically in the 1900s and now the Eastern barred bandicoot is isolated to a small area around Hamilton, numbering less than 200. This resulted from a change in environmental conditions (e.g. clearing of woodlands, growing exotic pasture grasses, grazing by domestic stock, introduction of rabbits and foxes), which severely reduced its available habitat in Victoria. Numbers of the Eastern barred bandicoot throughout most of Tasmania, however, are still healthy.

Conservation plans for the Eastern barred bandicoot depend heavily on how populations are classified. A **subspecies** is a level of classification below species, referring to races of a species that are fairly permanently geographically isolated from each other and may in future diverge to become two different species. Because of the relatively healthy bandicoot populations in Tasmania it is not regarded as an endangered species. If the Victorian population were identified as a different species, or subspecies, then it could be recognised independently for conservation purposes.

A number of studies have been conducted on the Victorian and Tasmanian populations in an attempt to protect the Victorian population. The bandicoots were trapped, small blood samples were taken and the animals were

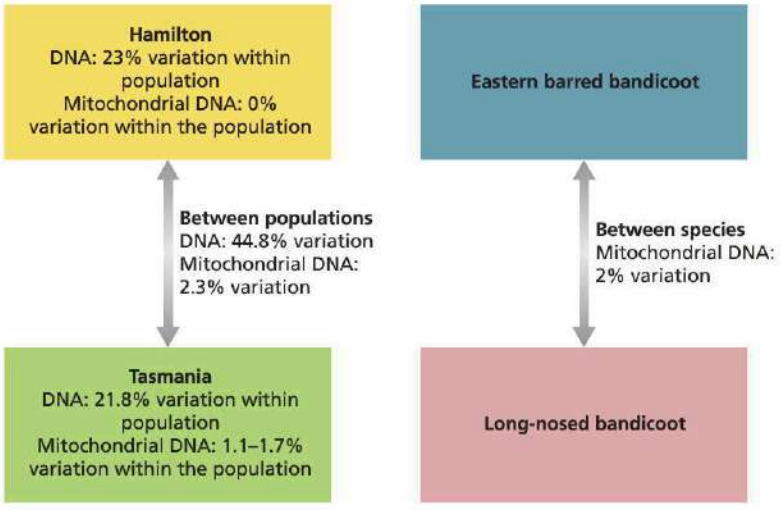


Figure 7.25 ▲
DNA variability in different populations of Eastern barred bandicoots. A 2% variation is the average difference between subspecies and closely related species of mammals.

released immediately into the same areas. The blood was snap frozen and later a DNA fingerprint was taken by analysing genomic variable nucleotide tandem repeats (VNTRs). The average percentage difference in VNTRs within the populations around Hamilton was found to be about 23%, and for those in Tasmania was 21.8%. The average percentage difference between the Hamilton and Tasmanian populations was 44.8%.

Further testing was done using mitochondrial DNA (MtDNA) restriction fragment length polymorphism (RFLP) analysis. This revealed a 0% nucleotide variation within the Tasmanian populations and a 1.1–1.7% variation for the Victorian populations. The percentage variation between the Victorian and Tasmanian populations was 2.3%. Variation of 2% is the average difference between subspecies of mammals.

There is no doubt that the two populations have diverged to some extent due to geographical isolation. But are the two populations separate subspecies? The answer to these questions is vital to how the conservation of these two populations of Eastern barred bandicoots is managed. Biologists currently use a variety of species concepts, all of which are based on the theory of evolution.

The biological species concept defines a species as a reproductive community of populations that occupies a specific niche in nature. The identification of species often uses phylogenetic data from genetic analysis. **DNA fingerprinting** is predominantly used to determine which groups are related – that is, share a gene pool – and which don't. A species defined according to this concept would be the smallest group of organisms that share a common ancestor not shared by any other organism.

The Australian Government, through the Department of Sustainability, Environment, Water, Population and Communities, lists two subspecies of *P. gunnii*. The following is an excerpt from the listing for the Eastern barred bandicoot.

> Scientific name: *Perameles gunnii* unnamed subspecies
> Common name: Eastern Barred Bandicoot (Mainland)
> The genetic diversity, as measured by the variable number of tandem repeat markers and mitochondrial DNA restriction fragment length polymorphisms, among specimens from Hamilton, Victoria was greater than that found in widespread populations of the Tasmanian subspecies (*Perameles gunnii gunnii*). The justification for considering the mainland form to be distinct is based in part on morphological comparisons of island and mainland forms, and that MtDNA data indicated separation of 270000–620000 years ago.

Aim

To investigate speciation in the Eastern barred bandicoot and relate this to conservation approaches

Questions

1. What species definition could be used to justify classifying the two populations as separate subspecies?
2. Does the recognition of two separate subspecies appear to be well accepted by the Australian Government at this stage?
3. What does the DNA evidence suggest about how the populations became separated? To what extent does this example illustrate the concept of allopatric speciation?
4. In your opinion, would the small genetic variability found in the Eastern barred bandicoot populations affect their survival? Explain.
5. Explain why the identification of the two possible subspecies of bandicoot is important for their conservation.

Conservation reserves

One approach to protecting species in danger of extinction is to set up reserves where they are protected from human influence and predation. However, it can be difficult to determine how these should be set up and managed. How large do they need to be and what area do they need to cover? Is a single large reserve required or is it preferable to set up several smaller reserves? (This is known as **SLOSS**: single large or several small.) What population size of the endangered species is needed to ensure long-term survival?

The yellow-bellied glider, *Petaurus australis*, is a rare, nocturnal marsupial. Its range extends from southwestern Victoria up along the eastern coast of Australia to the rainforests north of Cairns. The Federal Government initiated a National Recovery Plan, which has identified a number of areas that need to be researched. These include understanding how the fragmentation of habitats currently suitable for the glider affect the dispersal of gliders between these areas, and how this affects the gene flow through the whole population.

Studies have shown that these gliders live in small social groupings occupying individual home ranges of between 25 and 85 hectares. Also, individual habitats need to be able to accommodate at least 150 glider groups to be to be sustainable in the long term. Based on these calculations it was determined that a forest area of between 10 000 and 35 000 hectares would be required to sustain such groups. This is a large area and could be used as a guide to provide minimum sizes for forest reserves along the east coast of Australia to allow for the conservation of many other plants and animals, which may be able to survive in a range of smaller areas.

Figure 7.26 ▶ Retaining green corridors assists wildlife conservation by providing a chance for species movement and gene flow.

Shutterstock.com/Matthew Dixon

THE NATIONAL WILDLIFE CORRIDORS PLAN

Learn more about the Australian Government's Wildlife Corridors Plan that aims to support our country's biodiversity.

Small reserves and wildlife corridors

One possible way to overcome the problems of isolated conservation areas is to provide linking **wildlife corridors** (also known as habitat or green corridors) of natural landscape. These allow animals to move to new locations when resources become scarce, to facilitate seasonal migration and to permit interbreeding, ensuring that there is sufficient gene flow between different parts of the isolated populations. This solution is controversial as some environmental experts claim that green corridors will not be adequate for conservation of endangered species.

QUESTION SET 7.5

Remembering

1 Define the term 'mass extinction' and outline some events that have led to a mass extinction.

2 Outline how forest fragmentation can affect species diversity.

Understanding

3 Describe how wildlife corridors are intended to maintain species diversity. Use three new glossary terms in your answer.

4 Some organisms are phenotypically similar. Does this mean that they have a recent common ancestor? Explain your answer.

Case study

Too little, too late for the Leadbeater's possum?

Professor David Lindenmayer is one of Australia's leading landscape ecologists and conservation biologists. Based at the Australian National University in Canberra, he specialises in a broad range of areas including forest ecology and management and habitat fragmentation. He is also the leading expert on the Leadbeater's possum. He described how his research relates to possible future strategies for the conservation of Leadbeater's possum in an interview on the ABC radio program 'PM' in August, 2013.

Known only from a few collected specimens in the early 1900s, the Leadbeater's possum was declared extinct until it was rediscovered in the 1960s. Wildfire in 1939 created conditions that saw the isolated population grow to 7500 individuals in the 1980s. Then, bushfire in 2009 nearly wiped out all of their habitat and numbers. Down to less than an estimated 1000 individuals, this species is currently experiencing a genetic bottleneck and is threatened with extinction. Professor Lindenmayer has been studying the Leadbeater's possum for more than 30 years, in an attempt to develop an appropriate conservation plan.

Population modelling analysis has predicted that populations below 50 are unviable, whereas a population greater than 200 could survive 100 years. Metapopulation analysis was used to assess the effect of habitat patch size, connectivity between patches, fire and logging on species survival. Ideal needs for sustainable populations include patches of more than 50 hectares of mountain ash forest that is older than 120 years, with 6–12 tree hollows per 3 hectares. As mountain ash trees take decades to regenerate after fire or logging, a 10-year study trialled the provision of nest boxes in Leadbeater habitat, but the artificial hollows were not used. In addition, the last 30 years of work has shown that logging significantly degrades habitats for Leadbeater's Possum and makes areas of forest unsuitable for 150 to 200 years. The science is now 30 years in the making and it's very strong. The path forward is clear, and if we don't log strategically, Leadbeater's possum is going to go extinct.

Questions

1. Outline the main features essential in an area of forest suitable for the survival of the Leadbeater's possum remaining population.
2. Apply your understanding of selection pressures and describe those that are likely to be acting on the Leadbeater's possum population.
3. Fossil evidence shows a historical distribution up into New South Wales. Suggest how this species may have evolved and how its distribution became so limited and isolated.

Courtesy David Lindenmayer

▲ **Figure 7.27**
Professor David Lindenmayer in Victorian mountain ash forest. A possum hole is shown in the trunk of the tree (yellow arrow).

Auscape/Jean-Paul Ferrero

▲ **Figure 7.28**
Leadbeater's possum (*Gymnobelideus leadbeateri*) is a small possum currently restricted to small pockets of remaining old-growth ash forests in the Central Highlands of Victoria. It is an endangered species.

CHAPTER SUMMARY

- The collection of organisms on Earth has changed dramatically over geologic time scales in response to a range of different selection pressures. All organisms present on Earth today are the result of a process of descent with modification.
- The driving force for adaptive evolutionary change is natural selection. Natural selection acts on variation in a population. This variation is inheritable.
- The modern theory of evolution, taking into account all that we now understand of how our traits are inherited, is called the modern synthesis.
- A population is a group of individuals of the same species that live in the same geographic area and interbreed, producing fertile offspring.
- A population carries a range of different alleles. The sum total of all alleles present in a population is called the gene pool.
- Natural selection acts on the phenotype of individuals, but it is their genotype that is inherited by the next generation.
- Evolution through natural selection leads to adaptive evolution.
- Natural selection can be stabilising, directional or disruptive. In stable environments, stabilising selection often dominates.
- If the genetic makeup of a population changes over time, then it is evolving.
- Gene flow is the movement of genes between different populations
- A gradual change in the gene pool of a population is called microevolution.
- New alleles arise through mutations, which are a key source of variation within a population.
- Migration is the movement of individuals of a population into or out of a region.
- The bottleneck effect refers to a change in the gene pool of a species when a reduction in population numbers leads to a small genetic diversity.
- The founder effect is when a new population is established by a small number of individuals, leading to a population with limited genetic diversity.
- Sexual selection occurs when individuals with certain inherited characteristics are more successful than other individuals in finding mates.
- Population genetics is the study of allele frequencies in populations and how the frequencies change over time in response to various evolutionary processes.
- Rapidly developing techniques in genetic analysis have transformed our ability to study the genetic basis of evolutionary change.
- Populations often change over time so that species that exist today are clearly different to ancestral species.
- The formation of new species involves reproductive isolation combined with selection pressures, leading to a disruption of the flow of genes.
- The splitting of a single species into multiple new species usually involves physical or geographical isolation called allopatric speciation.
- Speciation always involves significant changes to the gene pool.
- New species sometimes evolve without physical isolation. This is called sympatric speciation.
- For every major group of organisms that exists today there was a single common ancestor.
- Major climatic and geological changes have led to the evolution of multiple new species from a single group, a process referred to as adaptive radiation.
- Severe changes to climate and other physical conditions have sometimes led to the extinction of many species, an event referred to as a mass extinction.
- Modern humans appear to be causing a sixth mass extinction event.
- Populations with reduced genetic diversity face increased risk of extinction.

CHAPTER GLOSSARY

adaptive evolution changes in populations of organisms that make that population better adapted to its environment over time

adaptive radiation a process where a lineage of organisms rapidly diversifies into many different forms and taxa with different adaptations; it can be triggered by many factors, such as changes to available resources, or other new challenges or opportunities; this is a type of divergent evolution

allopatric speciation speciation that occurs due to physical or geographic isolation

artificial selection the breeding of plants and animals to produce desirable traits in successive generations; also known as selective breeding

bottleneck effect when a catastrophic event or a period of adverse conditions drastically reduces the size of a population

descent with modification Darwin's terminology indicating that life today has descended and evolved from common ancestors that were generally different to their modern descendants

directional selection a form of selection that selects against one of two extremes and leads to a change in a trait over time

disruptive selection a form of selection that operates in favour of extremes and against intermediate forms

DNA fingerprinting also called DNA profiling; based on patterns of non-coding, repeating base sequences in the genome

fitness the capacity of an individual to survive and pass on viable offspring

founder effect a 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 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

genetic drift a change in the gene pool of a population as a result of chance; usually occurs in small populations

hybrid offspring from parents from two different species; some hybrids are also fertile and can produce further offspring

immutable unchanging; the idea (now considered incorrect) that species did not change over time

inheritable capable of being passed on to the next generation

isolating mechanism a mechanism that prevents organisms from mating or producing viable offspring

macroevolution the evolution of new groups of organisms comprising many related species through multiple speciation events; includes adaptive radiations

mass extinction extinction of many species over a relatively short (geological) period of time

megafauna generally refers to vertebrate species that were significantly larger than other species of the same type

microevolution any change in the gene pool of a single population over a short time

modern synthesis the theory of evolution incorporating our understanding of how traits are inherited

morphological species concept to define a species using measurable anatomical criteria and characteristics

mutation a gene or chromosome that 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 where individuals with certain inheritable traits survive and reproduce more successfully than other individuals, leading to evolutionary change in the population

niche an organism's habitat; or a way of life or function of an organism in its environment

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

population a group of individuals of the same species that live in the same area interbreed, producing fertile offspring

population genetics is the study of allele frequencies in populations and how they change over time in response to various evolutionary processes

post-reproductive isolating mechanism a mechanism that prevents fertilisation occurring or an embryo developing into viable offspring if fertilisation does occur

pre-reproductive isolating mechanism a mechanism that prevents organisms from being able to interact to reproduce

selection pressures factors that influence the survival of an individual within a population

sexual dimorphism the situation where males and females of a species have different morphologies, often in shape or size

SLOSS a term in conservation biology referring to the application of island biogeography theory to the establishment of conservation reserves being either single large or several small reserves

speciation the evolution of one or more new species from an ancestral species

stabilising selection natural selection that tends to advantage organisms similar to their parents; this usually occurs when the environment is very stable and unchanging and selects against extremes of phenotype

subspecies distinct populations of a species, which can interbreed but usually don't due to geographical isolation

sympatric speciation speciation that occurs without physical or geographic isolation

variable traits traits that vary in the population due to differences in alleles carried by different individuals

wildlife corridor a small area of preserved wilderness designed to connect larger reserves; also known as a habitat or green corridor

CHAPTER REVIEW QUESTIONS

Remembering

1 Define the following terms.
   a Gene pool
   b Allele frequency
   c Genetic drift
2 Draw a diagram to outline the founder effect.

Understanding

3 Explain the term bottleneck and what effect a bottleneck may have had on the human gene pool.
4 Draw a diagram to summarise the natural selection that occurred among the peppered moths of Great Britain as described in this chapter.
5 Provide an example of how an understanding of changing gene pools is important to understanding evolutionary change.
6 The founder effect leads to evolutionary change but is not 'adaptive'. Explain what this means.
7 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

8 Apply the definition of microevolution to discuss whether modern humans are still evolving.
9 Herbert Spencer used the phrase 'survival of the fittest' to describe Darwin's concept of natural selection. Outline the ways in which this term could be misleading.
10 In North America, species of fruit fly of the genus *Ragatosis* are confined to different species of apple trees and hawthorn bushes.
   a Describe how this could lead to speciation.
   b Would this be allopatric speciation? Explain.

Analysing

11 Construct a diagram that illustrates how recessive traits that are deleterious can survive in a population.
12 Identify the key difference between Darwin's original conception of adaptive evolution through natural selection and what is referred to as the 'modern synthesis of the theory of evolution'.
13 Explain why processes such as genetic drift, the founder effect and sexual selection are not regarded as examples of adaptive selection.
14 When a mutation occurs in large population it has very little effect on the population as a whole. Explain why mutations are still vital to the process of evolutionary change despite this small effect.
15 Artificial breeding of horses and cattle is not an example of natural selection but does lead to change in populations. Explain why Darwin still felt that artificial breeding was relevant to understanding evolution through natural selection.
16 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.
17 To establish the extent of relatedness between species, several techniques have proved to be useful. One of these measures the difference between the DNA of various species. When the DNA of the orangutan, gorilla, chimpanzee and humans were compared it was found that less than 1% of the total DNA of these

species differed. A rapidly evolving region of the genome was analysed and that showed less than 3.5% variation, as shown in the Table 7.1.

Table 7.1 Percentage divergence of nucleotide sequences in a rapidly evolving section of nuclear DNA in four primate species

		Compared with DNA from ...			
		Human	**Chimpanzee**	**Gorilla**	**Orangutan**
DNA from ...	**Human**	–	1.56	1.69	3.30
	Chimpanzee	1.56	–	1.82	3.42
	Gorilla	1.69	1.82	–	3.39
	Orangutan	3.30	3.42	3.39	–

a From the DNA data comparison, which primate(s) seem to be most closely related to humans?

b Of the non-human primates, which seem to be most closely related from this data?

c From the data, which pair of primates do you think shared the most recent common ancestor?

d Based on the data, construct a possible phylogenetic tree.

Evaluating

18 A group of biologists has proposed that the Tasmanian tiger (thylacine), extinct since the 1930s, can be brought back to life by cloning cells from a museum specimen. Comment on the desirability of this proposal in terms of genetic diversity and evolutionary theory.

19 The term population is widely used in biology, but there are difficulties in its application. Describe some situations where it may be difficult to identify whether groups of organisms comprise different populations or not.

20 The long-billed black cockatoo (*Calyptorhynchus baudinii*) is found in the south-west of Western Australia. It lives in the tall jarrah–karri forests and eats the seed capsules of eucalypts. Very similar birds, known as short-billed black cockatoos, live in the inner wheat belt around Geraldton and generally avoid forests. They eat pine seeds as well as hakea and banksia seeds. Some biologists classify these as the subspecies *Calyptorhynchus baudinii latirostris*. Bill length is described as an adaptation to the different environments that the birds occupy.

a What is meant by the term adaptation?

b What is meant by the term subspecies?

c Outline the steps that would need to take place for the two subspecies to result in two species.

Creating

21 Design a diagram that clearly summarises the different mechanisms leading to evolutionary change. Your table or diagram should indicate which changes contribute to evolutionary changes that lead populations to become better adapted to changing environments.

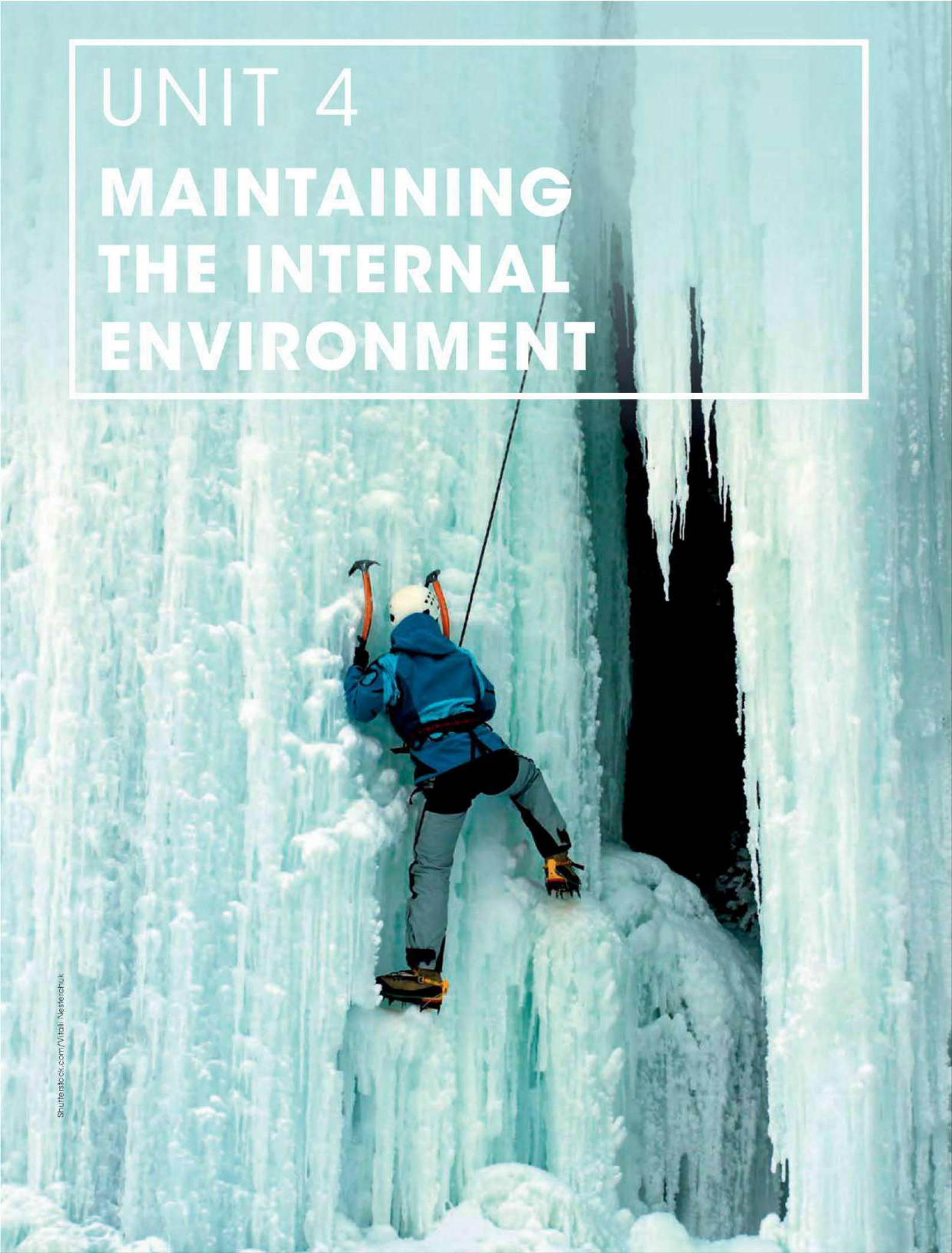
22 Two initiatives to conserve the unique flora and fauna of Eastern Australia include the National Wildlife Corridors Plan (see weblink on page 204) and the Great Eastern Ranges Initiative (weblink on this page). Devise a possible approach to conserving wilderness areas on the East Coast of Australia and compose a brief PowerPoint presentation to communicate this approach to the rest of your class.

GREAT EASTERN RANGES INITIATIVE

Learn about the Great Eastern Ranges Initiative in NSW.

UNIT 4

MAINTAINING THE INTERNAL ENVIRONMENT



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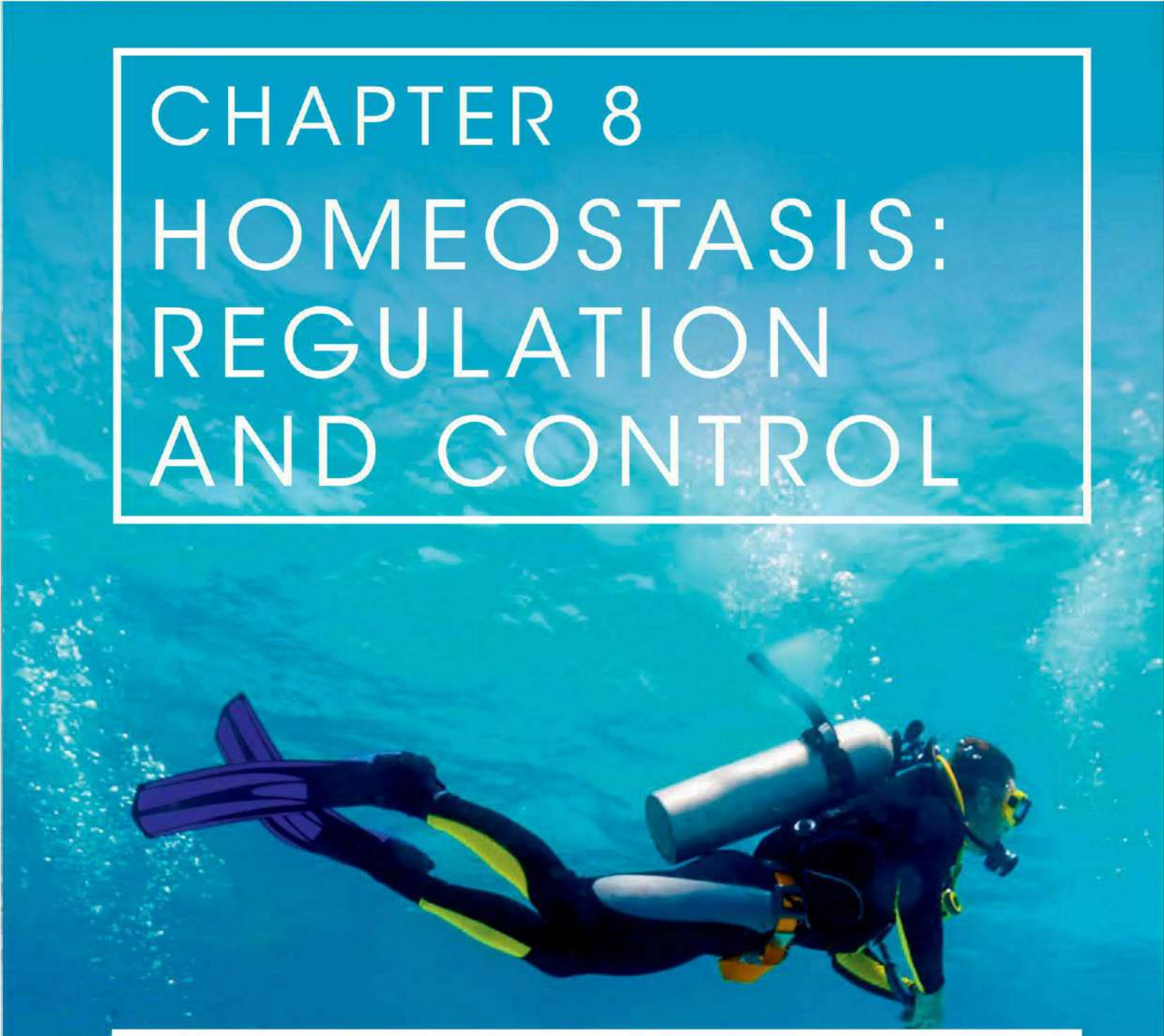
CHAPTER 8
HOMEOSTASIS: REGULATION AND CONTROL



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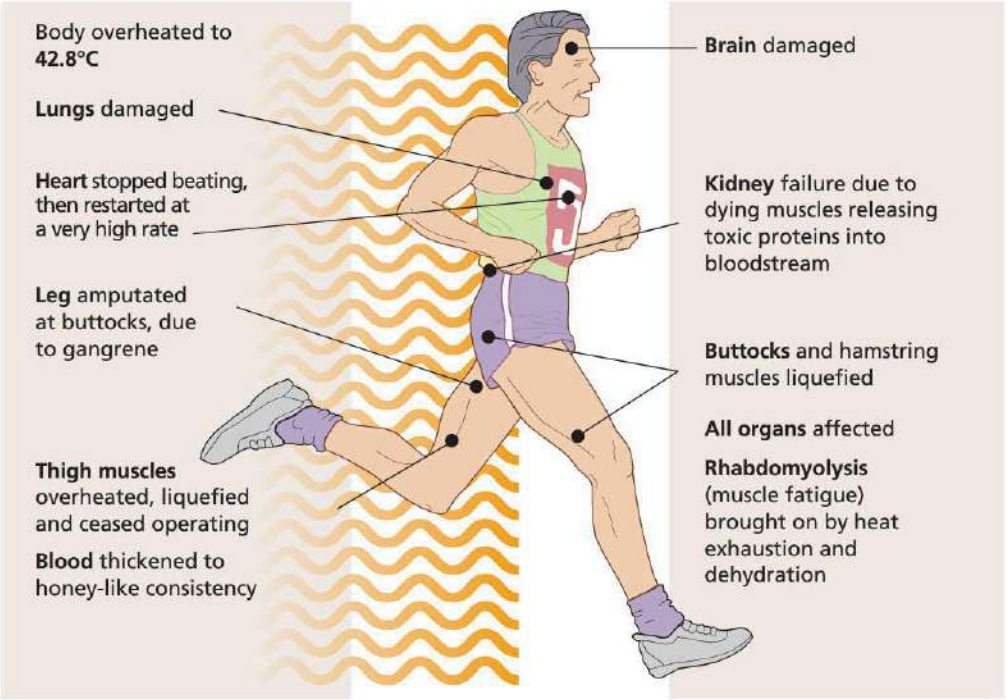
By the end of this chapter you will have covered the following material.

Science Understanding

- Homeostasis involves a stimulus–response model in which change in external or internal environmental conditions is detected and appropriate responses occur via negative feedback; in vertebrates, receptors and effectors are linked via a control centre by nervous and/or hormonal pathways (ACSBL110)
- Changes in an organism's metabolic activity, in addition to structural features and changes in physiological processes and behaviour, enable the organism to maintain its environment within tolerance limits (ACSBL111)
- Endothermic animals have varying thermoregulatory mechanisms that involve structural features, behavioural responses and physiological and homeostatic mechanisms to control heat exchange and metabolic activity (ACSBL114)
- Animals, whether osmoregulators or osmoconformers, and plants, have various mechanisms to maintain water balance that involve structural features, and behavioural, physiological and homeostatic responses (ACSBL115)

Figure 8.1 ▶
A near fatal mistake



In 1988 Mark Dorrity went on an 8 km run in extreme heat in New South Wales. During the run, his body overheated to 42.8°C and he neglected to drink water to stay hydrated. As a result, Mark's body could not regulate his temperature and water balance. His muscles generated more heat than could be lost from his body and Mark suffered a rare condition known as rhabdomyolysis. His thigh muscles liquefied and released toxic proteins into his blood causing kidney failure. Dehydration resulted in thickening of the blood to a point where it could not flow freely in some parts of the body. Every organ in his body was affected; he became delirious, brain damage occurred, his lungs barely functioned and his heart stopped at least once. Within an hour, he collapsed into a 3-month coma, during which he was on dialysis and had one leg amputated due to gangrene.

Under normal conditions Mark's body systems work together to enable him to function comfortably, but in the extreme environmental conditions his body's ability to maintain heat balance became impaired. His judgement and behaviour overrode the warning signs; his coordinating systems were unable to regulate his physiological responses to heat. The conditions in his internal environment became intolerable.

This chapter explores the structural features, behavioural responses and physiological mechanisms that aid organisms to maintain a relatively constant internal state – a state of **homeostasis** – and survive in their environment.

Detecting stimuli

Organisms communicate constantly with their environment, both internal and external. An organism and its cells receive different types of information and respond in many different ways. Principles governing communication in general can be applied to communication pathways between cells. The principles of communication involve:

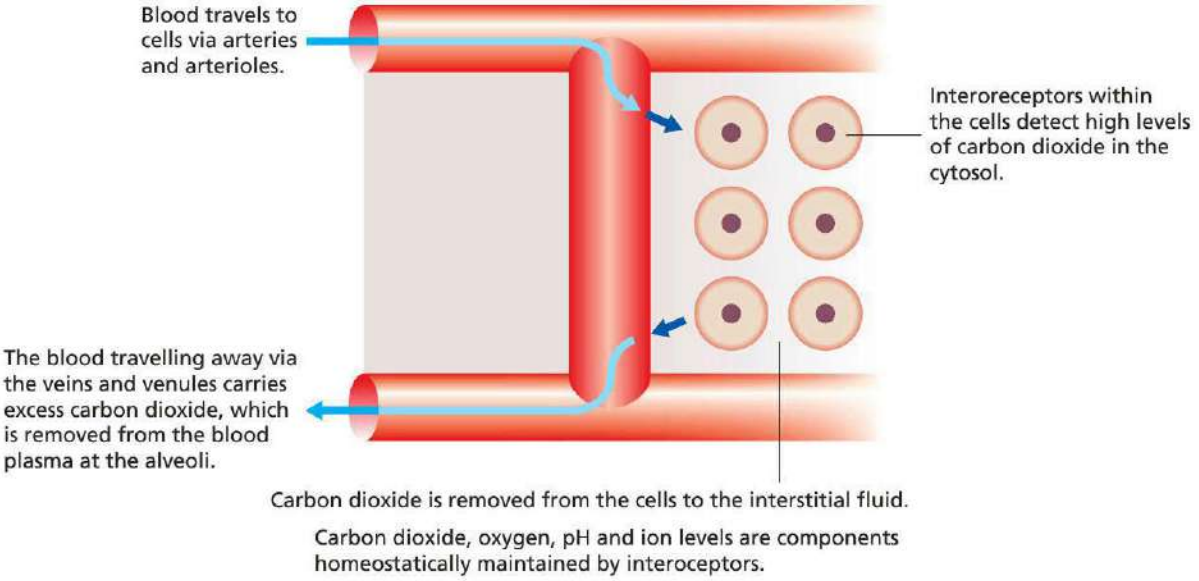
1 production of a signal that contains information to be transferred
2 detection of the signal
3 transfer of this signal until it reaches its target
4 a response to the signal by the target
5 switching off a signal once it has been responded to.

Signals may come from the external environment, other parts of the organism or from within the cell. **Stimuli** may be physical (light, heat, pressure) or chemical (hormones, neurotransmitters). There are millions of external and internal **receptors** that allow an organism to respond to stimuli. The five main types of receptors are: **chemoreceptors**, **mechanoreceptors**, **photoreceptors**, **thermoreceptors** and **pain receptors**.

Signals from the external and internal environments

Organisms detect signals from their external environment, interpret these signals and coordinate a response for survival or development. In multicellular organisms certain cells have **exteroceptors** that are highly specialised to receive signals from the external environment. They can work as individual receptors or together as a group, and are distributed evenly over the body (e.g. pain receptors), located in specialised areas (e.g. taste buds) or concentrated in organs (e.g. the eye). Exteroceptors work by receiving information and converting it to a chemical signal that can then be relayed between body cells.

Interoceptors receive signals from within the body's internal environment. Internal signals can be an increase in carbon dioxide concentration or low pH levels. The **interstitial fluid** that bathes the cells, and the blood plasma, creates the internal environment. Cells exchange substances across membranes via the interstitial fluid (Figure 8.2). With the aid of interoceptors, the internal environment is maintained within narrow limits allowing maximum cellular efficiency. Table 8.1 lists some examples of interoceptors and exteroceptors in the body.



▼ **Figure 8.2** The exchange of substances from blood plasma to interstitial fluid. Many substances are detected by interoceptors and homeostatically maintained.

Receptors can be broken down into five major groups: chemoreceptors, mechanoreceptors, photoreceptors, thermoreceptors and pain receptors. Receptors can detect internal signals (interoceptors) and external signals (exteroceptors).

WOW

The sensitivity of some receptors is amazing!

The hair-like mechanoreceptors of certain insects can detect disturbances of 3.6 nm, making them extremely sensitive to airborne vibrations. Chemoreceptors on the antennae of some moths can locate females about 10 km away from a single molecule.

Table 8.1 Some examples of exteroceptors and interoceptors

Type of receptor	Stimuli	Location in animals
Chemoreceptors	Exteroceptors: Substances that have smell (olfactory receptors) or taste	Nose, mouth
	Interoceptors: Detection of oxygen and ion levels	Aorta, carotid arteries
Mechanoreceptors	Exteroceptors and interoceptors: Pressure, touch, tension, sound vibrations, balance	Ear, skin
Photoreceptors	Exteroceptors: Light	Eyes, light-sensitive cells in body surface of some invertebrates
Thermoreceptors	Exteroceptors: External temperature variations	Skin
	Interoceptors: Internal temperature variations	Hypothalamus
Pain receptors	Exteroceptors and Interoceptors: Pain	Free nerve endings in the skin

QUESTION SET 8.1

Remembering

1 Name the five main types of receptors and provide an example of each.
2 What constitutes the internal environment?

Understanding

3 Distinguish between exteroceptors and interoceptors.
4 What measures could Mark Dorrity have taken to avoid his misfortune?
5 List five signals that a cow might receive from its external environment and five signals that would need responding to in its internal environment.

Fast control: the nervous system

Without food, water or being able to avoid danger, organisms are unlikely to survive. Two systems, the **nervous system** and the **endocrine system**, are responsible for monitoring changes and coordinating responses in complex organisms.

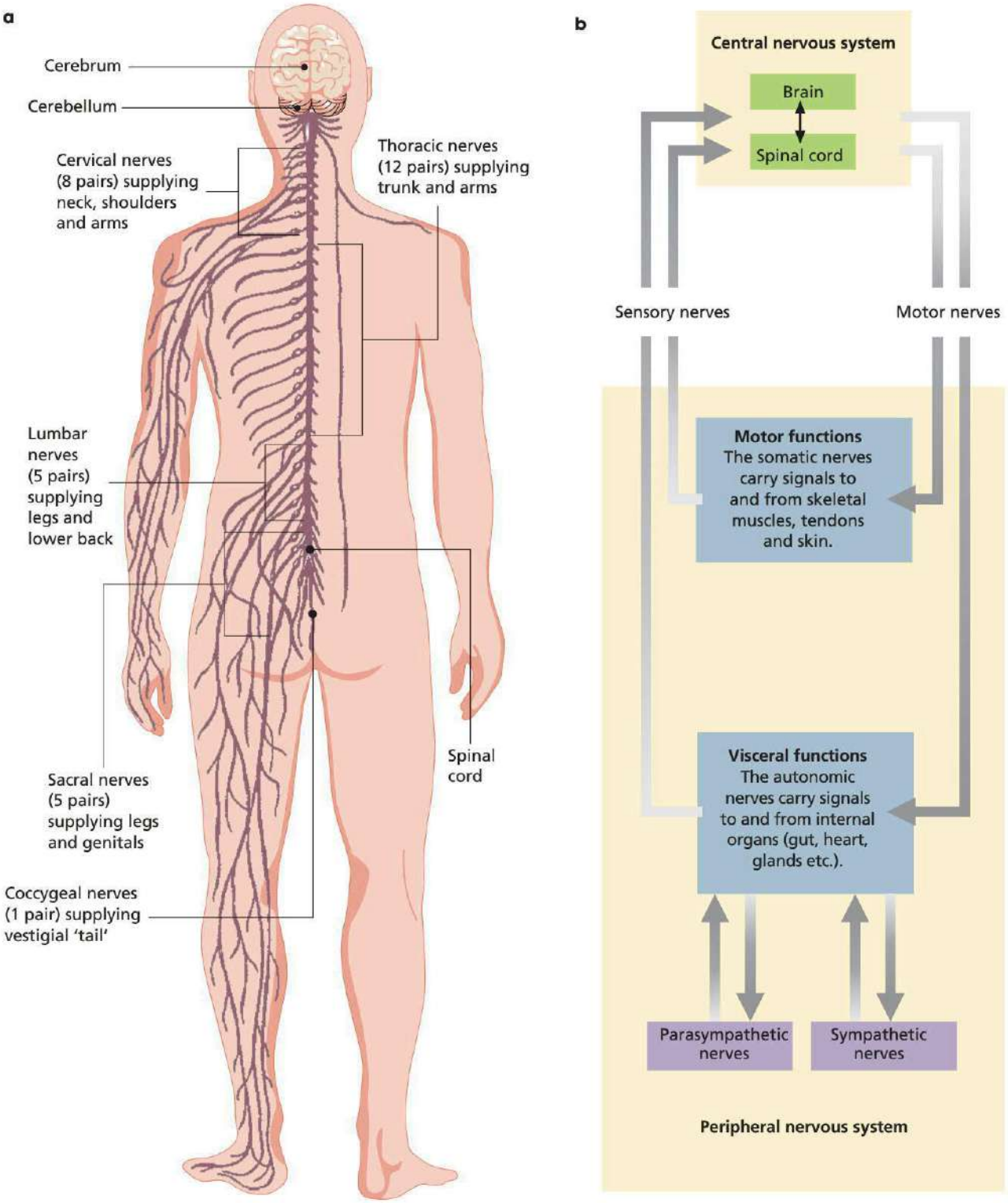
The transmission of signals through the nervous and endocrine systems is discussed in detail in Chapter 9.

The nervous system

The nervous system comprises the central nervous system (CNS) and peripheral nervous system (PNS). The brain and the spinal cord form the CNS, which is responsible for processing, storing and coordinating information. All the other neurons in the nervous system form the PNS, which is responsible for transmitting information to and from the CNS.

Nerve impulses travel along defined pathways. They follow the **sensory neurons** from the source of stimulation, via the PNS to the CNS. **Interconnecting neurons** located in the CNS relay the electrical impulses from sensory neurons to the appropriate motor neurons. From the CNS, **motor neurons** relay the signal via the PNS to the effectors along different pathways. **Effectors** are muscles or glands that respond to the stimuli.

▼ **Figure 8.3**
a) General view of the nervous system; b) Main divisions of the nervous system



Neurons are the basic units of the nervous system. Their structure is directly related to their function. They have extensions called fibres, along which nerve impulses travel. A bundle of nerve fibres comprises a nerve and each nerve is wrapped in a tube of connective tissue.

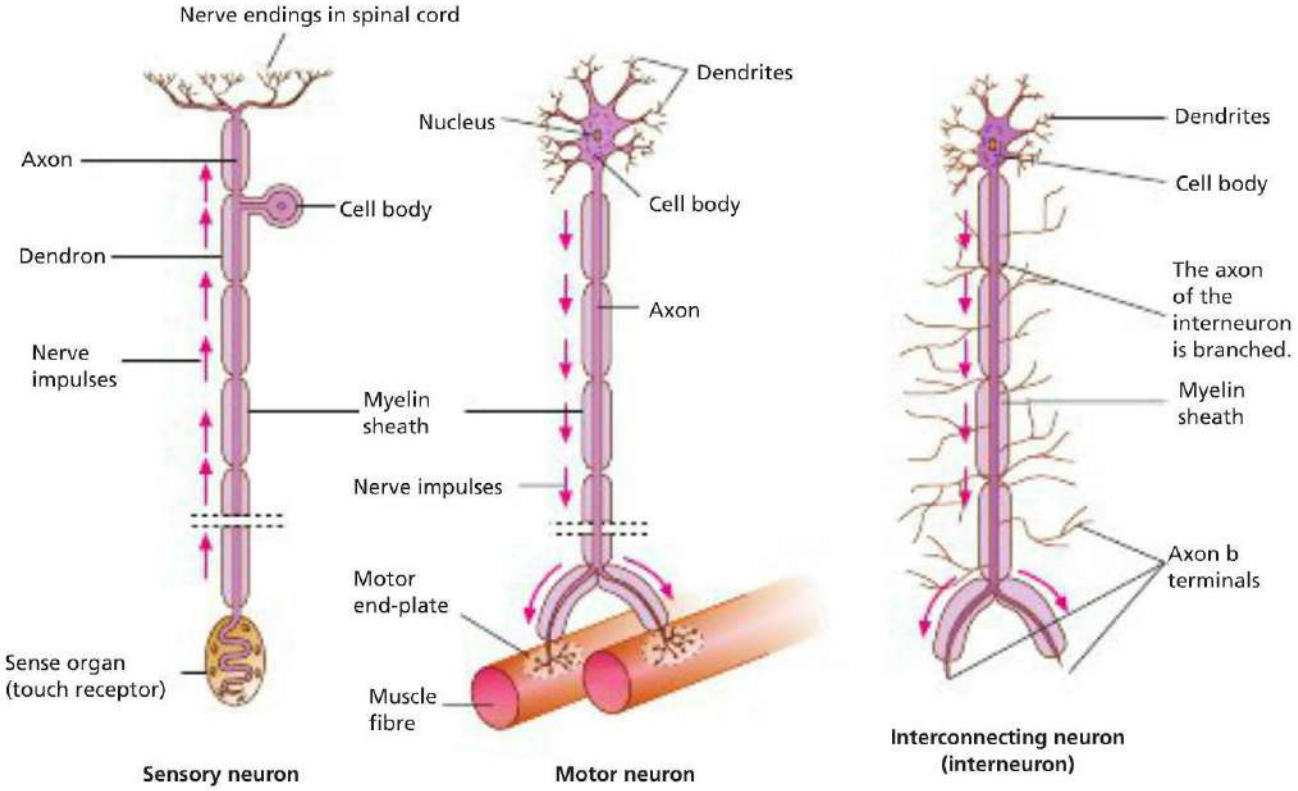
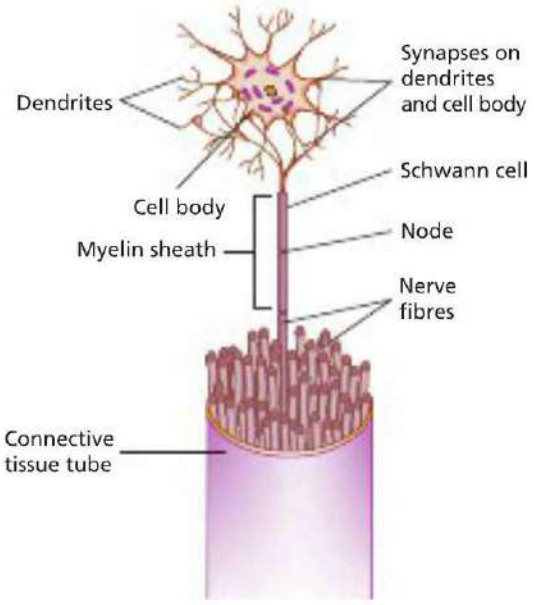


Figure 8.4 ▲ The generalised structure of sensory, motor and interconnecting neurons. All classes of neurons have a variety of shapes within them.

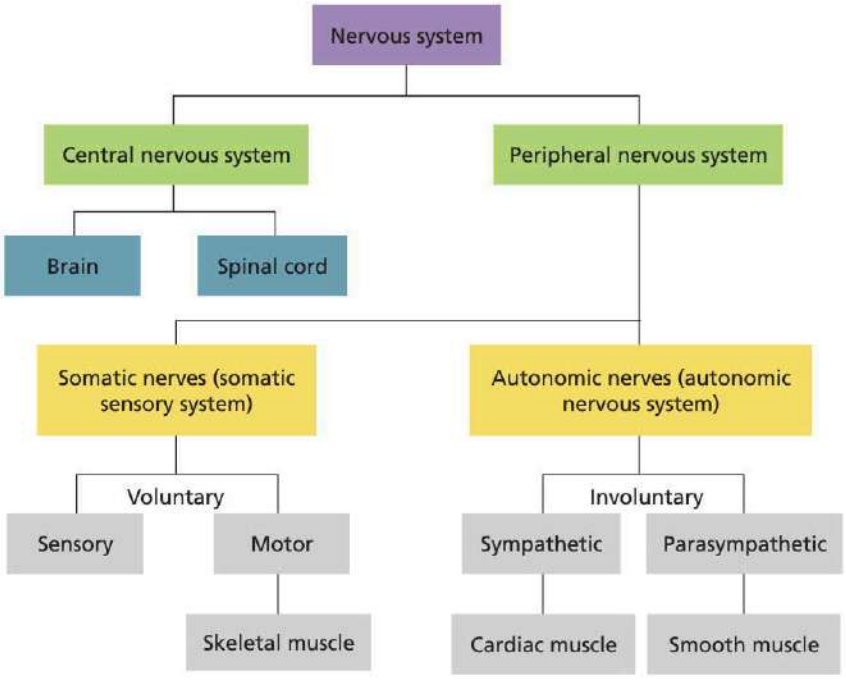
The peripheral nerves that connect directly to the brain are cranial nerves, and spinal nerves are those that connect to the spinal cord. Nerve fibres contain a tubular extension of the cell body, called the **axon**. This extension is enclosed in fatty material called the **myelin sheath**. Myelin is composed of thin Schwann cells that wrap around the fibre, leaving small **nodes** of bare axon in between the cells of myelin sheath. It is essential for proper nervous system function as it assists in the transmission of electrical impulses along sensory and motor neurons, acting like insulation. The electrical impulse is able to travel faster along the length of the nerve and the myelin also keeps the message from accidentally crossing over to adjacent neurons. Damage to the myelin sheath, as occurs in multiple sclerosis and other conditions, hinders the transmission of the nerve impulse and leads to debilitating consequences. Oligodendrocytes are the cells that create protecting nodes around the interconnecting neurons.

Figure 8.5 ▶ Structure of a nerve



Two systems in one

The nervous system is responsible for rapid responses to changes in the environment. It adjusts quickly to conditions or suffers consequences. Some responses are voluntary. Nerves involved in this kind of response, such as those going to the skeletal muscles, make up the **somatic system**. Other responses are involuntary. These belong to the **autonomic system**, such as the cardiac and smooth muscle systems.



◀ **Figure 8.6**
Two systems in one: the somatic and autonomic systems

Having to think about every breath before it is taken would leave little opportunity for thinking about anything else. The autonomic system plays a significant role in controlling these activities. Nerves of the autonomic system transmit impulses to glands, heart, gut and artery muscles. They cause involuntary responses such as breathing, secretion of hormones, the movement of food along the gut and sweating. They are essential to survival and usually only noticed when something goes wrong.

QUESTION SET 8.2

Remembering

1 Distinguish between nerve, neuron and nerve fibre.
2 Draw and complete a table that summarises the different kinds of neurons. Use the headings: Type of neuron, Simple labelled diagram, Function.

Understanding

3 Relate the structure of a neuron to its function.
4 Explain how myelin sheath is created and outline its function.
5 Distinguish between the somatic and autonomic nervous system. Suggest an advantage for having both systems.

Slow control: the endocrine system

THE ENDOCRINE SYSTEM

Read an overview of the endocrine system.

Not all changes in an organism's internal and external environment require an immediate response. Some take time and are under the control of hormones produced by the endocrine system.

Hormones are chemical substances, such as proteins, steroids, fatty acids and amino acids. In vertebrates they are secreted by **ductless glands** directly into the bloodstream. They target and activate particular cells and organs, causing a response (Table 8.2). Only the cells in the body that express receptors for a particular hormone will respond to it.

Table 8.2 Examples of human endocrine glands, a hormone they secrete, and its function

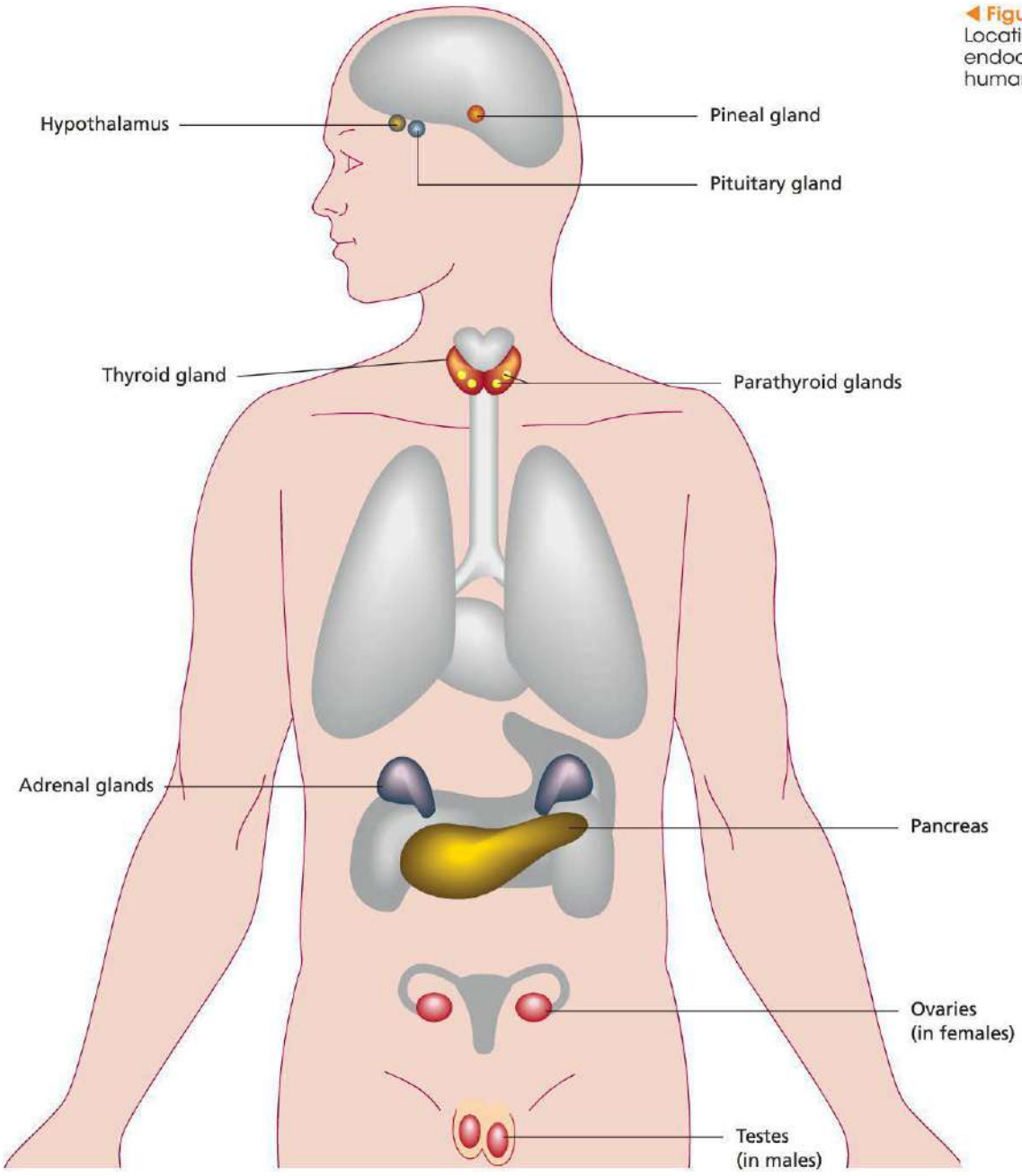
Endocrine gland	Hormone secreted	Target tissue/ organ	Function related to homeostasis
Posterior pituitary	Antidiuretic hormone	Kidney	Stimulates reabsorption of water
Adrenal	Adrenaline	Kidneys, liver, blood vessels	Constricts blood vessels in kidney and liver; stimulates liver to release more glucose; prepares for 'fight or flight'
	Cortisol	Many tissues	Prevents excessive immune response
Thyroid	Thyroxine	Nearly all tissues	Increases metabolic rate, therefore increases oxygen consumption and heat release
Beta cells of pancreas	Insulin	Most body cells	Lowers blood sugar level, increases glycogen storage by liver, stimulates protein synthesis
Alpha cells of pancreas	Glucagon	Liver	Stimulates conversion of glycogen to glucose and its release

A target tissue may be a long way from the gland that secretes the hormone (Figure 8.7). For example, antidiuretic hormone (ADH) is secreted from the pituitary gland in the brain and exerts its effects on the kidneys. It stimulates the reabsorption of water, helping maintain an appropriate water balance in the body.

Although minute quantities of a hormone are produced, they have considerable impact. Some effects are temporary, such as when adrenaline signals the release of glucose and increased heartbeat in the 'fight or flight' response. Regulatory mechanisms, such as those in foetal development, can have a longer effect.

Coordination of activities associated with the endocrine system is often connected to the pituitary gland. It's known as the master gland because it produces many hormones that affect hormone production by other endocrine glands.

Hormones and hormone-like substances occur in other organisms, including plants, and are essential to the regulation of a variety of activities. Female ring doves coo during courtship to stimulate the release of hormones that result in egg development. In plants, a light-sensitive hormone called auxin is responsible for plant growth towards light (**phototropism**) to maximise their photosynthetic ability.



◀ **Figure 8.7**
Location of the main endocrine organs in the human body

Why do cells and organisms need to respond?

Responding to signals for cells and organisms is essential for three main reasons: developmental processes, growth and reproduction; homestasis; and surviving challenges in the external environment.

1 Developmental processes, growth and reproduction

Mitotic divisions by a zygote results in a complex human body, with each cell having a specific function. Genes are switched on and off. Failure in these signalling pathways can lead to uncontrolled cell division and cancer. Apoptosis (programmed cell death) is important in foetal development. Failure to respond to signals for apoptosis can lead to malformations. During reproduction, particular cells differentiate to function differently; these processes all occur in response to certain signals both from the external and internal environments.

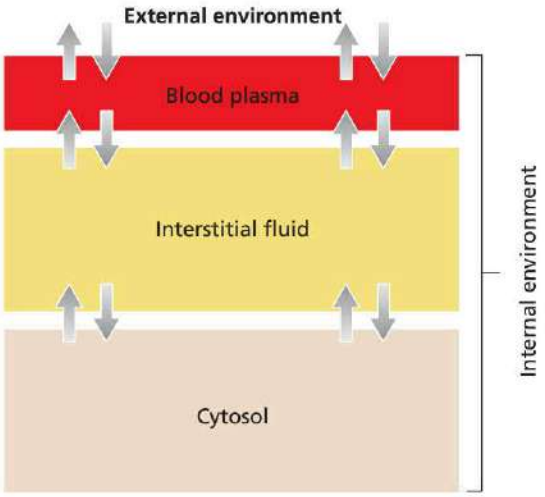


Figure 8.8 ▲ Organisms regulate their internal conditions despite changing internal conditions and external environmental conditions by constant movement of substances across membranes.

2 Homeostasis

Homeostasis refers to the maintenance of a relatively constant internal environment within small tolerance limits, despite changes in the internal or external environment. The concentration of substances such as glucose, carbon dioxide and hydrogen ions – in cells, interstitial fluid and blood plasma – all impact cell function. Movement of materials across membranes is affected by water concentration, nutrients and ions – such as sodium, potassium and chloride – on either side of the membrane (Figure 8.8).

The activities of enzymes are affected by body temperature and pH. Biochemical processes can be disrupted or cells killed by quite small changes. Responding to these changes and correcting them back to normal is essential. The processes involved in homeostasis are called homeostatic mechanisms and, in complex organisms, involve most body systems.

3 Surviving challenges in the external environment

Avoiding injury or death is clearly essential for the survival of the organism. As such, the cells of an organism coordinate a response to immediate threats in the external environment, such as a luring predator or falling rocks. A coordinated response requires signals from the external environment to be detected and conveyed to cells that then must respond appropriately.

QUESTION SET 8.3

Remembering

1 Identify the systems of the body that are largely responsible for monitoring and coordinating response mechanisms.

2 Identify four examples of hormones and where they originate.

Understanding

3 Define homeostasis and outline its purpose.

4 Explain why it is important for an organism to be able to detect changes in its external environment. Give an example.

5 Clarify the difference between an endocrine gland and a target organ. Give an example.

Applying

6 Account for the fact that growth in the human body is primarily regulated by the endocrine system rather than the nervous system.

Maintaining a balance

Fortunately, few people engage in the kind of activity that Mark Dorrity did in such extreme conditions, though for many organisms these conditions are a feature of their daily lives. Complex organisms have strategies and homeostatic mechanisms to keep internal conditions relatively stable. This allows for the necessary biochemical processes to be maintained.

The principle of feedback

Organisms have narrow ranges in which they keep internal temperature and fluid concentration. Minor fluctuations around this optimal level occur continually; however, considerable disturbances from the optimum level can also occur. Diseases, extremes in the internal environment, trauma, inherited disorders and toxic substances can interfere with homeostasis. All disturbances must be controlled quickly for cells to continue to function effectively.

Hormonal (endocrine) system
Hormones are released directly into the bloodstream and transported throughout the body. Target tissues involved in regulating cell activities respond.

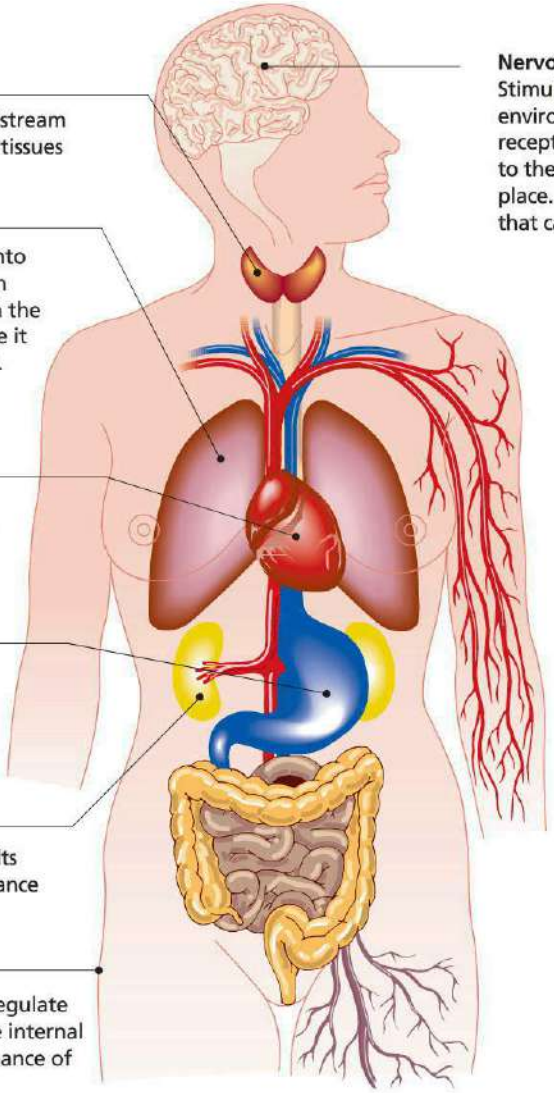
Respiratory system
Oxygen from the air diffuses from the lungs into the capillaries and is carried to all cells. Carbon dioxide from the cells is carried to the lungs in the blood and diffuses into the alveoli from where it is exhaled. Removal of CO_2 helps regulate pH.

Circulatory system
Blood distributes warmth, hormones, O_2, nutrients (including glucose, fatty acids and amino acids) to cells and removes wastes including CO_2.

Digestive system
The products of digestion (simple molecules) are absorbed into the blood and lymph vessels in the wall of the intestine, from where they are supplied to other parts of the body. Undigested material is eliminated.

Excretory system
Nitrogenous wastes (urea), excess water and salts are excreted from the body in urine. Water balance (osmoregulation) is regulated in this way.

Integumentary system (skin)
Evaporation of sweat from the surface helps regulate temperature. The skin is a barrier between the internal and external environments and reduces the chance of entry of micro-organisms.

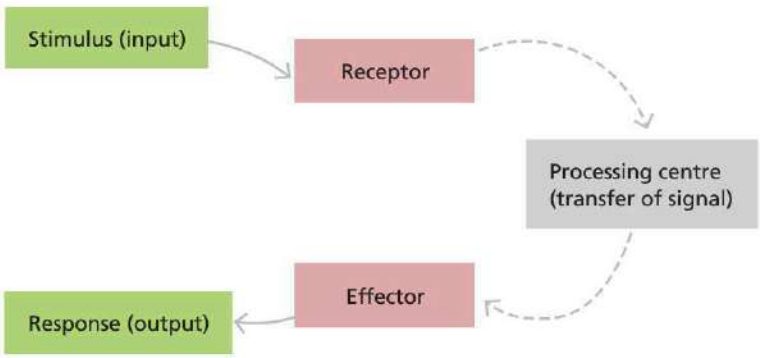


Nervous system
Stimuli in the external and internal environments are detected by receptors. Electrical impulses travel to the CNS where coordination takes place. Impulses are sent to effectors that carry out a response.

◀ **Figure 8.9**
Homeostasis and human body systems

Signals about disturbances are fed to a control centre. The centre interprets and coordinates a specific response that either counteracts or reinforces the disturbance. These processes are referred to as **feedback mechanisms**. A feedback mechanism is triggered when a **stimulus** is detected by a **receptor**. The information is then processed and a message is conveyed to an effector, which carries out the **response** to the stimulus.

In analysing how cells and organisms respond to signals it is useful to apply a stimulus–response model. This model represents feedback mechanisms. In animals, the main body organs that respond to signals are glands or muscles. Since the receptor organs are different from the effector organs, communication between cells to coordinate a response is essential.



▲ **Figure 8.10**
The stimulus–response model relies on the transfer of information between the receptor and effector.

Feedback mechanisms are processes that respond to small disturbances to keep concentrations of substances within narrow limits for optimal cellular function.

Controlling the response

Once a signal has been responded to, it is important that it is switched off. An over-response is a waste of energy and can often lead to cell damage. Three main strategies used by cells and organisms are:

1 disrupting the signal transduction pathway
2 removal of the original stimulus
3 responding in a way that alters the original signal, described as feedback.

Negative feedback

When a bar of chocolate is ingested, blood glucose levels rise rapidly above the optimal level known as the set point. The body's response is to lower the blood glucose level to normal by removing glucose from the blood and converting it to **glycogen**. The level of glucose in the blood drops as it is stored away. Mechanisms that counteract the stimulus are referred to as **negative feedback** (Figure 8.11). On the other hand, exercising vigorously or fasting decreases blood glucose levels below the set point. The body responds by breaking down glycogen stores and returning blood glucose levels to normal (Figure 8.12).

Negative feedback is extremely important in homeostasis as the response always aims to restore the internal environment to a constant set of conditions.

Figure 8.11 ▶ Negative feedback occurs when a response cancels or counteracts the original stimulus.

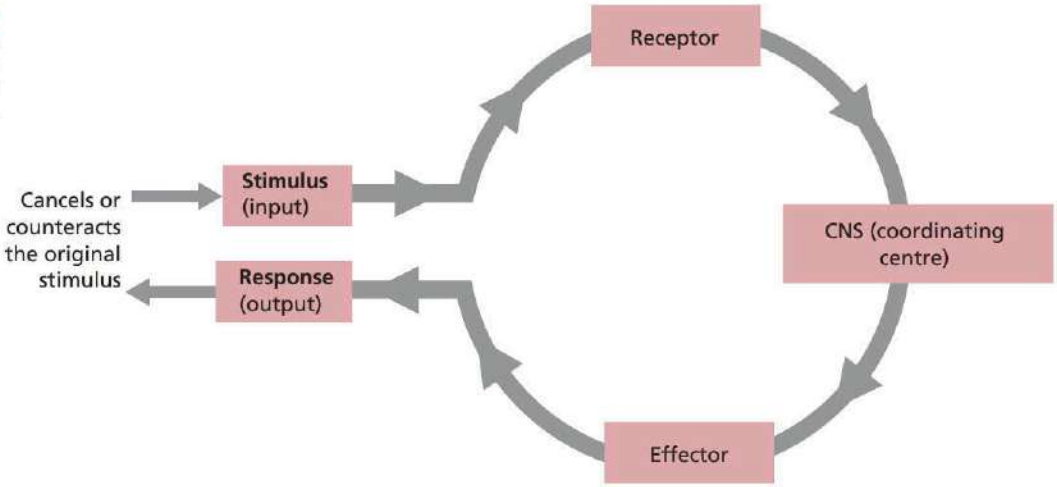
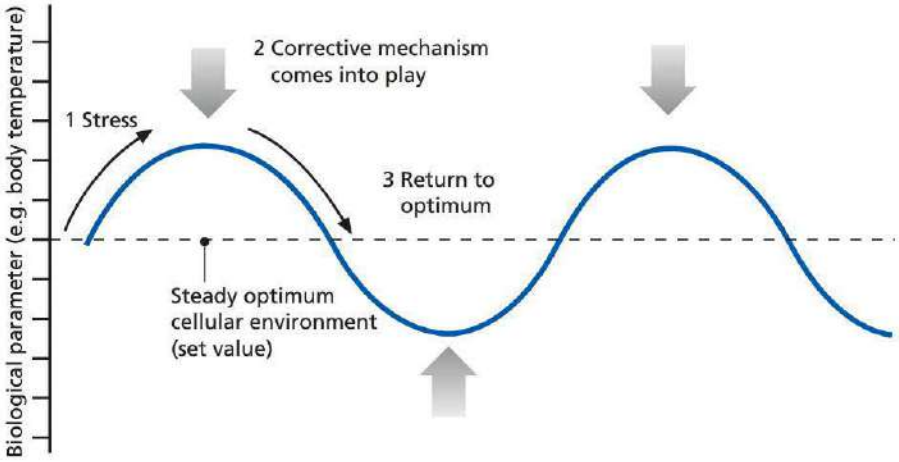


Figure 8.12 ▶ Set points and optimum conditions are maintained through negative feedback.



HOMEOSTASIS AND FEEDBACK

Read an overview of homeostasis and feedback mechanisms

Positive feedback

If adjustments are made that reinforce the original stimulus, the mechanism is referred to as **positive feedback**. Positive feedback is necessary during some developmental processes. For example, the development of frogs and toads is controlled by the hormone thyroxine. Just before the tadpole changes (metamorphoses) into an adult frog, negative feedback is changed into positive feedback. The concentration of thyroxine rises and triggers metamorphosis.

In terms of homeostasis, positive feedback can be harmful. When human body temperature rises during fever, a new and higher set point for temperature is established and the person may suffer from heatstroke. If the resetting of the set point continues upwards, cell function is impaired.

EXPERIMENT 8.1

INVESTIGATING HOMEOSTASIS: TEMPERATURE REGULATION

An organism whose temperature is constant, despite variations in the environment, must have mechanisms regulating its body temperature. Under normal circumstances, humans maintain a body core temperature around 37°C.

Aim

To investigate the responses of the human body to extremes of ambient temperature

Materials

Each group will require:

- oral thermometer
- skin thermometer
- alcohol swabs
- buckets or tubs
- hot and cold water
- ice cubes
- spray bottle
- stopwatch
- electric fan
- heater
- sleeping bag
- bathing suit
- hat, scarf gloves

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
Volunteer may start to feel unwell when experiencing extremes in temperature.	Cease activity immediately and assist volunteer in regaining normal body temperature. Note: Do not try to correct hypothermia with rapid temperature change. Try to increase the temperature gradually.

Procedure

1 Read the procedure and formulate a hypothesis about the effects that extreme ambient temperatures (after exposure for 20 minutes) will have on core temperature (as measured by an oral thermometer) and skin temperature in humans.

2 Identify the variable, the control and the factors that will be kept constant.

3 Work in groups of three or four.

Cold subjects:

4a Record skin and core temperatures of the subject before beginning (Table 8.3).

5a Make the subject cold as possible by one or more of the following means.

- Wearing bathers
- Placing in a cold breezeway
- Placing feet in buckets of icy water
- Exposing the body to ice water from a spray bottle

Hot subjects:

4b Record skin and core temperatures of the subject before beginning (Table 8.3).

5b Make the subject as hot as possible one or more of the following means.

- Wrapping in a sleeping bag
- Wearing hat, scarf and gloves
- Sitting in front of a heater

6 For both cold and hot subjects, record the core and skin temperature every 2 minutes, as well as any other observation (Table 8.3).

7 Record the class results (Table 8.4).

8 Using a spreadsheet, graph the temperature readings for core and skin temperature against time for your subjects.

Results

Table 8.3 Individual subject results

Time (mins)	Skin temperature (°C)	Core temperature (°C)	Ambient temperature for treatment (°C)	Observations
0				
2				
4				
...				
20				
Average				

Table 8.4 Class average results

	Skin temperature (°C)	Core temperature (°C)
Cold subjects		
Hot subjects		
Cold ambient temperature		
Hot ambient temperature		

Analysis of results

1 List all the changes observed in the hot subjects.
2 List all the changes observed in the cold subjects.
3 Briefly describe the trends shown in the graphs for your subject.
4 State the maximum variation in core temperature for the class.
5 State the maximum variation in skin temperature for the class.

Discussion

1 Which, if any, of the changes observed in the hot subjects might act to reduce the body temperature? Explain.
2 Which, if any, of the changes observed in the cold subjects might act to increase the body temperature? Explain.
3 From your answers to Questions **1** and **2**, copy and complete Table 8.5 with one example for each cell.

Table 8.5 Physiological and behavioural responses at different temperatures

	Physiological response	Behavioural response
Reduced core temperature		
Increased core temperature		

4 Summarise the corresponding changes observed in the subjects.
5 Was your hypothesis supported by the data? Restate or modify your hypothesis in light of the results.
6 Explain the probable mechanisms in your subjects that brought about the above changes.
7 Explain how the validity and reliability of the experiment could be improved.

QUESTION SET 8.4

Remembering

1 Outline the stimulus–response model.

2 Define 'stimulus', 'receptor', 'effector' and 'response'.

Understanding

3 With the use of an example, explain negative feedback.

4 Clarify how positive and negative feedback are different. Suggest why positive feedback is generally not associated with homeostasis.

Applying

5 Draw a stimulus–response model that demonstrates how glucose levels are maintained in the body.

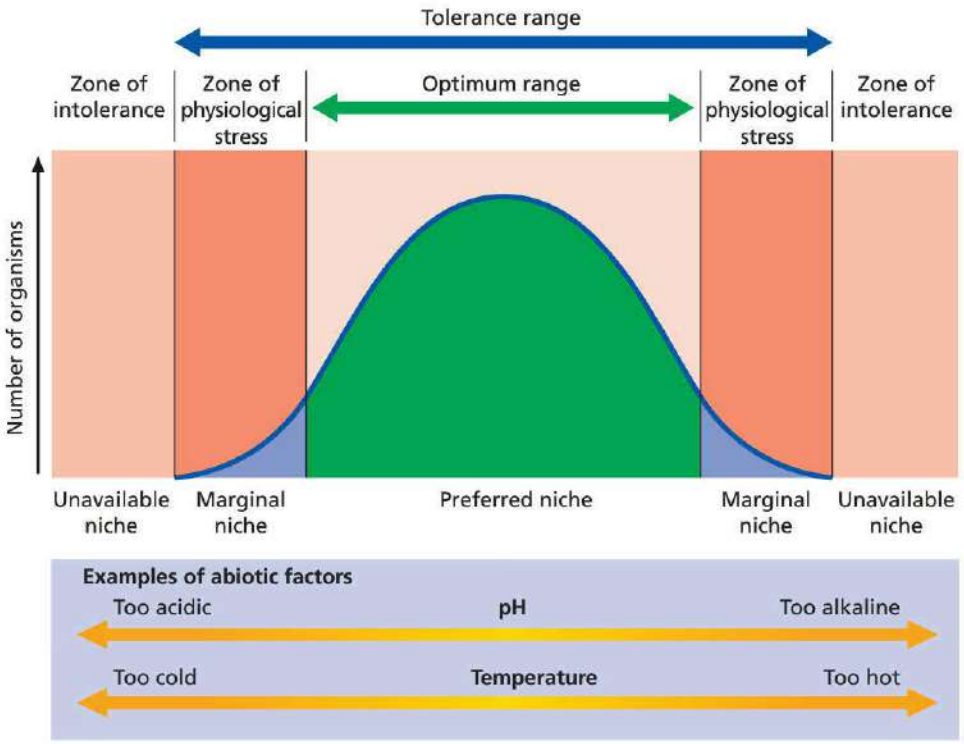
6 Explain why increase in the temperature set point affects cellular function and homeostasis. Include the effect of temperature on protein structure in your explanation.

Tolerance limits

Each organism has a set range in which they tolerate different levels of organic and inorganic materials, pressure and temperature. This is known as the **tolerance range** (Figure 8.13). Homeostasis maintains the levels within the **optimum range**. If homeostasis fails and the level of pH, for example, becomes too high, the organism can fall into a state of **physiological stress**, affecting its function. This section explores how humans can utilise changes in metabolic activity, physiological processes, structural features and behaviour to maintain their internal environment within the tolerance range.

EFFECT OF pH

Before reading this section, investigate the effect of pH on enzyme activity.



◀ **Figure 8.13**
The tolerance range for an abiotic factor is the range within which an organism functions best.

Scientific literacy: Enhancement of space shuttle operations by aid of human thermoregulation models

Thermoregulation is essential for human survival and is controlled by negative feedback. In extreme environments thermoregulation for humans is difficult. If internal temperatures rise above or fall below the set point, individuals start to show impaired cognitive and motor skills.

The environment within a space shuttle re-entering Earth's atmosphere is extremely hostile. It is essential for human function and survival that 'Earth-like' conditions are achieved within the cabin. This partially occurs through the use of the advance crew escape suit (ACES) and a liquid-cooled ventilation garment (LCVG). Under the ACES, astronauts wear additional anti-gravity pressure bladders on their legs and abdomen to prevent pooling of blood in the lower extremities. The LCVG has cooled fluid circulating through flexible tubing. This tubing is in direct contact with the astronaut's skin and removes excess heat.

During an Endeavour mission, the Thermal Control System failed. Fortunately, backup controllers were able to maintain the cabin temperature to allow optimal function by astronauts. This system failure prompted a group of NASA scientists to investigate whether NASA flight rule A13-151, which required the wearing of the ACES in a hot-cabin re-entry, was sufficient if cabin temperature was compromised.

A number of elements had to be taken into consideration in the modelling of this scenario. Two human thermoregulation models were utilised: the 225-node Wissler and 41-node Metabolic Man. These models were validated by a series of tests, including quantitative assessment of results obtained from prior simulations, checks that assured inputs were acceptably interpreted and qualitative assessment of the output in regards to input. Assessment of output was also measured against human subjects. This included data sets obtained from fully-suited fire fighters during exercise in various moderate, warm and hot air environments. Also, individuals fitted with ACES and LCVG were placed in a chamber in which parameters for temperature, humidity and inlet water coolant temperature varied.

After configuration of the models, scientists carried out a series of tests to identify how astronauts respond when conditions such as those on the Endeavour are experienced. It was concluded that increased temperatures caused the symptoms outlined in Table 8.6.

Table 8.6 Responses to increased body temperature

Body temperature	Medical symptoms
37.7–39.2	Decrease in cognitive skill begins Heat stress Manual skilfulness decreases
38.2–39.2	Increase in judgement error Decrease in tracking skills Potential heat exhaustion
39.2–39.6	Functional limit of physical tasks is reached Likely heat exhaustion Potential heat stroke
>40	Probable heat stroke

Therefore, after using the models to analyse a variety of conditions, it was recommended that flight rule A13-151 be modified. It was determined that the ACES were to be removed if the cabin temperature exceeded 35°C.

Figure 8.14 ▲
The ACES

Questions

1 Recount why scientists re-evaluated NASA flight rule A13-151.

2 Identify how scientists used models to determine the necessary change needed to the flight rule under investigation.

3 **a** Discuss some ways that the thermoregulation models were validated.

b Suggest a benefit of using data from other studies.

c The information obtained from the study would be entered into a data pool. Outline the benefit from this.

Metabolic activity

Metabolism is the sum of chemical reactions that occur within an organism to maintain life. The majority of these reactions require the catalytic help of enzymes. Different enzymes function best at different pH concentrations and temperatures. Metabolic activity is not only responsible for the breakdown or synthesis of molecules; it also creates internal body heat. An increase in metabolic activity increases internal temperature from the energy released in the reactions and vice versa. However, there other factors that alter temperature and pH levels. For example, concentration of carbon dioxide in the internal environment can alter pH levels. If carbon dioxide concentrations increase from exercise, pH levels decrease. Decreasing the pH causes lower enzyme functionality. In turn metabolic activity is reduced, resulting in less heat energy. The body must maintain pH levels to ensure the supply of nutrients to cells is met and internal temperature remains constant.

Physiological processes

As muscles contract and release more rapidly, the demand for oxygen and glucose is higher. These two reactants are **substrates** for cellular respiration and supply cells with energy. Carbon dioxide is a by-product of this **catabolic reaction**, along with heat. As activity increases, carbon dioxide levels and internal temperature subsequently rise. Physiological mechanisms are in place to account for these changes. One way to reduce carbon dioxide concentration is by increasing the breathing rate. This mechanism passes more blood through the lungs, releasing the carbon dioxide into the external environment. The blood is also oxygenated to maintain cellular respiration throughout the activity.

The changed internal temperature is detected by thermoreceptors in the hypothalamus, which signals for the sweat glands to operate, removing excess body heat.

Structure and behaviour

Metabolic activity and physiological mechanisms are not the only processes that regulate the body during exercise. Structural features and behaviour also come into account. The removal of clothing or moving into the shade are behaviours seen when an individual notices an increased body temperature. Additionally, exercise pace will slow accommodating breathing rate and removal of carbon dioxide. These actions change the metabolic rate and heat energy produced. Structurally the vast capillary network over the alveoli creates a large surface area for the carbon dioxide–oxygen exchange to work efficiently.

QUESTION SET 8.5

Remembering

1 Define the following terms.
   - a Tolerance range
   - b Optimum range
   - c Physiological stress

2 What are the by-products of cellular respiration?

Understanding

3 With the aid of an example, explain how anatomical structure helps to regulate the internal environment.

4 Explain the difference between cellular respiration and metabolic activity.

Applying

5 Create a stimulus–response model for exercise and pH level.

Keeping in the comfort zone: thermoregulation

Different animals have slightly different internal temperatures. These temperatures are where their enzymes work efficiently. If internal temperatures rise much above the set point, the enzymes denature, metabolic processes fail and the individual suffers from **hyperthermia**. Conversely, if body temperatures fall, enzyme function slows significantly and the individual suffers from **hypothermia**.

Ectotherms and endotherms

Some moths and beetles can raise their body temperature for short periods by vigorous flapping of their wings, generating heat by muscular activity. These moths and beetles and other animals such as mammals, birds and fast-swimming fish (e.g. yellow fin tuna) retain the heat generated by metabolic activity within their bodies. They are described as **endotherms**. If the animal can maintain a relatively constant temperature, it is also described as **homeothermic**. Animals such as reptiles and the majority of fish depend on absorbing heat from external sources. They are described as **ectotherms**. Many of these organisms cannot control their internal temperature, which fluctuates with their surroundings; they are **poikilothermic**. Most organisms are homeothermic endotherms or poikilothermic ectotherms, though a few organisms are classified as homeothermic ectotherms or poikilothermic endotherms. Table 8.7 gives examples of endotherms and ecotherms that are either homeothermic or poikilothermic.

Table 8.7 Examples of endotherms, ecotherms, homeothermic and poikilothermic organisms

	Homethermic	Poikilothermic
Endotherms	Kookaburras, penguins, emus, koala, humans, wombats Salmon shark	Fast-swimming fish (yellow fin tuna, billfish, most sharks) Naked mole rat, bees, butterflies, hibernating animals (bears, squirrels)
Ectotherms	Desert lizards, tropical marine invertebrates (blood lobster, sea apple, cleaner shrimp), desert pupfish	Snakes, lizards, frogs, toads Invertebrates (spiders, starfish, snails), fish (flathead, silver perch)

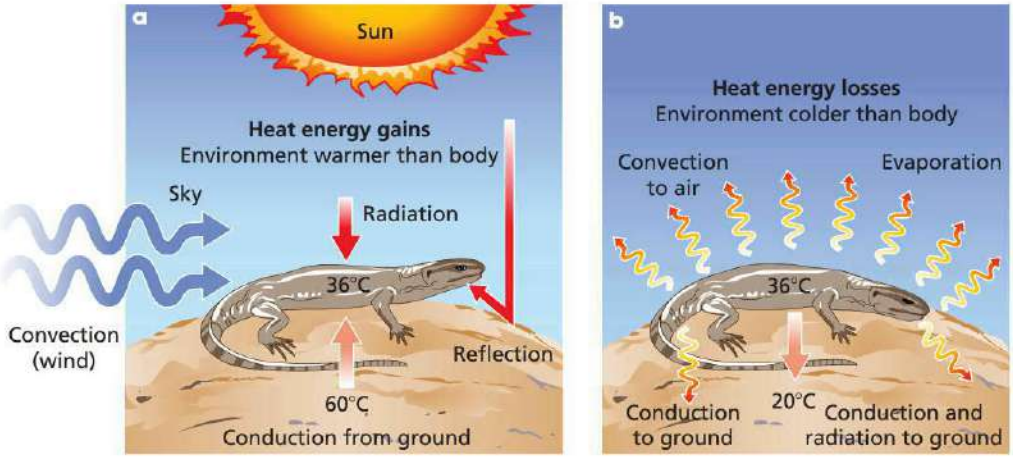
Auscape/Roger Brown

Figure 8.15 ▶ Crocodiles are ectothermic. Behaviours such as mouth-gaping and moving in and out of water help them thermoregulate.

Strategies and adaptations for thermoregulation

To understand how organisms regulate their temperature, it is necessary to understand how heat is transferred. Put simply, if an organism is too hot, it must lose heat. If an organism is too cold, it must gain heat. Heat transfer depends on the temperature gradient between the internal and external environments. When there is a balance between heat gain and heat loss, the organism is said to be in heat balance, which is the purpose of thermoregulation. Different organisms manage thermoregulation in different ways that usually involve the interaction of physiology (the structures they have to facilitate the process) and their behaviour.

An organism that is hotter than its surroundings may lose heat energy in a combination of four ways, and an organism that is cooler than its surroundings will gain heat in the same ways: **conduction**, **convection**, **evaporation** and **radiation**. Conduction is the transfer of heat energy from a hotter object to a cooler object by direct contact. Convection transfers heat when hot air or water rises and is replaced by cooler air or water. Evaporation (in relation to organisms) is when water or sweat turns to vapour, cooling the skin. When heat is transferred from an object by infra-red waves, it is called radiation.



◀ **Figure 8.16**
Heat transfer for a lizard during a) the day and b) the night.

WOW

Thermoregulation in plants

It is understandable to think of animals first when considering organisms that can regulate their body temperature. However, some plants do too. *Nelumbo nucifera*, found in the Northern Territory, is able to warm up and regulate its temperature. A bud starts heating until it reaches approximately 32°C. As petals start to open, its temperature will remain constant for 2–4 days despite fluctuating external temperatures. Just as would happen in a mammal, in the cool of night the plant increases its metabolic heat production and in the heat of the day evaporative cooling comes into play.

Thermoregulation is essential for an organism's survival. Heat energy can be lost or gained in one of the following four ways: conduction, convection, evaporation or radiation.

Staying cool in the heat

For endothermic homeotherms living in high-temperature environments, the problem is how to reduce heat gain and increase heat loss. In low-temperature environments, the problem is how to increase heat gain and reduce heat loss. Apart from homeostatic mechanisms that involve the physiology of the animal, behaviours and structures can also contribute to maintaining a relatively stable body temperature.

Figure 8.17 shows various mechanisms a mammal uses to maintain its heat balance. A number of thermoregulatory adaptations will be explored in the next section.

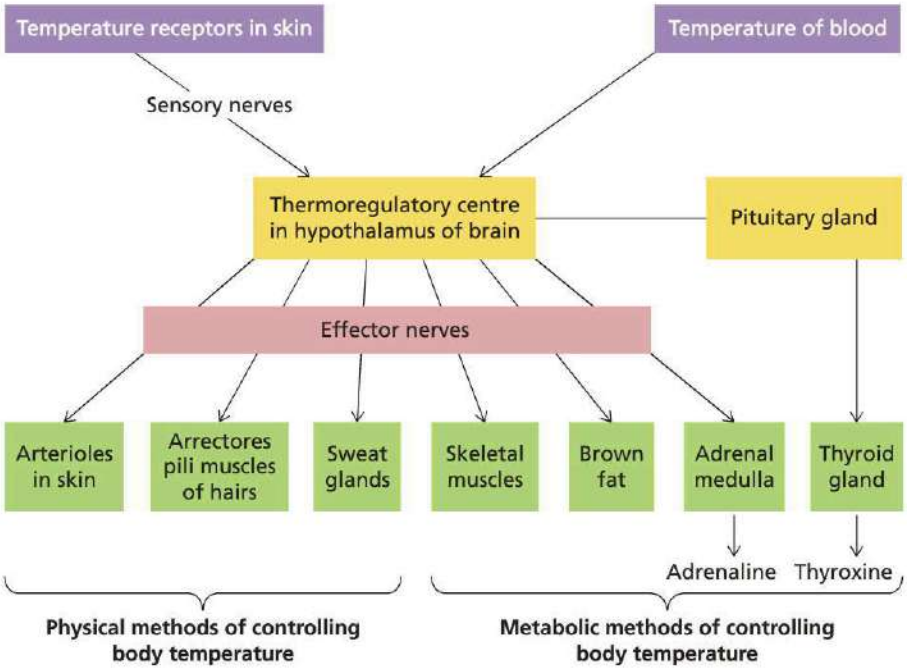


Figure 8.17 ▶ Mechanisms of controlling body temperature in a mammal

Physiological adaptations

Organisms achieve heat loss in a variety of ways. Exposing a large surface area to the environment allows heat to escape the body. Nerve impulses stimulate the arterioles to dilate (**vasodilation**), which allows lots of blood to flow close to the skin's surface, and heat to escape through the skin. Organisms also sweat in the heat – sweat glands open to release water and salt onto the skin, which evaporates and cools the skin.

Behavioural adaptations

To reduce heat gain, dingoes, birds and rock wallabies normally shelter from high temperatures, only emerging to feed in the relative cool of dusk and dawn. Various wallabies and kangaroos lick their wrists where the blood vessels form a dense network close to the surface. Even though this means loss of precious water, the evaporation has a cooling effect.

Crocodiles shelter in cool vegetation lining the river banks or submerge themselves in the water. They also open their mouths, enabling evaporation from internal surfaces. During cool seasons they bask in the sunshine before they get hot enough to digest their meals.

Figure 8.18 ▼ The behaviour of a) red kangaroos and b) dingoes helps them thermoregulate in the heat of Australia.

Auscape/Jean-Paul Ferrero

Nature Picture Library/Simon King

QUESTION SET 8.6

Remembering

1 Copy and complete the table to compare a mammal's physiological response to temperature.

Table 8.8 Simple stimulus–response mechanisms involved in thermoregulation

Stimulus	Physiological response	Effect
Increase in temperature		More heat lost through radiation
	Hairs flatten on skin, trapping less air	
Decrease in temperature	Constriction of blood vessels on the skin	
		Less heat loss through conduction
	Shivering	

2 Define the ways in which heat energy is transferred.

3 Explain how heat balance is achieved.

Understanding

4 What is vasodilation? Explain how it helps to maintain internal temperature.

5 Distinguish between 'endotherm', 'ectotherm', 'homeotherm' and 'poikilotherm', and use named examples in each definition.

Applying

6 Apply the negative feedback model to explain thermoregulation in mammals.

a Draw a simple annotated diagram to help with your explanation.

b Which organs and systems are involved in this control?

Keeping warm in the cold

The feathers of mutton-birds and the fur of polar bears aid thermoregulation by trapping an insulating layer of air close to the skin. A polar bear is so well insulated that heat loss is practically nil. The emperor penguin is also well insulated by several layers of scale-like feathers, and it takes a strong wind to ruffle them. Although they don't have feathers under their feet, emperor penguins are able to stand on ice for long periods (Figure 8.19). For details of an adaptation that allows this, see Figure 8.23 on page 235.

In hot climates, fur can insulate animals from radiant heat or hot air around them. For example, the hair on the top of the camel's hump reflects heat.

Variation in fur thickness and periods of moulting are other adaptations that assist thermoregulation in challenging environmental conditions.

▼ **Figure 8.19** Emperor penguins have physiological and behavioural adaptations to survive in the freezing Antarctic temperatures.

Corbis/Tim Davis

EXPERIMENT 8.2

THE SKIN AND TEMPERATURE CONTROL

Mammal body temperature varies little. What are some of the adaptations that help mammals maintain a fairly constant body temperature?

Aim

To model and investigate heat loss from an exposed surface

Materials

- Four test tubes
- Four thermometers
- Four beakers
- Funnel
- Measuring cylinders
- Cotton wool (or some other insulating material)
- Cardboard cylinder (such as from a toilet roll)
- Timer
- Fan
- Spray bottle of warm water

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
Hot water can burn.	Use a funnel and fill test tubes carefully.

Procedure

Part A: Effect of insulation on heat loss

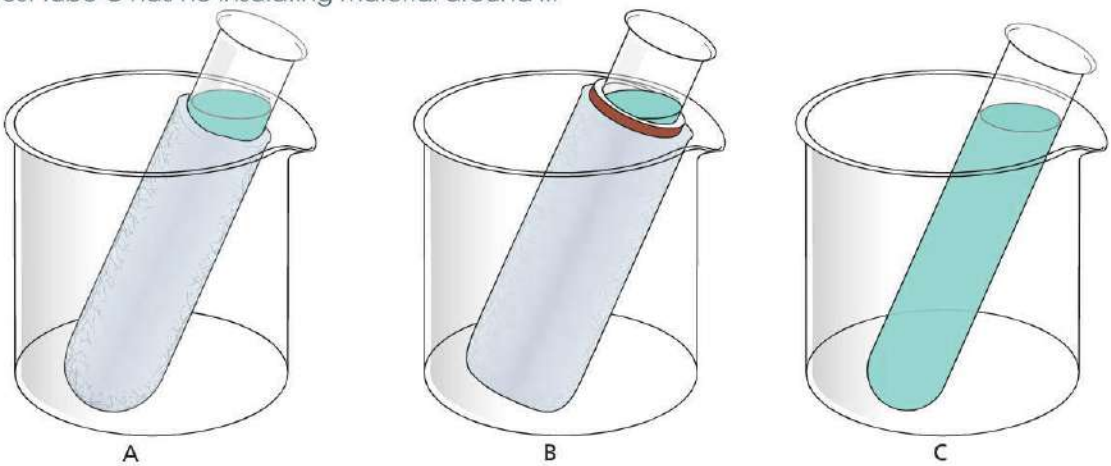
1 Take three test tubes, label them A, B and C, and place each one into a separate beaker.

2 Surround test tube A with cotton wool or some other insulating material.

3 Place test tube B in a cardboard cylinder and wrap the outside of the cylinder with the same amount of insulating material as you used for tube A (so that there is a layer of air between the test tube and the insulation).

4 Cover the top of the cylinder so the air is trapped.

5 Test tube C has no insulating material around it.



▲ **Figure 8.20** Experimental set-up to investigate the effect of insulation on heat loss

6 Fill each of the three test tubes with 20 mL water at 80°C.

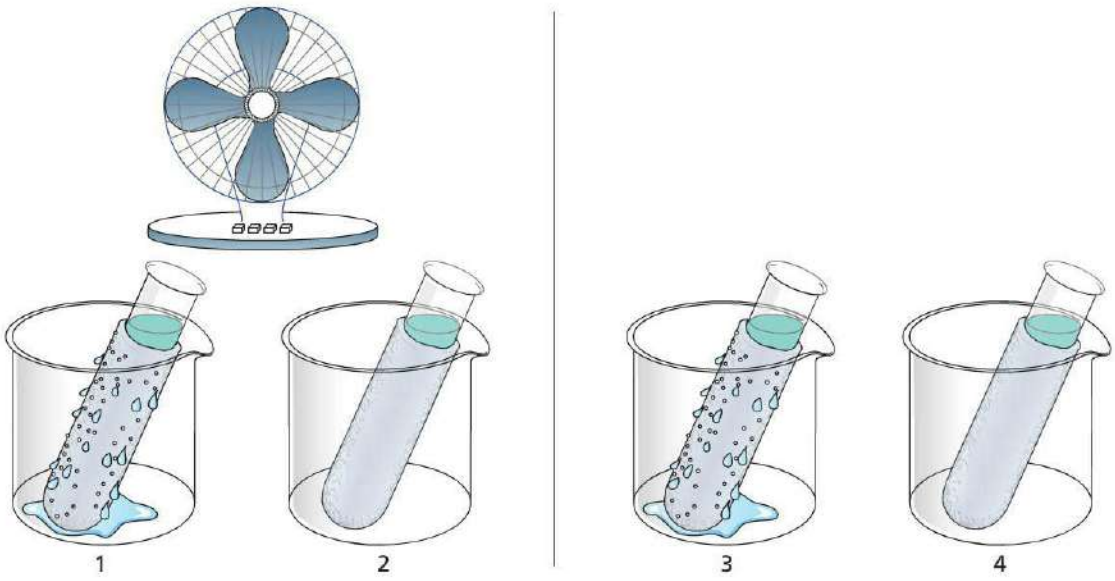
7 Insert a thermometer in each test tube and record the temperature as soon as possible after the water is added. In a table record the temperature every minute for 10 minutes.

8 Graph your results.

Part B: Effect of moisture on heat loss

1 Take four test tubes that have been wrapped in cotton wool and place each one in a separate beaker. Label them 1, 2, 3 and 4.

2 Spray the outside of test tubes 1 and 3 with warm water.
3 Place test tubes 1 and 2 in front of a fan, and test tubes 3 and 4 in an area without air movement.
4 Fill each of the four test tubes with 20mL water at 80°C.
5 Insert a thermometer in each test tube and record the temperature as soon as possible after the water is added. In a table, record the temperature every minute for 10 minutes.
6 Graph your results.



▲ **Figure 8.21** Effect of moisture on heat loss

Results

Observations are to be recorded in tables and then graphed.

Analysis of results

1 Which test tube in Part A was the most effective at reducing heat loss? Suggest what makes this set-up most effective at reducing heat loss.
2 Which test tube in Part B was the most effective at increasing heat loss?

Discussion

1 What structural feature of mammals is the cotton wool simulating?
2 How can an insulating layer of air be achieved in mammals?
3 How can the results from test tube B be used to explain the observation that a cat looks larger on colder days?
4 Based on the results, suggest why an individual feels cooler on a hot windy day compared with a hot still day.
5 Using the observations collected in this experiment, explain why panting in dogs is an effective way of losing body heat.
6 Why are animals like frogs at greater risk of perishing on a hot windy day? Use the experimental results to support your answer.

Taking it further

1 Which part of the experiment modelled the role of perspiration in maintaining body temperature?
2 Were any experimental controls used in Part A and Part B of this experiment? If so, explain what these were and discuss their importance.
3 Draw a diagram of a negative feedback model, using the examples of thermoregulation investigated in this experiment. Are all components of a feedback model completely demonstrated in this experimental set-up? Explain your answer.

4 When the body temperature in mammals starts to drop, a number of things happen. Describe some of these physiological and behavioural responses. Are any of these responses being modelled in this experimental set-up? Explain.

5 When the body temperature in mammals starts to increase, different physiological and behavioural responses occur. Describe these responses. Are any of these responses being modelled in this experimental set-up? Explain.

Extension

1 Devise a method to test the effects of shivering on heat regulation. Use a method similar to the one in this experiment.

2 Explain why a person shivers during a fever even though their body temperature is above 37°C.

3 Why would a small mammal shiver more than a large mammal on a cold day?

4 A small mammal was found to eat more than its body weight in food in a 24-hour period compared with a larger mammal, which ate less than its body weight in food in the same time period. Explain why.

Case study

The use of phase-changing materials in textiles to mitigate hypothermia

Scientists from CSIRO have worked for years to design a textile to be deployed in an emergency to alleviate the symptoms of hyperthermia or hypothermia. Mr Robin Cranston, an Honorary Research Fellow at CSIRO, worked in this field. His interest emerged from the need for emergency services to have an effective garment to utilise on patients at risk of hypothermia.

Burns victims are at high risk of contracting hypothermia due to loss of skin and its protective function. Doctors and scientists know that in cases of trauma the blood withdraws to the core, which means that temperature regulation in the limbs gradually retreats from fingers and toes back toward the body core. Current methods include using a space blanket or convection blanket at the incident site and a Bair Hugger™ (hot air blanket) at the hospital. The development of a garment to be used by paramedics could decrease the incidence rate of burn patients having hypothermic episodes.

Mr Cranston, who worked in the CSIRO Materials Science and Engineering faculty, investigated the use of phase-changing materials (PCM) in textiles and their ability to alleviate hypothermic tendencies. A PCM either absorbs or releases heat energy when it changes state. With the aid of thermal imaging he investigated whether temperature regulation can be achieved by PCMs. Unfortunately, PCMs only maintain temperature for the time it takes to change state, generally only a few minutes. 'To be effective in reversing any patient tendency to hypothermia actually requires that a textile can add heat effectively and for hours not minutes,' states Mr Cranston. To improve the effect of PCMs in reducing the susceptibility of hypothermia requires advanced fibre-making techniques to dramatically increase the PCM loading into the textile.

Discussions between CSIRO, paramedics, clinicians and the burns unit at the Royal Brisbane Hospital led to a theoretical prototype garment that could be used at the time of patient retrieval. It was designed to remain on the patient, during transport, surgery and recovery, to help stabilise core body temperature. The project proposal, although strongly supported by clinicians, was eventually not funded so the research required to identify the energy input and PCM loading remains unknown.

Questions

1 PCMs are being used as thermal insulation in firefighting protective gear. Predict how they would work.

2 Sportwool™ by CSIRO is a material that helps to maintain internal body temperature. Explain how it aids athletic endurance.

3 CSIRO has recently developed fibres containing thermochromic materials that transform colour to correspond to temperature. They can be tuned to very accurately measure skin temperature. These fibres have an application in measuring inflammation at the skin surface, mainly for acute wounds. Explain how this textile can help monitor, assess and evaluate a patient's infection.

WOW

Black polar bears

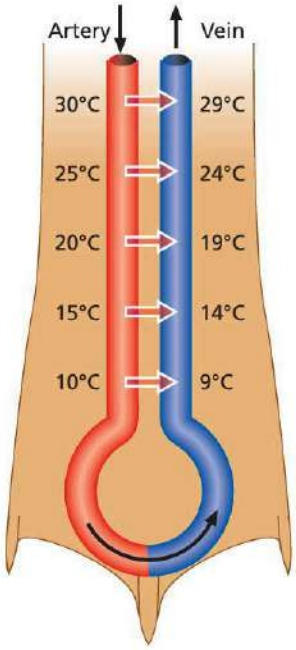
The fur of polar bears looks white but it is actually colourless; when photographed with film sensitive to ultraviolet light, polar bears appear black. Each strand of hair has a hollow shaft that scatters and reflects visible light, much like ice and snow does. The hollow shaft led to the belief that the hair acts like an optic fibre, conducting ultraviolet light to the black skin beneath. Experimentation proved this long-standing idea to be wrong; it is now thought that the keratin of the hair absorbs ultraviolet light; that is, it does not reach the skin.

Shutterstock.com/La Nau de Fotografia

Figure 8.22 ▲
A polar bear

Countercurrent heat exchange

Aquatic birds and mammals have a very effective system of keeping their extremities warm – the **countercurrent** heat exchange. Blood travelling in the arteries to the foot or fin warms the blood returning to the body in the adjacent veins. The outgoing blood to the extremity is cooled in the process but not enough to affect cell activities. As the temperature gradient between the extremity and the surroundings is reduced, heat loss is minimised (Figure 8.23).



▲ Figure 8.23
A model of countercurrent heat exchange in the foot of an emperor penguin

Shape and size

The shape of an organism helps to maintain homeostasis and internal temperature. Adaptations that reduce the surface-area-to-volume ratio reduce heat loss. For example, some bird species in Tasmania tend to be larger than those of their counterparts on the warmer mainland. The ears and limbs of Arctic foxes are more rounded than those of their relatives elsewhere (Figure 8.24). On the other hand, the large surface area of an elephant's ears assist heat loss. Some animals reduce heat loss by their behaviour. For example, by huddling together, penguins reduce the group's overall surface-area-to-volume ratio. (They move around within the huddle to prevent any individual from being exposed to the harsh environment for an extended period of time.)

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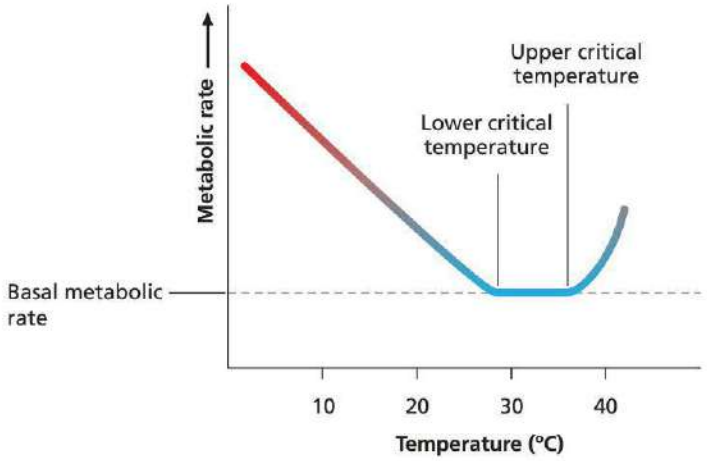
Nature Picture Library/Kevin Schafer

▲ Figure 8.24
Ear shape and size differ between the arctic fox (far left) and its relatives.

When all else fails, shut down

Sometimes behaviours and physical features are inadequate in stabilising temperature. At particular external temperatures the metabolic rate of an animal begins to rise, increasing heat output. The external temperature at which the metabolic rate begins to rise is the **lower critical temperature**, which varies according to species (Figure 8.25). The increase in metabolic activity requires a supply of energy which for some animals proves difficult if food is scarce.

Figure 8.25 ▶
The effect of environmental temperature on the metabolic rate of a generalised mammal

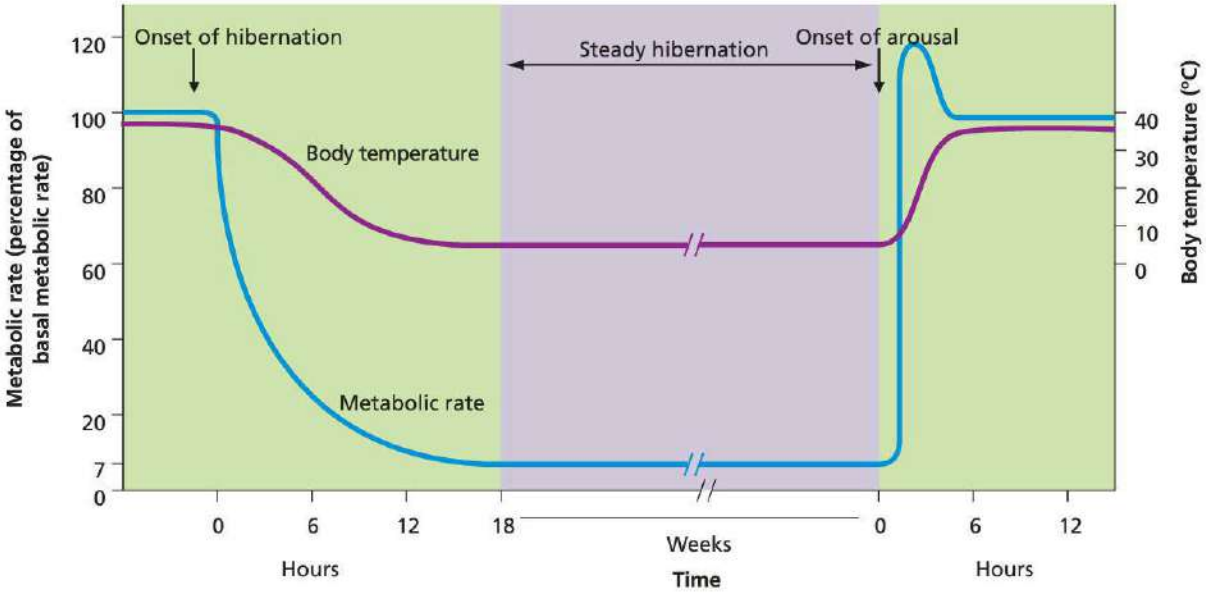


In very cold conditions, the increase in metabolic rate may be insufficient to maintain body temperature within tolerance limits. Many animals in these conditions **hibernate**. During hibernation, the metabolic rate falls to a level that just sustains life; the set point is lowered considerably – an excellent mechanism for conserving energy (Figure 8.26).

The **upper critical temperature** is the external temperature at which the body's cooling mechanisms fail and the metabolic rate increases with the rise in external temperature.

Another kind of seasonal dormancy is **aestivation**. This describes what some animals do in very dry conditions, but not necessarily in summer. The garden snail retreats into its shell and seals itself off; some earthworms coil into balls wrapped in mucus that dries out. Lungfish burrow in mud that hardens, and there they remain until the next rainy season some months later.

Figure 8.26 ▼
Metabolic rate and body temperature of a ground squirrel before, during and after hibernation



WOW

The longest sleep

It was reported that when a dried specimen of moss that had been in a museum for 120 years became moist, it generated living tardigrades, a small group of organisms thought to be related to the annelids and arthropods.

The larva of a beetle, *Buprestis aurulenta*, has shown delayed emergence of specimens between 26–51 years.

The pupa of the gall midge can lie dormant for 18 years.

QUESTION SET 8.7

Remembering

1 Explain the difference between lower and upper critical temperature.
2 Identify the difference between hibernation and aestivation.

Understanding

3 How does countercurrent heat exchange help to maintain internal temperature?

Applying

4 Explain how the shape of an organism aids thermoregulation.

Water: essential to life

Water is the universal **solvent** and essential to life. Most salts and minerals in organisms are dissolved and broken into ions by water. Water forms weak bonds with hydrophilic compounds, pulling them apart. Looking at the dissociation of sodium chloride, the negative oxygen atom of the water molecule attracts the positive sodium atom. The positive hydrogen atoms attract the negative chlorine atom, pulling the molecule apart. This results in an aqueous solution of sodium and chlorine ions ready for metabolic processes.

Metabolic reactions occur in a solution composed mainly of water. The blood plasma that transports the products is approximately 90% water. Blood not only supplies nutrients to cells, but also transports waste products for removal. The main wastes that require removal are carbon dioxide, via the lungs, and nitrogenous compounds, via the kidneys.

Water balance requires continual homeostatic control, or **osmoregulation**. If the supply of water does not meet what is lost, the relative concentrations of solute and solvent in tissue fluids become difficult to regulate. Physiological functions are therefore affected. A loss in blood volume results in a blood pressure drop; toxic wastes cannot be excreted effectively and enzyme function is affected. Severe dehydration can lead to death. In plants, loss of water can mean collapse of shoot systems and interference in cellular functioning.

See Nelson Biology Units 1 & 2 for the Australian Curriculum, Chapter 11 for more information about the removal of carbon dioxide via the lungs.

The kidneys

The kidneys are essential organs, involved in maintaining a constant internal environment. Specifically, they play an important role in osmoregulation. Their osmoregulatory function includes:

- removal of nitrogenous wastes
- regulation of water concentration in blood
- maintaining ion levels in the blood.

The elimination of nitrogenous wastes formed from the synthesis of protein molecules is essential. Nitrogenous wastes, such as **ammonia**, are toxic. A build-up of ammonia in cells can affect their pH severely. Different organisms have different ways of coping with this waste product.

Freshwater fish produce abundant amounts of dilute urine containing ammonia. It is excreted quickly and continuously. On the other hand, marine fish and terrestrial mammals must quickly convert the ammonia to the less toxic substance, **urea**. It is then released as concentrated urine containing less water. Other organisms, such as reptiles and birds, produce **uric acid**, which is the least toxic form of nitrogenous waste and contains very little water.

FORMATION OF URINE

This website contains an animated tutorial and quiz summarising the structures and function of the kidney.

How the kidneys regulate water balance

The feedback mechanism for the maintenance of water balance in humans is controlled by an antidiuretic (urine-reducing) hormone called **vasopressin**. It is secreted from neurosecretory cells in the hypothalamus when osmoreceptors detect an increase in blood solutes. Vasopressin increases the permeability of the distal tubules of the kidney, increasing water reabsorption. As water concentration increases in the blood plasma, negative feedback decreases the release of vasopressin.

Figure 8.27 ▶
A comparison of urine concentration between animals

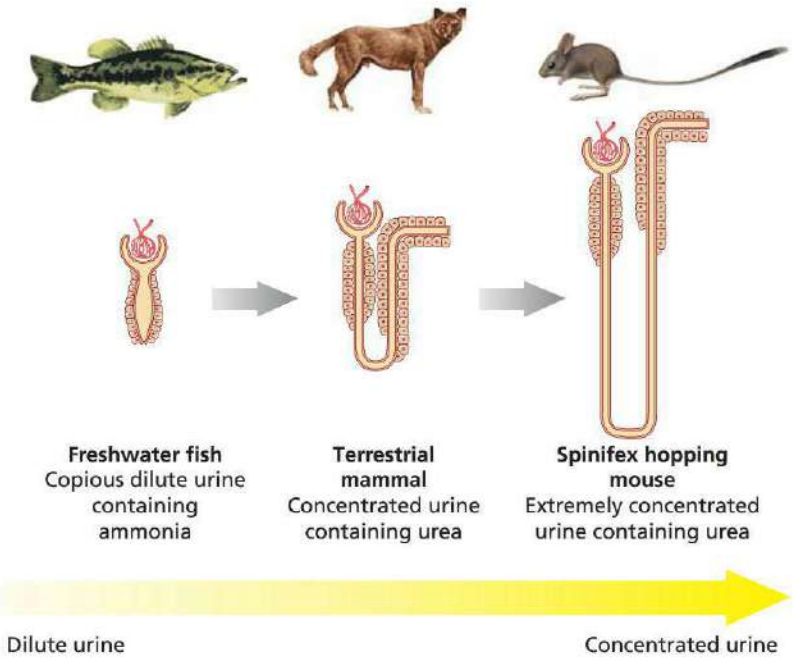
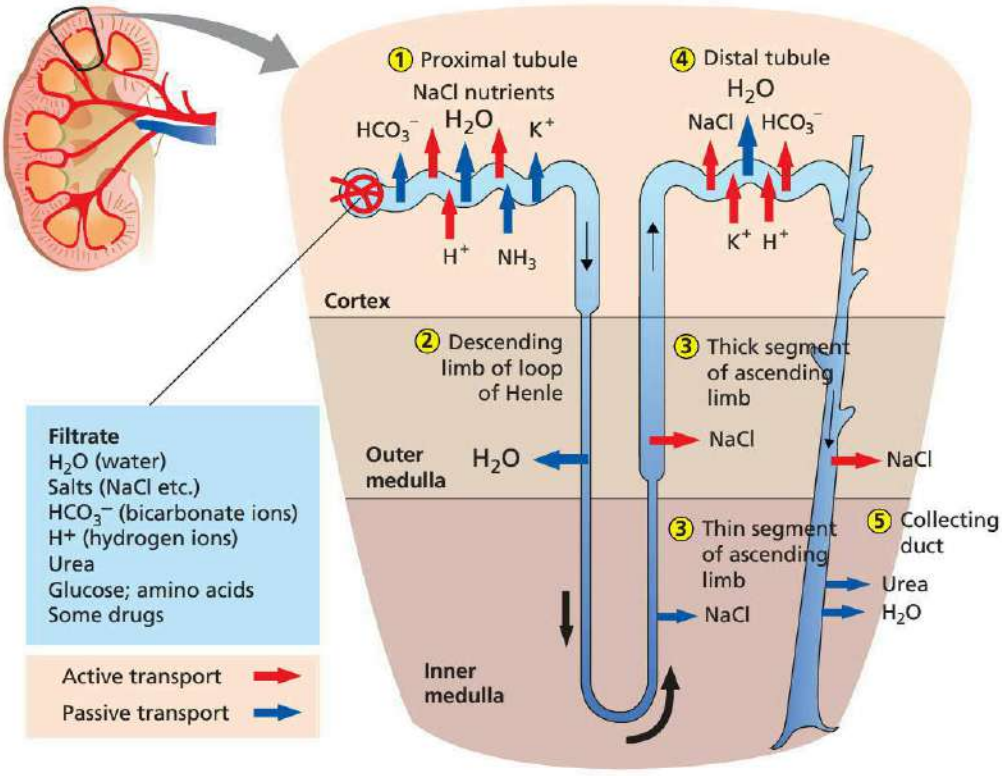


Figure 8.28 ▶
A mammalian kidney. Water is conserved when it is removed in the descending portion of the loop of Henle. The longer the loop of Henle, the more concentrated the urine and the more water saved.



QUESTION SET 8.8

Remembering

1 Why is water essential to life?

2 What are the three types of nitrogenous waste excreted by organisms? Explain why it is essential to remove these wastes.

Understanding

3 a Explain the difference between solute and solvent.

b Why is water known as the universal solvent?

c How does water act as a solvent? Use an annotated diagram in your answer.

Applying

4 Create an annotated diagram that demonstrates the role of vasopressin in human water balance.

Osmoregulators versus osmoconformers

Organisms have various mechanisms to maintain water balance. Some regulate their osmotic concentration to be either higher or lower than their external environment; these organisms are called **osmoregulators**. Others allow their osmotic concentration to be equal to the concentration of the external environment; these organisms are called **osmoconformers**.

Osmoregulators

Structural features, as well as behavioural and physiological responses, aid water balance maintenance in osmoregulators. Dingoes pant, losing water vapour from the tongue, air passages and the lining of the mouth. Other animals have high densities of sweat pores in certain areas, which are exposed as body temperature rises. These are effective cooling behaviours but involve water loss by evaporation. Fortunately, a thirst response is experienced as the concentration of blood solutes increases, and animals respond by drinking water. Thermoregulation and osmoregulation are intricately bound with each other. For many terrestrial organisms, a water supply is not always available. Animals living in dry areas have a range of structural, physiological and behavioural adaptations to maintain their water balance.

Structural features of the osmoregulator

A waterproof or impermeable outer layer (integument) can reduce water loss. For example, the scales of reptiles, the hair of mammals, the feathers of birds and the upper part of the epidermis contain keratin, a protein that hardens and waterproofs the body surface.

Plants are also osmoregulators. Water is essential for photosynthesis and the survival of the plant. However, it is continually being lost to the environment via stomatal pores of the leaf surface – and clearly plants cannot move to seek the additional water they require! Plants have a range of features that help obtain or retain water to maintain their balance.

- Thick waxy cuticle on the leaf surface
- Reduced numbers of stomata on the top of the leaf and increased numbers on the bottom of the leaf
- Sunken stomata
- Cylindrical or rolled leaves
- Reduced leaf numbers or no leaves
- Hairs on leaves
- Tap roots

TRANSPIRATION IN PLANTS

Learn how the stomata and guard cells work to change the rate of transpiration.

Thinkstock/Cathy Keifer

Thinkstock/jsmcqueen

Figure 8.29 ▲
The scales of reptiles and feathers of birds contain keratin.

Physiological processes of the osmoregulator

Many reptiles and birds reabsorb water from their cloaca, the cavity into which their rectum and ureter open. Excreting nitrogenous waste as uric acid is effective in saving water. Many terrestrial vertebrates, such as the Australian desert frog *Chiroleptes*, slow down the production of urine by reducing the rate of glomerular filtration. The frog, swelling up like a ball, retains urine in its bladder for use in the dry season. The desert hopping mouse, *Notomys alexis*, can concentrate its urine more than any other known rodent (see Figure 8.27). Water is conserved when it is removed in the descending portion of the loop of Henle. The longer the loop of Henle, the more concentrated the urine and the more water saved. The desert hopping mouse has a very long loop of Henle to maximise water conservation.

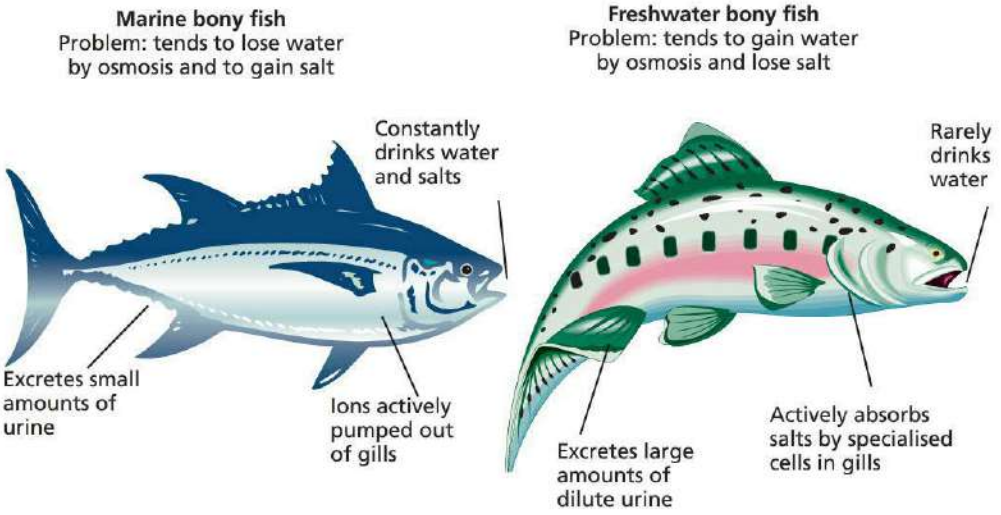
Camels are renowned for their ability to go for several weeks without drinking water. Camels can produce water by metabolising the fat in their hump; however, it is not enough to compensate for the water lost by evaporation. As water is lost, the camel's body fluids become more concentrated but the camel's tissues are extremely tolerant of this condition.

Figure 8.30 ▼
The desert hopping mouse, *Notomys alexis*

Auscape/Kathie Atkinson

Marine vertebrates have body fluids that tend to be **hypotonic** to their surroundings; that is, their body fluids are of a lower concentration compared with the medium in which they live. Water is lost via osmosis from the gill surfaces. Therefore marine fish drink copious amounts of sea water. The problem this creates is the additional salt intake. Marine vertebrates are able to solve this problem by the active removal of salts by special chloride secretory cells in the gills. In addition, a slow filtration rate and the excretion of concentrated nitrogenous waste help them reduce water loss. Some marine animals, such as sharks and rays, tolerate high levels of urea in their body tissues thereby reducing their water loss. In this way, the internal solute concentration of their tissues becomes slightly higher, or **hypertonic**, compared to the surrounding water. The water that consequently moves in by osmosis is easily removed by the kidneys.

Freshwater vertebrates have a concentration of ions in their tissues higher than the surrounding water. They have a high kidney filtration and produce copious amounts of dilute urine. Freshwater fish must actively absorb salts from their external environment in order to maintain their high ion concentration levels.



◀ **Figure 8.31**
Solving the problem of water balance

WOW

The problem of osmosis

Simple unicellular organisms, such as *Amoeba*, solve the problem of water gain from osmosis by accumulating the excess water in little bubbles in their cytoplasm. These contractile vacuoles swell to bursting point and the surplus water is expelled as the vesicular membrane contracts suddenly.

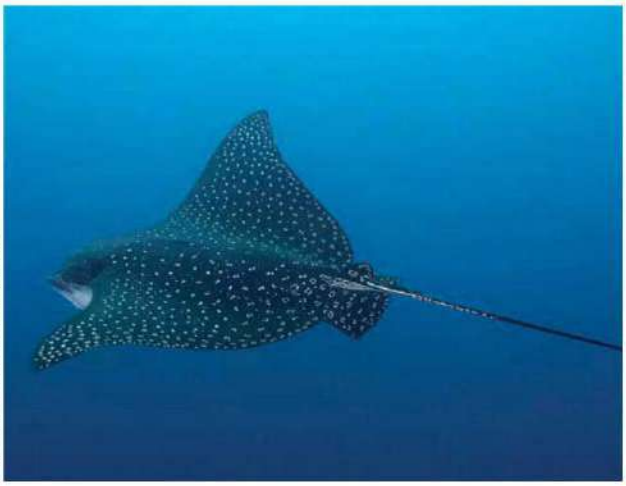
Behaviours of the osmoregulator – aestivation and burrowing

Desert frogs have adaptations, ranging from producing highly concentrated urine to burrowing in the desert sands for several months at a time. For example, the water-holding frog, *Cyclorana platycephala*, tucks itself in a water-conserving cocoon created from layers of skin. The frog's metabolic rate slows as it enters aestivation under the ground. It can survive in this way for many months.

Other desert animals spend a large proportion of time in burrows. Burrows have lower temperatures and higher humidity than the open air, so water loss is reduced. The desert hopping mouse, *Notomys alexis*, has a bushy end to its tail, which it wraps around its face. This interesting strategy reduces water loss by saturating the air between the hairs at its body surface and the air in the burrow with water vapour.

Auscape/Kathie Atkinson

▼ **Figure 8.32**
A water-holding frog, *Cyclorana platycephala*, breaking from its cocoon after rain



Nature Picture Library/Brandon Cole

Figure 8.33 ▲
Sharks and rays are osmoconformers.

Osmoconformers

Most marine invertebrates, such as cnidarians and molluscs, are osmoconformers. Their interstitial fluid concentration fluctuates to match the external environment. An organism whose body fluids are of the same concentration as the surrounding water is referred to as **isotonic**. Cartilaginous fish such as sharks and rays are also osmoconformers. They are able to concentrate urea in their bodies to maintain a high osmolarity, thus matching the ocean's high concentration of solutes. Some fish, such as sturgeon, sawfish and bull sharks, are capable of living in the brackish water found in **estuaries** and rivers. Organisms such as these that can tolerate the fluctuation in salinity within this mix of salt and freshwater are known as **euryhaline** species.

ACTIVITY 8.1

WATER BALANCE IN ANIMALS

Aim

To study water regulation in *Paramecium* and humans

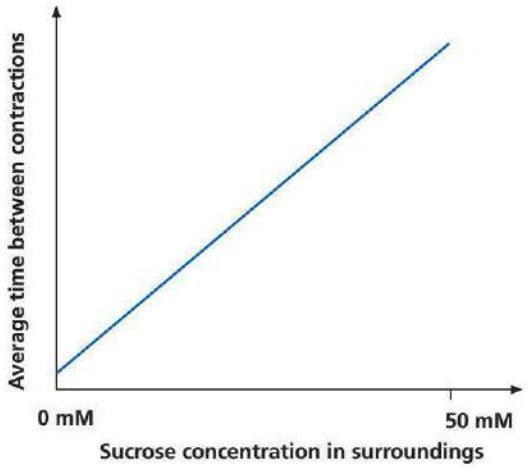
What to do

Read the following information and answer the set questions.

Part A

A culture of *Paramecium* was placed on a thin layer of petroleum jelly on a microscope slide. A coverslip was added and the cells were observed.

The anterior and posterior contractile vacuoles were located and the time between contractions was noted. The *Paramecium* culture was exposed to differing concentrations of sucrose from 0 to 50 mM (millimoles). A generalised relationship of the time between contractions and the concentration of the surrounding sucrose solution was displayed in a graph (Figure 8.34).



▲ Figure 8.34 A generalised relationship between time of contractile vacuole contractions and the concentration of surround solution

1. When a *Paramecium* lives in its normal fresh water environment, it is subjected to a continuous influx of water. Explain why.
2. Describe what happens to the time between vacuole contractions as the concentration of surrounding solution increases.
3. How would the rate of water expulsion from *Paramecium* change as the osmotic pressure of the surroundings increased?
4. How could you tell when *Paramecium* was in an isotonic solution?
5. Using information from the experiment, explain how the contractile vacuoles in *Paramecium* enable the cell to maintain a steady internal solute concentration.
6. Would this process of osmoregulation continue if the energy supply of the cell was cut off? Explain.

Part B

Antidiuretic hormone (ADH, also known as vasopressin) is a protein whose primary function is to retain water in the body of humans. Osmoreceptors in the hypothalamus detect the variations in the concentration of blood solutes, and the hypothalamus causes the release of ADH from the posterior pituitary gland. ADH diffuses into the capillaries and is carried to target tissues: the distal tubules and collecting ducts of the kidneys, the sweat glands, and the smooth muscles of small blood vessels in which it causes blood vessel constriction.

Table 8.9 Factors that affect concentration of ADH

Concentration of ADH (pg/mL)*	Output of urine (litres per day)	Sweat gland activity	Blood pressure
0.5	15.0	High levels of sweat	Decreased
3.6	1.5	Moderate amounts of sweat	No change
4.7	0.5	Very little sweat	Increased

* pg/mL is picograms (1×10^{-12} grams) per millilitre

1. Various factors are found to affect the secretion of ADH. Vomiting, diarrhoea, stress and cigarette smoking all cause an increase in the levels of ADH.
   a. What effects on urine output, sweating and blood pressure would you expect during these times?
   b. Describe another everyday situation that would cause the same effects.
2. What effects would high alcohol consumption have on levels of ADH and consequent body functions?
3. Relate changes in blood pressure to the action of ADH, which contracts the smooth muscle in small blood vessels.
4. Describe what happens to the ADH levels when the osmotic concentration of extracellular fluid is:
   a. high.
   b. low.
5. Explain why water balance in humans is described as a negative feedback mechanism.
6. Draw a diagram of a stimulus–response model to demonstrate water balance in humans. Include the stimulus, receptor, control centre, transmission of message, effectors and response.

QUESTION SET 8.9

Remembering

1. Identify the difference between an osmoconformer and osmoregulator.
2. Create a table summarises the adaptations osmoregulators use to maintain water balance.

Understanding

3. Distinguish between isotonic, hypotonic and hypertonic.
4. What is one benefit of being an osmoconformer above being an osmoregulator?

CHAPTER SUMMARY

- The principles of communication involve:
  1. production of a signal that contains information to be transferred
  2. detection of the signal
  3. transfer of this signal until it reaches its target
  4. a response to the signal by the target
  5. control of a signal, once it has been responded to, by switching it off.
- Multicellular organisms contain exteroceptors and interoceptors that are highly specialised to receive signals from the external and internal environments.
- The nervous and endocrine systems react to counteract stimuli.
- Reasons multicellular organisms must respond to stimulus include the following.
  1. Major developmental processes, growth and reproduction
  2. Homeostasis
  3. Surviving challenges in the external environment
- Homeostasis is the maintenance of a relatively stable internal environment within a small tolerance range, despite changes in the external environment
- Feedback mechanisms either counteract or reinforce a stimulus
- Negative feedback counteracts a stimulus to maintain internal pH, water and solute concentrations within narrow limits.
- Positive feedback reinforces a stimulus seen in developmental processes. Positive feedback can be harmful to homeostasis.
- Organisms must keep inorganic and organic materials, pressure and temperature within narrow limits for survival. These are known as tolerance limits. Each organism has an optimum range in which they function best; outside this range is the zone of physiological stress.
- Metabolic activity, physiological processes, structure and behaviour act to maintain an internal set of conditions within tolerance limits.
- Thermoregulation is essential in preventing hyperthermia and hypothermia. Vertebrates have physiological mechanisms, structural features and behavioural tendencies that aid the regulation of core body temperature.
- Organisms can be classified as homeothermic endotherms, poikilothermic endotherms, homeothermic ectotherms or poikilothermic ectotherms.
- Heat energy can be transferred in four ways: conduction, convection, evaporation and radiation.
- Water is essential to life and is known as the universal solvent. It plays a major role in the dissociation of molecules for metabolic activity.
- Organisms either osmoregulate or osmoconform. They can have a number of structural features or behavioural and physiological responses that aid their maintenance of water balance.
- Kidneys play a major role in osmoregulation in vertebrates and the removal of nitrogenous waste. Kidneys have adapted to dispose of different types of nitrogenous waste. There are three types of nitrogenous waste: ammonia, urea and uric acid.
- Organisms that live in estuaries are known as euryhaline species as they are able to tolerate a large fluctuation in salinity.

CHAPTER GLOSSARY

aestivation dormancy in some animals during periods of drought

ammonia a toxic chemical molecule produced as a by-product of protein synthesis

autonomic system part of the peripheral nervous system that deals with involuntary control

axon the extension from the cell body that aids the transfer of the electrical impulse along the nerve

catabolic reaction a chemical reaction whereby bonds in molecules are broken, releasing energy

chemoreceptor a sensory cell or organ that detects chemical stimuli

conduction the transfer of heat energy from a relatively hot object to a relatively cool object by direct contact

convection the transfer of heat by means of rising currents of warm air or water

countercurrent a current that follows in the opposite direction to another current

ductless gland a gland that secretes its product directly into the bloodstream

ectotherm an animal that depends on a source of external heat

effector an organ, cell or protein that acts in response to a stimulus

endocrine system the bodily system responsible for the production and secretion of hormones, which are released into the bloodstream to act on specific target cells and organs

endotherm an animal that retains heat generated by metabolic activity

estuary a transitional region where fresh water from a river meets salt water from the sea

euryhaline organisms that can tolerate a wide change in salinity

evaporation the process in which liquid water changes to water vapour through heating

exteroceptor a receptor that receives signals from the external environment

feedback mechanism a mechanism in which the output or response affects the input or stimulus

glycogen an important energy-storing polysaccharide

hibernate a period of dormancy over long periods of cold conditions

homeostasis the maintenance of a relatively constant internal environment within small tolerance limits, despite changes in the external environment

homeothermic the ability to maintain a relatively constant internal body temperature

hyperthermia a state in which the internal temperature rises above the set point

hypertonic describes a solution with a higher solute concentration compared with another solution

hypothermia a state in which the internal temperature drops below the set point

hypotonic a solution with a lower solute concentration compared with another solution

interconnecting neuron located in the CNS; transfers signals from sensory neurons to motor neurons

interoceptor a receptor that receives signals from the internal environment

interstitial fluid a fluid that lies in the spaces between cells; also known as tissue fluid

isotonic a solution with an equal concentration of solutes compared to another fluid

lower critical temperature the external temperature at which metabolic activity begins to rise, thereby increasing the output of heat

mechanoreceptor a sensory cell or organ that detects mechanical stimuli such as touch, pressure, vibration or tension

metabolism the sum of all chemical reactions occurring within an organism to maintain life

motor neuron a nerve that transmits impulses from the central nervous system to effector

myelin sheath the fatty layer surrounding and insulating the axons of many neurons; increases the speed at which electrical impulses travel along the nerve cell

negative feedback when a change of variable (stimulus) occurs, a response that reverses the direction of the change

nervous system the network of nerve cells and fibres that transmits nerve impulses to provide communication between parts of the body

node the small gap between two myelin cells

optimum range the narrow range within the tolerance range an organism has for a particular factor, at which the organism functions best

osmoconformer an organism in which the internal solute concentration changes with the concentration of solutes in the external environment

osmoregulation processes by which internal water and solute concentration are maintained despite fluctuations in the external environment

osmoregulator an organism that has specialised mechanisms for regulating internal water and solute concentrations, despite concentration changes in the external environment

pain receptor a sensory cell or organ that detects pain signals

photoreceptor a sensory cell or organ that detects light signals

phototropism a plant's hormonal response to light, whereby auxin accumulates on the darker side of the plant to stimulate cell elongation, bending the plant towards the light to increase its ability to photosynthesise

physiological stress stress caused when an organism experiences conditions outside its tolerance range

poikilothermic an organism whose body temperature changes with the temperature of its surroundings

positive feedback when a change of variable (stimulus) occurs, a response that changes the variable even more in the same direction

radiation the transfer of heat from a hot object by infrared waves

receptor a structure that detects or receives a stimulus

response the result of a stimulus

sensory neuron a nerve that transmits nerve impulses from the receptor towards the central nervous system

solvent a solution that causes a solid substance to dissolve

somatic system part of the peripheral nervous system associated with voluntary control

stimuli a the plural of stimulus

stimulus a signal that causes a response

substrate a substance that an enzyme acts on

thermoreceptor a sensory cell or organ that detects heat or cold

tolerance range the range within which an organism can function and reproduce

upper critical temperature the temperature at which the body's cooling mechanisms fail to keep the body temperature stable and the metabolic rate increases with rise in external temperature

urea a less toxic form of nitrogenous waste found in mammals as a result of protein breakdown

uric acid the least toxic form of nitrogenous waste produced by birds and some desert dwelling animals

vasodilation dilation (widening) of blood vessels, particularly arterioles

vasopressin an antidiuretic hormone responsible for increased permeability of the distal tubules of the kidney, increasing water reabsorption and reducing urine volume

CHAPTER REVIEW QUESTIONS

Remembering

1 Draw a labelled diagram of a motor neuron.

2 Using an example, explain the principle of positive feedback.

Understanding

3 Draw a table comparing features of the nervous and endocrine systems. Include details on the similarities, medium of transmission, speed of travel, effectors, duration of response and an example.

4 Tupong fish, small crustaceans and phytoplankton live in estuaries. Explain, in general terms, the mechanisms you would expect each organism to have for maintaining water balance.

5 Draw a table to summarise examples of structural, physiological and behavioural adaptations a mammal has to regulate temperature.

6 Referring to Figure 8.24, account for the differences shown in the size and shape of the ears of different species of fox.

7 Explain what would happen to the water balance of a marine fish if it were placed in fresh water.

Applying

8 Homeostasis maintains a constant internal environment necessary for survival. One factor it regulates is blood calcium concentration. Name another component that is under homeostatic control and explain why it must be regulated.

9 Compare the stimulus–response model of regulation with the negative feedback model of regulation. Use only annotated diagrams in your answer. Give one named example of each kind of regulation.

10 Name an animal that lives in conditions of either extreme cold or extreme heat. Draw a concept map to summarise the structural, physiological and behavioural adaptations it has to regulate its temperature.

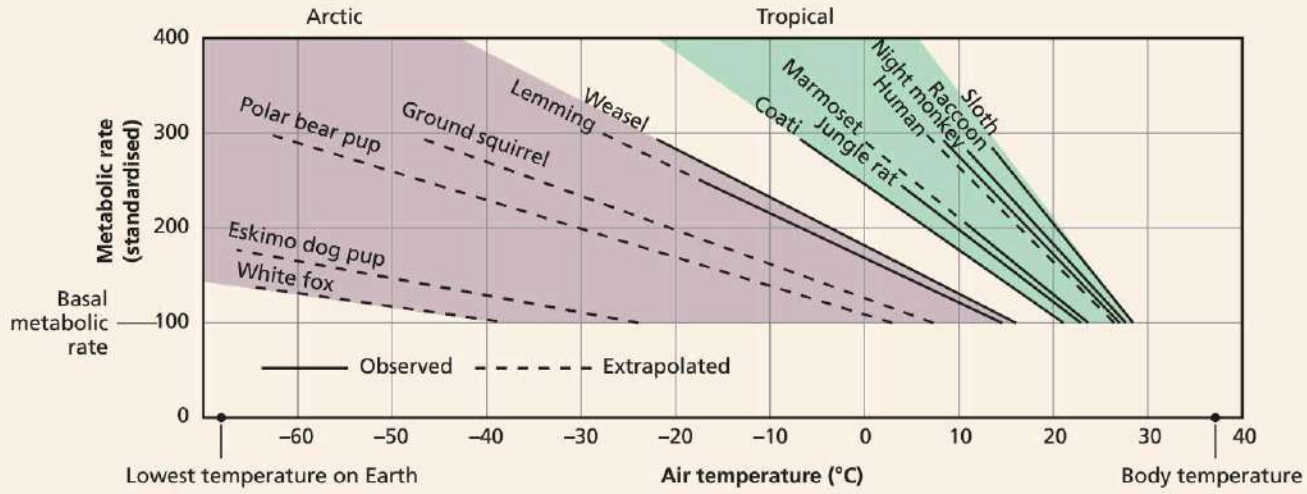
11 Explain the significance when exteroceptors and interoceptors fail.

Analysing

12 Why is the nervous system able to mediate much faster responses than the endocrine system?

13 Figure 8.35 shows the relationship between environmental temperature and metabolic rate of different animals. The basal metabolic rate for each animal is given a value of 100%. Any increase in metabolic rate is in relation to this value.

- a Explain what is meant by critical temperature.
- b Distinguish between critical temperature and set point for temperature.
- c At what external temperature does the metabolic rate of the Eskimo dog pup begin to increase?
- d At what external temperature does the metabolic rate of the sloth begin to increase?
- e Which animal represented in the graph has a critical temperature of about 4°C?



▲ **Figure 8.35** The relationship between environmental temperature and metabolic rate

f The gradients of the lines of the graph indicate the rate of increase in metabolic rate. Which animal, the ground squirrel or the polar bear cub, shows the greater rate of increase in metabolic rate?

g Analyse the information in the figure and compare species living in arctic conditions with species living in tropical conditions.

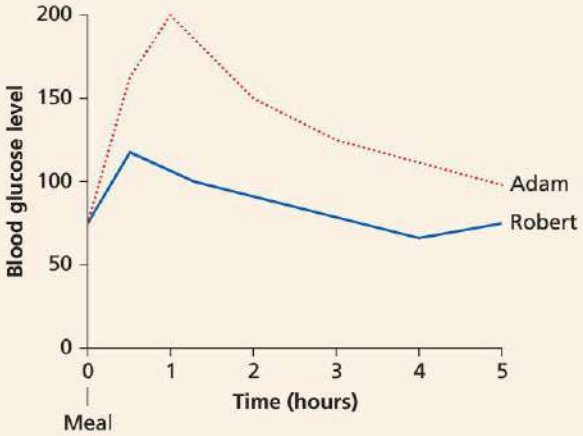
h What strategies do animals employ if they are unable to meet their energy needs?

14 The removal of waste products from the interstitial fluid is essential in maintaining optimal metabolic function. Justify this statement.

Evaluating

15 Two men were given a meal and their blood glucose level was recorded over a 5-hour period. Identify which individual's blood glucose was under homeostatic control. Justify your response.

▼ **Figure 8.36**
Blood glucose levels over a 5-hour period



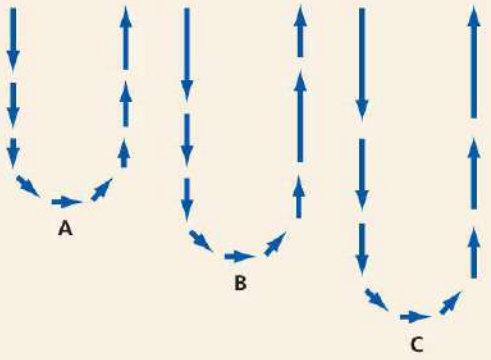
16 Figure 8.37 shows three different nephron structures, each with a varying loop of Henle length. Indicate and justify your choice of which nephron would be found in a:

a terrestrial mammal.

b freshwater fish.

c reptile.

Figure 8.37 ▼
Nephron structure from three different organisms

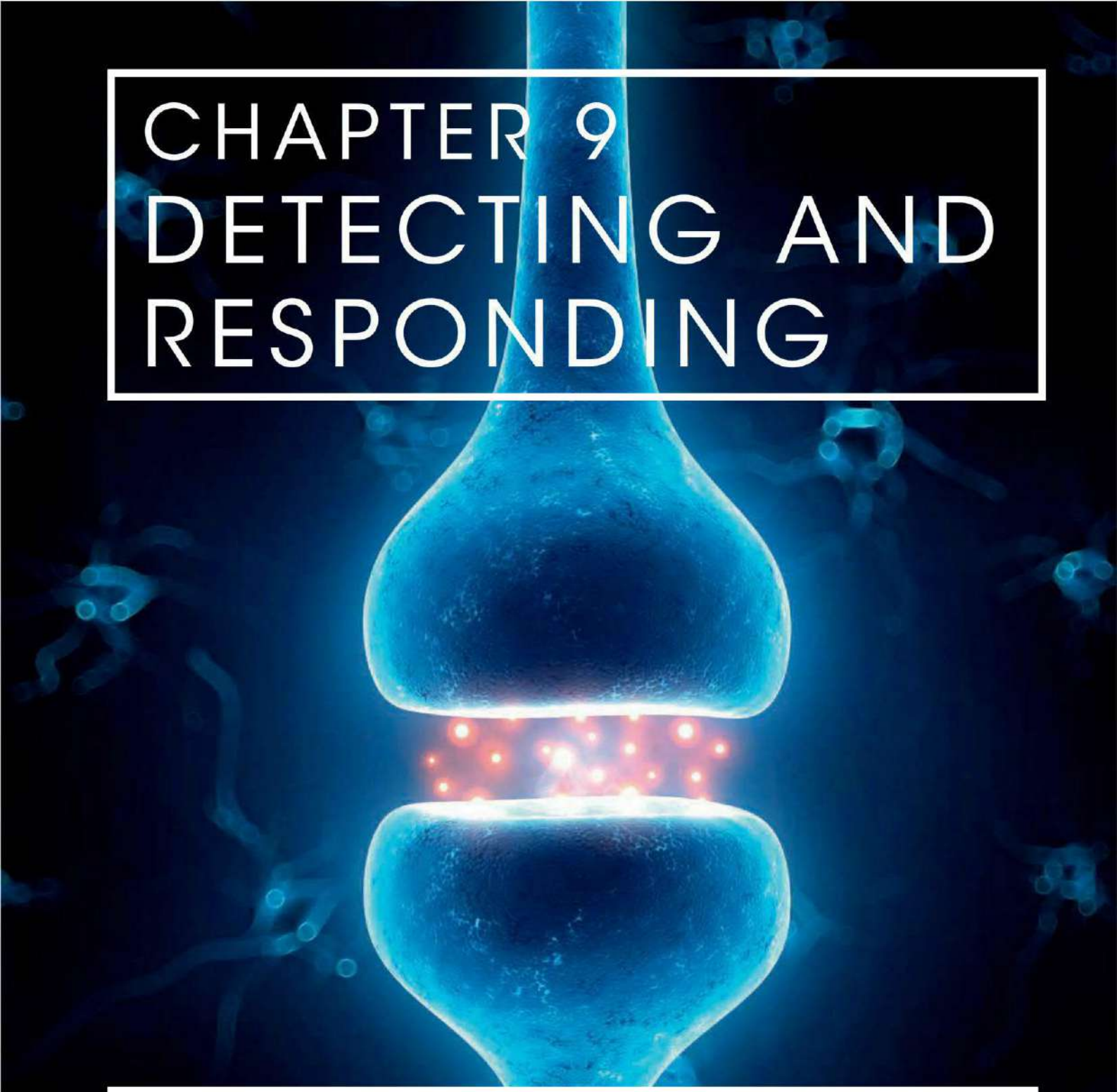


Creating

17 Design an experiment that would identify the tolerance range, optimal range, zone of physiological stress and zone of intolerance of a plant.

18 Humans must wear clothes in the depths of winter in order to survive, whereas other organisms do not. Propose some design modifications to the human body that would allow them to survive months of freezing temperatures without clothing.

CHAPTER 9
DETECTING AND RESPONDING

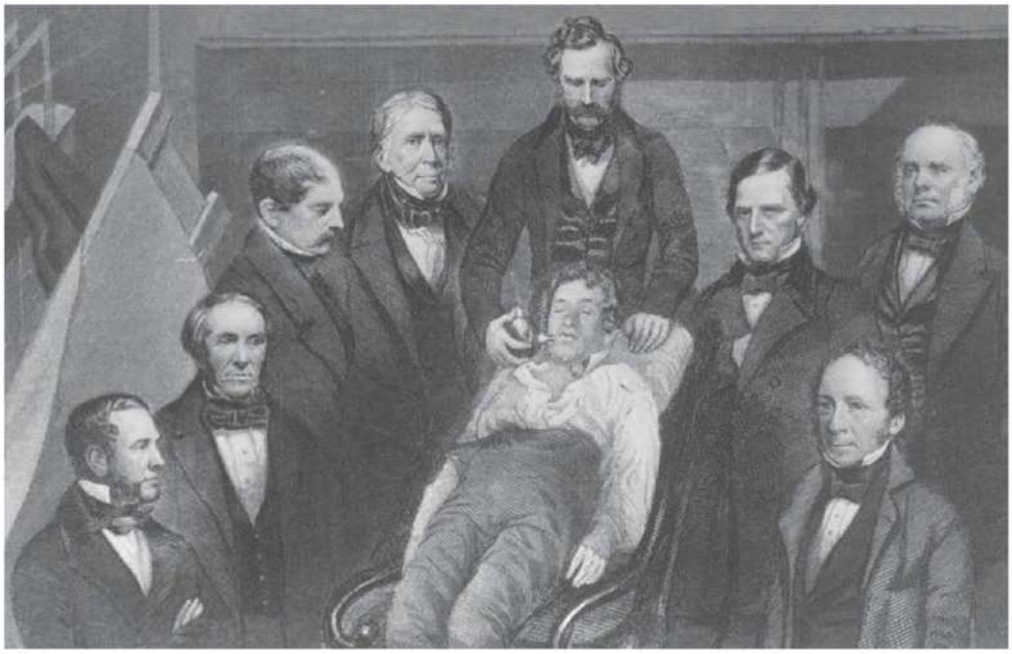


Shutterstock.com/Sebastian Kaulitzki

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

Science Understanding

- Neural pathways consist of cells that transport nerve impulses from sensory receptors to neurons and on to effectors; the passage of nerve impulses involves transmission of an action potential along a nerve axon and synaptic transmission by neurotransmitters and signal transduction (ACSBL112)
- Hormones alter the metabolism of target cells, tissue or organs by increasing or decreasing their activity; in animals, most hormones are produced in endocrine glands as a result of nervous or chemical stimulation, and travel via the circulatory or lymph system to the target cells, tissues or organs (ACSBL113)



Getty Images/Time Life Pictures

Figure 9.1 ▶ Nineteenth century revolutions in medicine: William Morton administering ether to a patient, enabling the painless removal of a neck tumour.

It's 1846 in Boston and an operation is about to be performed in an amphitheatre with medical professionals looking on. The surgeon, John Collins Warren, is about to remove a tumour from his patient's neck and, in the absence of pain killer, the patient is strapped down to prevent excessive movement. William Morton, a local dentist, strides in holding a glass inhaler containing ether. He asks the patient to breathe in the gas and the patient loses consciousness and feels no pain throughout his operation. This was the first successful public demonstration of the use of an anaesthetic. The ether, by disrupting **cell signalling** in the nervous system, rendered the patient insensitive to pain.

Modern research scientists use an understanding of cell signalling pathways and of molecular structures to design highly specific medicines to treat disease and pain. Understanding the mechanisms that cells utilise to produce, receive and respond to chemical signals is providing breakthroughs in the areas of health, agriculture and the environment that change the way we live and behave. This chapter is all about how cells communicate to coordinate cellular activities using chemical signalling.

Evolution of cell signalling systems

Ever since the first prokaryotic cell appeared on Earth billions of years ago, there has been a need to detect and respond to external environments to keep the internal environment safe. These early single-celled organisms bathed in a sea containing calcium (Ca^{2+}), potassium (K^{+}), sodium (Na^{+}) and chloride (Cl^{-}) ions. These charged ions are very small but they cannot cross cell membranes. To survive in these seas, the cells evolved **ion channels** to control the passage of ions across their cell membrane.

Calcium ions (Ca^{2+}) were integral to cell functioning in prokaryotes, but high concentrations were lethal. These primitive bacteria had to keep their **intracellular** Ca^{2+} levels 10 000 times lower than the **extracellular** environment. Through evolution, proteins arose to be highly sensitive to changes in Ca^{2+} concentrations. Small increases in intracellular Ca^{2+} levels could change the activity of the proteins inside the cell. This set the stage for the evolution of calcium signalling systems that are now found in all organisms. Ca^{2+} gradients are involved in regulating exocytosis, muscle contraction, gene regulation and more. **Ion gradients** have evolved to be enormously important in cell signalling.

Unicellular organisms socialise for group work

Communication systems continued to evolve as cells became more complex. **Prokaryotes** evolved the capacity to detect signals secreted by other cells. They started to socialise and coordinate group work, enhancing the survival of the species (see weblink, right). Some scientists believe that this **intercellular** signalling may have paved the way for the evolution of multicellular organisms. For example, the social slime mould (*Dictyostelium*) spends most of its time as a unicellular organism; however, when it is challenged by starvation the cells secrete a **signalling molecule** that attracts other slime mould cells by **chemotaxis**. This coordinates thousands of cells so that they group together to form a multicellular 'slug', capable of moving around in search of a new food source (Figure 9.2).

BONNIE BASSLER

Bonnie Bassler discusses how bacteria communicate using chemical signals to act as a coordinated unit. Her research is contributing to the development of new medicines.

Cell chatter in multicellular organisms

Becoming multicellular requires the coordination of many cells for survival. Cells become specialised to perform particular functions, and group together to form tissues, and ultimately organs and coordinated body systems. This type of complexity requires constant monitoring and adjusting and, therefore, cell communication.

Wikipedia/Bruno in Columbus

◀ **Figure 9.2**
A gathering of the social slime mould, *Dictyostelium*. Single cells can be seen migrating towards a central point by chemotaxis.

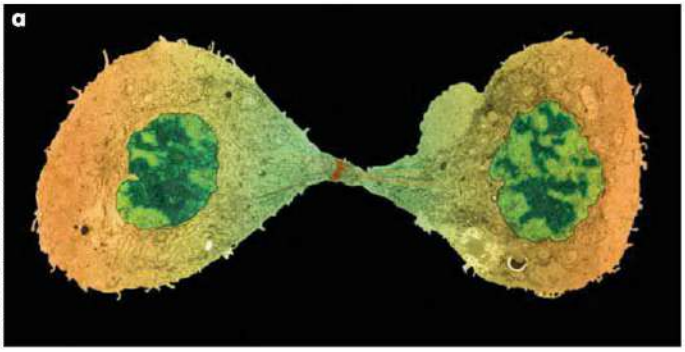
Cells specialise to perform particular functions

There are approximately 200 different types of cells that perform particular jobs in the human body. All of these cells arise from **stem cells**, precursor cells that receive signals that stimulate **cell differentiation**, so that they develop particular characteristics enabling them to perform specialised functions in our tissues. For example, many different types of blood cells arise from stem cells produced in the bone marrow.

Keeping cell numbers in balance

The length of a cell's life varies. White blood cells live for about 13 days, red blood cells 120 days and liver cells for 18 months. Every second, your body is producing more than one million red blood cells, so it makes sense that old and damaged red blood cells must be removed to balance the numbers. Chemical signalling controls the balance of cell numbers in multicellular organisms. Signals stimulating cell division increase numbers and those stimulating apoptosis (programmed cell death) decrease numbers.

▼ **Figure 9.3**
Regulating cell numbers through chemical signalling. a) A skin cell undergoing cell division to increase cell numbers; b) A damaged white blood cell undergoing apoptosis so it will be removed from the organism



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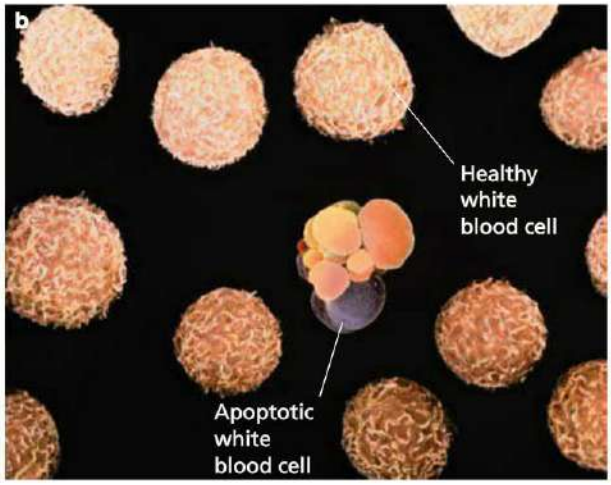


Science Photo Library/Dr Gopal Murti

Regulating cellular processes

Throughout each cell's life it releases and receives signals that regulate and coordinate everyday **cellular processes** for the benefit of the whole organism. These processes control whether a cell should move or stay in stasis, what molecules and structures to build and which ones to digest, whether to grow, what to engulf, and what to secrete. Table 9.1 provides examples of cellular processes that are coordinated to ensure the continued survival of the whole organism and its species.

THE DYNAMIC MACROPHAGE

Explore this animal cell to reveal the cellular processes controlled by chemical signalling.

Table 9.1 Cellular processes regulated by chemical signalling

Cellular process	Description of process
Gene expression	Proteins are the cellular machinery, carrying out diverse functions in cells. Cells regulate when a particular protein is produced by switching gene expression on and off.
Metabolism	The biochemical reactions required to maintain life are organised into metabolic pathways allowing for the transformation of molecules in steps. Catabolic (digestion) reactions provide energy for anabolic (synthesis) reactions.
Apoptosis	Allows removal of damaged and diseased cells and is involved in maintaining cell numbers.
Reproduction	Cells replicate for growth and development via mitosis and produce gametes via meiosis. In order to do this DNA replication must take place.
Cell communication	Cells need to communicate to coordinate functioning of the whole organism.
Molecule transport	Nutrients and molecules are transported between organelles and into and out of a cell through osmosis, diffusion, active transport, endocytosis and exocytosis.

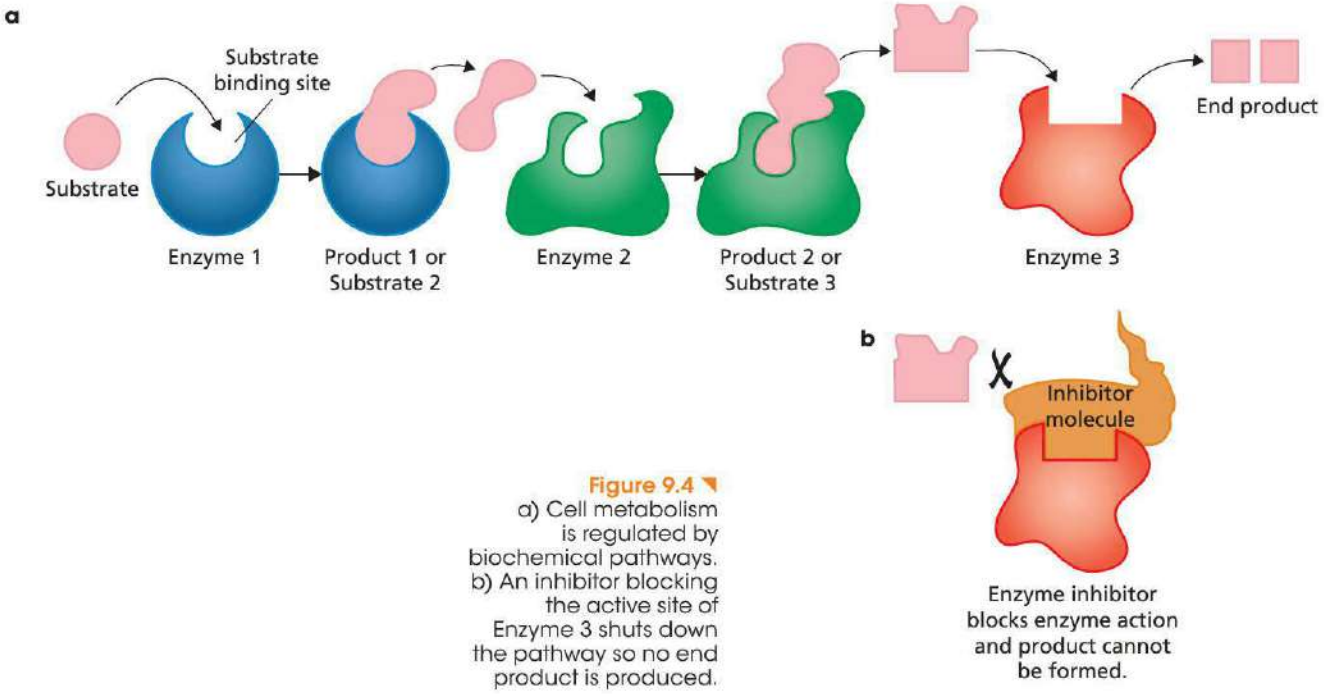


Figure 9.4 a) Cell metabolism is regulated by biochemical pathways. b) An inhibitor blocking the active site of Enzyme 3 shuts down the pathway so no end product is produced.

These cellular processes ultimately rely on **cell metabolism**, the collective biochemical reactions occurring in a cell to maintain homeostasis. As you can see in Figure 9.4, reactions are organised in **biochemical pathways**, with each reaction or step in the pathway controlled by an enzyme. At each step, substrate molecules are joined together, separated, or rearranged to produce new products. These reactions are monitored and balanced to meet supply and demand. Often a

pathway is **up-regulated** in response to increasing concentrations of substrate or to meet demand for a product. When the substrate becomes limiting, the pathway may be **down-regulated**. Sometimes the products of biochemical reactions cause down-regulation of a pathway in a process called **feedback inhibition**.

Cells regulate metabolism by controlling the production and activity of enzymes. To increase the amount of enzyme, the gene for that enzyme must be expressed. The activity of an enzyme can be altered by changing the **conformation** of the enzyme; for example, by adding a phosphate group. **Inhibitors** bind to enzymes, changing the conformation and rendering the enzyme inactive. When the inhibitor is removed, the enzyme becomes active once more. In a similar fashion, **activators** bind to enzymes to change their conformation so that they become active. By managing enzyme production and activity, and thus biochemical pathways, the cell can respond to changes in its environment and regulate its metabolism.

See Chapter 8 for more on homeostasis.

Many cell processes are controlled by cell metabolism that is regulated by enzymes. Chemical signals alter the action of enzymes and in doing so, regulate most cell functions.

QUESTION SET 9.1

Remembering

1 Name four charged ions utilised by cells in chemical signalling.
2 Ions cannot cross cell plasma membranes yet they are required for cell survival. Use an annotated diagram to illustrate how a primitive prokaryote regulates its internal calcium ion gradient.
3 Identify the cellular processes that maintain cell numbers in multicellular organisms.

Understanding

4 How did the evolution of ion pumps contribute to the development of cell signalling systems?
5 Draw a diagram to show how cell signalling can result in chemotaxis.
6 How has cell differentiation contributed to the evolution of multicellular organisms?
7 Draw a cell as a central part of a mind map and use branches to show the many different cellular processes that can occur in response to chemical signals.

Applying

8 Biochemical pathways in cells are used to maintain cell homeostasis. Figure 9.4 provides an example of a biochemical pathway that has three steps, each controlled by an enzyme. Copy this pathway and annotate it to show:
- the original substrate and the final product.
- how the final product can cause down-regulation of the pathway through feedback inhibition.
- how production, activation and inhibition of an enzyme can affect the amount of product produced.

Detecting and responding to signals

Cells detect and respond to external stimuli so they can activate changes in gene expression, enzyme activity and ion channel activity to generate **cellular responses** such as those seen in Table 9.1.

Signalling molecules are the key to controlling cell activities

Signalling molecules are effective in minute amounts and play important roles in homeostasis, growth and development, reproduction, behaviour, and energy production, storage and use. These

chemical signals can come into contact with many cells. But only specific **target cells** respond. What drives this specificity? For chemical signals to stimulate a response, they must bind to **receptors**, proteins that are located inside or on the surface of target cells. Just as you need the right-shaped key to open a lock, a specific signalling molecule can bind only to a specific receptor. Its shape and chemical properties allow it to fit into the **binding site** of the receptor protein. Once binding takes place, the receptor is activated and this triggers a cascade of processes inside the cell (Figure 9.5). The processes triggered depend on the receptor that has been activated. In this way the release of chemical signals controls specific responses in specific cells.

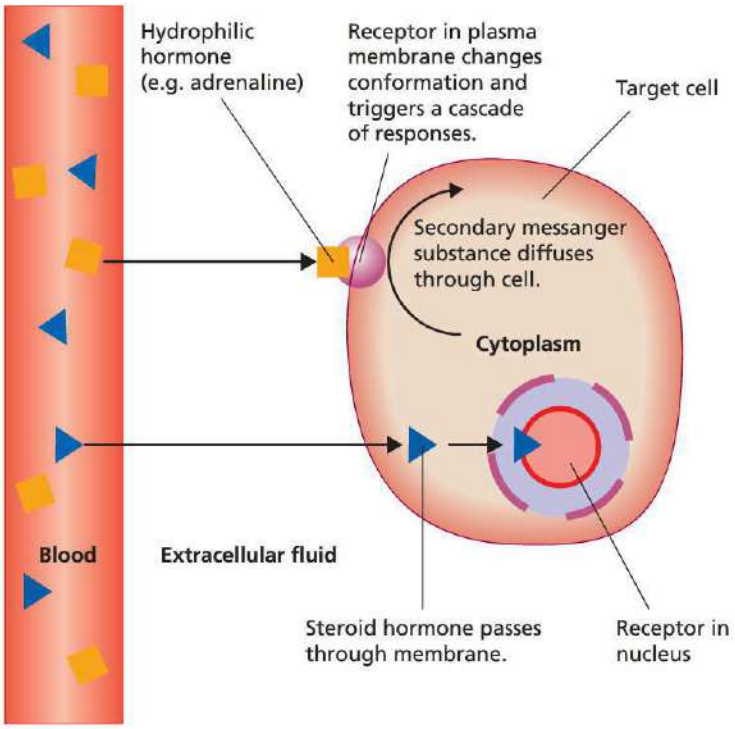


Figure 9.5 ▼ Hydrophilic hormones, such as adrenalin, bind to extracellular receptors to trigger a cascade of events inside the cell, leading to a cellular response. Hydrophobic hormones, such as the steroids, pass through cell membranes to bind to intracellular receptors leading to a change in gene expression.

Classifying chemical signals by their properties

Some chemical signals produced by multicellular organisms are **hormones**. How a hormone is produced and what it is made from govern its properties and behaviour. **Hydrophilic hormones** are water soluble and insoluble in lipids. **Hydrophobic hormones** (also called lipophilic hormones) are water insoluble and soluble in lipids. The solubility of a hormone determines how it is produced by cells, how it travels in fluids and how it exerts its effects at the target cell. Each target cell is surrounded by a plasma membrane, a phospholipid bilayer that serves as a boundary restricting the entry of large or hydrophilic hormones. The location of receptors on or within the cell varies depending upon the size of its binding hormone and whether the hormone is hydrophilic or hydrophobic and can readily pass through the plasma membrane.

Table 9.2 reveals how scientists categorise hormones into three groups based on their chemical structure: **steroid hormones**, **amine hormones** and **peptide hormones**.

Table 9.2 Hormones categorised into three groups based on their chemical properties and behaviours

Steroid hormones	Amine hormones	Peptide hormones
Synthesised from cholesterol in the gonads and adrenal gland	Synthesised from amino acids through the action of enzymes. Iodine is required so it must be supplied by the diet.	Chains of amino acids synthesised by ribosomes and packaged into secretory vesicles by the Golgi apparatus.
Hydrophobic, so they are lipid soluble and can cross cell membranes. They bind to intracellular receptors, travel in fluids attached to carrier proteins and cannot be stored, so are secreted as they are produced.	Most are hydrophilic and bind to extracellular receptors. The exception is the thyroxines, which are hydrophobic and bind to intracellular receptors.	Hydrophilic, so they cannot cross cell membranes. They bind to extracellular receptors.
The adrenal gland produces cortisol and aldosterone. The testes and ovaries produce testosterone and oestrogen.	Hydrophilic examples include adrenaline (known as epinephrine in the US) produced by the adrenal gland and by some nerve cells, dopamine produced by some nerve cells, and melatonin produced by the pineal gland. They also include a group of hydrophobic hormones called thyroxines that are produced by the thyroid gland.	Produced and secreted by exocytosis from cells in a range of tissues and glands including leptin by fat tissue, insulin by the pancreas, growth hormone by the pituitary, and antidiuretic hormone (ADH) produced in the hypothalamus and secreted from the pituitary.

The problem with this chemical classification system of hormones is how to categorise newly discovered chemical signals if they don't quite fit into existing categories. There is now mounting evidence that a fourth group of signalling molecules should be added to this list: the dissolved gases including nitric oxide, carbon monoxide and hydrogen sulfide. Scientists are still debating how to categorise this group of signalling molecules.

WOW

Preventing rotten egg gas production in cells may starve tumours

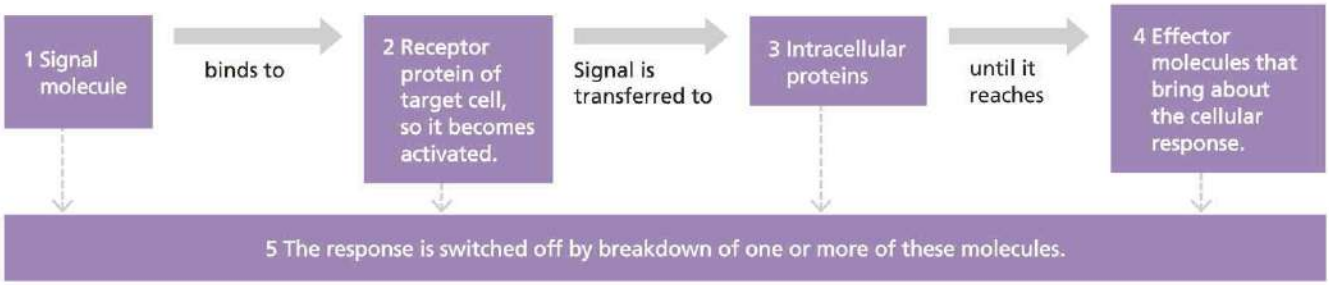
Incubating vascular cells with hydrogen sulphide gas (H_2S) ***in vitro*** stimulates an increase in cell division and migration to form new blood vessels. Some cells have an enzyme so they can produce the signalling molecule H_2S from the amino acid cysteine. Scientists are exploring the possibility of inhibiting the activity of this enzyme in tumour cells. By inhibiting the production of H_2S, scientists hope to stop the growth of new blood vessels that should starve the tumour cells and halt their growth.

Transduction of the signal

When a signal molecule binds to its receptor protein the receptor becomes activated. The activated receptor alters the activity of intracellular proteins, setting off an intracellular signalling cascade, so the message is transmitted through the cell in a process called **signal transduction**. The message is transmitted to **effector** proteins in the cell, which are stimulated to elicit a response.

Chemical signals initiate an effect on target cells by binding specifically to receptor proteins. When a signal binds to its receptor it causes a change in receptor conformation. This triggers a series of reactions in the cell, known as signal transduction, so the message is transmitted to effector proteins that cause a cellular response.

▼ **Figure 9.6**
The principles of signal transduction



Applying the principles of signal transduction to coral reproduction

Every year around the full moon in April the mass spawning of more than 250 species of coral occurs at Ningaloo reef in Western Australia. The corals simultaneously release their eggs and sperm, creating an underwater gamete storm. With so many eggs and sperm released from so many different species, how do the right eggs and sperm find each other?

Coral sperm are not able to move until an egg from the same species is nearby. The eggs secrete signalling molecules that will bind only with receptors on sperm of the same species. The signalling molecule has **binding specificity** for this particular receptor. Binding of the signal activates the receptor, the message passes into the cell and the activities of proteins inside the

APOPTOSIS

Learn about apoptosis, or programmed cell death for removal of damaged and diseased cells.

sperm are altered. This triggers a cascade of events so that the signal is transmitted to the protein machinery of the flagellum. The flagellum fires up to move the sperm towards the egg. In this case, the flagellum is the effector bringing about the desired response in the sperm. The sperm tracks the position of the egg by chemotaxis, following an increasing concentration gradient of signalling molecules secreted by the egg.

How cells respond to signals depends on the set of receptor and signal transduction proteins they each express, and the signalling pathways followed in the cell. The net effect of all of these signals is to regulate the function and behaviour of the cell (Figure 9.7).

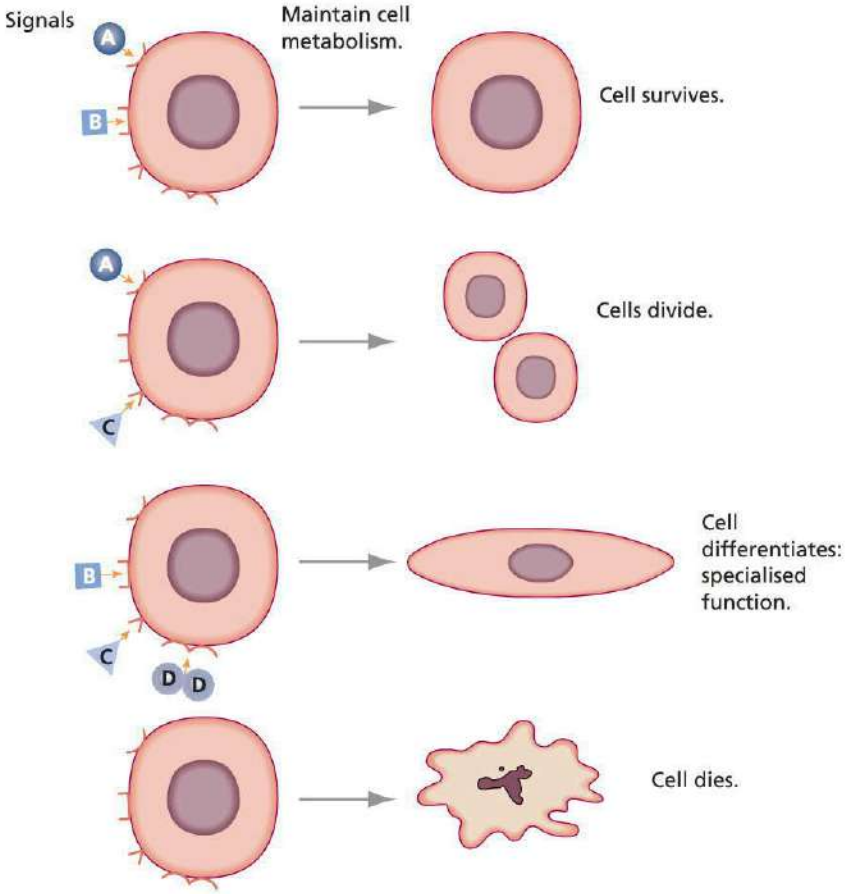


Figure 9.7 ▶ Signals act collectively to influence cell activity. Different combinations of signals activate different responses for maintenance and survival of the cell, cell division and differentiation. Withdrawal of some signalling molecules can induce apoptosis.

QUESTION SET 9.2

Remembering

1 Annotate each of two receptor proteins to indicate how the shape and properties of its binding site is complementary to its specific signal molecule.

2 Recount why signalling molecules can only exert an effect on target cells.

3 Recall the principles of signal transduction.

Understanding

4 Construct a chart to compare and contrast the three categories of hormones based on chemical structure. You should include how they are synthesised, whether they are hydrophilic or hydrophobic, and how they travel in the body fluids, and give examples of each category.

5 Use the principles of signal transduction to construct a flow chart that demonstrates how a sperm swims towards an egg from the same species of coral to coordinate fertilisation during mass spawning events.

6 Distinguish between the location of the cell receptor for a hydrophobic and a hydrophilic hormone.

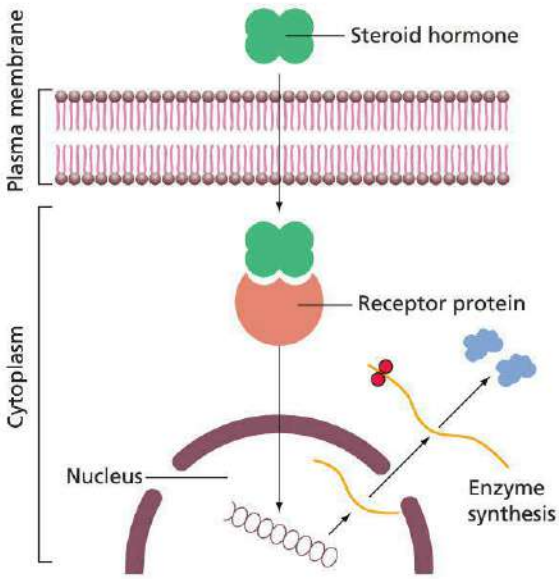
Hormone signalling pathways

Hydrophobic hormones such as steroid and thyroid hormones pass through cell membranes and bind to and activate **intracellular receptors** found in the cytoplasm and nucleus. These intracellular receptors are often transcription factors, which become active only when the hormone is bound, and can then bind to DNA and regulate gene expression.

Have you ever considered how carnivores get the glucose required to fuel respiration pathways when they don't eat starchy foods? Or how a fasting person can continue to respire even when glucose reserves have been depleted? During starvation, the steroid hormone cortisol is produced by the adrenal gland. This hormone exerts a range of actions to increase and maintain blood glucose levels. It stimulates glucose synthesis from amino acids and glycerol in the liver, stimulates release of fatty acids from adipose tissue, and inhibits glucose uptake by muscles and adipose tissue. How does cortisol exert so many different effects on different cells of the body?

In liver cells, cortisol binds to a transcription factor that regulates the expression of genes coding for enzymes that control biochemical pathways for building glucose from amino acids and glycerol. This binding changes the conformation of the transcription factor. It becomes activated to enter the nucleus and bind to its specific DNA region to switch on gene expression. Enzymes are then produced and the pathway is up-regulated (Figure 9.8). While cortisol switches on one particular set of genes in the liver, it binds to different transcription factors in adipose tissue; therefore, a different set of genes is switched on, a different set of enzymes is made and a different biochemical pathway is up-regulated. As a result, the response of adipose cells is different to the response of liver cells.

▼ **Figure 9.8**
The steroid hormone cortisol binds to a transcription factor in the liver cell, activating gene expression. The result is the production of a set of proteins needed to make glucose from amino acids and glycerol.



WOW

Supermice

A group of researchers has produced a strain of 'supermice' that are leaner, fitter and live longer than control mice. The supermice have increased production of an enzyme that allows them to use fatty acids as an energy source in muscle cells. Generally, gene expression of this enzyme is regulated by steroid hormones, so it is only produced in times of starvation when glucose levels are low. This signalling pathway is bypassed in the supermice and the gene is constantly expressed. The supermice can run for 6 hours while control mice only last 10 minutes. They eat 60% more food but weigh 40% less and they have one-third the amount of fat. They are also more aggressive.

Hydrophilic hormones bind to extracellular receptors

Peptide hormones and hydrophilic amines are signals that cannot pass across the cell membrane due to charge and size. They bind to **extracellular receptors** that are embedded in the cell membrane.

Extracellular receptors are generally grouped into three categories based on how they transfer a message into a cell, although some receptors cannot be categorised in this way.

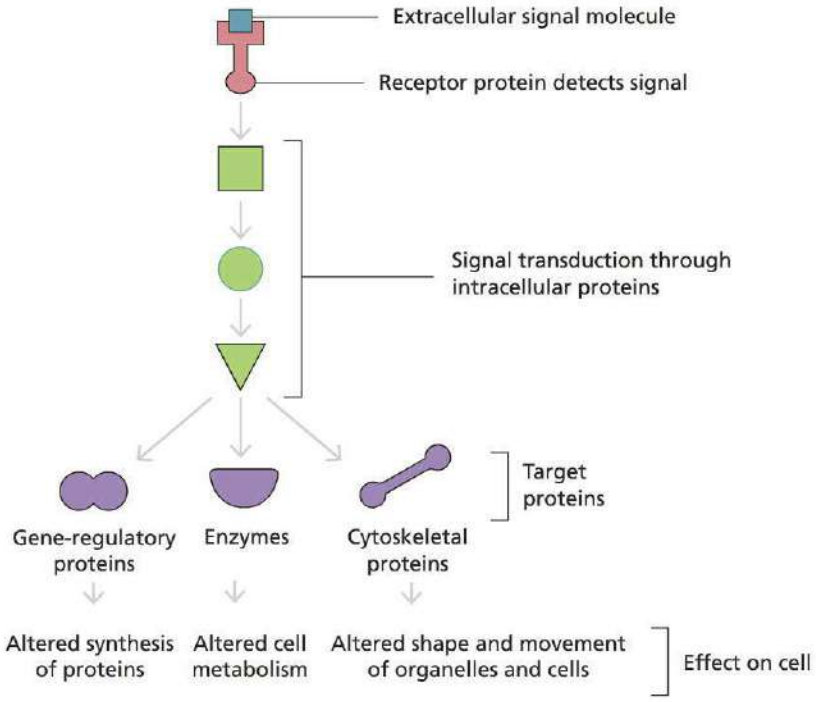
1 Ion channel receptors: When a signal binds to an ion channel protein, the channel opens so that ions can be transported across the cell membrane. This response is fast, as the cell responds quickly to changes in ion concentrations.

2 G protein-coupled receptors: These receptors have a protein called a G protein attached to them on the inside of the cell membrane. When a signal binds, the receptor changes conformation and the G protein is released and activates other proteins in the signal transduction pathway.

3 Tyrosine kinase receptors: These receptors are inactivated enzymes embedded in the cell membrane. Binding of a signal activates these enzymes by changing their conformation, usually by removing a phosphate group from an adenosine tripshosphate (ATP) molecule and adding it onto a protein (**phosphorylation**) that produces adenosine diphosphate (ADP) as a by-product. Protein phosphorylation switches on signal transduction pathways that ultimately lead to changes in gene expression inside the cell.

The activation of extracellular receptors triggers a cascade of modifications of cellular molecules and proteins, activating or inactivating them in the process called signal transduction (Figure 9.9).

Figure 9.9 ▶ Signal transduction pathway through a cell



Hydrophobic signals, steroids and thyroid hormones cross membranes to bind to intracellular receptors that regulate gene expression. Hydrophilic signals, peptides and hydrophilic amine hormones cannot cross membranes, so they bind to extracellular receptors that initiate a variety of responses in the cell.

Second-messenger systems relay and amplify the signal

The binding of hydrophilic hormones to extracellular receptors stimulates rapid production of a **second messenger** within the cytoplasm of the cell. With a domino effect, this second messenger then stimulates the activity of specific proteins within the cell that bring about its response.

Why is such a system advantageous to a cell? Imagine your neighbour's house is on fire. What do you do? Ring 000! The operator will then contact firefighters, police and paramedics, who will come to the scene. The second messenger in this scenario (the 000 operator) serves to spread the message to all the parties necessary to put out the fire and prevent further harm. Compare this to the responding cell. Only a small amount of hormone (maybe just one molecule) is necessary to notify the second messenger, which then leads to activation of a range of chemicals in the cell.

Second messengers are usually small molecules. The first one to be discovered was **cyclic adenosine monophosphate** (cyclic AMP or **cAMP**) produced in response to adrenaline binding to a G protein-coupled receptor on liver cells. On binding, the conformation of the receptor changes, leading to the activation of adenylate cyclase. This enzyme begins generating large amounts of cyclic AMP from ATP. The cAMP second messengers rapidly diffuse away to broadcast the signal to proteins in other parts of the cell so glucose is released from glycogen stores. cAMP is now known to be a second messenger in many signalling pathways, generating responses that can include switching genes on and off, moving vesicles around the cell or activating enzymes.

Just one hormone molecule can result in the production of many second messengers. This amplifies the response, which explains why hormones are active in such small quantities. The response triggered by second messengers depends upon the proteins that they interact with. In the case of cAMP, it activates enzymes called protein kinases. Kinases are enzymes that add phosphate groups to proteins. The cell response depends upon the proteins being phosphorylated. In this way, the same second-messenger system can produce a different response in one cell compared to another.

There are three major second-messenger systems now recognised in cells. These include phosopholipid derived molecules (e.g. inositol triphosphate), ions (e.g. Ca^{2+}) and cyclic nucleotides (e.g. cAMP).

Hormones are effective at very low concentrations, as binding of hormone to receptor initiates production of second messengers that amplify the response in a cell.

Effector proteins direct the cellular response

Activation of target proteins will lead to the cellular response, which can occur by one of two fundamental mechanisms: activation of proteins within the cell, or changing the expression of genes within the cell so that certain proteins are produced, or not produced (Figure 9.9). Effector proteins that elicit a response include gene regulators, ion channels, enzymes and parts of the cytoskeleton.

The signal is terminated

After signalling has been initiated and the message has been transduced to effect a response in the cell, the message must be switched off. This is important so that cells maintain their responsiveness and to avoid overstimulation that can lead to disease. One way of shutting off the response is to quickly clear up the second messenger. Another way is to dephosphorylate proteins by removing phosphate groups. Phosphatases are a group of enzymes that remove phosphate groups from proteins.

CELLS RESPONDING TO SIGNALS

Follow the journeys of selected steroid, amine and peptide hormones as they are produced and secreted, and then travel to exert an effect on their target cells.

QUESTION SET 9.3

Remembering

1. Create a timeline of events to recount how steroid hormones stimulate a response in cells.
2. Make a chart that identifies up to four cell responses and the effector proteins involved in coordinating these responses.

Understanding

3. Copy Figure 9.8 and provide a new caption that explains the different response stimulated by cortisol (a steroid hormone) in liver cells compared to fat cells.
4. Write a job description for a second messenger, outlining its responsibilities in cell signalling.
5. Construct a list that outlines the steps involved in signal transduction for a hydrophilic hormone initiating a response in a cell.
6. Discuss why only a small amount of hormone is required to initiate a response in a cell.

Signalling molecules in animals

Animals are multicellular organisms that use a variety of chemical and electrical signals in a communication network to coordinate individual cells to support the organism as a whole. Animal communication networks involve two systems: the nervous system and the endocrine system. Rather than being two separate systems, they are integrally related and follow the same principles of cell communication. They both rely on extracellular signalling molecules that are produced and released by cells to signal to other cells that will ultimately respond and effect cell behaviour.

Some chemical signals can be produced ahead of time and stored for later use while others cannot be stored and must be produced on demand. Similarly, cells can receive signals that stop the production and/or release of signal molecules. Signal molecules continue to stimulate target cells until the signal is deactivated or removed. Some signals degrade quickly so their effect is very short, while others have a prolonged effect.

Many signal molecules are secreted from cells by exocytosis while others diffuse across the plasma membrane through passive or facilitated transport. The chemical signal then diffuses through the extracellular fluid or is transported in the circulatory and lymphatic systems to arrive at target cells around the body. Some signal molecules remain tightly bound to the surface of the cell producing them, so they only influence other cells through direct cell to cell contact.

Classifying chemical signals by the distance they travel

Chemical signals can be classified according to the distance over which they travel to exert their effect (Figure 9.10).

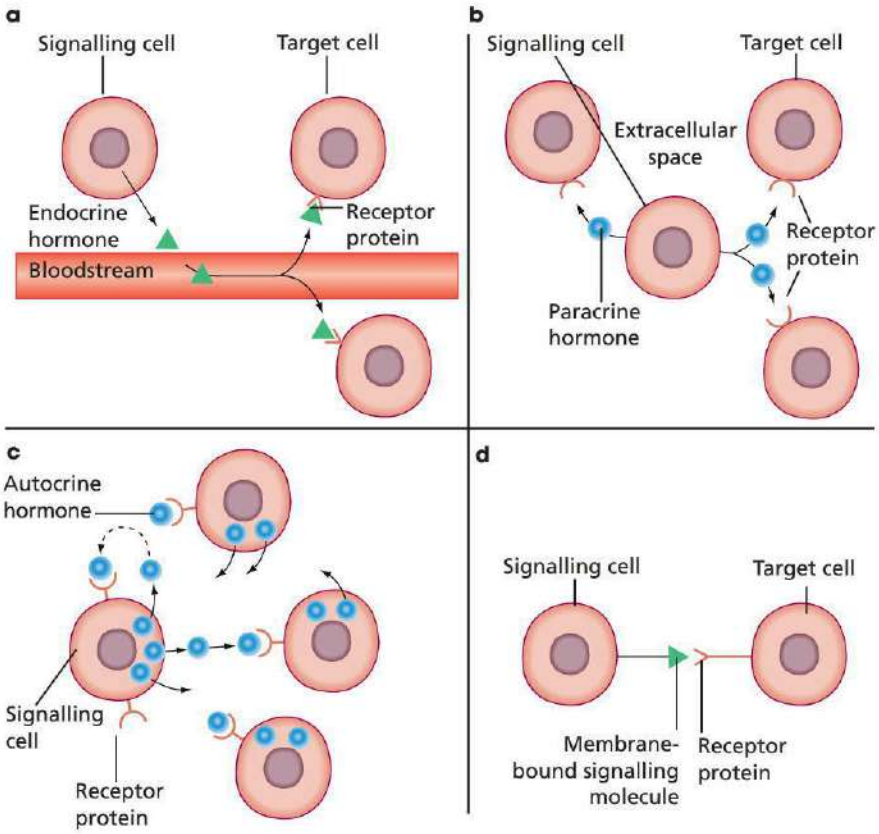


Figure 9.10 ▶ Chemical signals can be classified by the distance over which they travel to their target cells. a) Endocrine signals travel in the blood to reach target cells throughout the body. b) Paracrine signals act locally on neighbourhood cells. c) Autocrine signals act on the cell that secreted them or cells of the same type. d) Contact-dependent signalling requires cells to be in direct contact.

Contact-dependent signalling

As the name implies, **contact-dependent signalling** requires direct cell to cell contact (see Figure 9.10d). The signal molecule is anchored in the membrane of a cell and interacts with a receptor molecule located in the membrane of another cell. This type of contact-dependent signalling provides cross-talk between cells integral – for example, during embryonic development – for maintaining the size and architecture of adult organs, and for apoptosis.

In 1953 Abercrombie and Heaysman were interested in observing the social behavior of cells. They grew chicken cells in culture dishes. They noticed that when cells collided they did not crawl over one another. Rather, they behaved like dodgem cars, changing direction after a collision. As the number of cells in the dish increased, collisions became more frequent, causing cell motility and replication to slow and stop, with the cells forming a monolayer. This demonstrated that cells have a spatial awareness signalling system that operates through contact inhibition, mediated by a group of membrane proteins called the cadherins, **adhesion proteins** that influence the dynamics of the cell cytoskeleton required for a cell to move and replicate.

Contact inhibition of cell growth is essential for wound healing, to ensure cells move in and fill a gaping wound. It is also necessary for maintaining the size and shape of your organs. In cancer cells, signalling pathways for contact inhibition become disrupted, and cell division and movement become uncontrolled even when in contact with neighbouring cells. Cells no longer change direction or stop dividing on contact so they start to pile up and grow over each other, forming a tumour. Cells can also leave the tissue of origin and invade neighbouring tissues or travel to new organs around the body. This is called metastasis. These malignant cells acquire motility as they stop responding to migration inhibition signals exerted by neighbouring cells.

Local communication using autocrine and paracrine hormones

Autocrine hormones are secreted and act directly on the cell, or cells of the same type (see Figure 9.10c). Thus, they use a feedback mechanism to regulate their own activity. **Paracrine hormones** are local regulators that are used for communication between neighbouring cells (see Figure 9.10b). This signalling occurs when a cell secretes regulatory substances into the surrounding interstitial fluid, affecting only nearby target cells.

Prostaglandins are autocrine and paracrine hormones made from fatty acids. They are involved in regulating the contraction and relaxation of smooth muscles lining the blood vessels, gut, respiratory tract, bladder, uterus and eye. They induce a variety of signalling outcomes including the relaxation of blood vessels allowing for greater flow of blood, when needed, and the constriction of uterine muscles to induce labour. Prostaglandin action has also been implicated in less desirable outcomes, such as the constriction of bronchioles observed in asthma.

Long-distance communication using endocrine signalling

In many cases, the cells that produce the signal are not in the same part of the body as those that respond. Thus, information or signals must be transmitted to the cells that need to respond. How is this achieved? The endocrine system comprises a collection of glands that secrete chemical signals into the blood. The circulatory system and lymphatic system are used to transport these **endocrine hormones** throughout the body, but the hormones exert their effect only on specific target cells (see Figure 9.10a). Leptin is an endocrine signalling molecule produced and secreted by fat cells in adipose tissue. It travels in the blood to reach the brain where it helps to regulate food intake and body weight. Figure 9.11 shows the dramatic result observed when a mouse cannot produce leptin.

▼ **Figure 9.11**
The mouse on the left has inherited two copies of a mutant allele for an obesity gene, so it cannot produce the hormone leptin.

Science Photo Library/Oak Ridge National Laboratory

Hormones can be classified by the distance they travel. Contact-dependent signalling relies on direct cell to cell contact, autocrine and paracrine hormones are secreted into the interstitial fluid to stimulate cells locally, and endocrine hormones travel in the blood and lymph to arrive at and stimulate distant cells.

Scientific literacy: Increasing milk yield in dairy cattle

Somatotropin (ST) is a peptide growth hormone secreted by the pituitary gland of all mammals. It stimulates liver cells to produce and secrete a hormone called insulin-like growth factor (IGF-1). Circulating IGF-1 binds to receptors in target cells, activating signalling pathways that promote cell growth and proliferation, and inhibit apoptosis. In the late 1980s a synthetic version of bovine somatotropin (BST) was produced using recombinant DNA technology. This peptide, named rBST, is identical to the natural peptide found in cows. Injecting dairy cattle with rBST increases circulating levels of IGF-1. This hormone inhibits apoptosis of milk-producing cells and thus more milk is produced over an extended time.

Shutterstock.com/MarcelSchauer

Figure 9.12 ▲
International debate has raged for more than a decade as we try to decide whether synthetic growth hormone should be used to increase milk yield in dairy cattle. Its use is currently banned in Australia.

Amid much controversy the drug was approved by the US Food and Drug Administration (FDA) for use by dairy farmers in 1993. The controversy continues to this day. rBST is not approved for use in Australia, the United Kingdom, Canada and the European Union as there are some health and animal welfare concerns.

First, it is not known if rBST affects the health of consumers of milk. The rBST peptide has a different shape to human ST so it cannot bind to human ST receptors. Furthermore, both BST and rBST are denatured by the pasteurisation of milk and are digested by humans, so they would not enter the circulatory system. The main health concern is that cows treated with rBST have elevated levels of IGF-1 in their milk. IGF-1 survives pasteurisation and digestion, so it can enter the human circulatory system. As hormones are dose sensitive, there were concerns that elevated IGF-1 concentrations in milk could promote cancer in humans. In some studies elevated IGF-1 levels in humans have been implicated in increasing the growth rate of cancers. To date, scientific studies have not found a correlation between drinking the milk of rBST treated cows and the incidence of cancer. However, the public remains sceptical.

Second, there were concerns with animal welfare. Increasing milk yield in dairy cattle can lead to increased incidence of mastitis, a bacterial infection of the udder requiring treatment with antibiotics. Cattle treated with rBST can also suffer injection-site reactions, and are more likely to experience lameness and have reproductive problems. Again, the results of scientific studies into these concerns are conflicting.

Many argue that rBST would allow farmers to produce more milk or produce the same amount of milk using fewer cows. This would lower production costs and increase profit. This could also have a positive effect on the environment as fewer cows means less methane gas enters the atmosphere. Recent studies claim that rBST treated cattle also produce less gas.

Australian scientists are using selective breeding techniques to improve milk yield in dairy cattle. Variation in the gene coding for the BST receptor has been associated with milk yield. The scientists use single nucleotide polymorphism technology to select cows that have receptors that bind BST with high affinity. These cows are then bred so their offspring also have the more efficient receptor, resulting in increased milk production.

Questions

1 What is rBST?

2 Construct a flow chart that shows the mechanism of increasing milk yield by injecting cows with rBST.

3 Discuss concerns about producing milk with elevated levels of IFG-1 even though scientific studies have not revealed a correlation between drinking this milk and adverse health effects.

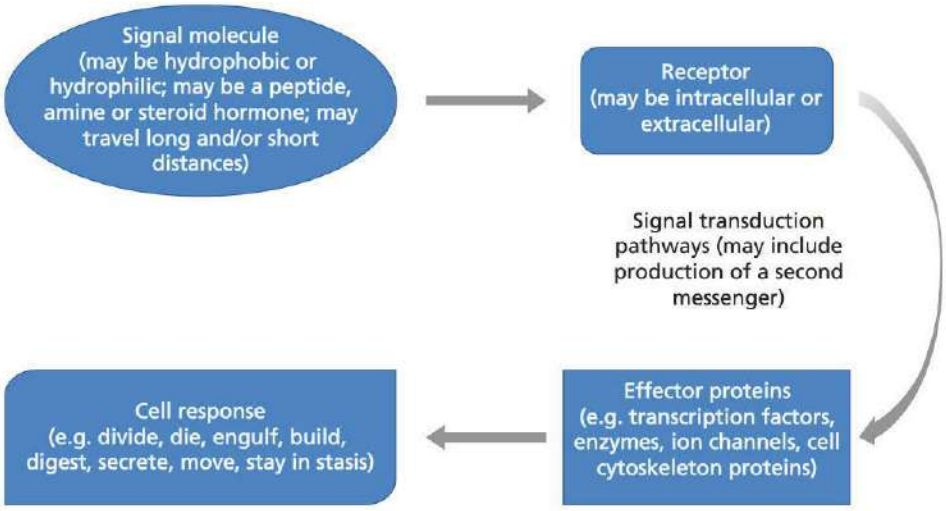
4 Some companies in the US started to label their milk as BST free. Discuss why this labelling is misleading and why it was banned by the FDA.

5 Would you approve the use of rBST in Australia? Construct a table to evaluate the risks and benefits from the perspective of the following, and then make your decision.
   - **a** Dairy cattle
   - **b** Consumers
   - **c** Economics
   - **d** Environment

6 In Australia, DNA technology can be used to select for dairy cattle with BST receptors that bind BST more efficiently. How does this trait lead to increased milk yield in cows? Does this practice eliminate all of the concerns associated with the use of rBST?

Putting it all together: using stimulus–response models to represent cell signalling pathways

Stimulus–response models can be applied to represent signalling pathways in cells.



◀ **Figure 9.13**
Stimulus–response models can be used to represent signalling at the cellular level.

Let's explore this by following the signal transduction pathway induced when antidiuretic hormone (ADH) reaches a target kidney cell. As we saw in Chapter 8, ADH is secreted from the pituitary gland when the hypothalamus senses a drop in blood volume. ADH travels in the blood to the kidneys where it acts to increase water absorption so blood volume increases. Let's follow the stimulus–response pathway stimulated by ADH in cells of the kidney collecting duct (Figure 9.14, page 264).

1 The stimulus, ADH, binds to an extracellular receptor, a G protein-coupled receptor called V2.
2 This binding causes a change in receptor conformation that leads to activation of an enzyme, adenylate cyclase, which is located on the inner side of the cell membrane. It starts to convert ATP molecules to cAMP in the cytoplasm. Many cAMP second messengers are produced here. The message is amplified.
3 The cAMP molecules (second messengers) initiate a signal transduction cascade by activating protein kinases. These enzymes phosphorylate target proteins that stimulate exocytosis of vesicles containing aquaporin channels. These water channels are inserted into the cell membrane so water leaves the kidney and moves into the blood.
4 After the cell has responded, intracellular enzymes degrade the cyclic AMP and, over time, the aquaporin channels break down.

Stimulus–response models can be applied at the cellular level to demonstrate a chemical signal (the stimulus) binding to its receptor, signal transduction (including any production of second messenger and changes to intracellular proteins), and stimulation of an effector to elicit a cellular response.

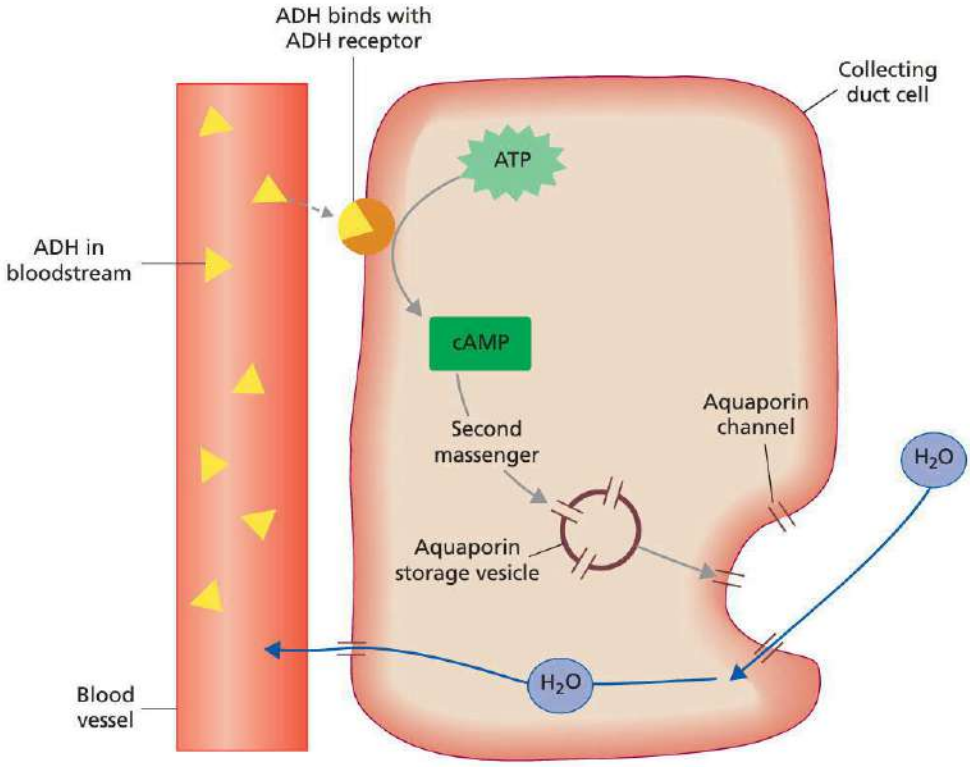


Figure 9.14 ▶ Antidiuretic hormone signals to cells in the collecting ducts of the kidney to reabsorb more water into the blood. Aquaporin channels are stored in vesicles in collecting tubule cells of the kidney. The signal transduction pathway is elicited when ADH binds to its receptor.

QUESTION SET 9.4

Remembering

1 Construct a table indicating how chemical signals can be classified by the distance they travel to reach target cells. Include some examples for each category in this table.

2 Distinguish between a receptor and an effector.

Understanding

3 Use a schematic to represent your understanding of how the production, storage, secretion and degradation of chemical signals affect the time taken before a cell will respond, and the length of time for which a cell will continue to respond.

4 Predict how prostaglandins could be employed to induce labour in a pregnant woman.

5 Describe how both contact-dependent signalling and paracrine signalling are important for the healing of wounds.

6 Refer to Figure 9.14 (showing the effect of ADH on cells in the collecting duct of the kidney) to explain the following.

- a Whether ADH is hydrophobic or hydrophilic
- b Whether ADH is an autocrine, paracrine or endocrine signalling molecule
- c The role of cyclic AMP in this pathway
- d The role of protein kinases in this pathway
- e The cell process stimulated to effect more water reabsorption into the blood

7 Construct a stimulus–response model to represent the chain of events that occur at the cellular level when ADH binds to receptors in a kidney cell.

Communication through electrical signals

One of the major components of an effective signalling system in higher order animals is the nervous system, a circuitry of specialised cells that transmit electrical impulses around the body. Peripheral nerves pick up messages and transmit them to the central nervous system (CNS) for processing. They also deliver messages to muscles lining glands, skeletal muscles, hairs and ducts in a response coordinated by the CNS. The brain alone contains one trillion **neurons** that are connected to form circuits. To function within this circuit a neuron must receive, process and relay signals to other neurons. Other cells in the brain are glial cells that provide a scaffold to support neurons. A constant dialogue between neurons and glial cells is essential for the computational operation of these circuits. For messages to be sent along the correct pathway some cells act to block transmission along one pathway while others act to transmit a message along another pathway.

SEE-THROUGH BRAINS

Scientists can now remove the fats from brains to make them transparent. This allows them to use molecular markers to distinguish between particular neurons, or to compare cells of diseased and healthy brains.

WOW

Can electricity revive the dead?

Prior to penning *Frankenstein*, Mary Shelley had been listening to scientific discussions about the work of Galvani who could use electricity to stimulate movement of a dissected frog's leg. This spurred philosophical discussions with her husband, the poet Percy Shelly, and their friend, Lord Byron, on using electricity to revive the dead. In her novel, a scientist creates a man by stitching together stolen parts of cadavers. He then harnesses electricity from lightning to bring the creature to life. Today there is a blurring of the lines between fact and science fiction. Think about this. Does electricity help us to revive the dead? Can we use knowledge of chemical signalling to build body parts?

The signal: initiating the electrical message

Many stimuli can initiate an electrical message; stimulation can come from an external receptor organ, certain chemicals or even physical stimulation, such as a pinch. In all cases, the message is initiated by opening up ion channels in the cell membrane, thus allowing the movement of sodium and potassium ions across the membrane. How does this then result in an electrical impulse?

BIOENGINEERED KIDNEY MAKES URINE

Scientists can strip cells out of donated organs, such as kidneys, leaving a scaffold. The scaffold is populated with stem cells under conditions mimicking those in the body. The end result is a functioning bioengineered kidney that can be transplanted into the stem cell donor.

The electrical impulse

Neurons transmit information in the form of an electrical impulse, much like electricity travels through wires to the various appliances in our house.

What happens to an axon when an impulse passes along it? To follow this we need to study the cell membrane of the axon. The cell membrane of an axon is polarised (i.e. there is a potential difference in charge between the inside and the outside of the cell). An axon that is at rest (not transmitting an impulse) is negatively charged inside relative to the outside. This is called a **resting potential**. To attain this polarised state, **sodium–potassium pumps** in the cell membrane actively pump sodium ions out of and potassium ions into the cell. The pump works in such a way that three sodium ions are expelled for every two potassium ions pumped in. Consequently, an excess of sodium ions accumulates outside and potassium ions inside. Although both sodium and potassium ions are positive, this difference results in the inside being negative relative to the outside.

What happens when an electrical impulse arrives? The membrane suddenly becomes permeable to sodium ions that, being about 10 times more concentrated on the outside than the inside, diffuse in rapidly. This depolarises the membrane, reversing the resting potential. The inside of the axon is now positive relative to the outside. This is called an **action potential** and takes place in a millisecond.

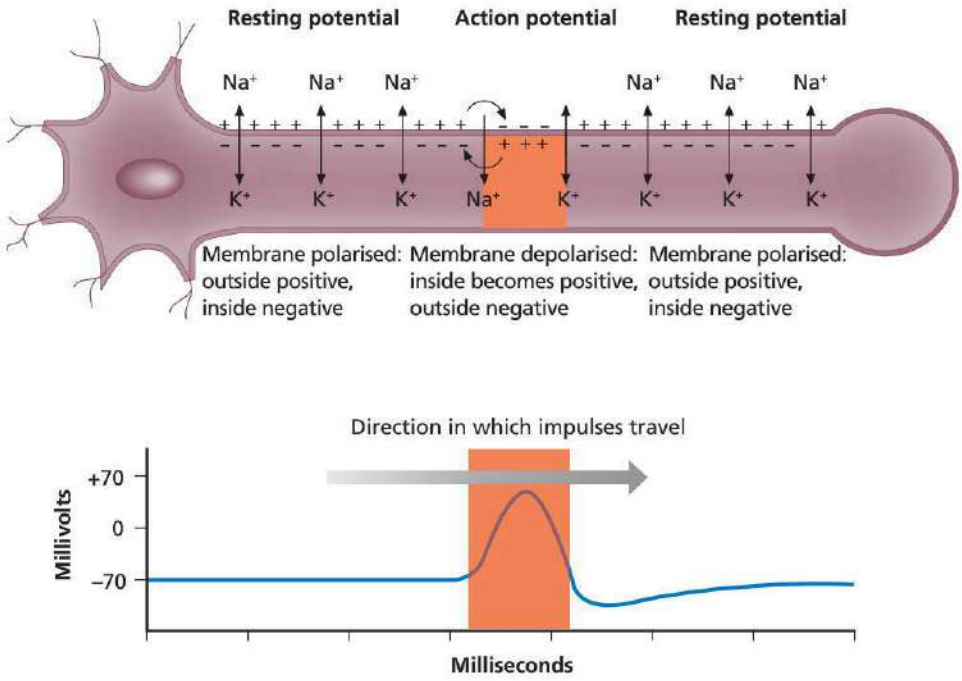


Figure 9.15 ▶ Transmission of impulses along a neuron

As the sodium ions enter, the potassium ions begin to leave: this is the start of the recovery process. The sodium–potassium pump mechanism now restores polarisation by pumping out sodium ions and pumping in potassium ions. As a result the distribution of ions that normally exists when the axon is at rest is reinstated; that is, the axon is returned to its resting potential.

Transferring the message

Once an action potential has been generated, it is necessary to relay that along the axon, which can be up to a metre long. What keeps the message going within the neuron itself? And once at the end of that neuron, how is the message transmitted to the next cell?

Relaying the signal along the axon

See page 216 in Chapter 8 for more information about transmission along the axon.

On depolarisation, specific channel proteins in the next part of the cell membrane open up, so sodium ions diffuse into the cell. Once depolarisation is complete, the channels close and the influx of sodium ions ceases. Thus, depolarisation moves progressively down the axon. When axons are protected by a **myelin sheath**, the depolarisation wave jumps from one gap of exposed axon membrane to the next. These gaps between Schwann cells (in the PNS, or oligodendrocytes in the CNS), called nodes of Ranvier, are the only part of the membrane that become depolarised and because of this jumping between nodes, the impulse travels much faster along a myelinated axon.

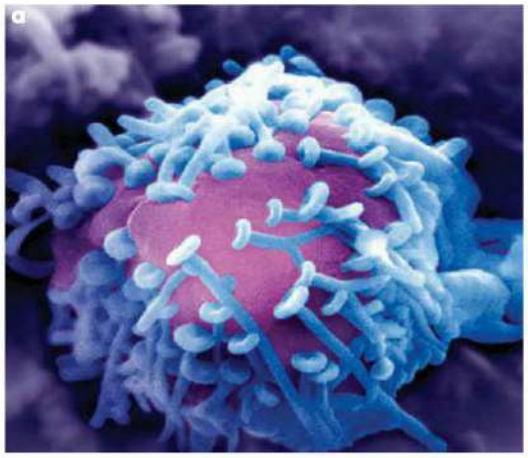
Neurotransmitters: bridging the gaps

An impulse travels to the end of the axon but neurons do not actually touch; there is a gap between them known as the **synaptic cleft**. The electrical message is transmitted to the next neuron through the use of chemical messengers.

The end of each axon is rounded to form a synaptic knob (Figure 9.16). The end of the synaptic knob is called the presynaptic membrane, the gap between the synaptic knob and the adjoining neuron is called the synaptic cleft, and the membrane on the far side is called the postsynaptic membrane. The cleft is approximately 20 nm across.

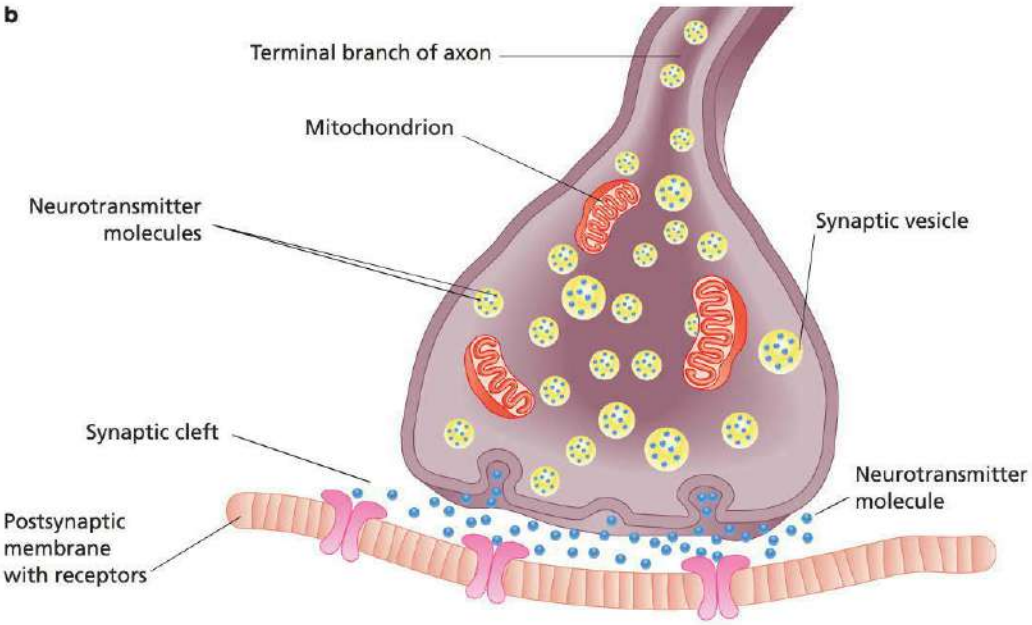
The presynaptic membrane contains numerous mitochondria and secretory vesicles that contain a chemical **neurotransmitter**. When an impulse arrives at the synaptic knob, it causes calcium ion channels in the cell membrane to open. Calcium ions diffuse into the synaptic knob from surrounding tissue fluid and stimulate exocytosis of secretory vesicles containing neurotransmitters. These vesicles merge with the presynaptic membrane and release their contents into the synaptic cleft. The neurotransmitters then diffuse across the cleft to bind

with specific receptors in neighbouring neurons. This causes ion channels to open up, allowing sodium ions to diffuse from the cleft into the postsynaptic neuron, causing partial depolarisation. If sufficient channels are opened, an action potential will be initiated. Once the neurotransmitter has activated the protein channels, it is important that it does not continue to stimulate the postsynaptic neuron. Thus, excess neurotransmitter in the synaptic cleft is deactivated by enzymes and recycled into the synaptic knob of the presynaptic neuron. This is summarised in Figure 9.17.



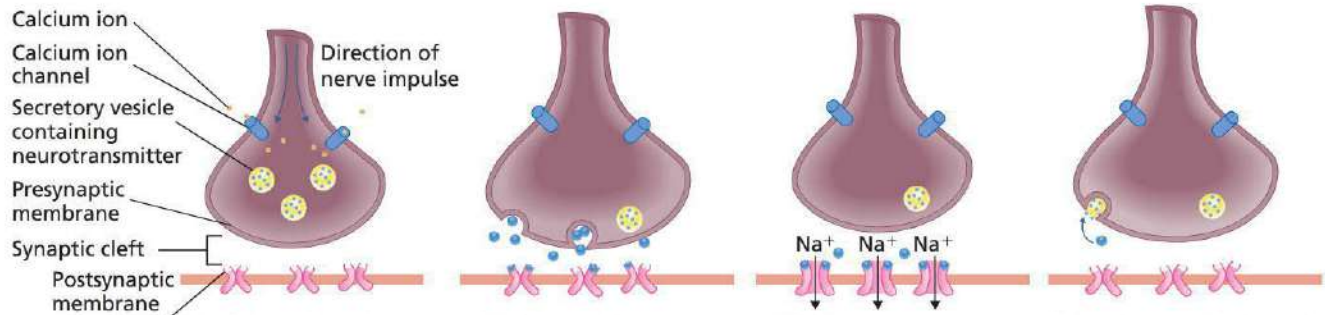
Visuals Unlimited/Science VU/Lewis-Everhart-Zeevi

◀ **Figure 9.16**
Neurons connect with each other by synapses. a) Scanning electron micrograph of synaptic knobs; b) The structure of a synapse



Generation of an action potential in a neuron results in an electrical impulse being relayed along its axon. The impulse is transmitted to other neurons or glands by neurotransmitters that are released into the synaptic cleft and bind with receptors in the postsynaptic membrane.

▼ **Figure 9.17**
Signal transduction across a synapse



1 An impulse arrives, stimulating calcium ion channels to open. Calcium ions diffuse into the synaptic knob and stimulate exocytosis of secretory vesicles containing neurotransmitters.

2 Vesicles release neurotransmitters into the synaptic cleft. The neurotransmitters bind to receptor binding sites on sodium channels.

3 Binding of neurotransmitters opens sodium channels so sodium flows into the postsynaptic cell, generating an action potential.

4 Neurotransmitter degrades so sodium channels close. Some neurotransmitter molecules are recycled back into the synaptic knob by endocytosis.

Neurotransmitters can control and coordinate responses

When neurons are excited, they usually fire through several **synapses** at once. A single synapse will not produce enough neurotransmitter for an action potential to be generated in the postsynaptic neuron.

Synapses are also sensitive to the amount of stimulation they receive. Some have limited amounts of neurotransmitter available and, once it has been secreted, supplies have to be restored before further impulses can be transmitted. During this period they will not work. Different synapses vary in how quickly they become fatigued.

Not all synapses are involved with transmitting an electrical impulse. Some are there to hinder the message, using neurotransmitters that cause the inside of the postsynaptic membrane to become more negative than usual and thus harder for it to become depolarised. This means that the nervous system is able to finely control any adjustments that need to be made to respond to a signal. For example, in homeostasis, a gland can be stimulated to secrete more of a substance and, when balance is restored, the gland is inhibited.

What determines when a synapse should be open or closed? Significant research is progressing here and the brain appears to be most important. Much is yet to be learned. One emerging research area that is casting light on nervous system function is optogenetics.

METHOD OF THE YEAR 2010: OPTOGENETICS–BY NATURE VIDEO

Scientists can genetically engineer organisms so they express light sensitive channel proteins in neural membranes. They can use light to activate or silence specific neurons to research nerve pathways and to treat mice with Parkinson's disease.

Neurons are connected in an intricate network, with particular pathways coordinating particular responses in the body. The pathway followed depends upon whether a neuron is an excitatory neuron or whether it is an inhibitory neuron.

Types of neurotransmitters

Scientists have isolated hundreds of neurotransmitters, many of them in the brain, and the list is still growing. Neurobiology is described by many as the frontier of medical research for the 21st century, illuminating the mysteries of the nervous system. By discovering the roles and natures of the chemicals within the brain, we hope to gain useful clues to the complex behaviour patterns that make humans unique as well as information for treatment of nervous and mental disorders.

A common neurotransmitter is **acetylcholine**, which is found in synapses and nerve–muscle junctions in the somatic nervous system. It is normally deactivated by the enzyme cholinesterase. Many insecticides work by destroying the enzyme cholinesterase. In this way, the insect's muscle cells receive continuous signals, resulting in muscle spasms.

Paralysis ticks, tiger snakes and other venomous animals produce chemicals that block the production or action of neurotransmitters at synapses. Some pain-killing tablets take effect because they block the transmission of impulses from pain receptors. Naturally occurring endorphins in the CNS relieve pain when they are released at times of stress. Unfortunately, chemical warfare makes use of neurotransmitters and nerve gases that affect the nervous system in a harmful way.

WOW

Bryan Grieg Fry: venom hunter!

With a tally of 26 snakebites, Bryan Grieg Fry is no stranger to venom. Bryan talks of the neurotoxic effects of a death adder bite. 'It shut down all voluntary control of muscles. My arms got very heavy and breathing was difficult. The world started getting very far away. Eventually full paralysis set in. Even opening my eyes was impossible. I could hear people but I had no way of communicating that I was alive in my limp body. Being trapped on the inside is the loneliest place on earth.'

Courtesy of Bryan Grieg Fry

▲ **Figure 9.18** Bryan Grieg Fry milks the venom from a deadly king cobra.

Case study

Researching venom to find new medicines

Associate Professor Bryan Grieg Fry has had a long relationship with toxins. At just 20 months of age, he suffered the searing pain of spinal meningitis, a deadly disease caused by bacteria. The bacteria produce toxins that cause severe neurological damage. Bryan survived but lost hearing in one ear. This experience led to his life's work researching toxins. His research has seen him chasing venomous fish in Norway, scorpions in the Amazon and deep-sea giant octopuses in Antarctica. Venoms are toxic proteins produced to immobilise predators and prey. Many venoms block neural signalling pathways. Other venoms exert their effect on the circulatory system, causing haemorrhaging, loss of blood pressure and kidney damage.

Venoms have been extraordinarily useful in drug design and development. Captopril, a drug used to treat hypertension, was developed from the venom of the South American lancehead viper. Byetta, a drug used to treat diabetes, was developed from the venom of lizards. Both medicines are multibillion-dollar wonder drugs that have saved countless lives. Bryan's work has led to similar discoveries, including a potent peptide from the venom of the world's most toxic snake, the inland taipan. This drug is showing great promise as a treatment for high blood pressure.

The global burden of snakebite

It is thought that more than 100000 snakebite deaths occur annually in Asia, where snakes are a common part of rural life. To date, the only treatment for snakebite is to inject anti-venom. Anti-venoms are produced by injecting small harmless amounts of venom into horses. This stimulates the immune system of the horse to produce antibody proteins that bind specifically to the venom to neutralise it. The antibodies released into the circulatory system of the horse are extracted and purified to produce anti-venom. The anti-venom is only effective against toxic proteins that very closely resemble the original venomous protein.

There is a need for global cooperation and investment to increase the availability of effective anti-venom in developing countries. Drug companies keep numerous snakes so they can milk their venom to produce anti-venom. One significant aspect of Bryan's research is investigating these anti-venoms to determine if they neutralise snake venoms for which there is no current treatment. 'In Pakistan, most anti-venoms for sale are produced in India. We tested many of these anti-venoms and found they were absolutely useless against Pakistan snakebites. We need to help Pakistan to produce anti-venom specific to its own deadly snakes.' With this in mind, Bryan travelled to one of the most dangerous places in the world, the Zamzama gas fields in the Sindh desert of Pakistan. The riches that pool beneath the sands make it an attractive target for insurgents. Gas-field workers are protected by armed guards but their quarters are not safe. Many workers are bitten by snakes, even when asleep in bed, and available anti-venoms are not always effective.

Bryan liaised with the site doctor at the Zamzama gas fields to evaluate their anti-venom stocks. Finding them ineffective against the venom of the krait and viper, Bryan milked venom from these snakes and gave it to the University of Karachi so they could produce effective anti-venom for the region. Bryan tells us that 'it is important to use a mix of venom from many representatives of the same species when making anti-venom as the range of toxins found within the venom of a species can vary widely'.

As venoms often contain a cocktail of proteins, the subsequent anti-venom produced in horses contains many different antibodies that often have low specificity for the bites they are used to treat. Large doses are required and this can trigger an allergic reaction that is dangerous for patients. Anti-venoms often don't work well if they are stored incorrectly, especially in hot countries. New research is exploring the possibility of using synthetic DNA to produce anti-venom. Scientists are using bioinformatics to identify the common regions of toxic proteins in specific venomous species. These sequences are then strung together and used as a DNA vaccine. The hope is that the body will first produce small parts of the toxic protein and then produce antibodies against that protein. In this way, the body acquires immunity to the venom. While this has been demonstrated to be effective in mice, the research is in its infancy. Bryan cautions that this method may be impractical as venoms are complex, and subtle changes in venom structure within and between species can have a huge impact.

Questions

1. Construct a flow chart to map the process currently used to produce anti-venom to treat snakebite.
2. Discuss the problems that arise when anti-venom produced in India is used to treat snakebites in Pakistan.
3. Discuss why incorrect storage of anti-venom, especially in hot countries, can cause it to become ineffective.
4. How can Australian science contribute to reducing the global snakebite burden?
5. What are the possible benefits and harms of using synthetic DNA to produce anti-venom?
6. Some people would like to see all venomous animals removed from earth. Compose some arguments to refute this statement.

Neurotransmitter or hormone?

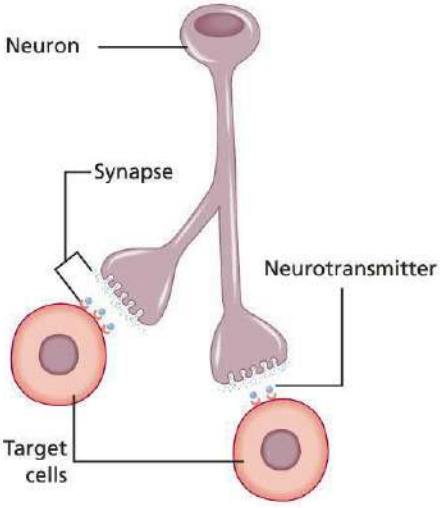


Figure 9.19 ▲ Neurotransmitters act like paracrine hormones, affecting cells in close proximity to them.

How different is nervous signalling from endocrine signalling? Both systems rely on chemical signalling molecules: hormones and neurotransmitters. Like paracrine hormones, neurotransmitters only affect cells in their local neighbourhood. Some neurotransmitters are very similar to hormones. For example, the neurotransmitter noradrenaline is almost chemically identical to the endocrine hormone adrenaline. Noradrenaline is associated with the sympathetic nervous system, and both adrenaline and noradrenaline are responsible for preparing our body to respond to stress. So, why is noradrenaline considered to be a neurotransmitter and not a hormone? The criteria defining a neurotransmitter are that it is released in response to presynaptic depolarisation and that its specific receptors must be located on the postsynaptic membrane.

However, some chemical signals can act as both a neurotransmitter and a hormone. ADH and oxytocin, two peptide hormones secreted into the blood by the pituitary gland, can also be found functioning as neurotransmitters at some synapses.

The endocrine and nervous systems are inextricably linked through the hypothalamus and pituitary gland. The hypothalamus, which is made up of nervous tissue, is located in the brain and connected to the pituitary gland via both nerves and blood. An example of the interplay between the endocrine and the nervous system is seen in the regulation of the concentration of water in the blood plasma. Osmoreceptors, sensory neurons that detect the water content of the blood, are located near the hypothalamus. If the water content of the blood is low, nerves of the hypothalamus are stimulated to produce ADH and secrete this from their axons into the pituitary. The pituitary then secretes ADH into the bloodstream, where it travels to affect cells in the kidneys.

When cell communication goes wrong

Cells are constantly communicating with each other using a huge array of chemical signals to stimulate a variety of different signalling pathways in different cells. But what happens if this tightly regulated communications system malfunctions? This can result in disease. Most diseases involve breakdowns in cell communication. Most disease treatments address this breakdown in communication in some way.

In type 1 diabetes the beta cells of the pancreas are destroyed by the immune system and no longer secrete insulin. This loss of signal leads to high blood glucose levels. Patients can inject themselves with synthetically produced insulin to overcome this problem. In type 2 diabetes, the insulin receptor in cells no longer responds to the insulin signal and the patient has become 'insulin resistant'. This is much harder to treat and patients have to manage their diet very carefully to make sure blood glucose levels don't spike.

Parkinson's disease occurs when a set of dopamine-producing nerve cells deep in the brain become impaired. Dopamine is a neurotransmitter required for controlled movement of muscles. When levels of dopamine become too low, the effects of another neurotransmitter, acetylcholine, are enhanced, creating a resultant tremor in parts of the body. The adult CNS has neural stem cells with the capacity to produce the major cell types of the brain. Scientists have stimulated these stem cells to produce neurons *in vitro*. Continued research may result in a treatment where stem cells in the brain can be stimulated to become dopamine-producing cells.

Multiple sclerosis is a disease that results when cells of the immune system destroy the protective myelin sheath of nerve cells. This slows down transmission of nerve impulses and disrupts signals. This nerve damage can lead to uncontrolled movements, blurred vision and depression.

Sometimes multiple breakdowns in cell communication need to occur for a disease to become manifest. Cancer is one such disease. It generally starts when the signals that control cell division are disrupted, so cells start to divide uncontrollably. This unregulated cell division should stimulate a signalling pathway in the cell to target it for apoptosis. If the cell no longer

DEEP BRAIN STIMULATION TO CORRECT DYSFUNCTION

Different brain circuits control different functions. Deep brain stimulation allows neurosurgeons to activate or suppress circuits to correct dysfunctions. Watch as a woman with Parkinson's disease instantly stops shaking, as severe depression is treated and as brain areas eroded by Alzheimer's disease are brought back to life.

responds to death signals then a tumour will develop. These tumour cells are greedy. They need huge supplies of oxygen and nutrients, so chemical signalling pathways are switched on to promote the growth of blood vessels throughout the tumour. Many treatments for cancer target malfunctions in chemical signalling by blocking or inducing proteins in signalling pathways.

ACTIVITY 9.1

SONIC HEDGEHOG, THE CYCLOPS LAMB AND CANCER: A CHEMICAL SIGNALLING RESEARCH DETECTIVE STORY

In the 1950s, a small group of Idaho sheep farmers noticed that lambs were being born with strange defects. Scientific studies revealed that during drought years, sheep grazed on an alpine plant called the corn lily. Pregnant ewes eating these plants during early gestation gave birth to lambs with one eye, a condition known as cyclopia. Something in the plant was interfering with the development of the foetus.

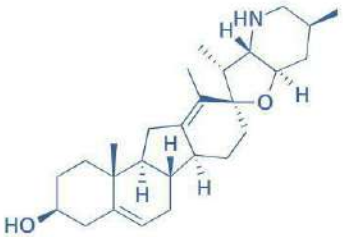
In the 1970s, scientists conducted experiments to discover how a fruit fly embryo, a ball of cells, knows where to grow a leg, a wing or a head. Investigating the effect of more than 1000 mutations revealed 50 genes essential for development: the genetic toolkit for building a fruit fly. When one particular gene was mutated, the fruit flies grew spines on their underbellies, so they called this the hedgehog gene.

Scientists started a search for homologous genes in mammals. A few hedgehog genes were found; the most important was named sonic hedgehog (Shh). Mutations in this gene resulted in deformities in mammals, including cyclopia.

Aim

To construct a stimulus–response model demonstrating how cells respond to Shh and to explain how an **endocrine disruptor** is showing promise as a treatment for cancer

Figure 9.20 ▶
Cyclopamine is a molecule found in the corn lily plant. It interferes with a protein in the sonic hedgehog developmental pathway, causing lambs to be born with fatal birth defects such as cyclopia.




Alamy/Robert Shantz

What to do

1 Use the following paragraph to construct a stimulus–response model that shows how Shh exerts an effect on cells.

When the Shh gene is expressed, Shh signalling protein is produced that diffuses out of the cell to affect nearby cells. These target cells have a receptor called Patched. Binding of the Shh signal activates Patched receptors to release a membrane protein called Smoothened into the cell. Smoothened activates a signal transduction pathway that leads to transcription of a set of proteins. These proteins drive the cell response to divide. In this way, Shh is a growth factor, stimulating growth through cell division.

Shh signals cells in a developing limb bud to switch on one set of genes while it signals for a different set of genes to be switched on in cells involved in nervous system development. Shh being produced in the wrong place at the wrong time can lead to extra digits being formed in the hand. If Shh isn't present when nerve buds for the eyes are developing, then only one eye develops, leading to cyclopia.

This hedgehog signalling pathway is integral to embryonic development, but it is also important for regenerating short-lived tissues such as skin cells and blood, stimulating cells to divide to replace dying cells. If this pathway is not regulated then cancers may develop as cells divide uncontrollably.

A connection was made back to the Idaho sheep. It was found that the corn lily produces a chemical, called cyclopamine, which binds to Smoothened protein c rendering it inactive. It is an endocrine disruptor as it interferes with normal signal transduction.

2 Draw and annotate a diagram of four large skin cells that reveals the following information.
   - **a** Sonic hedgehog (Shh) is a peptide growth hormone. Show the location of the Patched receptor in the cell and defend your decision.
   - **b** Indicate whether Shh is an endocrine, autocrine, paracrine or contact-dependent hormone by showing how it is secreted and travels to target cells.
   - **c** Indicate how signal transduction of the Shh message elicits a response in target cells.

3 On your diagram indicate how the hedgehog development pathway might be disrupted, resulting in a loss of regulation of cell division in skin cells and leading to the development of basal cell carcinoma.

4 Cyclopamine shows great promise in the treatment of basal-cell carcinoma tumours caused by inappropriate hedgehog pathway activation. Add to your diagram to show how cyclopamine may be used as a drug to treat basal cell carcinoma.

5 Construct a timeline of scientific discoveries that led to the development of cyclopamine as a promising cancer treatment.

6 What sort of warnings would be important to include with this drug if it were to be marketed?

QUESTION SET 9.5

Remembering

1 Make a simple annotated diagram to show how nerve impulses are transmitted from an axon terminal to a receiving neuron.

Understanding

2 Cut out 30 circles and label half of these as sodium ions and the other half as potassium ions. Use your ions to model transmission of the nerve impulse along an axon. Return the cell to its resting potential when you have finished.

3 Botox is a toxin produced by the bacteria *Clostridium botulinum*. It acts as a neurotransmitter inhibitor. Eating food contaminated with botox causes death. Yet many people are now getting botox injections to look younger. Draw an annotated diagram to demonstrate how botox disrupts nerve signalling pathways to relax facial muscles.

Applying

4 Use a stimulus–response model to explain how malfunctions in cell signalling can lead to disease.

CHAPTER SUMMARY

- The evolution of ion pumps to maintain internal ion gradients relative to external ion gradients was integral to the development of cell signalling systems.
- Unicellular organisms evolved to have intercellular signalling systems to coordinate group work.
- Multicellular organisms have complex signalling systems that coordinate cell processes for the survival of the whole organism.

- Multicellular organisms maintain cell numbers by regulating cell division and apoptosis.
- Cell differentiation in multicellular organisms led to tissues, organs and systems having specialist functions that are coordinated by signalling systems.
- Chemical signalling is used to regulate cellular processes for the survival of the whole organisms and its species.
- Cellular processes are coordinated by metabolism through regulation of biochemical pathways that are controlled by enzyme production, and the activation and inhibition of enzymes.
- All cell communication mechanisms follow the same principles: production of a signal, detection of the signal, transduction of the signal, activation of effectors to bring about a response and regulation of the response.
- Animal cells respond to hormones that are effective in minute amounts and are highly specific so that only target cells respond.
- Hormones differ in chemical properties that affect the way they are manufactured, the way they are transported and the position of their receptor in target cells.
- Hydrophobic hormones, including the steroid and thyroid hormones, bind to intracellular receptors to elicit a change in gene expression in the cell.
- Hydrophilic hormones, including peptide hormones and some amine hormones, bind to extracellular receptors to initiate a response that can include altering gene expression, altering the activity of enzymes, and stimulating vesicle transport or cell movement.
- Binding of a hydrophilic hormone to a receptor causes a change in conformation triggering signal transduction to elicit a cell response. This begins with the production of numerous second messengers that amplify the message as they transmit it to proteins in the cell.
- Stimulus–response pathways can be applied to demonstrate signalling pathways at the cellular level.
- Hormones can be classified by the distance they travel to stimulate target cells; they can be contact-dependent, autocrine, paracrine or endocrine hormones.
- Higher animal communication systems include a nervous system that transmits electrical impulses around the body at high speed.
- Neural signalling is initiated when ion channels in the membrane open, allowing an influx of sodium ions creating a more positively charged environment than the outside. This depolarising of the membrane generates an action potential.
- Sodium–potassium pumps in the axon membrane move sodium ions out of the neuron to return the neuron to its resting potential. The nerve cannot be stimulated again until the resting potential has been returned.
- A synaptic junction connects the axon of one nerve with a neighbouring dendrite, muscle or gland cell.
- A synaptic junction consists of presynaptic knob, a gap called the synaptic cleft and the postsynaptic membrane.
- When an impulse arrives at the synaptic junction, calcium ion channels open so the postsynaptic knob is flooded with calcium ions. These ions stimulate exocytosis of vesicles containing neurotransmitters. The neurotransmitters enter the synaptic cleft and bind to receptors on the postsynaptic membrane, transmitting the message to the receiving cell.
- A neurotransmitter is a chemical signal that is released in response to depolarisation at a presynaptic junction and binds specifically with receptors in the postsynaptic junction.
- Cell signalling systems can malfunction resulting in disease.
- Synthetic and natural endocrine disruptors can mimic chemical signals, resulting in disruption of normal cell signalling regulation.

CHAPTER GLOSSARY

acetylcholine a neurotransmitter in the human nervous system

action potential a brief change in the electrical potential on the surface of a nerve or muscle cell in response to stimulation, which results in the transmission of an electrical impulse

activator a regulatory protein that binds to an enzyme or DNA, causing a change of conformation so that enzymes become active, or activating gene expression

adhesion proteins proteins on the surface of cells (e.g. cadherins) that are involved in binding with other cells or to an extracellular matrix in a process called cell adhesion

amine hormone a hormone derived from amino acids; examples include epinephrine, dopamine and thyroxine

autocrine hormone a hormone whose target cell is the secretory cell itself or neighbouring cells of the same type

binding site a region on a protein, DNA or RNA molecule to which other specific molecules and ions bind through forming chemical interactions

binding specificity occurs when the shapes and charges of molecules allow them to selectively recognise and bind to each other

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 form a final product in a cell

cell differentiation the process by which a less specialised cell develops or matures to have more distinct characteristics and functions

cell metabolism the set of chemical transformations that take place in cells so they can grow and reproduce, maintain structures and respond to their environment

cell signalling a complex system of signal transduction pathways that governs basic cellular processes and coordinates cell actions

cellular process any process that is carried out at the cellular level but is not necessarily restricted to a single cell; for example, cell communication occurs among more than one cell but occurs at the cellular level

cellular response in chemical signalling, any process that results in a change in state or activity of a cell; for example, secretion, movement, gene expression, enzyme activation

chemotaxis the movement of an organism or cell along a chemical concentration gradient either towards (positive chemotaxis) or away from (negative chemotaxis) the chemical stimulus

conformation the shape of a molecule that is determined by the three-dimensional arrangement of its atoms and bonds; important for molecular functioning

contact-dependent signalling a type of cell signalling system where the signal molecule remains bound to the surface of a cell and will signal only to those cells that come into contact with it

cyclic adenosine monophosphate (cAMP) a second messenger formed from ATP that is responsible for the intracellular mediation of hormonal effects on various cellular processes

down-regulate (down-regulation) the process by which a cell decreases the quantity of a cellular component, such as RNA or protein

effector an organ, cell or protein that acts in response to a stimulus

endocrine disruptor synthetic or natural chemicals that mimic hormones and in doing so disrupt hormone regulation by interfering with cell signalling pathways

endocrine hormone hormone that is secreted into the bloodstream and can bind to distant target cells

extracellular occurring outside of a cell or cells

extracellular receptor a receptor molecule located in the cell membrane that has a binding site located outside of the cell to which hydrophilic signalling molecules bind

feedback inhibition the cellular control mechanism in which an enzyme that catalyses the production of a particular product is inhibited by the product, therefore balancing supply and demand of a product for a cell

hormone a chemical messenger secreted directly into the bloodstream, other body fluids, or into adjacent tissues, where they move to their target cells

hydrophilic hormone a hormone that is water soluble and binds to extracellular receptors to initiate a response in that cell; for example, peptide and some amine hormones

hydrophobic hormone a hormone that is water insoluble and binds to intracellular receptors; for example, steroid and thyroid hormones

in vitro when processes or reactions take place in a test tube, culture dish or elsewhere outside a living organism; as compared to *in vivo* where the process takes place in the living organism

inhibitor a substance that slows down or prevents a particular chemical reaction; by binding to proteins, inhibitors change the protein, conformation so it no longer performs its job

intercellular occurring between cells

intracellular occurring within a cell or cells

intracellular receptor a receptor molecule located inside the cell to which hydrophobic chemical signals bind; examples include transcription factors

ion channel a protein or protein complex that spans the cell membrane, forming a channel to facilitate the movement of ions across the membrane

ion gradient the concentration gradient of ions across a membrane; also referred to as an electrochemical potential

myelin sheath the fatty layer surrounding and insulating the axons of many neurons; increases the speed at which electrical impulses travel along the nerve cell

neuron a nerve cell

neurotransmitter a chemical substance that carries the action potential across a synaptic cleft

paracrine hormone a hormone for which the target cell is close to the signal releasing cell, and the hormone is broken down too quickly to be carried to other parts of the body

peptide hormone a hydrophilic hormone composed of a chain of amino acids so it can bind to extracellular receptors on target cells; for example, insulin and ADH

phosphorylation the addition of a phosphate group to a protein or other organic molecule

prokaryote a microscopic single-celled organism with no distinct nuclear membrane and no organelles except ribosomes

prostaglandins autocrine and paracrine hormones made from fatty acids

receptor a structure that detects or receives a stimulus

resting potential the electrical potential difference between the two sides of an unstimulated nerve cell's plasma membrane; when this potential exists, the cell is ready for action

second messenger small molecules that relay a signal from receptors on the cell surface to target molecules inside a cell

signalling molecule a chemical involved in transmitting information between cells; hormones and neurotransmitters are signalling molecules

signal transduction the process by which a cell converts one kind of signal into another; occurs when an extracellular signal binds to and activates a receptor, which, in turn, alters intracellular molecules to bring about a cell response

sodium–potassium pump a membrane protein that moves potassium ions into and sodium ions out of a cell, using active transport

stem cell an unspecialised cell with the potential to differentiate into many different kinds of cells

steroid hormones hydrophobic signal molecules found in plants and animals; these are produced from cholesterol, giving them a common chemical structure; examples include oestrogen, testosterone and cortisone; these signalling molecules are lipophilic so they can slip across the cell membrane and bind to intracellular receptors

synapse the point where an axon terminal meets another neuron, a muscle cell or a gland cell, separated by a synaptic cleft

synaptic cleft the space between the presynaptic cell and postsynaptic cell in a synapse, across which neurotransmitters diffuse to transmit a nerve impulse

target cell a cell that responds to a signalling molecule because it expresses specific receptors for that molecule

up-regulate (up-regulation) the process by which a cell increases the quantity of a cellular component, such as RNA or protein

vasoconstriction the constriction of blood vessels by the surrounding smooth muscle cells, which increases blood pressure and redirects blood flow away from the constricted vessel

vasodilation dilation (widening) of blood vessels, particularly arterioles

CHAPTER REVIEW QUESTIONS

Remembering

1. Describe the cellular processes that might be induced by chemical signalling in the life of a liver cell.
2. Describe how the sodium–potassium pump in a neuron restores its resting potential.
3. Both testosterone and growth hormone are involved in increasing muscle mass. Identify where each hormone is produced, how it travels in the blood and how it exerts its effect at target cells.
4. Distinguish between the resting potential and the action potential of a neuron.
5. Demonstrate what happens to a nerve impulse when it arrives at a synaptic junction.
6. What can you deduce about the properties and actions of a hormone if it is manufactured by joining amino acids together in a chain?

Understanding

7. Draw a diagram of a simple cell, illustrating the various types of chemical messages it may receive.
8. Responses to endocrine hormones are usually slower and longer lasting than those mediated by paracrine hormones. Why?
9. Are neurotransmitters hormones? Explain your understanding.
10. Protein hormones that use a second-messenger system often lead to a faster response than steroid hormones that bind with receptor proteins in the cytoplasm or nucleus of target cells. Explain why.
11. Use a diagram to represent how chemical signalling can control the enzymes that regulate cell metabolism.
12. Relate your understanding of protein conformation to discuss how hormones affect receptor molecules.

Applying

13. Smooth muscles lining arteries respond to chemical signals to contract and to relax, causing **vasoconstriction** or **vasodilation** of vessels. This controls blood flow through the vessels. Noradrenaline is a neurotransmitter that binds to receptors in smooth muscle to make them contract. Figure 9.21 shows nerve pathways to two different arteries in a person in a cold environment. Some nerves are stimulatory (green) and some are inhibitory (red). Defend your choice of which artery is found in the fingers and which artery is in the brain.

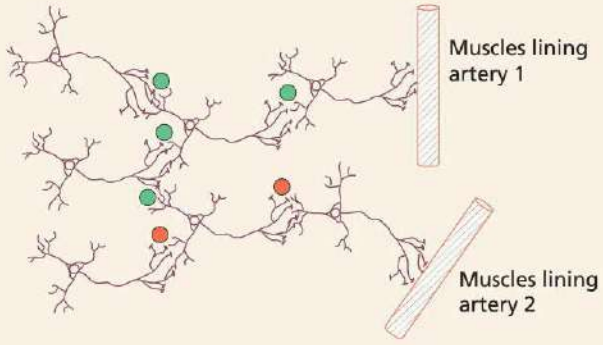


Figure 9.21 ▲
Nerve pathways to different arteries

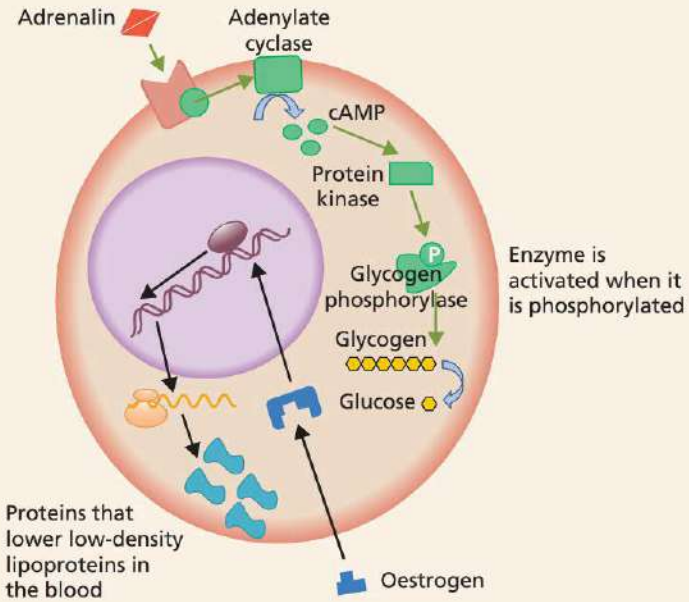


Figure 9.22 ▲
Adrenalin and oestrogen activate signal transduction pathways in a liver cell to elicit a response.

Analysing

14 Refer to Figure 9.22, which demonstrates the effect of the two hormones, adrenaline and oestrogen, on a human liver cell. Draw up a table to demonstrate the following for the signal transduction pathway triggered by each hormone.

- **a** Whether the hormone is hydrophobic or hydrophilic
- **b** Whether the receptor is intracellular or extracellular
- **c** A description of the signal transduction pathway
- **d** The effector molecule
- **e** The response

15 A toxic molecule from the venom of Chinese redheaded centipedes shows promise as a treatment for chronic pain that does not cause addiction. The molecule is selective for one of the nine different types of voltage-gated sodium (Nav) channels that humans have in their neurons.

- **a** Draw an annotated diagram that illustrates the role of Nav channels in transmission of nerve impulses.
- **b** Nav channels are important for pain perception, as well as heart and muscle function. Discuss why it is important that the toxic centipede molecule does not exert the same effect on all nine Nav channels.
- **c** People with a mutation that affects the gene for one of the Nav channels, known as Nav 1.7, are unable to experience pain. Discuss how this knowledge would help scientists to find an effective pain therapy.
- **d** The toxin from the Chinese redheaded centipede is a peptide called Ssm6a. Use an annotated diagram to propose how this toxin exerts its effect to inhibit pain transmission.
- **e** The toxin has been tested on mouse models that displayed minimal response to pain and had no side effects or change in blood pressure. Discuss if this is also guaranteed when human trials begin.
- **f** Most pain therapy drugs act by blocking pain receptors, making them addictive. Discuss why Ssm6a toxin should not be addictive.

Evaluating

16 Referring to chemical signalling, critique this comment made by Jacques Monod: 'What is true for *Escherichia coli* is also true for the elephant.'

Creating

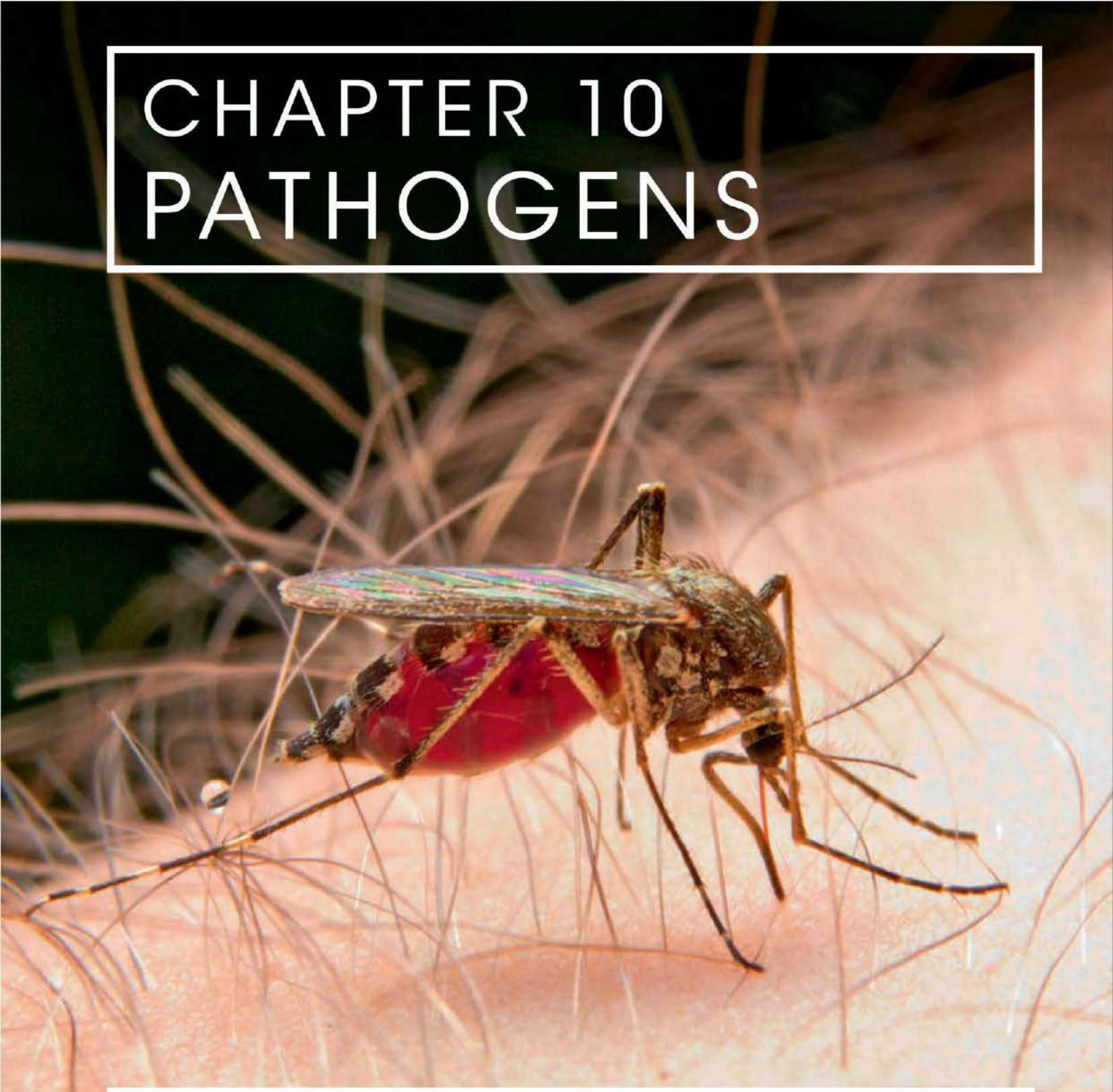
17 Design an antibiotic that will disrupt intercellular signalling in bacteria. Use a diagram to illustrate how this antibiotic will work.

18 On a large piece of paper draw a diagram of an animal cell, including a phospholipid membrane, a large nucleus and organelles. This cell is the central point for a mind map. Add branches to the cell to incorporate interesting facts about how cells communicate. Refer to the summary points and glossary as you build your mind map.

Reflecting

19 Consider the tools of summary tables, flow charts, timelines and annotated diagrams you have used in this chapter. Assess which are the most effective to enhance your understanding.

CHAPTER 10 PATHOGENS



Shutterstock.com/Mikhail Nabotov Popov

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

Science Understanding

- Infectious disease differs from other disease (for example, genetic and lifestyle diseases) in that it is caused by invasion by a pathogen and can be transmitted from one host to another (ACSBL116)
- Pathogens include prions, viruses, bacteria, fungi, protists and parasites (ACSBL117)
- Pathogens have adaptations that facilitate their entry into cells and tissues and their transmission between hosts; transmission occurs by various mechanisms including through direct contact, contact with body fluids, and via contaminated food, water or disease-specific vectors (ACSBL118)

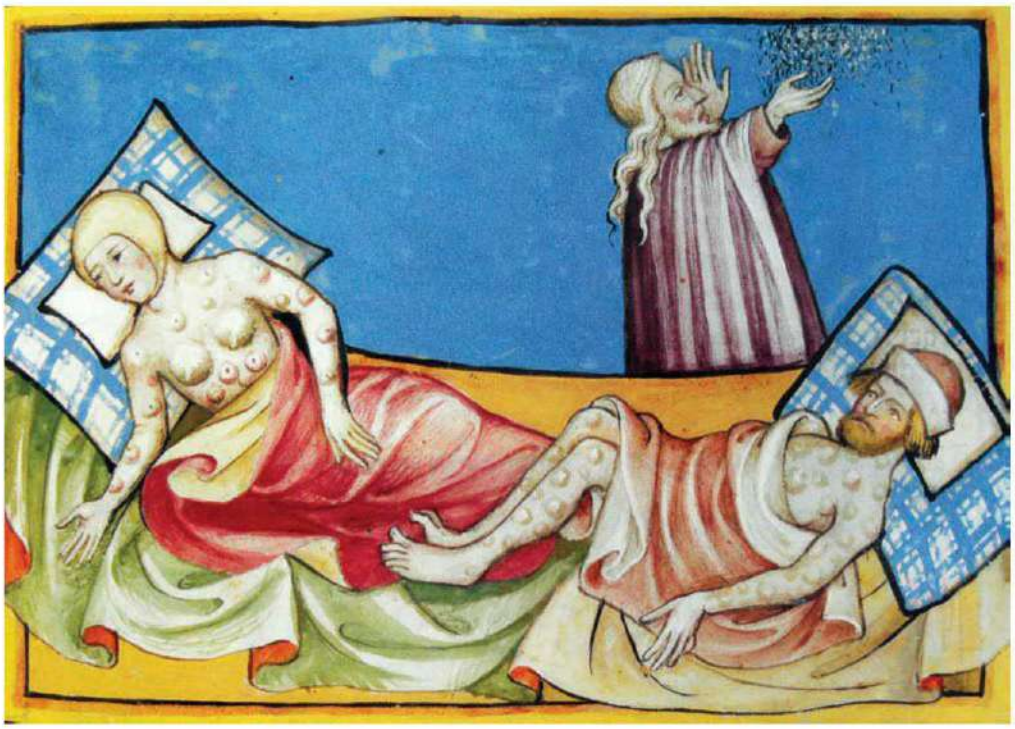


Figure 10.1 ▶ The plague (depicted here in the pages of the Toggenburg Bible of 1411), swept through Europe in the Middle Ages.

Corbis

History tells of how diseases have repeatedly ravaged human populations. Tuberculosis (TB) has been the scourge of humans for many thousands of years and evidence of its presence has been found in Egyptian mummies dating from 1000 BCE. Cholera and smallpox epidemics were rife for centuries and the plague threatened to wipe out most of Europe during the 14th century.

Emerging diseases – infections new to humans – are cause for concern; for example, Middle East respiratory syndrome, severe acute respiratory syndrome (SARS), Ebola virus disease and avian influenza (bird flu). Millions of people worldwide suffer from the acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), one of the top **infectious** killers in the world. When epidemics sweep through a population, the impact can change the course of history.

What is disease?

A disease is any condition that interferes with how an organism, or any part of it, functions, hence the name 'dis' 'ease'. Diseases are described as **endemic** if they are common in a population but at a low level. If there is a sudden increase in the incidence of the disease in the population of a species, it is referred to as an **outbreak**.

Diseases can be grouped according to their cause. Infectious diseases, such as TB, are caused by an agent that can be passed from one organism to another. The infected organism is the **host**. An infectious agent that causes disease is called a **pathogen**. Pathogens include prions, **viruses**, bacteria, fungi, protists and **parasites**. **Micro-organisms** such as bacteria, viruses and fungi cause familiar infections like sore throats, colds and tinea. Protists are the disease-causing agents in malaria, amoebic dysentery and giardiasis, while prions cause kuru and mad cow disease. A parasite is an organism that lives on or in its host for all or part of its life, causing harm and gaining nutrition from the host. Multicellular parasites, such as tapeworms, roundworms and flukes, cause untold misery and illness throughout the world.

Figure 10.2 ▼ Short-duration flash photograph of a sneeze, showing the number of droplets expelled. Each droplet may contain thousands of bacterial or viral pathogens.

Science Photo Library/Dr John Brackenbury

Transmission is the passing of an infectious disease from an infected host to another individual. Pathogens have a variety of adaptations that enable transmission from host to host in a number of ways. Diseases that are easily transmitted by close contact with an infected organism or their secretions are called **contagious**.

Non-infectious diseases are those that are not caused by pathogens and are not **communicable,** or transmitted from one individual to another. These include nutritional diseases, such as obesity, malnutrition or beri beri, and

degenerative diseases, such as diabetes, osteoporosis and Alzheimer's disease. Factors in the environment may also cause disease. Intake of heavy metal particles such as lead interferes with the development of the nervous system, and overexposure to sunlight can trigger skin cancer.

Genetic diseases or disorders are those that are due to mutations inherited from parents. They are numerous and include cystic fibrosis, phenylketonuria and haemophilia.

The **immune system** is responsible for detecting the difference between its own cells and those of other organisms. This does not always function as effectively as it should. When tissues are attacked by the body's own defense system, **autoimmune diseases** ('auto' = self) such as rheumatoid arthritis, multiple sclerosis and lupus can result.

Autoimmune diseases are discussed with the adaptive immune response in Chapter 12.

QUESTION SET 10.1

Remembering

1 Define 'disease', 'pathogen' and 'contagious'.
2 List the types of organisms that can cause disease.

Understanding

3 Predict some forms of disease transmission that involve pathogens.
4 Using examples, outline the difference between an infectious disease and a non-infectious disease.

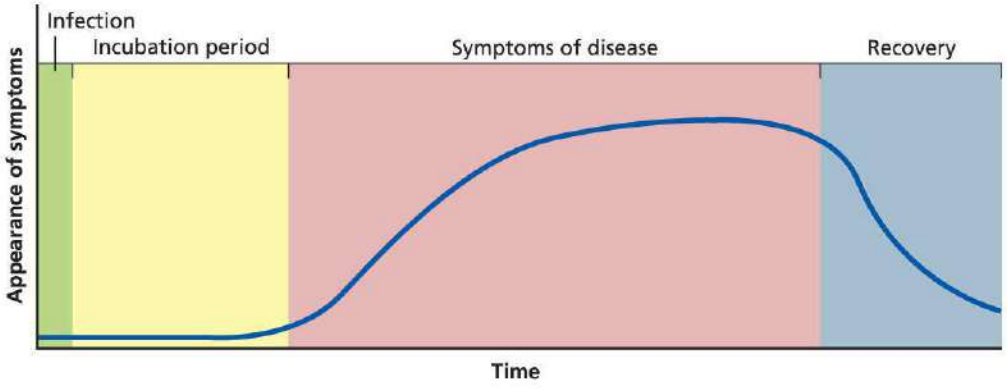
The nature of disease

Most micro-organisms are not pathogens. The fact that a micro-organism is pathogenic is due to special characteristics of the organism. This includes the ability to stick to or invade a particular cell type, produce toxins, or cope with or avoid the host immune system. As parasitism is a relationship between organisms where one organism, the parasite, benefits at the expense of the host; by definition all parasites are pathogens. Pathogens differ in their disease-causing capacity or **pathogenicity**. The intensity of the effect of the pathogen is called its **virulence**.

Individuals vary in their **susceptibility** to a pathogen; some have greater **resistance** than others. For example, if a cold is spreading through family and friends, every person does not necessarily become ill. Almost certainly every person in contact with the sufferer will have contact with the cold virus, but not everyone will develop cold symptoms. An individual's ability to avoid being affected by a pathogen depends on a number of factors, such as their age, state of health and their natural resistance to that particular pathogen.

Symptoms are the effects the pathogen has on the body of the host. For example, a high temperature and a rash develop in cases of measles and an annoying cough and sore throat may be early signs of a TB infection. Diseases usually have characteristic symptoms and these are useful to doctors trying to diagnose the cause of the disease without actually isolating the pathogen itself.

For many pathogens, symptoms of the disease do not appear immediately on infection. The time between infection and the onset of symptoms is known as the **incubation period**. This time lag (Figure 10.3) may occur for a number of reasons. For example, the pathogen may have to divide many times to reach numbers sufficient to cause disease or it may take time to reach



◀ **Figure 10.3**
Graph illustrating the various phases of an infection. Note the time lag between the time of infection and the onset of symptoms. This is known as the incubation period.

the target tissues that are susceptible to that particular pathogen. Toxins produced by bacteria as wastes of metabolic activity take time to accumulate to a level that affects the host. Diseases are often contagious before the onset of symptoms. This means that the pathogen can be passed on before the person even knows they have it. This incubation period may be an adaptation of the pathogen, allowing it to be transmitted before the host is incapacitated by symptoms.

Chapters 11 and 12 cover the defence mechanisms of hosts.

In most cases, the defence mechanisms of the host organism will fight off the pathogen and the host will recover. If this does not happen, disability or death may occur.

Infectious diseases are caused by pathogens that can be passed from one organism to another. Symptoms are signs of disease in the host. They can be used to diagnose the pathogen.

QUESTION SET 10.2

Remembering

1 Define 'susceptibility' and 'incubation period'.

2 List three factors that determine the pathogenicity of an organism.

Understanding

3 Describe how doctors use symptoms to help treat their patients.

4 Explain the difference between pathogenicity and virulence.

WHAT'S THE DIFFERENCE BETWEEN A COLD AND THE FLU?

Watch the video and draw a table to compare symptoms of a cold and the flu.

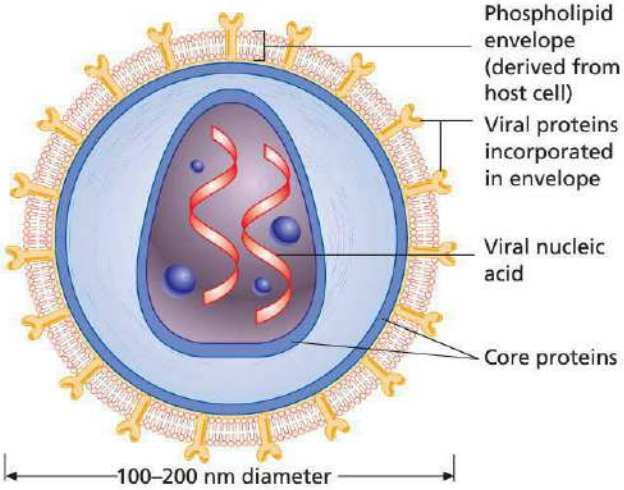
Non-cellular agents as pathogens

Tobacco mosaic virus, a highly infectious plant pathogen that infects tobacco, tomato, cucumbers and a number of ornamental flowers, was the first **virus** to be discovered. Late in the 19th century, researchers showed that infected sap remained infectious, even after filtering through the finest filter available. They had found tiny infectious agents, too small to be bacteria, that were able to reproduce and multiply in the host cells of tobacco plants. We now know that viruses are not cells, as they possess no metabolic machinery for processes such as cellular respiration. We will see that prions are even simpler, non-cellular pathogens.

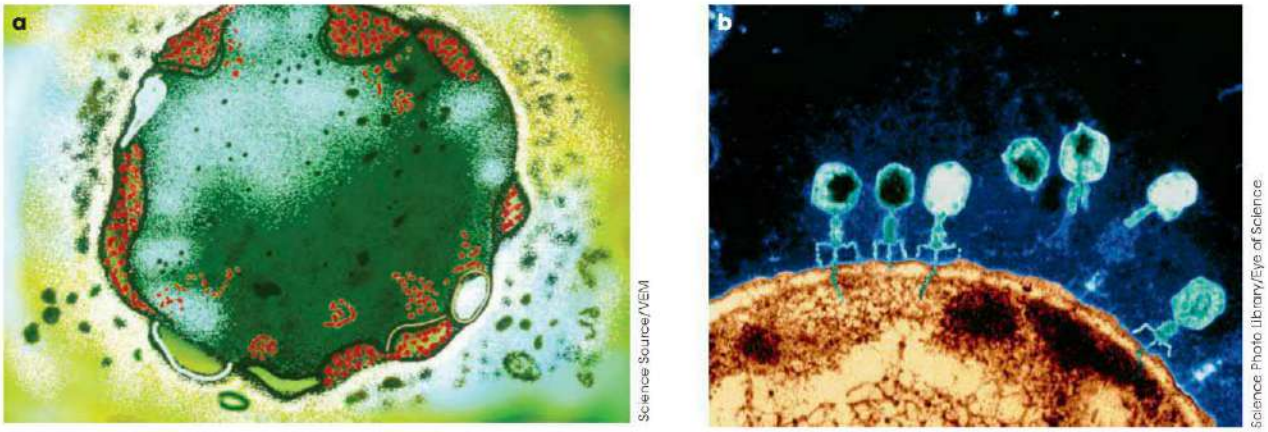
Viruses

It is a common misconception that you can catch a cold if you go out on cold, wet days. The common cold is caused by a virus, not by becoming cold and damp. A virus is a non-cellular agent composed of a protein coat and nucleic acid (Figure 10.4), either DNA or RNA (Figure 10.5), but never both. When a virus infects an organism, it injects its nucleic acid into a host cell. Once inside, the viral nucleic acid takes over the host cell and directs it to make multiple copies of the viral protein coat and nucleic acid. These then assemble into new viruses and are released when the host cell undergoes **lysis**, or splits open. This releases many more viral particles, which can infect other cells within the host (Figure 10.6). Exposure to cold and wet conditions might lower a person's resistance to the virus but it is not the cause of the disease.

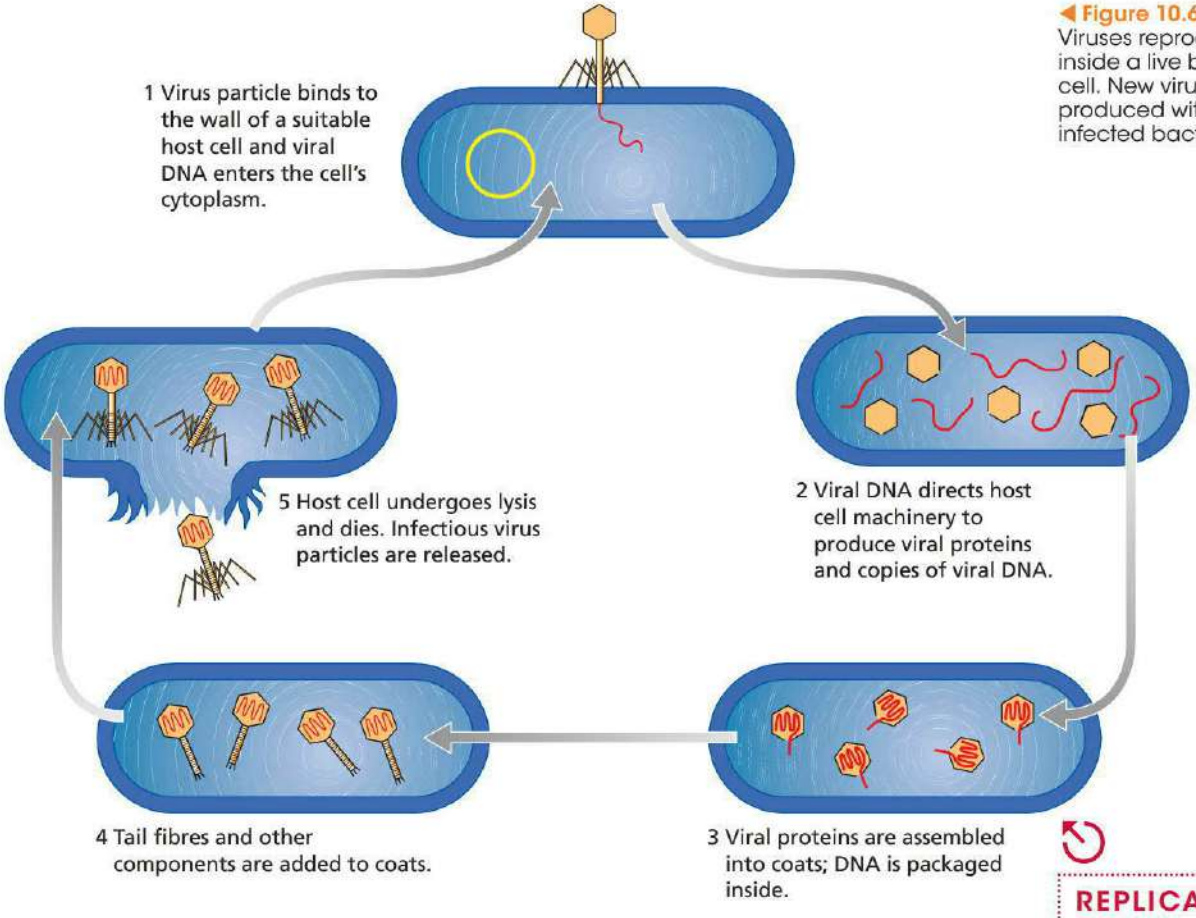
Figure 10.4 ▼ Viruses consist of a nucleic acid core surrounded by a protein coat.



All viruses cause some type of disease, as they rely totally on host cells for their reproduction. A virus is often referred to as an **obligate** parasite because it cannot function outside the host cell. This means that, unlike bacteria, viruses cannot be grown and studied outside live cells. This trait poses limitations on viral research.



▲ **Figure 10.5**
a) The DNA virus that causes herpes in humans; b) A coloured TEM 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 tails of genetic material (DNA) are being injected into the bacterium.



◀ **Figure 10.6**
Viruses reproducing inside a live bacterial cell. New viruses are produced within the infected bacterium.

Some viruses do not cause their host cell to produce multiple viruses immediately. Instead, they enter a **lysogenic phase** in which their nucleic acid becomes integrated into the chromosome of their host. It is replicated as the host cell divides. In this way the virus can remain dormant for a long time and avoid detection by the defence mechanisms of the host organism. The only way to remove the virus is to kill off the host cell before the virus has been replicated. At some point, usually in response to an environmental stimulus, the latent virus may exit the host's genome and once again become a separate entity within the cell. It will then replicate its components, package them into viral particles and lyse the host cell. This is called the **lytic phase**.

Each virus is usually highly specific to the host cell or organism it can infect. For example, an adenovirus specifically infects lung epithelial cells, causing the common cold. This is because the virus is able to recognise and bind to receptors that are expressed only on lung epithelium.

REPLICATION OF HIV

View the narrated animation and describe the ways in which the virus uses host cells for its own purposes.

Surface molecules as identification markers are discussed in Chapters 11 and 12.

Visuals Unlimited/Nigel Cattlin

Figure 10.7 ▶ This apple tree is afflicted with the apple mosaic virus, one of the oldest known and most widespread apple viruses.

Virtually every type of organism on Earth is susceptible to viral infection. Viruses are significant pathogens of many plants, sometimes resulting in the loss of crops such as potato, tobacco, corn and apples (Figure 10.7). Even bacteria have their own group of viral pathogens, known as **bacteriophages** (refer back to Figure 10.6).

Table 10.1 Some diseases caused by viruses

Virus	Disease	Symptoms
Herpes simplex type I	Cold sores	Recurring blisters on skin, usually around mouth
Herpes simplex type II	Genital herpes	Recurring blisters in genital area; affects both males and females
Varicella zoster	Chickenpox	Fever, pink spots that blister and burst
Hepatitis A	Hepatitis A	Inflammation of liver, kidney, spleen; jaundice, fatigue, aching limbs, headache
HIV	AIDS	Fatigue, loss of appetite and weight, immune system impaired so prone to many infections
Adenoviruses	Respiratory infections	Sore throat, coughing, sneezing
Flaviviruses	Yellow fever, dengue fever	Fever, chills, jaundice, severe muscle pain
Rhinoviruses	Common cold	Sore throat, sneezing, coughing, headache

Prions

In the United Kingdom in the late 1980s there was an outbreak of what was called mad cow disease. This had serious consequences for the British beef industry, and resulted in the slaughter of more than 4 million cattle. Mad cow disease is also known as bovine spongiform encephalopathy (BSE) because it belongs to a group of diseases called transmissible spongiform encephalopathies (TSE). The name comes from 'encephalo' meaning brain, 'pathy' meaning disease and 'spongiform' meaning sponge-like, due to the degeneration of brain tissue that makes it look like a sponge. These diseases are caused by a small infectious protein called a **prion** (pronounced pree-on) that brings about degeneration of the nervous system and ultimately death. TSEs are a disease of humans and other mammals, such as sheep, cattle, cats and even mink.

Getty Images/Science Photo Library

▲ **Figure 10.8**
This cow is infected with mad cow disease, or BSE, which is caused by a prion.

Consumption of cattle products, infected with the prion protein that causes BSE, has now been linked to the occurrence of a new variant of the human TSE, variant Creutzfeldt–Jakob disease (vCJD). This disease is characterised by gradual loss of motor coordination, dementia and paralysis, eventually leading to death. More than 200 deaths in Britain and Europe have been attributed to this disease. Cattle passports were created to travel with each animal to track their movements from birth. This paperwork has been used to improve consumer confidence in the wake of the mad cow disease outbreak.

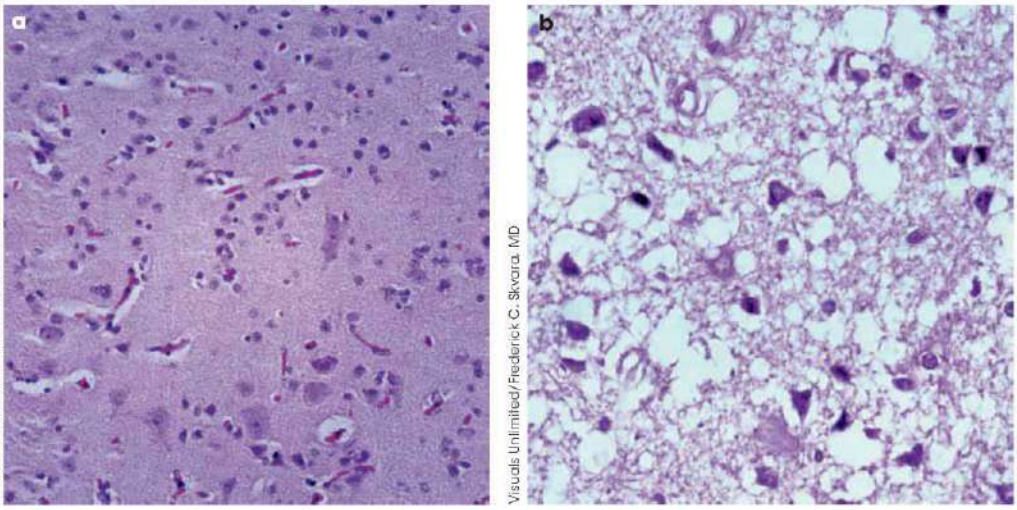
The earliest recorded TSE, noted in the 1700s, was commonly referred to as scrapie, as the infected sheep and goats became irritable and experienced an intense itchiness, causing them to scrape off their wool or hair. The victims showed a gradual loss of muscular coordination so that they could no longer stand and eventually died. Although it is not common, scrapie still occurs today.

Prions are unique among pathogenic agents as they do not possess any genetic material, neither DNA nor RNA. They are much smaller than even viruses. So, how can such an agent cause infectious disease?

Prion proteins actually exist in our bodies normally and play important roles in memory, learning and passing signals from cell to cell. They are often found at the surface of neurons. There are two forms: the normal prion protein cellular form, denoted PrP^{c} and the disease-causing prion protein scrapie form, PrP^{Sc}. When a PrP^{Sc} protein molecule encounters a normal PrP^{c} form, it converts it to the harmful form, which in turn converts other normal forms to harmful forms and so on. When there are sufficient numbers of the pathogenic PrP^{Sc} form, they aggregate to form filaments. These fibres kill brain cells (Figure 10.9) consequently affecting muscle coordination and brain function.

REPLICATION OF PRION PROTEINS

Watch the animation and describe the process in your own words.



Visuals Unlimited/Frederick C. Skvara, MD

Visuals Unlimited/Frederick C. Skvara, MD

◀ **Figure 10.9**
a) Healthy brain tissue; b) Brain tissue from a victim of Creutzfeldt-Jakob disease. Note the plaques (holes) giving the brain tissue a spongy appearance.

The PrP^{Sc} protein is very resistant to high temperatures, strong enzymes and ultraviolet radiation, making it extremely difficult for the immune system to combat. Researchers have not yet discovered a chemical that is effective in destroying the harmful protein and is also safe enough to administer to a patient. The PrP^{Sc} form of the protein can arise spontaneously as a mutation in the gene that codes for the normal protein, but it is usually transmitted by entering the body in infected food, most commonly brain tissue from an infected animal.

The incidence of prions in humans is not a recent occurrence. Kuru is a similar disease that has been recorded in the Fore people in the Eastern Highlands of Papua New Guinea and was first documented in 1957 by Vincent Zigas and Dr Carleton Gajdusek. The transmission of this disease was traced to practices of ritual cannibalism, where the Fore people would honour their dead by eating soup prepared from the deceased's brain. The incidence of kuru declined dramatically when this ritual was stopped and now rarely occurs. This form of TSE has been found exclusively in only one tribe; other tribes in this region of Papua New Guinea have no recorded cases of kuru.

Viruses and prions are non-cellular infectious agents that are always pathogenic.

QUESTION SET 10.3

Remembering

1 List four diseases that are classified as TSEs.
2 Define 'obligate parasite'.
3 Outline how altered prion proteins affect the brain.

Understanding

4 All viruses are pathogens. Justify this statement.
5 Describe the unique feature of a prion that distinguishes it from other non-cellular infectious agents.
6 Describe two changes to the structure of prion proteins that lead to Creutzfeldt–Jakob disease.
7 Viruses infect only specific host cells. Explain how this specificity comes about.
8 Outline the steps involved for a virus to reproduce.

Applying

9 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 they manage to enter the bloodstream without being digested. Provide evidence to support your answer.

Cellular agents as pathogens

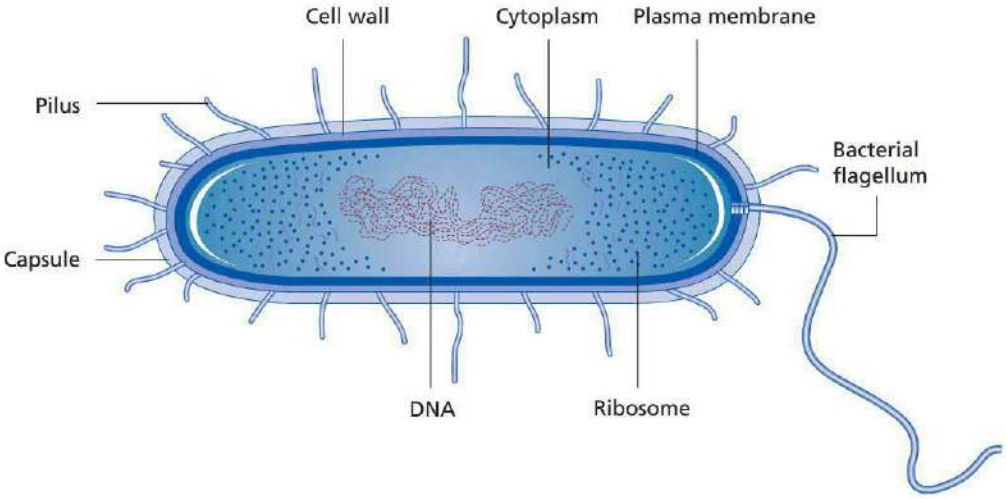
All living organisms are made of cells and are characterised by the ability to grow, reproduce and respond to stimuli. In this section, we are not considering the vast majority of organisms on Earth; instead we focus on those few living organisms that cause disease: the pathogens.

Bacteria

Bacteria may have been the first 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. There are billions of bacteria living on our skin and in our bodies that are not pathogenic and are often beneficial.

Bacterial pathogens: structures and adaptations

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 (Figure 10.10). As they are prokaryotes they have no membrane-bound organelles or nucleus; however, bacteria do possess ribosomes and a single circular chromosome. Most bacteria have a cell wall outside their plasma membrane made of **peptidoglycan** (a protein–carbohydrate compound).

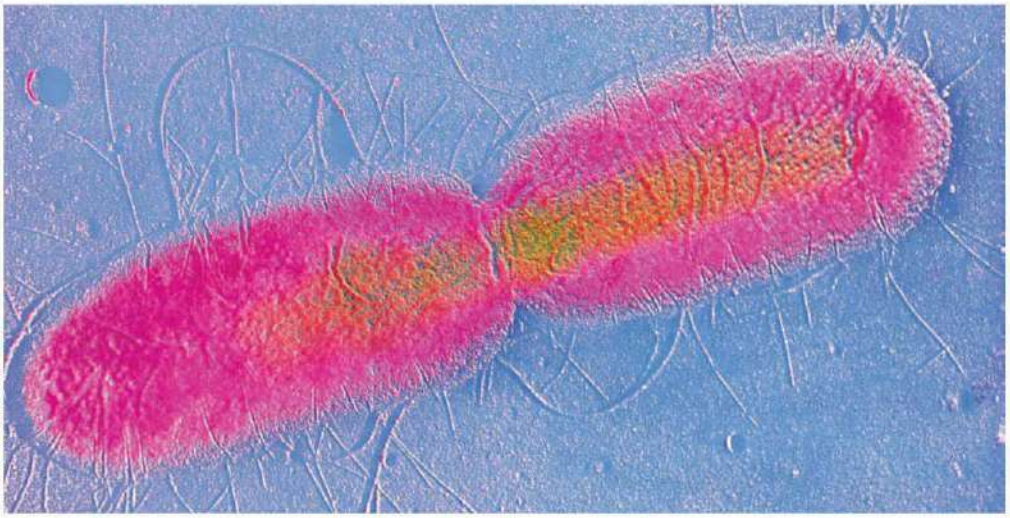


◀ **Figure 10.10**
Generalised structure of a bacterium

Some bacteria possess a **flagellum**, which helps them to move about. Another adaptation found only in some species is a slimy **bacterial capsule**, which may be used to help the bacteria stick to surfaces, such as teeth or mucous membranes. The capsule is a large, well-organised layer sitting outside the cell wall. It usually increases the virulence of a species, as it makes it harder for the body's immune system or antibiotics to attack the inner bacterium.

Many bacteria are capable of forming tough, dormant structures called **endospores**, which are resistant to extreme temperatures, chemicals and drying out. This adaptation helps bacteria resist unfavourable conditions and facilitates dispersal to new hosts.

Some bacteria reproduce by **binary fission** (Figure 10.11), in which one cell splits into two. Others reproduce by budding off spores. These asexual forms of reproduction allow bacteria to reproduce very rapidly in favourable conditions. Some species can reproduce every 20 minutes. For such a species, one bacterium could give rise to a colony of 4.7×10^{21} individuals in just 24 hours. (That is 4 700 000 000 000 000 000 000 bacteria in a single colony!) *Mycobacterium tuberculosis*, however, has a much slower reproductive rate, taking 12 hours to divide.



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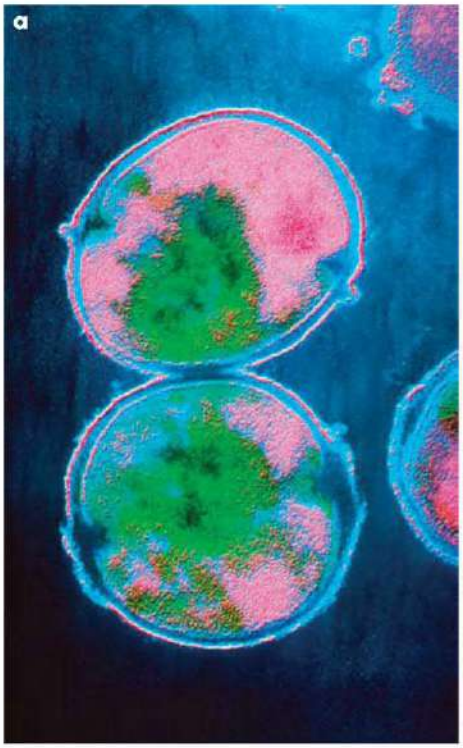
◀ **Figure 10.11**
Transmission electron micrograph of *E. coli* dividing into two by binary fission

EXPONENTIAL GROWTH USING BINARY FISSION

Follow the instructions to simulate the rapid growth of a population of bacteria.

Figure 10.12 ▶
a) Transmission electron micrograph of a cocci-shaped bacterium, *Streptococcus pneumoniae* (magnification × 30000); b) A rod-shaped bacterium, *Bacillus anthracis*, which causes anthrax in sheep and cattle (magnification ×10500); c) Transmission electron micrograph of a spirally shaped bacterium, *Leptospira* (magnification ×4400)



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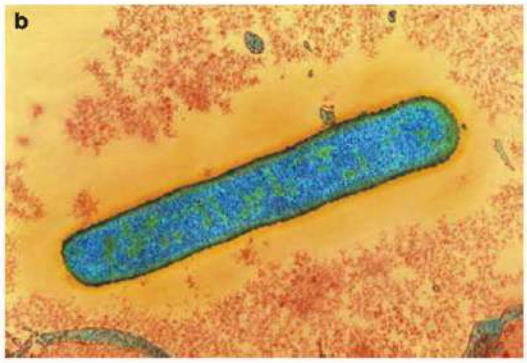
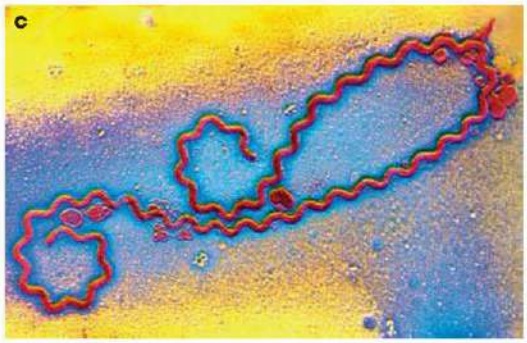


Science Photo Library/A Dowsett, Health Protection Agency



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Classification and identification of bacteria

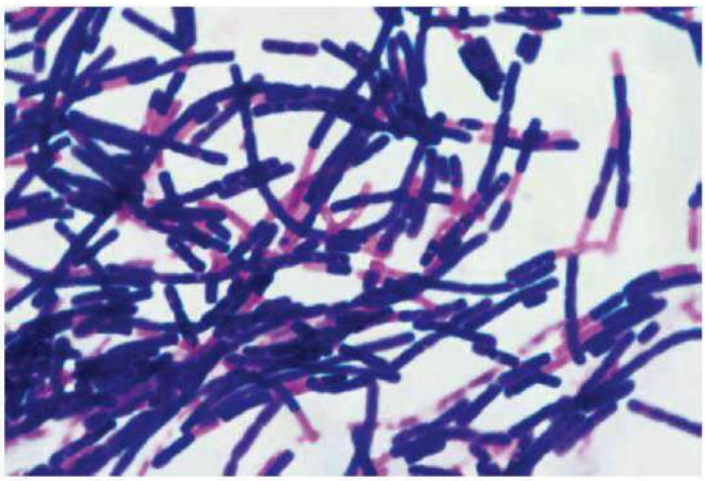
To study bacteria in detail, it is necessary to view them under a powerful electron microscope. However, useful information can still be seen using a light microscope after staining. This reveals a variety of different shapes of bacteria, namely:

- spherical, known as coccus (plural cocci) (Figure 10.12a)
- rod-shaped bacillus (plural bacilli) (Figure 10.12b)
- spiral (plural spirilli) (Figure 10.12c)
- vibrio, rather like a comma.

Bacteria are unicellular organisms but individuals of some species cluster together.

- Diplococci are spherical bacteria that occur in pairs.
- Streptococci exist as chains of bacteria.
- Staphylococci occur in clusters.

Figure 10.13 ▼
These bacteria have been stained by the Gram stain technique. The Gram-positive bacteria stain purple and the Gram-negative bacteria stain pink (magnification ×1000).


Science Photo Library/CNRI

It is difficult to distinguish between the different strains of each shape. A pathogenic bacillus may look no different from a bacillus involved in cheese production. There is one feature that can be a useful tool in classifying them (Figure 10.13). Many strains of bacteria have differences in the structure and composition of their cell walls causing them to respond differently to stains and dyes, for example, the Gram stain.

As most bacteria are able to exist as free-living organisms, it is possible to grow colonies of them. This is done by inoculating a small number of a particular strain into a medium containing all their nutrient needs. This medium may be a liquid broth or a solid gel called agar (Figure 10.14). When one bacterium is inoculated on to a plate, it divides many times to form a visible colony. The appearance of these colonies can differ in

colour, texture and shape, depending on the particular strain. An advantage of growing colonies on a solid medium is that individual strains can be isolated and grown in pure culture. This allows microbiologists to distinguish between benign and pathogenic strains of bacteria according to their appearance and response to antibiotics and chemicals.

How bacteria cause disease

Bacteria can be transmitted from one host to another in a number of 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 divide rapidly. Some damage host tissues directly, while others produce powerful 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 parts of the bacterial cell, are highly pathogenic to the host. External molecules such as **lipopolysaccahriades** (a lipid – carbohydrate compound) or peptidoglycans are examples. This is because they stimulate immune responses that are sometimes so strong that they damage host cells and tissues. Other bacterial strains interfere with the host's immune system, making the host susceptible to other pathogens.

Science Photo Library/CC Studio

▲ **Figure 10.14** Haemolytic bacterial pathogens infect blood cells, so they must be grown on agar plates that contain blood.

Chapters 11 and 12 discuss the ways in which organisms detect and respond to pathogens.

Table 10.2 Some diseases caused by bacteria and their symptoms

Bacteria	Disease	Symptoms
Clostridium tetani	Tetanus	Sustained, severe muscle contractions due to blocking of nerve impulses by tetanus toxin
Legionella pneumophila	Legionnaire's disease	Fever, coughing, lung congestion
Vibrio cholera	Cholera	Severe dehydration, diarrhoea
Yersinia pestis	Plague	Swollen lymph nodes, fever, ulcers
Corynebacterium diphtheriae	Diphtheria	Headache, vomiting, spots on throat and tonsils
Mycobacterium leprae	Leprosy	Lesions on skin, loss of pigmentation, nerve damage that causes numbness – leads to muscle weakening and damage to affected areas due to lack of feeling
Bacillus anthracis	Anthrax (in cattle)	Boil-like lesions on skin, swelling of lymph glands, respiratory distress, fever, possibly death

WOW

Drawing battle lines

New methods of battling infection without the use of antibiotics involve pitting one microbe against another. Deadly enzymes, made naturally by viruses called bacteriophages, are synthesised and mixed in a saline buffer solution. The solution is then squirted into the nasal passages. The enzymes kill the bacteria by punching holes in their cell wall, causing them to explode. Researchers predict that a single squirt of the bacteria-killing enzyme may keep an infected person from sneezing out bacteria for up to week.

QUESTION SET 10.4

Remembering

1 State three ways that a bacterial pathogen can harm its host.
2 Define 'binary fission'.

Understanding

3 Describe the advantages of bacteria:
   a having a capsule.
   b forming endospores.
4 Describe the methods by which different strains of bacteria can be identified.

Science Photo Library/Dr. Jeremy Burgess

Figure 10.15 ▲ These grapes are infected with the fungus *Botrytis cinerea*.

Fungi

The fungal world includes large organisms, such as mushrooms and toadstools, as well as minute forms that were only revealed with the invention of the microscope. These microscopic fungi include unicellular yeasts and moulds. Fungi are eukaryotes that reproduce using spores and possess cell walls made of **chitin**, rather than cellulose. Microscopic fungi are generally larger than bacteria. Some of them are pathogenic, causing disease in a wide range of organisms, including plants (Figure 10.15) and animals. Like bacteria, not all fungi cause disease.

Most fungal diseases in animals are external, where they irritate and inflame the skin. 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, and 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 as they can remain alive for years in bedding, furniture and grooming tools, germinating when conditions are suitable.

Internal fungal infections in animals are fairly rare. *Aspergillus* is a common microscopic fungus that is normally harmless to humans, but to people who have a suppressed immune system, such as sufferers of AIDS, it may be deadly. Tangles of fungal filaments can fill space in the lungs and limit breathing, or can spread in the bloodstream and infect major organs such as the heart and kidneys.

Fungal infections in plants can cause serious disease, for example, *Phytophthora cinnamomi*, the cinnamon fungus, is devastating jarrah forests in Western Australia and Tasmania. Rusts, which infect crops like wheat and barley, are significant pathogens. The hyphae of fungi can penetrate the external surface of the plant and extend into its phloem, depriving it of valuable nutrients and reducing crop yield. Part of the fungal life cycle includes the production and release of large quantities of spores that effectively transmit these fungal pathogens to new hosts.

MANAGING A PLANT FUNGAL DISEASE

Make practical plant health management decisions by following the instructions and devising a disease control plan for a plant nursery.

WOW

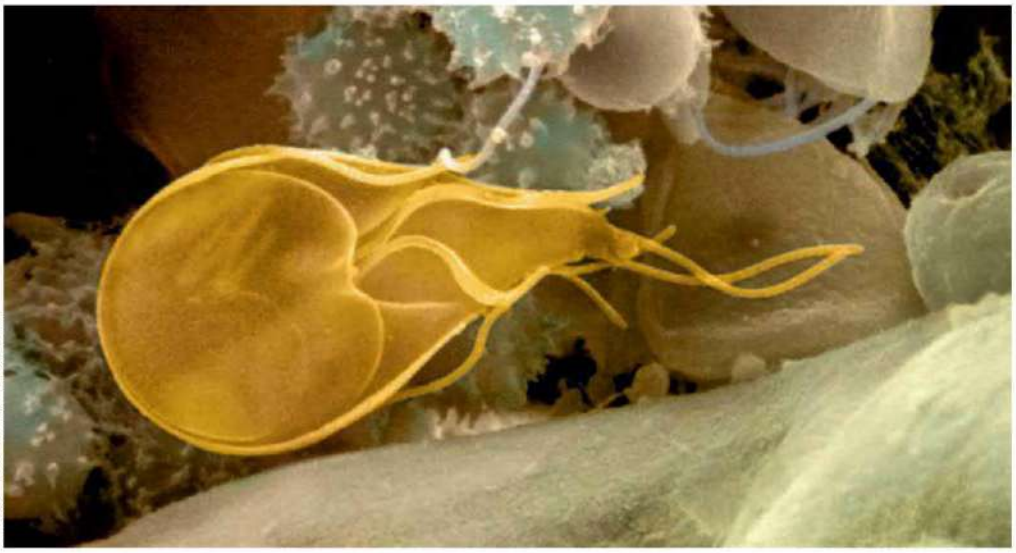
Deadly fungi

While most external fungal diseases in animals are not life threatening, platypus fungal disease, termed mucormycosis, is lethal to the Tasmanian platypus. The infection causes ugly skin lesions on various parts of the body including their backs, tails and legs. These ulcers lead to death as they reduce the animals' ability to maintain body temperature and forage efficiently, and they often develop secondary bacterial infections. An unusual feature of this disease is that it affects platypuses only in Tasmania and not on the Australian mainland, where the same pathogen infects frogs and toads. As there are no records of Tasmanian frogs infected with the fungus, it is believed to have been introduced into Tasmania either via green tree frogs among banana shipments from Queensland or from the illegal introduction of frogs as pets.

Protists

Protists are unicellular, eukaryotic organisms. They reproduce both sexually and asexually. Of the 65 000 known species of protists, less than 24 species cause diseases in humans, but these few infect hundreds of millions of people each year. To date, we still do not have effective preventatives against many of them and the treatment drugs we have are limited in their effectiveness.

In July 1998, the people of Sydney were horrified to hear that their water was undrinkable. The situation was blamed on an outbreak of *Giardia lamblia* (Figure 10.16). This flagellated protist can cause mild intestinal upsets, such as diarrhoea, but may also have more severe effects in the young or the elderly. It is often found in the bodies of cattle or wild animals and usually leaves them in the form of a cyst in the faeces. People become infected if they drink water that contains these cysts. In Australia, this has not usually been a problem as our sewage system is well isolated from our drinking water. However, it is a major problem in many developing countries, where travellers are advised never to drink water that is not bottled or boiled. Cryptosporidiosis and amoebic dysentery are also caused by pathogenic protists.



Science Photo Library/Dr Tony Brain

◀ **Figure 10.16**
Scanning electron micrograph of *Giardia lamblia* (yellow) in the human small intestine. This flagellated protist contaminates drinking water, causing intestinal upsets.

Malaria and its pathogen

Malaria has been plaguing the human species for many thousands of years. It is caused by protists from the *Plasmodium* genus. These are transmitted to the host by the bite of a female *Anopheles* mosquito. Sporozoite stages of the parasite are injected into the bloodstream as she feeds (Figure 10.17). After invading liver cells, sporozoites divide repeatedly to produce thousands of merozoites. These then leave the liver and enter the bloodstream, where they infect red blood cells and divide again. The life cycle of *Plasmodium* is completed when these merozoites form male and female gametocytes. At night, the *Anopheles* mosquito may bite an infected human, ingesting the gameocytes. Inside the mosquito, the gametocytes fuse to form zygotes that burrow through the wall of the mosquito stomach and form cysts. Sporozoites form within the cysts and migrate to the salivary glands of the mosquito, ready to infect a new host.

Infected red blood cells in the host eventually rupture, releasing merozoites 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 merozoites. If left untreated, the host may develop enlargement of the liver and spleen or, in the case of cerebral malaria, brain injury leading to death in severe cases.

Today, malaria is one of the most infectious diseases in tropical and subtropical countries. People planning to travel to countries where malaria is prevalent are encouraged to use antimalarial drugs, such as mefloquine. However, strains of *Plasmodium* have evolved that are resistant to these drugs and the incidence of malaria is rising. Effective vaccines have not yet been developed, making it extremely difficult to control the spread of this disease.

THE MALARIA LIFE CYCLE

Watch the video and draw a flow chart to show stages in the life cycle of *Plasmodium*.

WHO MALARIA FACT SHEET

A summary of current malaria information by the WHO.

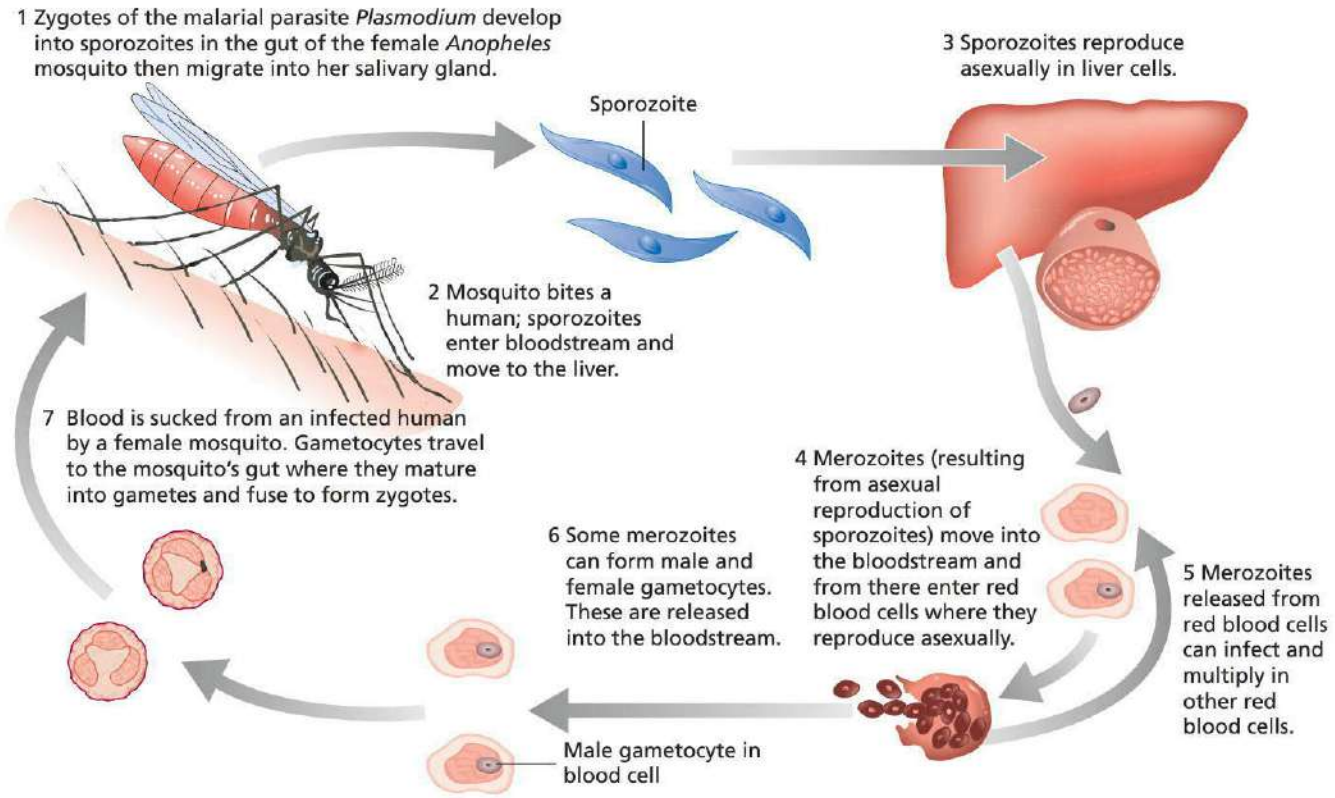


Figure 10.17 ▲ Life cycle of *Plasmodium*, the pathogen that causes malaria

Endoparasites

Many parasitic flatworms, roundworms and flukes call the human body home. The majority live in our intestines, where they consume nutrients, reproduce and release huge quantities of eggs into the faeces. A female *Ascaris* roundworm, living in the intestine for 12–18 months, can produce 25 million eggs, at an average daily output of 200 000. Often, they cause significant illness and blood loss. For example, hookworms parasitise more than 900 million people worldwide, causing daily combined blood loss of 7 million litres.

Science Photo Library/David Scharf

Figure 10.18 ▲ Hookworms are so-called because of their 'hooks' – tooth-like structures that enable them to cling to the host's bowel.

Some tapeworms that parasitise humans use other animals such as pigs, freshwater fish or cattle as **intermediate hosts**, that is a host in which they undergo development, but do not reach sexual maturity. In the intermediate host, eggs develop into juvenile stages that migrate and form cysts in the tissues. This stage can be especially dangerous to the intermediate host, as cysts can form in the eyes or brain (e.g. the pork tapeworm *Taenia solium*). Humans become infected when they eat pork, fish or beef that is raw or improperly cooked and contaminated with cysts containing tapeworm larvae. Once the parasites reach the intestine, they mature, and fertilised eggs are excreted in the faeces. Humans are called the **definitive host** as the adult phase of the parasite produces gametes in us. When eggs are eaten by grazing animals, they become intermediate hosts, completing the life cycle.

Hookworms, a type of nematode transmitted from contaminated soil, are especially serious in impoverished parts of the tropics and subtropics. Adult hookworms live in the small intestine of humans and other mammals, where they feed on blood and other tissues (Figure 10.18), causing anemia and protein deficiency. Adult females, about a centimetre long, can release a thousand eggs a day. These leave the body in faeces and then hatch into juveniles that live on the ground. These juveniles may penetrate the skin of a person walking barefoot. Once inside a host, the parasite travels through the bloodstream to the lungs, where it works its way into the alveoli. After

moving up the trachea, the parasite is swallowed. Soon it reaches the small intestine where it matures and may live for several years.

The roundworm *Trichinella spiralis* causes painful, sometimes fatal symptoms. It has a **direct life cycle**, as it completes its development in a single host. Adults live in the lining of the small intestine of pigs. Females release juveniles into blood vessels where they feed and travel to muscles where they **encyst** (produce a covering around themselves and enter a resting stage). Humans become infected mostly by eating insufficiently cooked meat from pigs or certain game animals. The presence of encysted juveniles cannot easily be detected when fresh meat is examined, even in a slaughterhouse.

Scientific literacy: What is the best way to defeat malaria?

This infectious disease poses a risk to approximately half of the world's population with more than 200 million cases of malaria and nearly 700000 deaths each year. Most malaria cases occur in Sub-Saharan Africa, Asia and Latin America and deaths occur mostly in children. In addition to its health toll, malaria places a heavy economic burden on these countries.

Early attempts to control the disease relied on the control of mosquito numbers by removing or poisoning their breeding grounds or the aquatic habitats of larval stages. In the 1940s, control was revolutionised by the targeted use of the insecticide dichlorodiphenyltrichloroethane (DDT).

Despite the success, the awareness of DDT's persistence in the environment, carcinogenic effects and its biological magnification up the food chain led to bans in many countries. Although DDT was still permitted in small quantities for the control of mosquitoes, the World Health Organization (WHO) changed its focus. It recommended the use of diagnosis and medical treatment, like anti-malarial drugs, to contain the disease, moving malaria control into public healthcare systems.

In the last decade, increasing numbers of illnesses and deaths from malaria have prompted WHO to reverse its position on the use of DDT and recommend wider use of this pesticide to control mosquitoes. They advocated an indoor residual spraying (IRS) program of spraying DDT on the interior walls of homes in malaria affected areas. Its action is threefold. Initially, it works as a spatial repellent, preventing the entry of mosquitoes into houses. It is also a contact irritant, repelling insects that land on the walls and thirdly, it kills mosquitoes on contact. WHO cites its high insecticidal activity, low acute mammalian toxicity, wide spectrum use, low price, long duration of activity, easy storage and transport and relative safety for the sprayer as benefits.

The call to increase DDT usage has been strenuously opposed by environmental groups such as the World Wildlife Fund (WWF), which is calling for the total ban of this pesticide. WWF advocates the use of alternative but more expensive insecticides, such as the pyrethroids permethrin and deltamethrin and the use of insecticide-treated bed nets. These chemicals must be reapplied more often than DDT to remain effective.

At the 6th annual MIM Pan African Malaria Conference in October 2013, South African delegates reported that they had lowered the mortality rate from malaria by 85% over the past 12 years. After the DDT ban in 1996, local infection rates tripled over the following 4 years, so in 2000, they reintroduced the use of DDT. In 2012, there were only 70 deaths in South Africa.

Mathematical modelling has long been applied to many aspects of malaria research, such as control and prevention strategies. Models can use a vast array of data and are highly useful for informing decision makers. They have the potential to determine the optimal strategies for malaria elimination in different epidemiological settings.

Questions

1. Describe three ways that DDT prevents people being bitten by mosquitoes.
2. Create a table that demonstrates the benefits and limitations of using DDT to control the malaria parasite.
3. Considering the evidence provided, suggest why pharmaceutical and chemical companies have joined the call to ban DDT.
4. Evaluate the claims made by the two sides in this debate and decide whether or not you support the use of DDT for malaria control. Use reasoning to construct scientific arguments to support your recommendation and present these in a letter to WHO.

CONTROLLING MALARIA

Use different control measures in the simulation to find the best way to reduce malaria in the area.

Science Source/Dr P Marazzi

Figure 10.19 ▲
A tick feeding on a dog. Ticks and fleas usually cause only minor skin irritations, but they may also transmit dangerous pathogens.

Ectoparasites

Ectoparasites are parasites that live on the surface of another organism. The most common group are arthropods and include fleas, ticks (Figure 10.19) and lice. While their biting may cause discomfort at times, most create only minor symptoms that can be easily treated. However, in many cases, they are **vectors** for the real villains. These organisms are capable of carrying some fearsome microscopic pathogens, including bacteria that cause plague and Lyme disease. Despite most people thinking that the plague is a disease of the Middle Ages, there are still cases of infection and death to this day. Asia and the USA continue to see cases, but most occur in African nations. Madagascar reports 300–600 cases per year and the disease caused 32 deaths in one outbreak in late 2013.

Cellular pathogens include bacteria, fungi, protists, endoparasites and ectoparasites.

QUESTION SET 10.5

Remembering

1 Describe the way in which fungi feed.
2 Name and describe two fungal diseases of plants.
3 Describe both the route of infection and symptoms caused by *Giardia lamblia*.
4 Distinguish between ectoparasites and endoparasites. Give two examples of each.

Understanding

5 Identify two benefits to a parasite of having an intermediate host.
6 Distinguish between the features of a fungal pathogen and a bacterial pathogen.
7 Describe the difference between malaria and *Plasmodium*.

Forms of transmission

To be able to persist and survive, pathogens must follow a repeating cycle of transmission from current to future host. This cycle can simply be direct transmission from one host to the next, or may involve one or more steps through an intermediate host or a vector. The first step requires the pathogen to escape from the body of its current host. It must then gain transport to a suitable new host, enter their body, establish itself in their tissues and finally ensure it is once again passed to a new host. Understanding their infectious cycles is critical to be able to identify suitable strategies to control pathogens.

Table 10.3 Common infectious diseases and their general method of transmission

Disease	Pathogen	Name of pathogen	Method of transmission
Common cold	Virus	Rhinoviruses and coronaviruses	Airborne: coughs and sneezes
Tinea (athlete's foot)	Fungus	*Trichophyton rubrum*	Direct contact
Plague	Bacteria	*Yersinia pestis*	Insect vector: flea
Influenza	Virus	Influenza virus	Airborne: coughs and sneezes

(continued)

Table 10.3 CONT.

Disease	Pathogen	Name of pathogen	Method of transmission
Ringworm	Fungus	*Microsporum canis*	Direct contact
Typhoid	Bacteria	*Salmonella typhi*	Food, water
AIDS	Virus	HIV	Body fluids
Food poisoning	Bacteria	*Staphylococcus aureus* and *Salmonella*	Contaminated food
Cold sores	Virus	*Herpes* simplex	Direct contact
Giardiasis	Protozoan	*Giardia lamblia*	Contaminated food
Gonorrhoea	Bacteria	*Neisseria gonorrhoeae*	Direct contact during sexual intercourse

Pathogens demonstrate a variety of adaptations that exploit different aspects of their transmission from one host to the next. For example, some pathogens like *Giardia* and *Shigella* are able to establish a foothold in their new host from a very low dose (Table 10.4). Pathogens differ in their ability to survive outside the body of a host. For example, HIV lives for only a few hours outside the host. On the other hand, the bacterium *Clostridium tetani*, which causes tetanus, can last for years as an inert spore. This adaptation means that *Clostridium* has an increased time for possible transmission to a new host. Other pathogens, including larger multicellular parasites such as tapeworms, have developed quite complex life cycles, often involving the use of intermediate hosts, as adaptations to ensure transmission.

Table 10.4 Examples of infective doses of some faecal–oral diseases

Disease	Infectious dose (in number of pathogens)
Shigellosis dysentery	10–100
Giardiasis	10–100
Rotaviral enteritis	100–1000
Salmonella	$>10^5$
Cholera	Usually 10^6–10^8
Typhoid	10^3–10^9

ACTIVITY 10.1

WHAT DOES A MILLION LOOK LIKE?

You will need

- small quantity of rice
- balance

What to do

1. Weigh out 1 g of rice.
2. Count the grains.
3. Calculate the mass of rice that would provide one million grains.

What did you discover?

Were you surprised by what a million looks like?

The most common human intestinal worm infection in Australia is the pinworm nematode, *Enterobius vermicularis*. Parents are most likely to be alerted to their child's infection by the symptom of an itchy bottom, which in reality is an adaptation for transmission. When mature, the adult female moves down the large intestine and exits the host via their anus to lay a batch of eggs on the surrounding skin. She then dies. The eggs cause intense itching, especially at night, so when people scratch their anus, they scrape eggs under their fingernails and onto their hands. As the eggs can survive for several days in the right conditions, the host can easily reinfect themselves or others by transferring eggs to their mouths or onto food. The whole life cycle of the pinworm then starts again.

Transmission by direct contact

Many pathogens have adaptations to ensure they are transmitted from one host to another when the skin of the two hosts comes into direct contact. Examples of these types of diseases are cold sores (*Herpes simplex* virus), chicken pox (*Varicella zoster* virus) and impetigo (*Staphylococcus aureus* or *Streptococcus pyrogenes* bacteria). One important adaptation for this mode of transmission is that reproduction of the pathogen in the skin often causes the formation of fluid-filled lesions teeming with millions of copies of the pathogen. These lesions resemble small blisters on the skin and are often very itchy. This prompts scratching, which allows the spread of infection to new areas or to a new host. Similarly, conjunctivitis makes a person's eyes itchy, and scratching them puts the pathogen on their hands from where it is easily spread.

Another adaptation to transmission is that asymptomatic shedding of the virus may occur. This may happen early in the infection by a pathogen, before any signs or symptoms of the disease are apparent or, in the case of herpes, between the occurrence of visible sores. Skin-to-skin contact at this time can still lead to transmission, even though the host is unaware that they are contagious.

Scabies, an infestation of the skin by a tiny mite called *Sarcoptes scabiei*, is another disease characterised by intense itching. This skin disease develops when a pregnant female mite burrows into a person's skin and lays her eggs. Larvae escape the tunnel and wander on the skin. They start new burrows and mature there to continue the cycle. This pathogen has an adaptation of provoking an allergic response that causes intense itching. Scratching releases eggs onto the skin surface, where they are easily transmitted to a new host. The result is that it is very contagious, spreading rapidly in crowded locations such as nursing homes and child-care facilities, where people spend extended periods of time in close contact with one another.

A **zoonotic** disease can be transmitted between animals and humans. While ringworm can be spread between humans, it is also commonly transmitted to people from pets such as cats and dogs, and domesticated animals such as sheep and cattle. This fungal infection gives rise to raised red rings on the skin. Like impetigo and other skin diseases, ringworm spores can also be transmitted during close contact sports such as rugby and wrestling.

In contrast, an example of transmission that does not use direct contact is when a **fomite** is involved. This is a non-living object such as bedding, towels, coins, toys or barbed wire that can carry disease-causing organisms. For example, the fungus *Trichophyton* that causes athlete's foot can be spread indirectly through towels and changing room floors. An adaptation for this type of transmission is the formation of resistant spores that can survive for extended periods on the fomite.

WOW

Do antibacterial soaps work?

Controlled studies have found that the use of antibacterial soap, kitchen spray and laundry powder is unnecessary. Households using them reported just as many coughs and colds, and just as much fever, vomiting and diarrhoea as those households that did not use them. Often it is the hands, that have been sneezed or coughed on and have then touched surfaces, that actually spread the disease. Studies show that it is the soap used in the process of handwashing that lifts and removes the bacteria, and controls the spread of infection.

Case study

How can mathematical modelling help the Tasmanian devil?

Tasmanian devils are suffering from a new disease that is decimating the population. Devil facial tumour disease (DFTD), as it is called, is an infectious disease that emerged in the mid-1990s. Scientists were initially baffled by the disease, taking some time to work out that DFTD is a very unusual and very rare contagious cancer. Cancer is not usually something you catch; it is something which dies with its host.

See Chapter 3, page 81, for more on DFTD.

In this cancer, the tumour cells themselves are the infectious agent and they are spread from devil to devil by biting, especially during mating. Once in the new host, the cancer cells grow into new tumours that block the eyes, mouths and ears, causing the animals to starve to death. This devastating disease has brought about devil population declines in excess of 90% in many areas of Tasmania.

In his work at the University of Tasmania, Dr Nick Beeton is using mathematical modelling to determine the best way to control this important wildlife disease. He finds this research compelling because it is a unique problem that no-one else has tackled before and answers are required urgently.

Dr Beeton says: 'The main output so far has been my work on modelling the possible effects of removing infected devils as a means of disease suppression; this method was trialled in the Forestier Peninsula in Tasmania's south-east but proved ineffective and was eventually dropped. My work demonstrated that even if more time and resources had been allocated to the project, a prohibitively high rate of removal would have been needed to make disease suppression effective.

'So far my work has helped the Save the Tasmanian Devil Team to determine the way in which they perform management, such as how often to trap, how much effort is needed ... and whether projects are worth starting or continuing based on the models' predictions.

'As an applied mathematician, I use a range of different mathematical and computational techniques to solve problems, sometimes using multiple computers concurrently to run the needed simulations; colleagues of mine also performing devil-based modelling tasks have also used supercomputers for this purpose. Information and communication technology is an important part of the work I do: often I'm dealing with large datasets, either publicly available online or directly from other researchers all over the world, and use a variety of techniques to obtain this information. Communication is particularly important as many of my collaborators are outside Tasmania and being able to effectively communicate with them online is vital.'

Courtesy Nick Beeton; Photographer: Kaitlyn Higgins

▲ **Figure 10.20**
Dr Nick Beeton is using mathematical modelling to determine the best way to control the devil facial tumour disease.

Questions

1. The cancer cells referred to here have been described as a very unusual pathogen. Use your knowledge of infectious disease and pathogens to argue either for or against this description.
2. Describe some potential benefits of developing mathematical models to describe the spread of disease.
3. A quick look at one of Dr Beeton's scientific papers shows graphs, numbers and equations. Discuss the value of information and communication technology and other technologies in managing data sets.
4. Colonies of uninfected devils have been set up on the mainland. How important is this investment in the long-term survival of devils to you personally?

EXPERIMENT 10.1

FOMITES AND THE SPREAD OF PATHOGENS

Some bacteria can survive for days or even weeks on surfaces such as handrails, chopping boards and bathroom sinks. In this activity, you will test the degree of contamination of four different fomites, by swabbing the objects and assessing the amount of bacterial colonies that grow on agar plates.

Aim

To compare the degree of contamination of four different fomites

Materials

Class requires:

- incubator set to 25°C

Each group requires:

- four nutrient agar plates
- marking pen
- unopened box of sterile cotton swabs
- sticky tape
- disinfectant solution

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately.
Disinfectants may damage clothes and cause skin irritation.	Wear gloves and lab coats.

Procedure

Note: To minimise contamination, wipe the bench down with bleach or alcohol before you start.

1. Choose four objects, such as a doorknob, chopping board or coin, that you think may be covered with bacteria. Write a hypothesis to predict degree of contamination of your four different fomites.
2. Sample one of your objects by rubbing a sterile swab tip across its surface.
3. Open the lid of the agar plate and, starting at the top, gently drag the swab in a zig-zag motion down and across the agar, taking care not to gouge the surface.
4. Replace the lid quickly, seal the plate with sticky tape and label it with your group's name and the name of the object.
5. Repeat steps 2 to 4 using your other fomites.
6. Place plates in an incubator at 25°C for 24–48 hours.
7. Ensure the bench is wiped down with bleach or alcohol and wash your hands thoroughly.
8. Devise a way of scoring the amount of bacterial growth on each plate (e.g. no coverage, partial coverage, complete coverage etc.)
9. The next day, do not open the plates. Use your scoring system to record the amount of bacterial growth on each of the plates.
10. Dispose of your plates as instructed by your teacher, ensure the bench is wiped down with bleach or alcohol and wash hands thoroughly.

Results

1. Record your results for each fomite in a suitable table.

Analysis of results

1. Which fomite grew more colonies? Why do you think this was the case?
2. Describe the pattern observed in the size and number of colonies in the streaking on each plate.

Discussion

1. Was there a control in this experiment? Explain why this is, or is not, important.
2. List four factors that you would need to control to make this a fair test.
3. Identify any possible limitations in the data by considering the sample size and measurement errors.
4. Write a conclusion, ensuring that you refer back to your hypothesis.

Transmission via body fluids

A cold can be caught by shaking the hand of a person who has a cold and who has just used their hand to wipe their dripping nose. The mucus from their nose will be teeming with cold virus particles. Once the hands of the second person are contaminated with cold viruses, they can be transferred into their nose or mouth by their fingers. This is transmission via a **body fluid**. Body fluids are any liquids that come from inside the body, including sweat, tears, vomit, nasal secretions, blood, saliva and urine. An adaptation to this form of transmission includes the ability to survive outside the body for substantial periods of time.

Have you ever shared drink bottles, eating utensils or lip balms? If so, you are exposing yourself to diseases such as glandular fever, cytomegalovirus and the common cold. The Epstein–Barr virus that causes glandular fever (or kissing disease) most commonly affects young adults and leads to fever, sore throat and swollen lymph nodes. Given there is no treatment, avoiding infection is the best option; however, the diseased person often has no symptoms and is unaware that they are infected. In the case of glandular fever, symptoms do not develop until 4–6 weeks after infection with the virus. Moreover, secretion of the virus from the throat can occur for months, or even years, after infection, with some healthy adults becoming long-term carriers. These are clever adaptations of the pathogen to enhance its spread to a new host. One way to reduce transmission is to treat all body fluids, including saliva, as potentially infectious.

A wide variety of pathogens can be spread when body fluids are exchanged during sexual contact. Sexually transmitted infections (STIs) caused by viruses include HIV, hepatitis B virus, human papillomaviruses (HPV) and herpes simplex virus. STIs caused by bacteria include syphilis (*Treponema pallidum*), gonorrhoea (*Neisseria gonorrhoeae*) and chlamydia (*Chlamydia trachomatis*). STIs cause significant illness and contribute greatly to increasing healthcare costs. In many cases, the infected person remains contagious but asymptomatic, an adaptation that would favour the transmission of infection.

Women may suffer severe complications from these STIs, such as pelvic inflammatory disease caused by bacterial infections and cervical cancer caused by HPV infection. With its national school-based HPV Vaccination Program, Australia has led the world in providing the vaccine to all males and females aged 12–13 years.

Foodborne transmission

One of the easiest ways for a pathogen to gain entry into the body is via the gastrointestinal tract, often by hitching a ride on our food. Bacteria such as *Salmonella*, *Campylobacter*, *Escherichia coli* and *Staphylococcus aureus* and viruses such as norovirus, hepatitis A and rotavirus cause these foodborne illnesses, commonly referred to as food poisoning. Although they have been recognised as diseases of humans for thousands of years, they are still quite common, affecting an estimated 5.4 million Australians each year. Sometimes it is the toxins and sometimes the pathogen itself that give rise to the disease.

In some cases, pathogens are spread to food from the faeces of an infected person. Their key adaptation to transmission is that they produce symptoms of watery diarrhoea, nausea and vomiting. A person with rotavirus gastroenteritis can excrete 10 000 million (that is 10^{10}) virus particles per millilitre of faeces. When a person visits the toilet or changes the nappy of an infected infant, their hands can easily become contaminated with pathogens. As the infective dose is only 100 to 10 000 virus particles, enough pathogen can be transmitted via food unless very strict handwashing procedures are followed. Moreover, harmful microbes, living elsewhere on the bodies of people, can be transferred to food by sneezing or coughing or if an infected person touches their nose or mouth and handles food without washing their hands.

Bacteria, in contrast to viruses, have the advantage of being able to reproduce to an infective dose outside the host. This makes it important to keep food out of the **temperature danger zone** of between 5°C and 60°C, where most bacteria can grow and reproduce. It is not uncommon for meat, poultry and eggs to be contaminated with *Salmonella* bacteria from the animals' intestines. Thorough cooking at temperatures above 60°C kills the pathogen. However, undercooked food may contain live bacteria that transmit the disease to humans. Food can also be contaminated after cooking. Luke-warm temperatures encourage bacterial growth to harmful levels, especially in food such as eggs, cream and mayonnaise that support multiplication of bacteria. This means that food needs to be kept in the fridge below 5°C.

The growing trend toward consuming take-away meals makes food poisoning an increasingly important problem in the developed world. Food should be eaten immediately after purchase or kept hot enough to kill bacteria. If it is to be stored, the food must be cooled very quickly to prevent the growth of bacteria that could cause food poisoning.

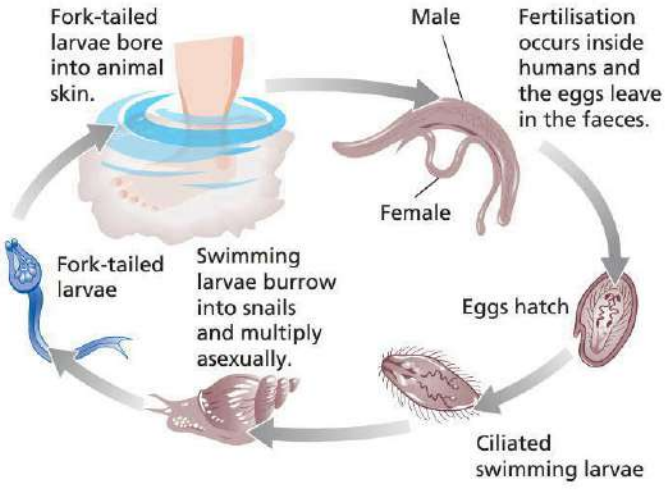
The evolutionary battle between host and pathogen is clearly illustrated by the changes noted in *Salmonella* over the last 50 years. External faecal contamination of egg shells commonly caused food poisoning after contaminating hands, cutting boards, knives and other utensils. Stringent procedures for cleaning and inspecting eggs, that were implemented in the 1970s, made this type of food poisoning extremely rare for decades. Lately, however, eggs have once again been found to be contaminated with *Salmonella*. Unlike egg-borne problems of the past, the current epidemic is due to intact and disinfected eggs. It seems that changes in human behaviour, such as cleaning and inspecting eggs, has selected a strain of pathogen that can migrate up the oviducts of chickens, infect their ovaries and contaminate eggs before the shells are formed around them.

Waterborne transmission

When travelling overseas to Africa or Asia, why are you told not to drink the tap water? In many developing countries untreated sewage is discharged into the environment or onto cropland. This may lead to the faecal–oral route of transmission of disease, when pathogens in human or animal faeces are introduced into the oral cavity of another host. Several important diseases are transmitted in contaminated water in this way. These include bacterial diseases such as typhoid, cholera and Shigella, and viral diseases such as Hepatitis A. Protists including *Giardia*, *Cryptosporidium* and *Entamoeba*, and endoparasites such as intestinal worms, can also be transmitted in contaminated water.

The South-East Asian blood fluke, *Schistosoma japonicum,* is carried by more than 200 million people and is one of several species of *Schistosoma* that causes the chronic, parasitic disease called schistosomiasis, the second most prevalent tropical disease in the world. Figure 10.21 shows its life cycle.

Figure 10.21 ▼ Life cycle of the blood fluke, *Schistosoma japonicum*, which causes schistosomiasis



The life cycle requires a human definitive host and an aquatic snail, the intermediate host. The blood flukes reproduce sexually and their fertilised eggs enter the intestines and leave the human body in faeces or urine. On contact with fresh water, they hatch into ciliated swimming larvae that burrow into a snail and multiply there asexually. In time, fork-tailed larvae leave the snail and swim until they contact human skin. They secrete enzymes that break down the proteins in the skin, an adaptation enabling their entry into host tissues. After migrating to veins near the intestine, they begin to feed on red blood cells and the cycle begins anew.

These blood flukes have many adaptations to ensure their transmission to their next host. With a lifespan of more than 4 years and producing up to 3000 eggs per day, they have a huge reproductive potential. They also reproduce asexually in the snail. The aquatic larvae can live for some time in water while searching for a human host. The worms live in male–female pairs, an adaptation for overcoming the difficulties in finding a mate. Another adaptation to evade the host's immune system is to incorporate human proteins into their surface structures, so that most people produce little or no immune response to the adults.

Clean water for drinking, washing and bathing, improved hygiene including handwashing, and good sewerage systems help to prevent the transmission of waterborne diseases. Water can be disinfected, by irradiation or with chemicals such as chlorine, and sanitation systems can be installed. Control of schistosomiasis is based on drug treatment, improved sanitation, health education and snail control that includes draining swamps because the flukes depend on water to complete their life cycles.

Airborne transmission

In 1976, a new human disease emerged from obscurity because of the use of a new technology: air conditioning. Have you ever seen health warnings on bags of potting soil? These warnings tell you to wear a mask to prevent inhalation of dust from the soil. What is dangerous about using potting mix and air conditioners? Both activities can transmit *Legionella* bacteria, the cause of an acute respiratory disease that can be fatal.

First identified in 1976 at an American Legion Convention in Philadelphia, Legionnaire's disease is now recognised as a relatively common cause of pneumonia. The pathogen lives in water, particularly in evaporative air conditioning systems, such as those commonly found in hotels, hospitals and large office buildings. More recently, the bacteria have been found in shower heads, hot tubs and other devices that produce fine water droplets called aerosols.

The disease is spread in the air and not by person-to-person contact. The case of Legionnaire's disease illustrates the link between a host's lifestyle and the transmission of a pathogen. Legionellosis seems to be a disease of human progress, brought about by devices that maintain water at warm temperatures and produce aerosols. Contamination of water sources has been associated with numerous outbreaks in settings ranging from inner-city hospitals to luxury cruise liners.

Many other pathogens have adaptations to enable transmission from one host to another in air. Examples include measles, mumps, SARS, TB, colds and influenza. A cough or a sneeze can release millions of microbes into the air in droplets of mucus or saliva that are so small they remain airborne for extended periods of time. If a droplet lands on the mucous membranes of a person's mouth, nose or eyes, they may catch the disease. Sometimes talking, singing or just breathing out is enough to allow pathogens to leave the host and become airborne in aerosols. These tiny particles can travel considerable distances in air currents. Crowded, indoor environments may promote the chances of airborne transmission, which explains the increase in respiratory infections during winter months.

Some pathogens increase their chances of transmission by stimulating excessive nasal secretions loaded with a new generation of pathogens. If the infection also irritates the mucous membranes, the subsequent coughing and sneezing expels droplets containing millions of copies of the virus or bacterium. Because these aerosols remain airborne, they can be carried over large distances, which may create a potential for long-range infections. Airborne transmission is highly effective; for example, 90% of people without immunity to measles will catch the disease if they share a living space with an infected person.

Transmission by vectors

A vector is a living organism that transmits pathogens from one host to another. Sometimes, the pathogen is dependent on the vector for the completion of its life cycle. Using a vector is an important adaptation for transmission because a pathogen may not otherwise come into contact with a new host. A vector may also enable a pathogen to penetrate the outer defences of the host in a way that would not be possible unassisted. For example, viruses would be unable to penetrate the cellulose cell walls of plant cells without the help of insects such as aphids. Mosquitoes, ticks, fleas, lice and flies are other examples of vectors.

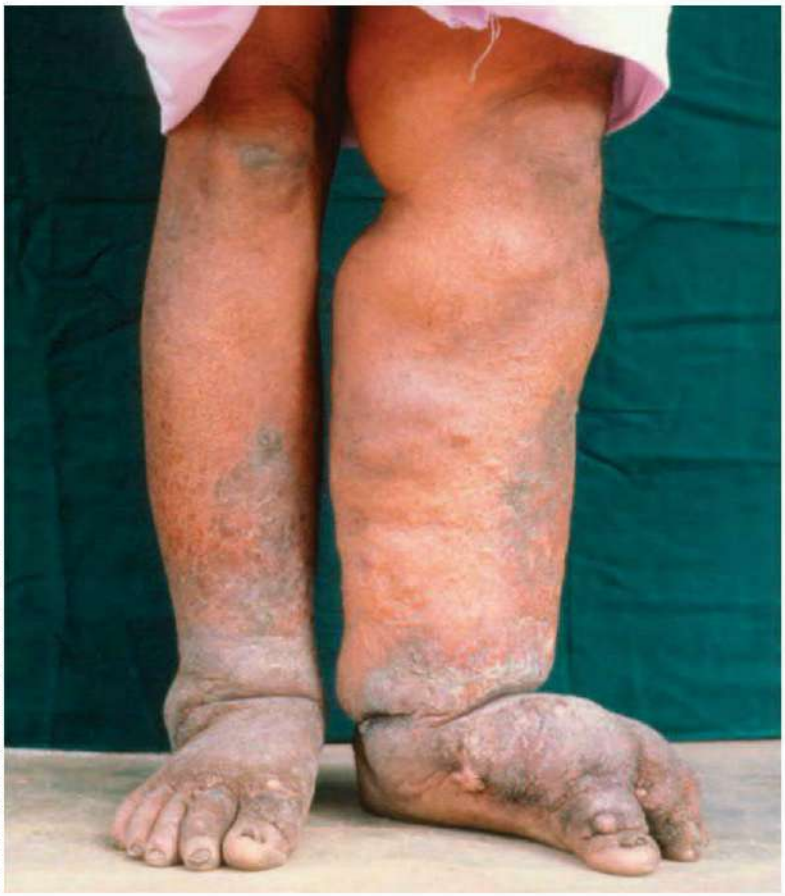
Bats, which are numerous in Australia, act as vectors for the *Hendra* virus. This virus causes a potentially fatal disease when humans become infected through close contact with the body fluids of an infected horse. Scientists believe fruit bats are the natural hosts of the *Hendra* virus. It is not known how horses become infected by the bats, but it seems likely to be via food, contaminated with bat urine or faeces.

FLU ATTACK! HOW A VIRUS INVADES YOUR BODY

Using a flow chart, summarise how a virus infects a throat cell and replicates inside that cell.

Biting insects are vectors for many diseases. The plague (also known as the Black Death) is often thought to be a disease of the past, but each year 1000–2500 cases occur globally. It is a bacterial disease of rodents caused by *Yersinia pestis* that can be spread to humans and other animals by infected rat fleas. Deer ticks may carry the bacterium that causes Lyme disease and female mosquitoes may carry malaria parasites and dengue fever.

The mosquito is also a vector for *Wuchereria bancrofti*, a filarial roundworm. Figure 10.22 shows the results of prolonged, repeated infections by this parasite. Adult worms become lodged in the body's lymph nodes, where they obstruct the flow of lymph, which normally trickles back into the bloodstream. When the obstruction causes fluid to accumulate, legs and other body regions undergo grotesque enlargement, called elephantiasis. Female *Wuchereria* produce young that move actively through the bloodstream at night. If a mosquito sucks blood from an infected human, the juveniles may enter the insect's tissues. In time, they move near the insect's proboscis, ready to enter a new host when the mosquito bites another human.

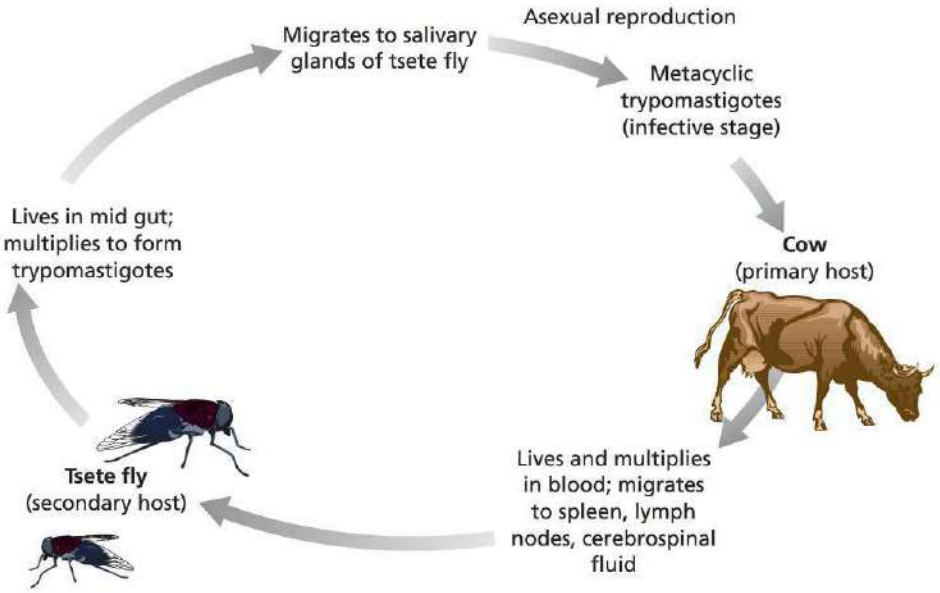


Science Photo Library/P. Umesh Chandran, TDR, WHO

Figure 10.22 ▶ Elephantiasis is a result of infection with the roundworm *Wuchereria bancrofti*, which results in gross enlargement of the legs and external genitals, and the formation of ulcers and tubercles.

African sleeping sickness is a debilitating and, for humans, ultimately fatal disease caused by a flagellated protist called *Trypanosoma brucei* that is transmitted by the tsetse fly. In the first stage of infection by *Trypanosoma*, invasion of the circulatory and lymphatic systems causes intense headaches, joint pains, fever and enlarged lymph nodes. The common name of the disease originates from the host's desire to sleep. This symptom occurs after the parasite invades the nervous system, disrupting the host's sleep cycle. *Trypanosoma* also infects African herd animals, such as antelopes and wildebeest, but these species have developed a high resistance to the parasite and suffer far less serious symptoms than humans.

Humans or cattle are first infected with *T. brucei* when bitten by the tsetse fly. Within the human host, the parasite multiplies in the blood and lymph nodes and may move into the cerebrospinal fluid. If the host is then bitten by another tsetse fly, the infective stages are ingested by the fly and multiply in its gut, doing it little harm. The parasite then moves to the salivary glands of the fly, multiplies and waits to be injected with saliva into the blood of another unsuspecting victim. This life cycle is summarised in Figure 10.23.



◀ Figure 10.23
Life cycle of *Trypanosoma brucei*, which causes African sleeping sickness

Knowledge of the pathogen's life cycle equips us with strategies to control the spread of this disease. Spraying insecticides to kill the tsetse flies and clearing habitats where they breed has helped. Another simple measure has been to prevent the tsetse fly biting either infected or uninfected people, using insect repellents as well as wearing protective clothing, and sleeping with nets and screens. Infected people need to be treated and, in some areas, game animals such as the antelope have been removed to prevent them acting as a reservoir for the pathogen.

The transmission of a pathogen from current to future host may occur directly from one host to the next, or may involve one or more steps through an intermediate host or a vector.

Pathogens have adaptations that facilitate their transmission by various mechanisms, including through direct contact, contact with bodily fluids, through the air and via contaminated food, water or disease-specific vectors.

QUESTION SET 10.6

Remembering

1 Summarise the six forms of disease transmission by copying and completing Table 10.5. One has already been done for you.

Table 10.5 Forms of disease transmission

Form of transmission	Pathogen adaptations	Example of pathogen
Direct contact	Reproduction in skin causing itchy skin lesions Asymptomatic virus shedding	*Varicella zoster* virus *Herpes simplex* virus

2 Define 'fomite' and give three examples of fomites.

3 Describe the ways in which a lack of running water, sanitation and garbage collection could increase the spread of disease.

4 Define 'body fluid' and give four examples of human body fluids.

Understanding

5 Distinguish between the features of an intermediate host and a vector.

6 Use an example to describe the transmission of a zoonotic disease.

7 Identify and describe two benefits to a parasite of using a vector.

CHAPTER SUMMARY

- Disease is any condition that interferes with the proper functioning of an organism.
- Pathogens are disease-causing agents. Cellular pathogens include bacteria, fungi, protists, endoparasites and ectoparasites. Viruses and prions are non-cellular infectious agents that are always pathogenic.
- Pathogens have adaptations to ease their entry into cells of vectors, intermediate hosts and final host.
- Infectious diseases are caused by any agent that can be transmitted from one organism to another.
- Non-infectious diseases include nutritional diseases, genetic diseases and diseases that arise from an interaction of genetic and environmental influences.
- Specific diseases are characterised by their virulence, incubation period and through recognisable symptoms.
- People differ in their susceptibility to different diseases.
- Viruses and certain parasites are host-specific.
- Pathogens have adaptations to facilitate their transmission between hosts. Examples of such adaptations include long-lasting resistant spores (or similar) to remain dormant outside a host, use of a vector, and ability to exist in water.
- Transmission of disease occurs by various mechanisms including through direct contact, contact with body fluids, through the air and via contaminated food, water or disease-specific vectors.
- Mathematical modelling can be used to simulate the spread of disease and help to formulate suitable interventions to curb the spread of the disease.

CHAPTER GLOSSARY

autoimmune disease a condition where the immune system attacks the body's own tissues

bacterial capsule a slimy layer sitting outside the cell wall of some species of bacteria

bacteriophage a virus that invades bacteria

binary fission the division of a cell into two without mitosis; a prokaryotic cell splits to form two daughter cells

body fluid any liquid that comes from inside the body

chitin a polysaccharide that is the main component of fungal cell walls and the exoskeletons of insects and other arthropods

communicable able to be communicated (transmitted) from one organism to another

contagious able to be transferred by direct contact

definitive host a host in which the adult phase of a parasite produces gametes

direct life cycle the life cycle where a parasite completes its development in a single host

ectoparasite a parasite that lives on the surface of another organism

encyst when organisms produce a covering around themselves and enter a resting stage

endemic broadly, common to a particular area; specifically, a pathogen that is prevalent at a constant rate within a population

endospore tough, dormant structures formed by many bacteria to help them resist unfavourable conditions and disperse to new hosts

fomite an inanimate object that can be contaminated with a pathogen

flagellum a whip-like appendage that helps bacteria move

genetic disease a disease arising from mutations inherited from parents

host an organism that is infected by a pathogen

immune system a complex network of cells, tissues and organs in the body that detects differences between 'self' and foreign organisms, and mounts an immune response

incubation period the time between infection and the onset of symptoms

infectious an agent that can be transmitted from one organism to another

intermediate host an organism in which a pathogen or parasite undergoes development and spends a small portion of its life cycle

lipopolysaccharide a lipid-sugar compound forming the outer surface of some types of bacteria

lysis the process of a cell bursting (verb: to lyse)

lysogenic phase part of life cycle of a virus in which the nucleic acid of the virus is integrated into host cell's DNA

lytic phase part of life cycle of a virus in which viral components are replicated and packaged to form new viruses that lyse the host cell

micro-organism a microscopic organism; for example, bacteria

non-infectious disease a disease that is not transmitted from one organism to another

obligate describes an organism that can survive only in another organism; it is 'obliged' to live there

outbreak an increase in the occurrence of a particular disease above the baseline level for that population

parasite an organism that lives on or in its host for all or part of its life, causing harm and gaining nutrition from the host

pathogen a disease-causing agent

pathogenicity the capacity of a pathogen to cause disease

peptidoglycan a protein–carbohydrate compound that forms the cell wall of bacteria

prion a small infectious protein

resistance describes the extent to which an organism is or is not affected by an agent such as a pathogen or chemical toxin

susceptibility describes the level of response by an organism to a pathogen; that is, its resistance

symptoms characteristic effects of a pathogen on the body

temperature danger zone the range of temperatures at which harmful bacteria can grow and reproduce in food

transmission the passing of an infectious disease from an infected host to another individual

vector a living organism that transmits pathogens from one host to another; a vehicle used to transfer DNA sequences from one organism to another

virulence refers to the ability of a pathogen to cause severe disease within its host

virus a non-cellular pathogenic agent with either DNA or RNA that can only reproduce inside a living host cell

zoonotic describes a disease that animals pass to humans; infections that are naturally transmitted between vertebrate animals and humans

CHAPTER REVIEW QUESTIONS

Remembering

1 Identify two adaptations that aid the entry and transmission of fungal pathogens to a new host.
2 Define 'non-infectious disease'.
3 Describe what is meant by cross contamination during food preparation.
4 State two important differences between a bacterium and a virus. Give two examples of diseases that are caused by each of these pathogens.
5 List five diseases that can be spread by direct contact during sexual contact.
6 State two diseases caused by each of the following pathogens: fungi, protists and endoparasites.
7 Describe two ways in which the spread of malaria could be reduced.

Understanding

8 Describe the reasoning behind keeping cold food below 5°C and hot food above 60°C.
9 *Staphylococcus aureus* causes food poisoning by releasing a toxin. Describe the effect of reheating food on the potential of this pathogen to cause food poisoning.
10 You should cook eggs well, and should not use dirty or cracked eggs. Provide an explanation for each of these precautions.
11 Gonorrhoea and genital chlamydia infections can have similar symptoms. Suggest how a microbiologist would determine whether a patient was infected with gonorrhoea, chlamydia, both or neither.
12 *Giardia lamblia* is a waterborne pathogen that can form resistant cysts. It is often found in the bodies of cattle or wild animals and usually leaves them in the form of a cyst in the faeces. Cysts have a tough resistant coat enabling them to survive for long periods in the environment under cool, moist conditions. People become infected if they drink water containing as few as 10 of these cysts. Explain how this adaptation aids the pathogen's:
    a survival.
    b transmission.
    c entry into to a new host.
13 Explain the reasoning behind each of the following statements.
    a Wash hands immediately after going to the toilet or handling raw foods and before handling cooked or ready-to-eat food.
    b Use different chopping boards, trays, utensils and plates when preparing raw foods and ready-to-eat food. If you have only one chopping board, wash it well in hot soapy water before reuse.

c Thoroughly wash all soil off any raw vegetables and fruits before preparing and eating them.

d Dry dishes with a different cloth to that used for wiping hands or bench tops.

e Wash dishcloths regularly.

14 An intermediate host often acts as a vector for the parasite. Name the intermediate host of *Plasmodium* and describe its role in the life cycle of this pathogen.

15 Describe two adaptations that help waterborne pathogens to be transmitted to a new host.

Applying

16 Draw a diagram to illustrate the life cycle of *Wuchereria bancrofti*. Clearly indicate the primary and secondary hosts. Use the diagram to:

a identify adaptations that enhance the transmission and entry of this parasite into its hosts.

b describe two methods to control this disease.

17 Using an example to justify your answer, explain why an understanding of a parasite's life cycle is essential in limiting the transmission of that parasite.

18 Figure 10.24 shows the life cycle of the liver fluke, *Fasciola hepatica*.

a In what group of organisms would you classify this pathogen?

b Look at the diagram of the adult fluke. Describe two adaptations of the fluke that suit its parasitic way of life.

c Name the definitive host of *Fasciola hepatica*.

d How does the liver fluke enter its definitive host?

e Describe the advantage of the liver fluke in having a free-swimming stage.

f The adult liver fluke is hermaphroditic; that is, it possesses both male and female reproductive organs. Describe the advantage of this feature for the parasite.

g Describe the advantages for the liver fluke in having an intermediate host.

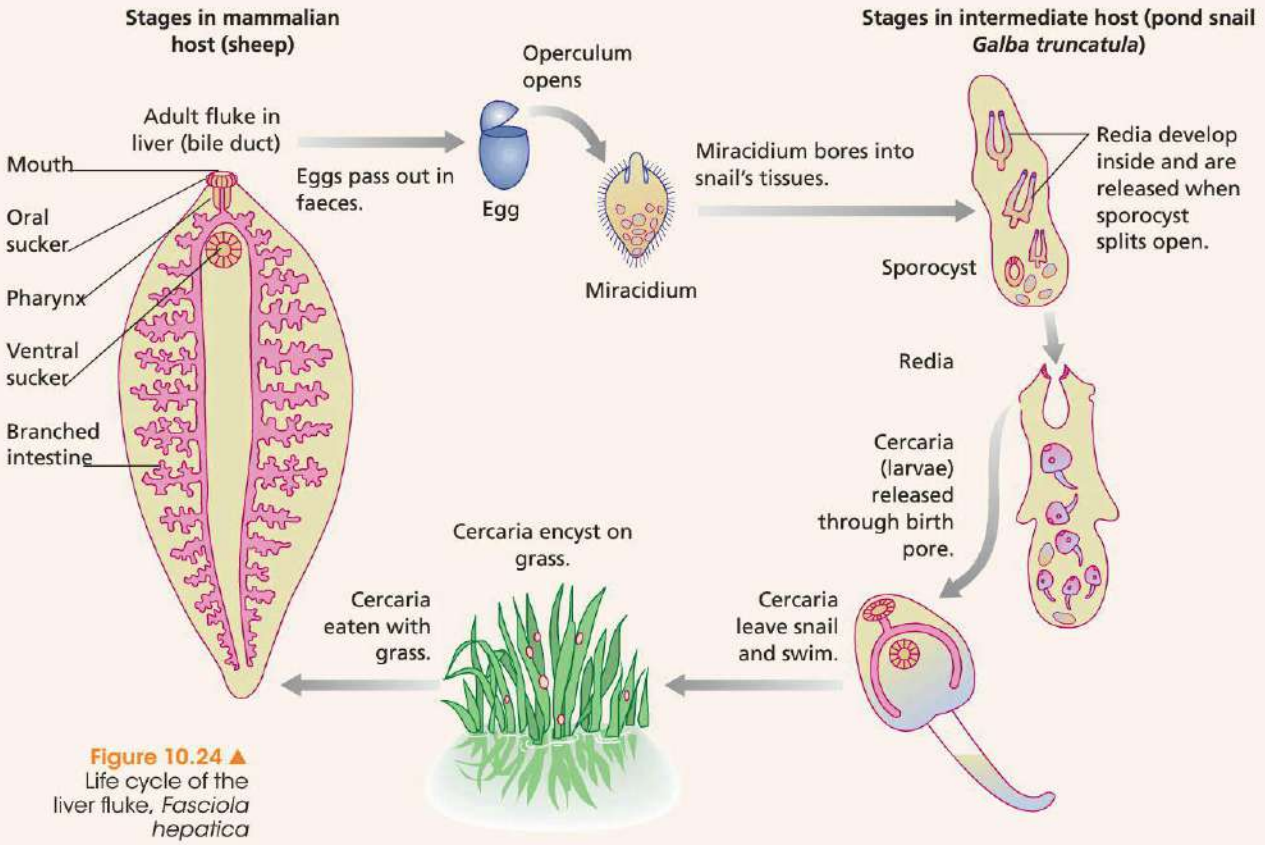
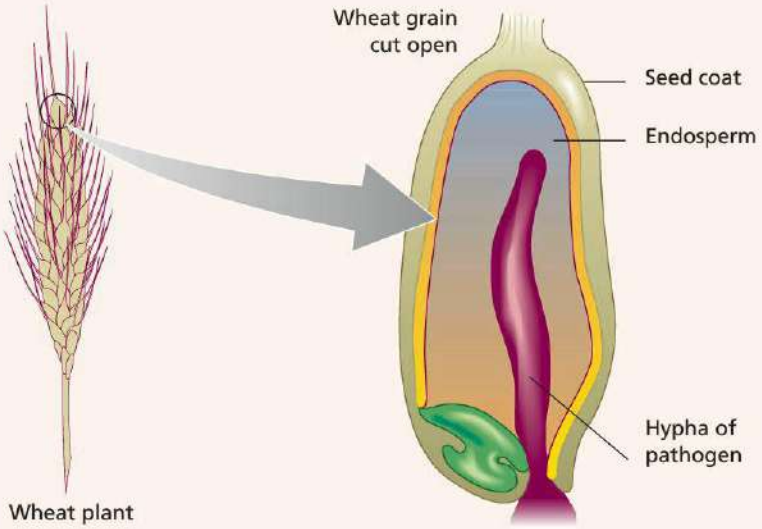


Figure 10.24 ▲ Life cycle of the liver fluke, *Fasciola hepatica*

Analysing

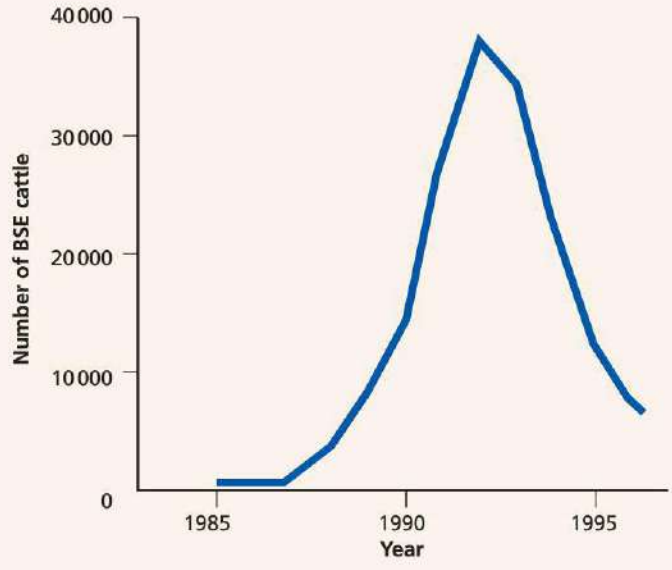
19 Figure 10.25 shows infection by a pathogen responsible for rust in wheat and rye.

- **a** State the major classification group to which this pathogen belongs.
- **b** Identify the part of the plant it probably gains access through.
- **c** Describe the damage it causes to its host.
- **d** Predict, with reasons, whether antibiotics would be useful in controlling its spread. Design a controlled experiment to test your hypothesis.
- **e** Describe two methods that could be employed to control the pathogen.



◀ **Figure 10.25**
Wheat plant and magnified cut wheat grain, showing infection by a pathogen

20 Figure 10.26 shows the number of cattle infected with the prion causing BSE (also known as mad cow disease) in Britain for the years 1985–95. Since 1992, feedstuff containing sheep offal has been banned.



◀ **Figure 10.26**
Number of cattle infected with BSE from 1985 to 1995

- **a** Describe the trend in numbers of BSE-infected cattle in Britain 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 1992.
- **d** There are fears that the infectious agent causing BSE is now infecting humans, causing Creutzfeld–Jakob disease. Describe measures that could reduce the transmission of this disease.

21 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.

22 Compare and contrast the ways in which prions and viruses reproduce.

Evaluating

23 Imagine you are able to remove one group of pathogens from Earth. Justify your choice by evaluating the effect of different pathogens on the quality of human life.

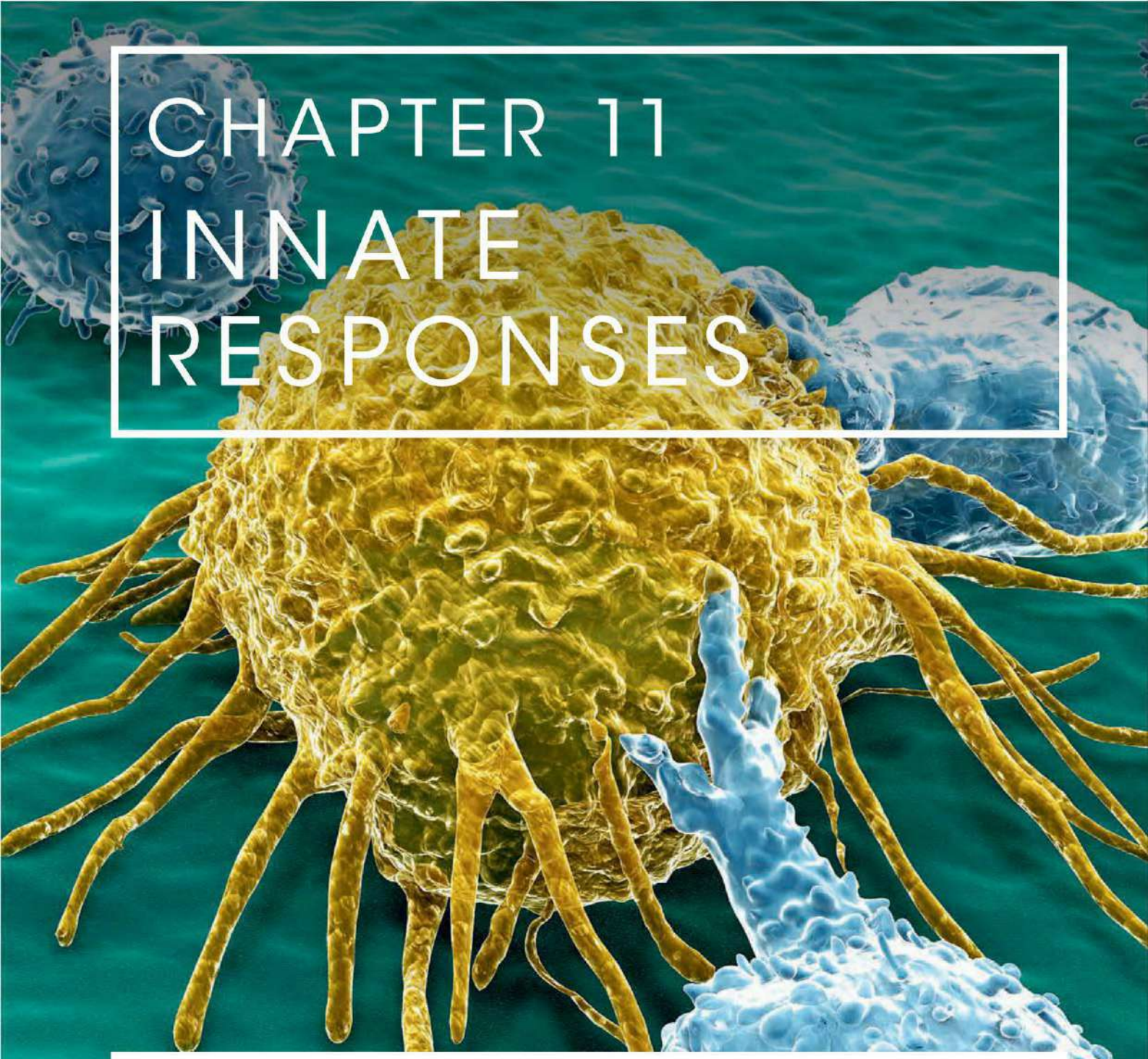
Creating

24 Many cleaning products have been designed to decontaminate surfaces such as handrails, doorknobs and bathroom sinks. Create a design for an investigation to test how well four substances disinfect a fomite such as a kitchen sink or bench top. Be sure to construct a hypothesis, devise a detailed method (including a control), and complete a risk assessment. Record your results in a table.

Reflecting

25 Some bacteria may be beneficial in recycling nutrients, while others can be pathogens causing disease. After reflecting on these two roles and judging their value, describe which role is more important in our world today.

CHAPTER 11
INNATE RESPONSES



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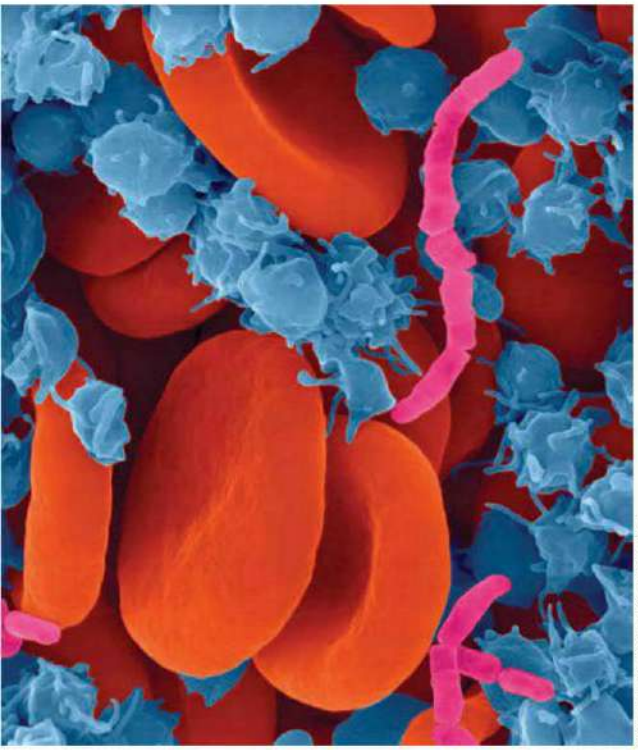
By the end of this chapter you will have covered the following material.

Science Understanding

- When a pathogen enters a host, it causes physical or chemical changes (for example, the introduction of foreign chemicals via the surface of the pathogen, or the production of toxins) in the cells or tissues; these changes stimulate the host immune responses (ACSBL119)
- All plants and animals have innate (general) immune responses to the presence of pathogens; vertebrates also have adaptive immune responses (ACSBL120)
- Innate responses in animals target pathogens, including through the inflammation response, which involves the actions of phagocytes, defensins and the complement system (ACSBL121)



Visuals Unlimited/Dr Dennis Kunkel

Figure 11.1 ▲
A rod-shaped bacterium (pink) is shown circulating among red blood cells and platelets (blue). When pathogenic bacteria enter the bloodstream the infection is known as blood poisoning, or septicemia.

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 invaders 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. In what has been referred to as an evolutionary arms race between pathogens and their hosts, all organisms have evolved various types of defence mechanisms to inhibit the entry of pathogens and deal with them should they gain a foothold. Even simple, single-celled organisms such as bacteria can defend themselves by producing enzymes to destroy invading viruses (bacteriophages). In this chapter and Chapter 12, the ways in which plants and animals detect and respond to attack by pathogens are discussed.

Preventing entry: keeping pathogens out

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. The first line of defence against disease comprises various physical, chemical and biological barriers designed to stop the entry of pathogens and other foreign substances. The scales of reptiles (Figure 11.2), 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.

HOW BLOOD CLOTS

View the animation and draw a flow chart to describe the events of clotting to seal a wound.

The skin: a tough physical barrier

As the largest organ in the human body, the skin 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**. After becoming **keratinised**, a process in which the structural protein **keratin** is deposited, the epithelial cells form a hard outer layer of the skin that is impervious to water and micro-organisms (Figure 11.3). 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 due to multiple infections caused by invading micro-organisms that overwhelm the immune system.

Figure 11.2 ▼
Reptiles, such as this iguana, have tough, scaly skin that is helpful for defence against some pathogens.

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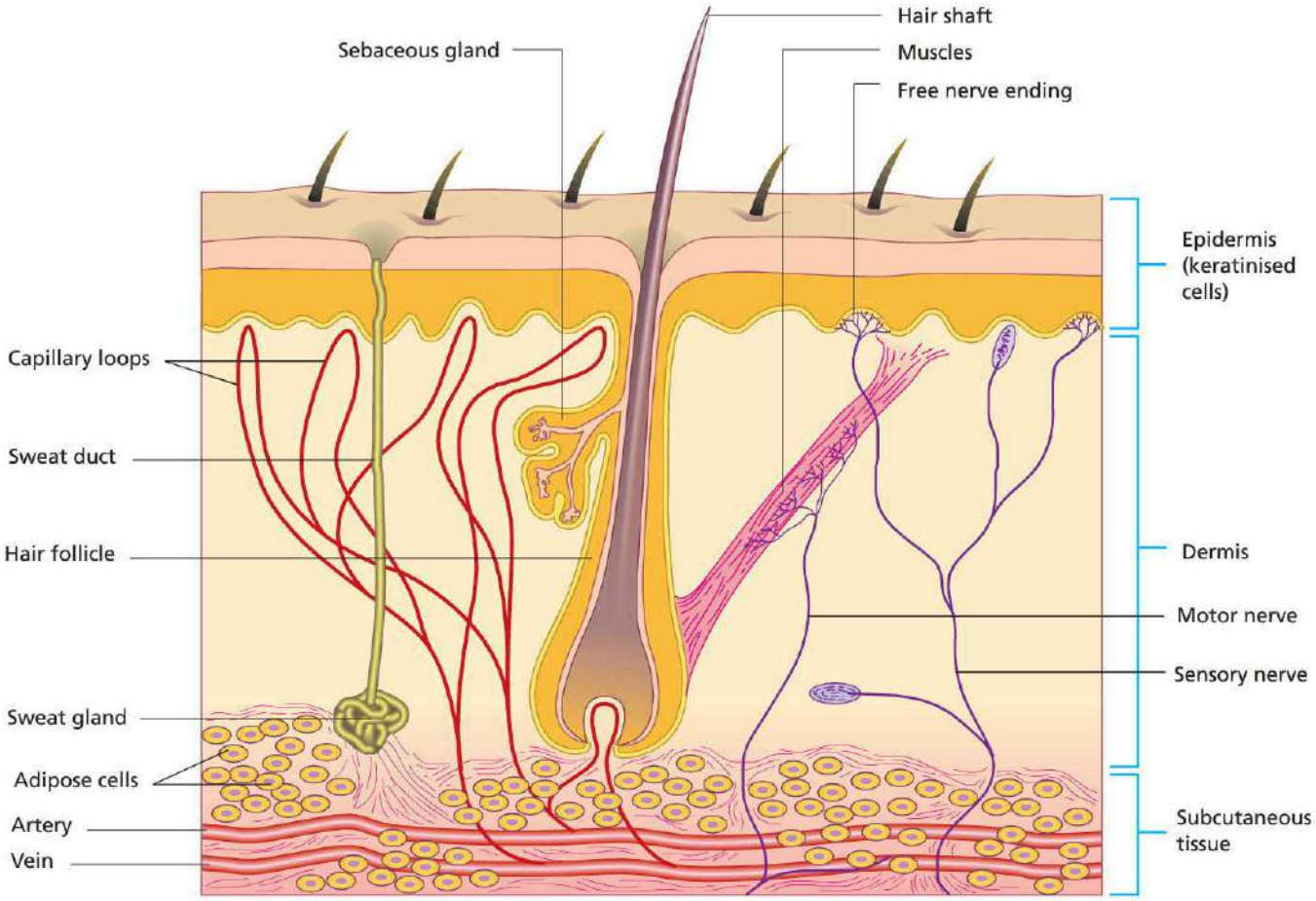
Damaged skin can become an ideal site for infection. In addition to burns, other injuries such as cuts and abrasions provide a potential site for the entry of pathogens. When the skin is cut and blood vessels are damaged, cell fragments in the blood, called **platelets**, are quickly attracted to the 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.

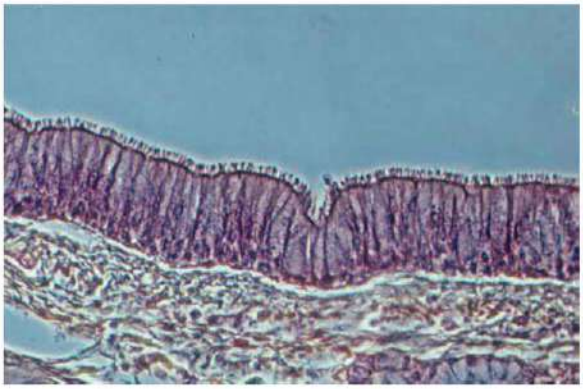


▲ **Figure 11.3**
A cross-section of human skin

Flushing out pathogens

As long as it remains unbroken, our tough waterproof skin is an effective barrier against invaders; however, the external openings of the respiratory, digestive, excretory and reproductive systems provide ideal entry points into any organism. Various mechanisms exist to physically trap and expel invading micro-organisms and other foreign particles.

The human respiratory, gastrointestinal and reproductive tracts are lined with epithelial cells that secrete mucus, which traps invaders. For this reason they are called **mucus membranes**. Slender hair-like structures called **cilia** line the respiratory tract (Figure 11.4). Their beating pushes mucus up to the throat, where it can be coughed out or swallowed. The effectiveness of mucus flow in clearing infection is illustrated by people with defective mucus secretion or inhibition of ciliary movement. They frequently develop lung infections caused by bacteria colonising the epithelial surfaces.



▲ **Figure 11.4**
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.

Coughing and sneezing can help to physically remove potentially harmful micro-organisms and foreign substances from the nasal passages and upper respiratory tract. Passing urine has a flushing effect on micro-organisms that are trying to enter the body via the urethra. Tears also help to flush out micro-organisms, preventing them from settling on the surface of the eyes. 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.

QUESTION SET 11.1

Remembering

1 Name the three types of barriers that form the first line of defence against disease.
2 List three openings in the skin that can allow the entry of pathogens.
3 Outline the role of mucus membranes.

Understanding

4 Describe three ways in which the body is able to flush out micro-organisms.
5 Recount the role of platelets in blood clotting.

Chemical defences

The epithelial surfaces of skin and the respiratory, digestive, excretory and reproductive systems are more than mere physical barriers to infection. They also produce chemical substances that destroy or inhibit the growth of micro-organisms (Table 11.1). This can be shown by spreading an equal number of typhoid bacteria on a person's skin and on a glass plate. Those on the skin die much more quickly than those on the plate. Skin secretions such as sweat and oil give the skin a pH ranging from 3 to 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 contained in food and drinks, as do the digestive enzymes secreted by the stomach and small intestine. **Lysozyme**, which is an enzyme contained 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** (burst).

Table 11.1 Summary of human defence barriers

Point of entry for pathogen	Mode of transmission for pathogen	Barriers or mechanisms to prevent entry of pathogen
Skin	Direct contact	Keratinised skin cells, rapid blood clotting, rapid wound healing, **antiseptic** action of acidic secretions
Digestive system	Ingested food and drink	Lysozyme in saliva and mucus, enzymes and strong acids in stomach
Respiratory system	Water droplets in air	Mucus traps dirt and small pathogens; cilia lining trachea move this upwards.
Reproductive tract	Sexual contact	Mucus contains acids; moving fluids flush out pathogens.
Urinary tract	Bacterial entry into urethra	Urine flushes out pathogens and its acidity inhibits bacterial growth.
Sense organs	Direct contact	Ear wax and hairs, eyelashes and nostril hairs trap pathogens; tears wash away pathogens and contain lysozyme.
Bloodstream	Pathogens use a vector organism (e.g. a mosquito) to inject themselves directly into the bloodstream	As these pathogens avoid the first lines of defence, they are subject to the host's immune system internally.

EXPERIMENT 11.1

SECOND-HAND DATA ANALYSIS: IS LYSOZYME AN EFFECTIVE BARRIER AGAINST BACTERIA?

This experiment is designed to compare the antibacterial effectiveness of lysozyme from tears, with an antiseptic and a disinfectant. The method and results from the experiment are presented for the students to interpret and analyse.

Introduction

With their warmth and moisture, the eyes are an ideal entry point for bacteria into the human body. Tears contain a powerful antibacterial enzyme called lysozyme that is able to rapidly destroy pathogens by lysing them. This experiment uses agar plates, spread with a culture of bacteria, to compare the bactericidal effectiveness of lysozyme with an antiseptic and a **disinfectant**. Bacteria can be grown on agar plates to produce a bacterial 'lawn'; a cloudy film of millions of bacteria on the surface of the agar plate. If paper discs containing antibacterial substances are placed on the agar, they produce clear areas where bacteria cannot grow.

Aim

To compare the antibacterial effectiveness of lysozyme from tears, with an antiseptic and a disinfectant

Materials

Class requires:

- broth culture of *Escherichia coli*
- incubator set to 25°C
- lab coats
- safety glasses
- gloves

Each group requires:

- three nutrient agar plates
- one filter paper
- one sterile 5 mL pipette
- forceps
- glass spreader
- onion
- 10 mL each of disinfectant, antiseptic and distilled water
- Bunsen burner
- sticky tape
- ruler
- dilute disinfectant solution; for example, bleach

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
While lab strains are usually harmless, bacteria may cause disease, so assume them to be pathogenic.	Wear lab coats, safety glasses and gloves; wash hands thoroughly at end. Decontaminate benches before and after activity. Flood spills with bleach.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately after autoclaving.
Onions contain substances that irritate the eyes and nose.	Ensure onion is held close to eyes, but does not actually come in contact with face or eyes.
Ethanol may be used to sterilise the bench top and is highly flammable.	Be careful to avoid ignition of ethanol liquid or fumes when using the Bunsen burner.

Method

Note: To minimise contamination, wipe the bench down with bleach or ethanol before you start.

1. Fold a piece of filter paper into quarters and, using a hole punch, make four filter-paper discs.
2. Label the base of the plate with date and name of group, and then divide into four quarters. Near the edge of the plate, label each of the four quarters: water, lysozyme, antiseptic and disinfectant.
3. Remove 1 mL of *E. coli* culture with the pipette, lift the lid off the labelled plate and transfer the bacteria to the surface of the agar.

4 Either replace the lid quickly and spread the liquid evenly by swirling, or spread the liquid evenly with the glass spreader, then replace the lid. Leave on bench for 2 minutes to allow bacteria to penetrate agar.
5 Make your eyes water by holding a cut onion near them, and blink to get tears to run down your face.
6 Sterilise the forceps in the Bunsen burner flame, allow them to cool, then pick up a filter-paper disc and carefully dip it into one of the tears. Quickly touch the edge of the disc to the remains of the folded filter paper to blot, then gently place the disc on the quarter of agar plate labelled lysozyme.
7 Make small quantities (10 mL) of disinfectant and antiseptic solutions by diluting according to directions on the bottles.
8 Resterilise the forceps and moisten a disc by dipping it into antiseptic and blotting, then gently place the disc on the correctly labelled quarter of the agar plate.
9 Repeat step 8 for disinfectant and for distilled water.
10 Repeat steps 1 to 9 twice more to make a total of three replicates.
11 Seal the plates with sticky tape and incubate at 25°C for 24 hours.
12 Ensure the bench is wiped down with bleach and wash hands thoroughly.
13 The next day, observe for the presence or absence of growth near the discs.
14 Measure the diameter of the zone of inhibition, which is the clear area around each disc. This shows the degree of sensitivity of the bacteria to each substance.

Results

The following table shows the data that one group of students obtained when following the above method. Calculate the mean values and draw a suitable graph to represent the data.

	Diameter of zone of inhibition (mm) for each substance			
Trial	**Lysozyme**	**Antiseptic**	**Disinfectant**	**Water**
1	11	13	15	6
2	16	17	13	8
3	12	12	16	7
Mean				

Analysis of method

1 What steps were taken in the method to ensure there was no cross-contamination?
2 Explain the role of the disc dipped in water.
3 Explain the purpose of the three agar plates.
4 Identify one other risk and how you would manage it.

Analysis of results

Describe the results by stating the order of effectiveness of each of the solutions as bactericides.

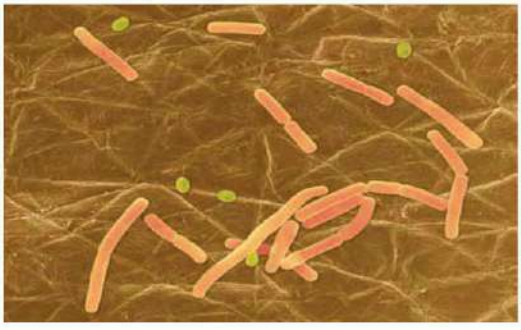
Discussion

Compose a discussion of your findings (minimum 300 words) as per the scientific method.

WOW

Are we people or bacteria?

An adult human body consists of approximately 10 trillion eukaryotic cells. An additional 100 trillion prokaryotic micro-organisms live on the surface of the skin and the mucus membranes lining the digestive, respiratory and reproductive tracts. This means there are 10 microbial cells for every human cell in our body!



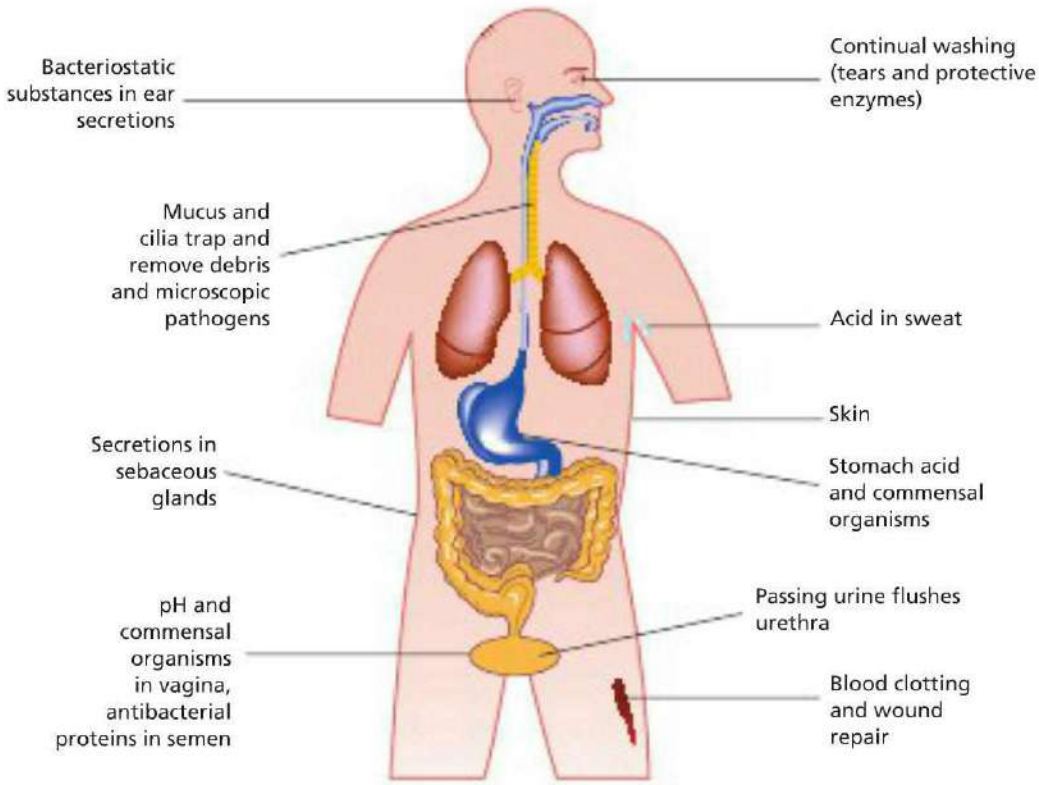
Visuals Unlimited/Dr Dennis Kunkel

Figure 11.5 ▶ Bacteria living on the surface of the skin

Biological barriers that prevent the entry of pathogens

During the birth process, a baby acquires various micro-organisms that will become permanently associated with it. The symbiotic micro-organisms that live on and in our bodies, but do not cause disease, are our normal **microflora**. By taking up space and using nutrients, our normal microflora prevent colonisation by other pathogenic micro-organisms. When non-pathogenic bacteria are killed by **antibiotic** treatment, any pathogenic micro-organisms with antibiotic resistance may replace them and cause disease.

Structural, chemical and biological features can act as barriers to pathogens as a first line of defence.



◀ **Figure 11.6**
Summary of the physical and chemical barriers to pathogenic infections in a human

QUESTION SET 11.2

Remembering

1 Outline three ways in which chemicals defend the body.
2 State three places in the body where low pH kills pathogens.
3 Outline the role of enzymes in defending the body from pathogens in food.

Understanding

4 Describe two ways in which tears protect the body from disease.
5 Identify two advantages of hosting our own microflora on our skin.

Scientific literacy: Introducing the poo transplant

Some wily species of bacteria have evolved to take advantage of gut microflora that has been thrown out of balance. The diarrhoea-causing bacterium, *Clostridium difficile*, is one such organism that flourishes in the power vacuum that results after antibiotic treatment. The unsavoury, yet highly effective treatment that has been adopted as an alternative to antibiotics is the faecal microbiota transplant, aka the poo transplant. A poo transplant is exactly as it sounds: taking faeces from a healthy donor, and transferring it, usually via enema, to a willing recipient. By replacing a depleted, out-of-balance gut ecosystem with a robust and healthy one, balance is restored. *C. difficile* becomes out-competed by friendly bacteria and the diarrhoea ceases. Unlike blood infusions and tissue transplants, faecal transplants require no immunological typing (tests to determine donor-recipient compatibility) to prevent rejection.

Faecal transplants are not new. A Denver surgeon, Dr Ben Eiseman, and his colleagues published the first report of the procedure in 1958. And once again, doctors are discovering what Eiseman did 50 years ago – that poo transplants work. A recent review of all reported studies of faecal transplants to treat *C. difficile* infection found poo transplants to be effective in more than 90% of cases. Recurrence of infection is rare and there has not been a single report of adverse side effects.

As we grapple with the complexity of our microbial ecology, perhaps we will discover which specific microbes are responsible for reigning in *C. difficile* during a faecal transplant. By identifying the microbes responsible, the poo transplant could eventually be replaced with a probiotic pill containing only the necessary species required to right the system. The 'yuck' factor would be removed.

Poo transplants are the ultimate in probiotics. Although consuming a tub of *Lactobacillus*-laden yoghurt is easier to swallow than the idea of a faecal enema, the principles are essentially the same.

Adapted from Lewis, D. (2012) 'Trading chemistry for ecology with poo transplants', *The Conversation* online, 6 December.

Questions

1. Discuss your reaction to this article and its contents, and whether or not you would undertake this treatment if you were sick.
2. Assess the science behind this treatment and discuss the validity of this application with respect to our immune system as you currently understand it.

The immune system

In all organisms, the first line of defence is provided by effective physical, chemical and biological barriers that reduce the chance of pathogens gaining entry. If a pathogen breeches the 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 that particular pathogen invades the body. This response is called the **innate immune response** and is sometimes described as being the second line of defence. This chapter will mainly discuss the innate immune response. The third line of defence is the **adaptive immune response**, which develops into a potent action against a pathogen and involves the activation of specific 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.

The adaptive immune system is discussed in detail in Chapter 12.

Organs, tissues and cells of the immune system

The **primary lymphoid organs**, the bone marrow and thymus, are responsible for the production and development of the cells of the immune system. The **secondary lymphoid organs** harbour matured cells of the immune system and provide the environment for the initiation of the immune response. Table 11.2 provides a summary of the main organs of the immune system.

Table 11.2 Organs and tissues of the human immune system

Organ or tissue	Role
Primary lymphoid organs	These organs are responsible for the production and maturation of the cells of the immune system.
Bone marrow	Bone marrow is found in the central shaft of certain bones, including the thigh and pelvic bones. All blood cells develop from bone marrow stem cells. These stem cells are multipotent because they can develop into all types of blood cells.
Thymus	The thymus sits inside the ribcage and is made up of two pinkish grey lobes. It is involved in the development of mature T cells (a type of white blood cell) and shrinks (involutes) with age, beginning soon after birth.
Secondary lymphoid organs	Secondary lymphoid tissue provides the environment for the initiation and progression of the immune response.
Lymph nodes	These small, bean-shaped structures are found in specific locations throughout the body, including the throat, armpits, groin, abdomen and chest. They filter the extracellular fluid (lymph) that drains from limbs and mucosal tissues, trapping foreign material, and are sites where lymphocytes can come across pathogens and begin to respond to them.
Spleen	The spleen is a large, purple-coloured organ located just above the stomach. Functions include filtering the blood, recognising and destroying old and faulty red blood cells, detecting foreign invaders and producing antibodies. The spleen is 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 respiratory, 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 immune 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.

Cells of the immune system play an important role defending the body. Like all blood cells, they are produced in the bone marrow from blood stem cells and are sometimes described using the general term white blood cells. There are various types of immune cells. 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 and destroy invading pathogens and other foreign material.

The lymphoid organs of the immune system are discussed in more detail in Chapter 12.

Recognising 'self' from 'non-self'

Despite effective barriers to infection, some pathogens are able to gain access to their host. Both plants and animals are alerted to this invasion by physical and chemical changes that occur in their cells or tissues, which enable them to recognise **'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.

The host immune response is triggered by foreign molecules on the surface of pathogens or the chemicals they release. Destruction and removal follows.

FLIES, SPACE AND DEFENCE

Compare and contrast the detection of invaders by toll-like receptors in fruit flies and humans.

Cells of the immune system recognise molecular patterns that are characteristic of microbes, but are not found on host cells, as 'non-self'. These molecules include

EPITOPES AND ANTIGENS

Watch the animation and draw a diagram to demonstrate your understanding of the concept of the difference between an epitope and an antigen.

lipopolysaccharides (LPS), peptidoglycans, chitin, some glycoproteins and particular protein sequences. Immune cells have evolved to recognise these molecules because they are unique to pathogens and have remained largely unchanged during evolution.

These molecules are called **pathogen-associated molecular patterns (PAMPs)**. The receptors that recognise PAMPs are called **pattern recognition receptors (PRRs)** and are found on the surface or in the cytoplasm of a variety of body cells including white blood cells and epithelial cells. PRRs include **toll-like receptors (TLRs)** in membranes and **NOD-like receptors (NLRs)** in the cytoplasm.

A particular PRR can recognise a variety of different pathogens if all of them display the same molecular pattern (PAMP). For example, the material that makes up bacterial flagella, called flagellin, is a PAMP found in a wide variety of bacteria. This enables the PRR for flagellin to recognise many different types of bacteria as invaders. Similarly, double-stranded RNA is another PAMP that enables detection of a variety of viruses that have their genomes encoded in dsRNA, a form of RNA not naturally found in vertebrates. Although this system of recognition has the advantage of activating a rapid response to invaders, it lacks a high degree of specificity and some pathogens have evolved ways to evade detection by the system.

Other components of the immune system are able to recognise and react specifically to one particular pathogen. **Antigens** are usually proteins or polysaccharides and their name comes from their role as 'antibody generators'. PAMPs; toxins and enzymes secreted by bacteria and fungi; and substances produced by an organism, such as snake venom, can be antigenic. Recognition occurs when antigens bind to receptors on the cell surface of various immune cells, especially macrophages, and leads to the beginning of an immune response.

The details of the roles of epitopes, antibodies and antigens in the adaptive immune response are discussed in Chapter 12.

Even a small section of a molecule, such as a toxin or PAMP, can generate an immune response. Part of a molecule that is recognised by the immune system and binds with receptors is called an **epitope**. As only a small peptide length may be potentially antigenic, most proteins have several epitopes, each of which is recognised by a different **leukocyte** and induces the production of a different antibody. Each different epitope is a specific chemical group or structure.

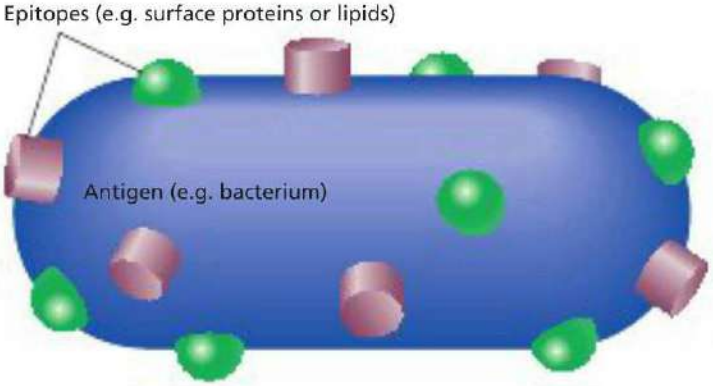


Figure 11.7 ▶ The distinction between an antigen and an epitope. A large antigen, such as a bacterium or a large protein complex, may have several different antigenic determinants, called epitopes. The different epitopes are specific chemical groups or structures.

Immune cells, such as macrophages, begin the immune response after their receptor sites bind with foreign antigens.

QUESTION SET 11.3

Remembering

1 Give the full name of PAMPs and describe their role in defence.
2 Provide three examples of the types of molecules that act as PAMPs.
3 Outline the role of PRRs and give two examples of these receptors.
4 State the collective name for white blood cells.

Understanding

5 Describe the importance of an organism being able to distinguish between 'self' and 'non-self'.

Case study

Pain and the immune system

Two specialist pharmacologists from the University of Adelaide's School of Medical Sciences have found a link between our immune system and how we feel pain. Their research shows that while the brain and nerves play a role in pain signalling, the immune system is involved too. Costing in excess of $12 billion per annum, pain and its associated suffering is the fourth most prevalent health issue in Australia and has the single biggest societal impact. Dr Mark Hutchinson and Professor Paul Rolan are collaborating in a search for ways to not just treat chronic pain, but to prevent and cure it.

Ninety per cent of the cells in the brain and spinal cord are immune-like cells. The question was 'how did the immune cells detect the pain?' Dr Hutchinson says. 'Work conducted in the USA first implicated the innate immune system receptors, toll-like receptor 4 (TLR4), in chronic pain. We extended this work to demonstrate that drugs that can block TLR4 are capable of reversing chronic pain. This work was extended even further when we showed that some types of pain killers, that are known to have significant side effects, could activate the same TLR4 signalling and this contributed significantly to their unwanted actions. We are now developing new drugs to target this system in the hope we can treat pain more effectively and more safely.'

Courtesy of Dr Mark Hutchinson

▲ **Figure 11.8**
Dr Mark Hutchinson from the University of Adelaide's School of Medical Sciences

Dr Hutchinson commented on the acceptance of his novel findings: 'Interestingly, the scientific community were much more resistant to some of the ideas than the medical community. This has changed gradually over time, but established concepts are hard to modify, even if you have the data to prove it.'

Questions

1. Write a letter to the Australian Research Council in which you use reasoning to construct scientific arguments to support the funding of Dr Hutchinson's current and future research.
2. Given that the acceptance of scientific knowledge can be influenced by the social, economic and cultural context in which it is considered, create an argument as to why scientists may be more resistant than doctors to new theories.

Host immune responses to the presence of invaders

When the immune system detects a pathogen it responds in a variety of different ways. Innate immune responses are **non-specific** and are inborn features of the way the body works. Adaptive immune responses are specific and retain memory acquired from previous experience of that specific pathogen. The two types of response are closely linked: the detection of 'danger signals' such as PAMPs and subsequent initiation of an innate immune response is required for an adaptive immune response to occur, and signalling molecules produced in an adaptive response can further stimulate an ongoing innate response (Figure 11.9).

Innate immune responses are non-specific and are inborn features of the body. Adaptive immune responses are specific and can retain a 'memory' of previous pathogens.

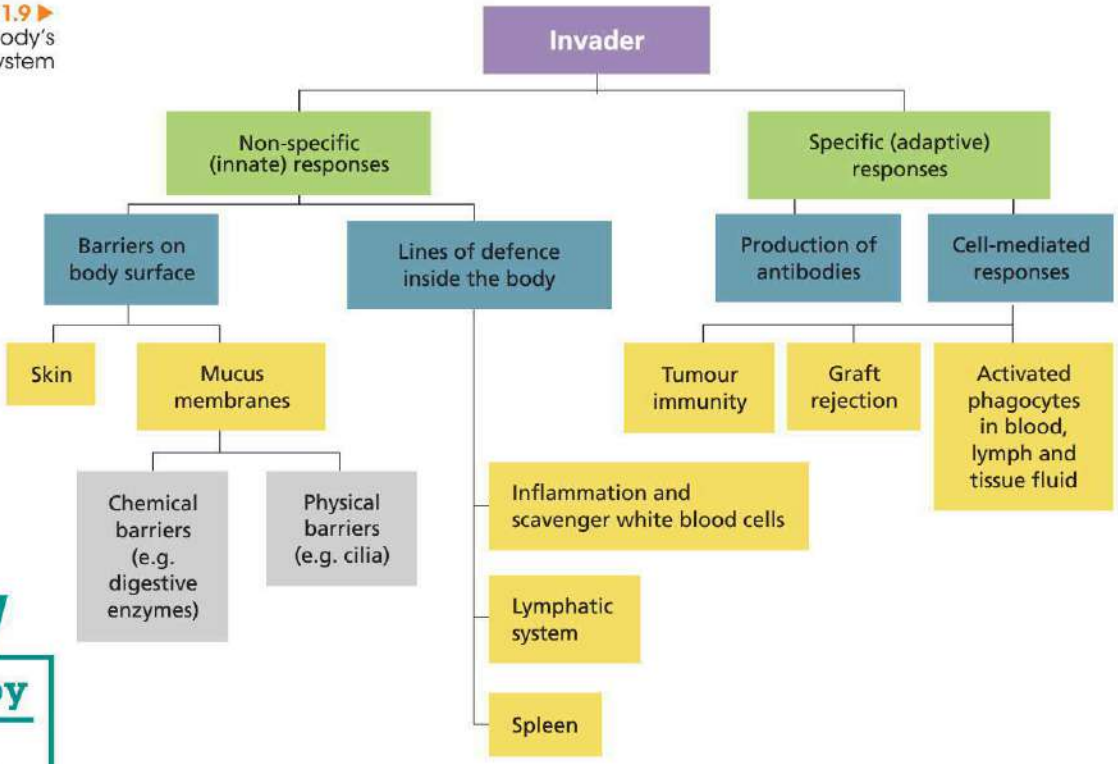
Adaptive responses

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 as they attack only the organism that stimulated the response. Because of this specificity, the body requires some time to tailor its custom-made response, meaning that adaptive responses are not as rapid as innate responses.

Chapter 12 covers the adaptive immune responses of vertebrates in detail.

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.

Figure 11.9 ▶ The human body's defence system



WOW Biotherapy

Many soldiers of Napoleon's army owed their lives to blowfly maggot infestations in their wounds. As they hatched, the maggots ate away only the dead tissue and helped reduce bacterial infection. With the increasing problem of antibiotic resistance, doctors have now returned to this form of biotherapy. Legislation, passed in 2004 in both the USA and Great Britain, regulates the use of maggots for cleaning non-healing necrotic skin and soft tissue wounds.

Innate responses

Innate immune responses are non-specific. This means that the immune system responds to any invader rapidly and regardless of its type. The responses are not learned and not influenced by our own past experience, although they have been shaped by evolution, following past experiences of our ancestors. This type of immunity is the natural resistance with which any organism is born. As with all genetically determined traits, there is significant individual variation in the effectiveness of the innate immune responses. This explains the significant variation in natural resistance, and hence disease susceptibility, between individuals, whether they are people, plants, insects or other organisms.

All plants and animals mount innate immune responses to pathogens, but only vertebrates have evolved adaptive immune responses.

The innate responses of plants, invertebrates and mammals are remarkably similar. It seems likely that this similarity reflects a common ancestry. In fact, scientists believe that the mechanisms evolved hundreds of millions of years ago in ancient eukaryotes and remain in the same defensive role in their modern descendants. Although the innate responses to infection have ancient origins, they are still highly effective at preventing an infection from being established. It is difficult to know how many infections are repelled in this way, because the body defeats the invader before there are any symptoms of disease.

QUESTION SET 11.4

Remembering

1 List the major types of organisms that show innate responses to infection.

2 Define 'adaptive responses to infection' and state the type of organism that can generate these responses.

Understanding

3 Describe one similarity and one difference between innate and adaptive immune responses.

Inflammation

The first signs of infection in a cut finger are usually swelling, redness, heat and pain. These are the physical signs of **inflammation**. This process 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.

Inflammation can be triggered in two main ways. If pathogens enter the body, they are likely to be encountered by white blood cells (macrophages) and dendritic cells. Small numbers of these cells are strategically distributed in all body tissues, especially in the liver, lungs, kidneys, spleen and lymph nodes, where they act as resident sentinels. They are large cells, with a non-granular cytoplasm, that engulf invaders by **phagocytosis**. Macrophages tend to survive for a long period of time, sometimes for months. They develop from **monocytes** that have left the blood vessels and entered the tissues in response to signs of infection. Body cells at the site of infection can release signalling molecules that attract macrophages, helping to initiate the process.

As potent activators of other immune cells, macrophages are specialised to switch on inflammation. Macrophages have PRRs that recognise PAMPs on the surface of invaders. Macrophages activated in this way produce a wide range of cell signalling molecules called **cytokines**. Cytokines, which are important for the orchestration of an appropriate inflammatory response, include **interferons** and various **interleukins**. Macrophages also double up as cleaners for the body, destroying pathogens and clearing apoptotic cells and damaged tissue.

Infection by a pathogen is not essential for inflammation. Intracellular molecules, which are usually hidden from the immune system, can be released during injury or tissue damage and can be detected as **damage- or danger-associated molecular patterns (DAMPs)**. These molecules, including adenosine triphosphate (ATP), DNA and some intracellular proteins, can also be detected by PRRs and may trigger **sterile inflammation** (inflammation arising in the absence of infection).

Physical damage that ruptures body cells can stimulate **mast cells**, a member of a group of white blood cells called **granulocytes**. Granulocytes are so called because their cytoplasm is packed with intracellular granules containing powerful, defensive chemicals. Mast cells are located in the tissues. When activated, they release their granules, which are loaded with **histamine**, a major stimulus for the inflammatory response. Similarly, closely related cells called **basophils** circulate in the blood and also secrete histamines when damaged.

Histamine, together with cytokines 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. 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 further enhances swelling and causes some pain. Feeling pain is an important process, as it reduces voluntary movement in that area, thus speeding up the repair process.

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 the **lymphatic system**. The lymphatic system (Figure 11.10) consists of lymphoid organs, discussed earlier (see Table 11.2), 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, named according to where it is found.

Unlike the blood circulatory system, the lymphatic system has no pump. It relies on muscle contraction and one-way valves to move the lymph towards the heart. Lying along the course of lymphatic vessels, sometimes in chains, are **lymph nodes**. Humans have approximately 500–600 lymph nodes distributed throughout the body, with clusters found in the underarms, groin, neck and chest, and abdomen. These collect and monitor material drained from the arms, legs, oral and nasal passages, 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. As lymph moves along the lymph vessels, the lymph nodes act as filters or traps for foreign particles and invading pathogens. Lymph vessels coming from the tissues eventually join up with the circulatory system by draining into the bloodstream near the neck.

Cells of the immune system travel in a process of directed migration called **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 molecules called **chemokines**, which are any molecules that induce chemotaxis. Chemokines include molecules released by micro-organisms, activated macrophages and other immune cells.

In response to chemokines, two types of leukocytes, monocytes and **neutrophils**, squeeze out through the capillary walls into the tissues (Figure 11.11). After monocytes enter the tissues they mature into macrophages. A neutrophil is a type of granulocyte that is abundant in blood, and has irregular, multi-lobed nuclei and a granular cytoplasm. As neutrophils rarely survive longer than a few days, reinforcements from the blood are constantly required. At least 80 million of these cells are produced by our bone marrow every minute. Like macrophages, neutrophils carry out phagocytosis (and so these cells are sometimes collectively called **phagocytes**). They produce a wide-range of cytokines that are capable of inducing chemotaxis and can trigger further release of histamine by mast cells.

KILL THE INVADER!

Read the instructions, then test your skill in killing the pathogens before they multiply.

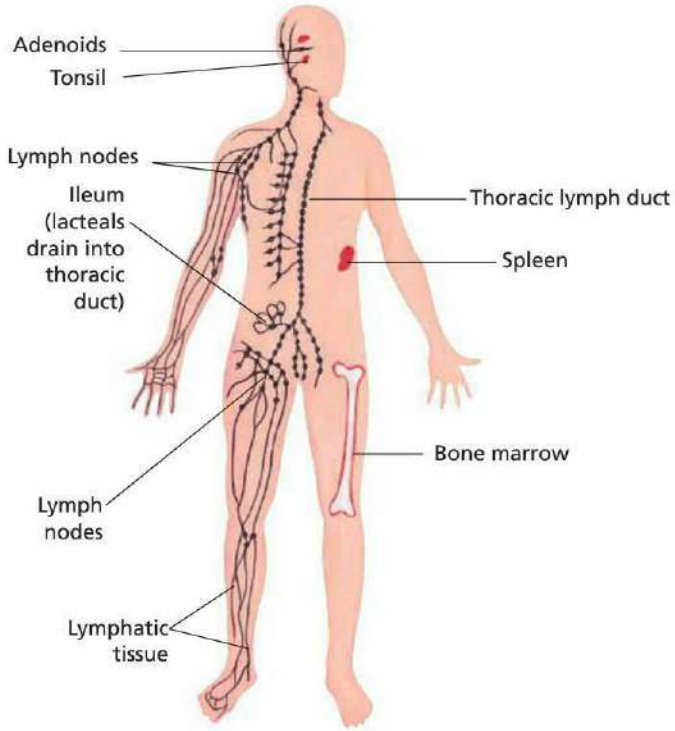


Figure 11.10 ▲
The location of organs and tissues involved in the lymphatic system in the human body

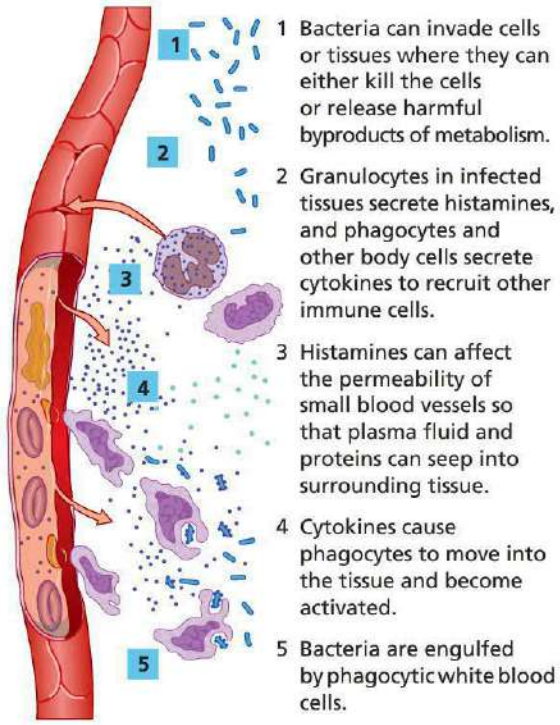
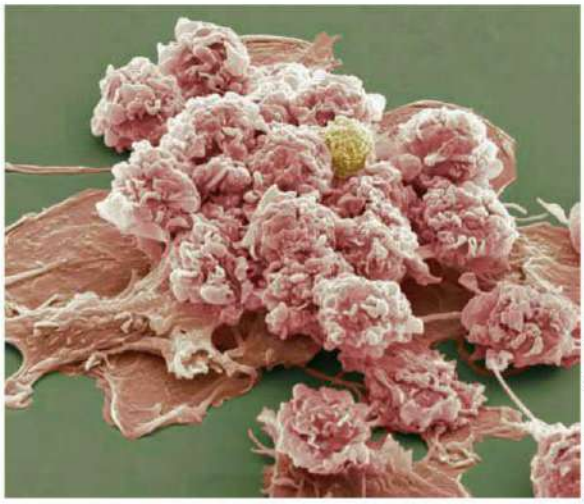


Figure 11.11 ▲
The steps that occur in acute inflammation after invasion by a bacterial pathogen

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 11.12).

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** that becomes increasingly acidic. An array of digestive enzymes and antimicrobial compounds, often including a burst of highly reactive oxygen molecules, helps to destroy the invader (Figure 11.13).



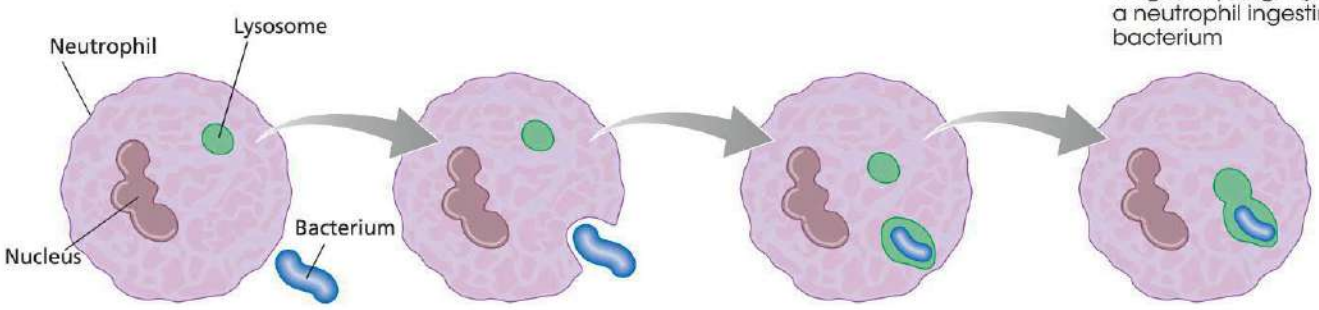
Science Source/Steve Gschmeissner

◀ **Figure 11.12**
Scanning electron micrograph of a macrophage with cytoplasmic extensions. It uses these to engulf the foreign particle (yellow) that it encounters.

THE INFLAMMATORY RESPONSE

Draw a flow chart to show major steps in inflammation.

▼ **Figure 11.13**
Stages of phagocytosis: a neutrophil ingesting a bacterium



1 Bacterium comes into contact with neutrophil.

2 Cell membrane of neutrophil indents around the bacterium.

3 The bacterium is encased in a vesicle called the phagosome.

4 The phagosome fuses with the lysosome to become the phagolysosome, which now contains digestive enzymes and low pH.

An important behavior of neutrophils is their death; 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. Massive neutrophil death at the site of inflammation is the basis of pus formation. 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, releasing their lethal contents into the cell, killing the phagocyte and releasing the pathogen.

Other leukocytes with a role in innate defence are **eosinophils** and **natural killer (NK) cells**, both granulocytes. Eosinophils secrete powerful enzymes that are capable of making holes in multicellular pathogens, such as the blood flukes and parasitic worms. NK cells circulate around the body acting like security guards. They check the credentials of the cells they encounter by looking for suitable surface markers, which identify the cell as 'self'. Any suspicious cells, such as those infected with a virus or transformed by cancer, are destroyed by an attack on their plasma membranes. This leads to **apoptosis** (programed cell death), ensuring the destruction of both the cell and the virus inside. The importance of NK cells in the initial response to infection by a virus is shown by patients deficient in NK cells being highly susceptible to the early phases of *Herpes* infection. Table 11.3 and Figure 11.14 summarise the actions of cells of the immune system including eosinophils and NK cells. Cells involved in the adaptive immune system, including subsets of lymphocytes, are discussed in more detail in Chapter 13.

THE ACTION OF LYSOSOMES

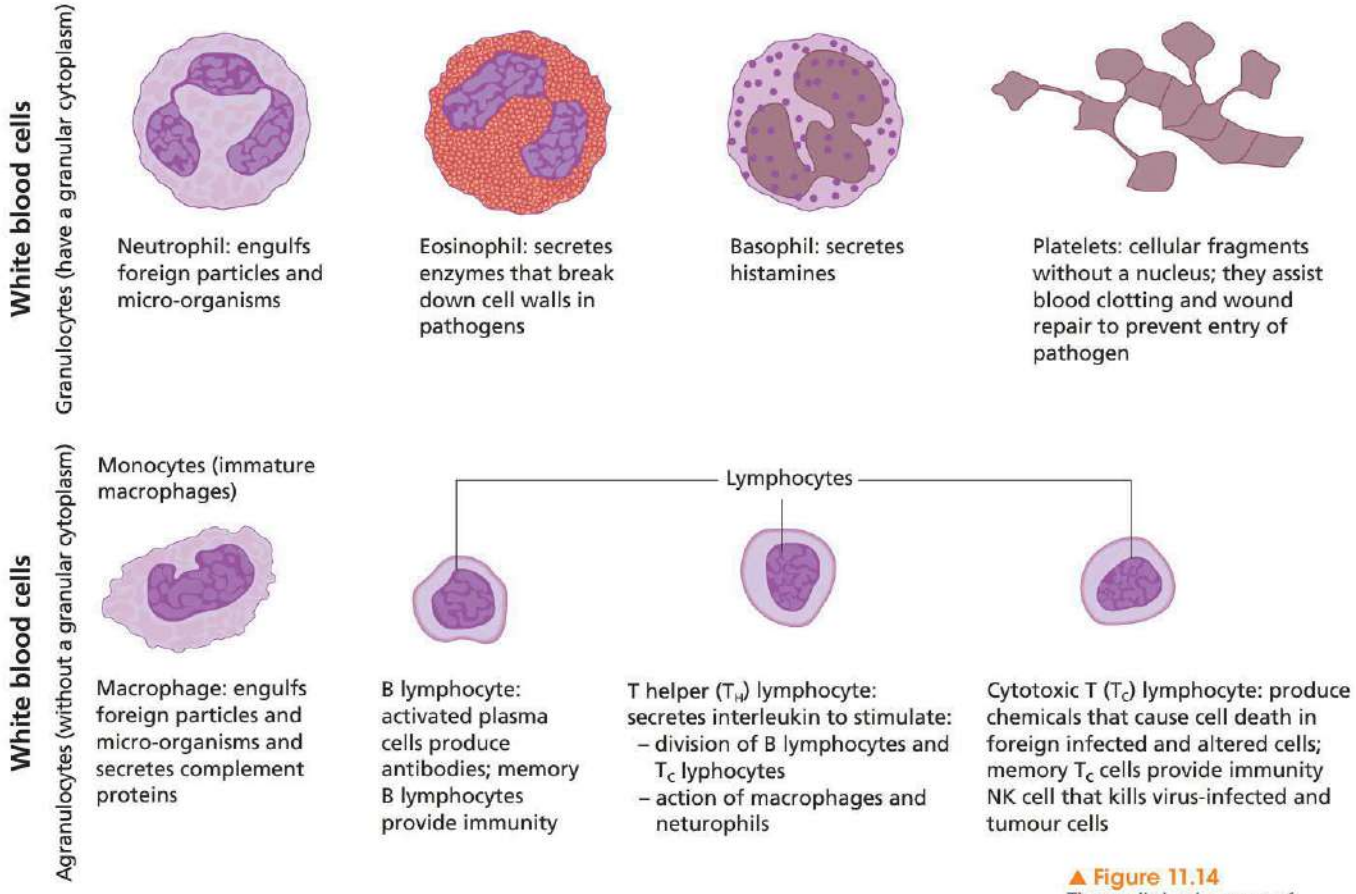
After watching the animation, draw a flow chart to show the action of lysosomes and complete the quiz.

WHEN PHAGOCYTOSIS IS SABOTAGED!

Explore the interactive, ensuring you scroll over all of the information buttons, then describe how each of the pathogens avoids phagocytosis.

Table 11.3 A summary of immune cells and their functions

Cell	Function
Leukocytes	General term describing white blood cells. Includes all of the cells listed below.
Phagocytes	General term describing white blood cells that engulf and digest foreign pathogens in a process called phagocytosis. Neutrophils and macrophages are phagocytes.
Granulocytes	General term describing white blood cells that are granulated (neutrophils, basophils, and eosinophils). They have a granular cytoplasm due to the presence of secretory vesicles that contain powerful chemicals.
Macrophages	Macrophages are found in the body tissues. They are large phagocytic cells that become powerful stimulators of an immune response when they engulf a pathogen.
Monocytes	White blood cells that circulate in the blood. They grow in the bone marrow and are released into the bloodstream. They respond to chemical mediators of inflammation and squeeze through the walls of the capillaries into the tissues, where they become macrophages.
Neutrophils	White blood cells found in the blood and tissues. They are granulated and phagocytic. They rapidly enter sites of pathogen entry, engulfing the pathogen and then dying *en masse*. This is the basis of pus formation.
Eosinophils	Granulated white blood cells. Their secretory vesicles contain powerful enzymes that rupture (lyse) cell walls of pathogens. They are important in combating parasites, such as worms and flukes. Their chemicals are toxic to the tissues of parasites and host.
Mast cells	Granulated cells that release histamines; also involved in healing wounds. Mast cells are concentrated within the respiratory and gastrointestinal tracts, and within the deep layers of the skin.
Basophils	White blood cells with granulated cytoplasm that secrete chemicals, including histamines. These cells circulate in the blood and play a role in inflammatory and allergic reactions.
Platelets	Cell fragments that assist blood clotting and wound repair, preventing the entry of micro-organisms into the body
Dendritic cells	Cells with membranous extensions that phagocytose pathogens, process them and present them to other cells of the immune system.
Lymphocytes	This is a general term for a range of specialised white blood cells that respond to specific antigens in the process of adaptive immunity.
B lymphocytes	White blood cells that are produced and mature in bone marrow, and travel to the spleen and lymph nodes. They produce specialised proteins called antibodies, which bind to specific foreign material, thereby labelling it for engulfment and destruction by other white blood cells, such as macrophages.
Plasma cells	Plasma cells are B lymphocytes that secrete specific antibodies.
T lymphocytes	White blood cells that originate in the bone marrow, then travel to the thymus where they mature. T lymphocytes contribute to the immune system in a variety of ways.
NK cells	Granulated lymphocytes that secrete 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.
Cytotoxic T cells	T cells that contain lethal chemicals that destroy foreign, infected and altered cells
Helper T cells	T cells that help or activate other cells of the immune system
Regulatory T cells	T cells that suppress or turn off the activity of other cells once the threat has passed



▲ **Figure 11.14**
The cellular heroes of the immune system

Inflammation – characterised by swelling, redness, heat and pain – is the key weapon of the innate immune response, destroying invading pathogens before they can establish an infection.

Fever is another symptom we commonly associate with an infection. What is its role in defending our body against infection? As macrophages attack an invader, the interleukins they secrete send a message to the hypothalamus, the region of the brain that controls body temperature. As a result, the body's temperature is set at a higher point, about 39°C. The higher temperature can restrict the functioning and reproduction of many pathogens, making it easier for other components of our immune system to act. In addition, some cytokines also cause drowsiness, thus lowering general body activity and allowing more energy to be used for destroying the pathogen and repairing damaged tissue.

CELLS OF THE IMMUNE SYSTEM

Watch the animation and compare and contrast the roles of monocytes and NK cells.

QUESTION SET 11.5

Remembering

1 Describe the role of phagocytic leukocytes in the immune system.
2 List one similarity and one difference between:
   a macrophages and eosinophils.
   b neutrophils and basophils.
3 Some white blood cells are called granulocytes. Describe:
   a one feature that distinguishes them from other white blood cells.
   b their function.
4 Describe the role of the lymphatic system in defending the body.

Understanding

5 Suggest why phagocytes, such as macrophages, typically contain large numbers of ribosomes and lysosomes.

6 Describe the role of fever in defending the body against pathogens.

7 Arrange the following points in order, to illustrate the sequence of events that would occur when a macrophage encounters a bacterium.

- Lysosome fuses with vacuole.
- Macrophage recognises bacterial surface molecules as 'non-self'.
- Powerful enzymes digest bacterium.
- Vacuole forms around bacterium.
- Macrophage envelops bacterium with its cell membrane.

Applying

8 People on long-haul aeroplane flights are advised to walk around every hour or so, to prevent getting swollen ankles. Use your understanding of the lymphatic system to explain this advice.

Analysing

9 Prepare a flow chart to summarise the steps involved in the inflammatory response.

Evaluating

10 Evaluate the hypothesis that phagocytic cells such as macrophages have their origins in the unicellular eukaryotic amoeba.

The role of the complement system

ACTIVATION OF COMPLEMENT

Watch the second half of the animation, then draw a flow chart to show the results of the activation of complement.

The **complement system** consists of a number of small proteins with an important role in inflammation. Approximately 20 different kinds of complement proteins circulate in the blood as inactive precursors. They are secreted principally by the liver, but also by macrophages, monocytes and other body cells. The inactive precursor proteins become activated when they encounter a foreign body, such as an invading bacterium. Activation of one complement protein has a cascading effect, stimulating the activation of other complement proteins, which then activate other proteins in turn. These proteins produce a range of effects for defending the body (Figure 11.15).

In a process called **opsonisation**, complement proteins bind to the surface of pathogens, in particular yeasts and bacteria, acting as a tag to facilitate their detection and uptake by phagocytes, which have complement receptors on their surface. They induce chemotaxis by creating concentration gradients that recruit and 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. Complement also stimulates mast cells to release mediators such as histamine. An important product of the complement cascade is the membrane attack complex (MAC). The MAC forms pores in the membranes of target cells, disrupting the phospholipid bilayer. With membrane integrity destroyed, osmotic cell lysis and death follows.

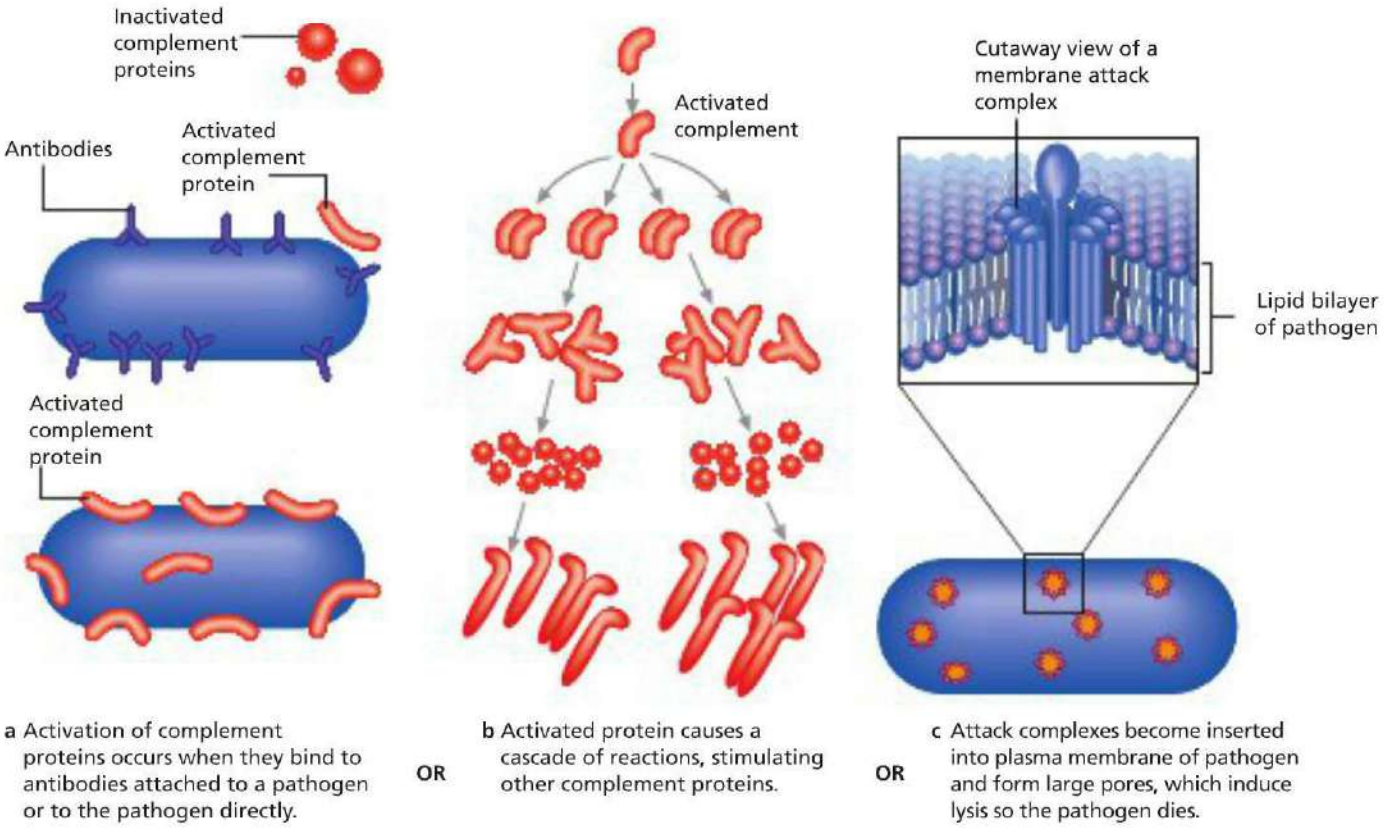
With its powerful and potentially dangerous effects, the complement system must be subject to tight regulation. One important safeguard is that activated complement proteins are rapidly inactivated unless they bind to the surface of a pathogen. In addition, MAC-inhibitory proteins exist on all body cells. 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.

INNATE IMMUNITY

Watch the animation and complete the quiz.

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



▲ **Figure 11.15** Activation of complement proteins can: a) encourage phagocytosis, b) enhance inflammation or c) cause pathogens to lyse.

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.

Because of their many and varied effects on cells, interferons have proved useful in the treatment of a number of diseases including 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.

Cytokines such as interferons also play an important role in the adaptive immune response. This will be discussed in Chapter 12.

Defensins

The expression of another set of chemicals called **defensins** has been shown to increase during infection and inflammation. Defensins belong to a group of small (25–45 amino acids long) antimicrobial peptides. They bring about a number of responses that serve to stimulate innate host inflammatory defences against microbial invasion. All the epithelial surfaces, including the skin and the mucosal linings of the nose, throat, lungs, gastrointestinal tract and genitourinary tract, are protected by defensins. With their activity against a broad range of pathogens, including antimicrobial, antiviral and antitoxic properties, they are powerful natural antibiotics.

Defensins are also found in granules of neutrophils and to a lesser extent in macrophages, where their role is to enhance phagocytosis and assist in killing the pathogen once it is engulfed. Their antimicrobial activity depends on their chemical structure, which allows them to disrupt pathogen membranes. As defensins can stimulate mast cells to release histamine, they promote the recruitment and accumulation of neutrophils at inflammatory sites.

Defensins are secreted by a wide variety of organisms to protect them from invasion by bacteria and other pathogens, with more than 300 defensins having been identified in mammals, birds, invertebrates, plants and fungi. Virtually every species studied, including the evolutionarily ancient horseshoe crab, has some type of defensin.

Chemicals – including complement, cytokines and defensins – stimulate, coordinate and carry out the destruction of invading pathogens.

Resolving inflammation

It used to be thought that inflammation passively dissolves after an infection has died down. That is far from the case. Resolution of inflammation is a highly coordinated, active process that is controlled by several factors including cytokines and other signalling molecules. One set of pro-resolving and anti-inflammatory mediators, called **resolvins**, are synthesised from dietary omega-3 fatty acid precursors. The discovery of resolvins has been a major breakthrough in understanding the processes involved in resolution of inflammation.

Resolvins 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 accumulated at the site of infection. This includes neutrophils that have done their job and died; fibrin; and exudate, 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. Whereas apoptosis is an immunologically 'silent' form of cell death, which doesn't normally activate the immune system, necrosis stimulates inflammation, and macrophages release cytokines that further enhance inflammation rather than suppressing it.

Successful resolution of inflammation by cytokines and resolvins 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 Crohn's disease and rheumatoid arthritis, result in ongoing tissue damage.

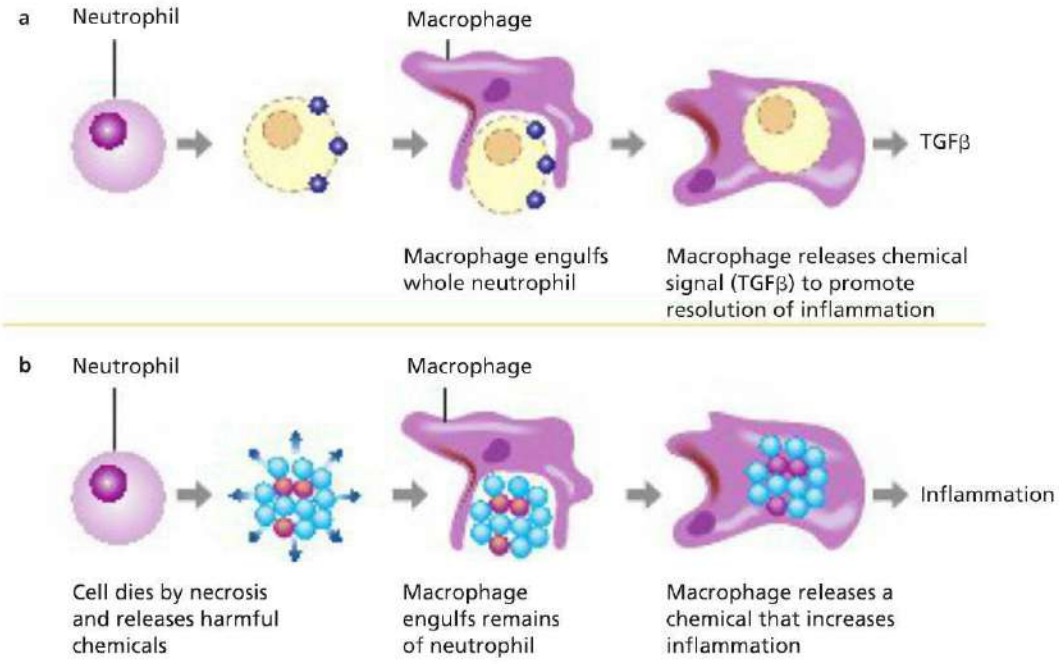


Figure 11.16 ▶ Solving the problem of inflammation? Different signals, from a) apoptosis and b) necrosis, can resolve or prolong inflammation.

Table 11.4 The role of chemicals in the innate immune response

Chemical	Source	Role
Chemokines	Micro-organisms, activated macrophages and other immune cells	Induce chemotaxis to recruit other immune cells from the blood to sites of infection or tissue damage
Complement proteins	Principally liver cells, also macrophages, monocytes and other body cells	Facilitate uptake and destruction of pathogens by phagocytes, attract phagocytes, form pores in pathogen membranes, leading to lysis
Cytokines	Macrophages, neutrophils, other cells of the immune system and body cells.	Cell signalling molecules with diverse roles
Defensins	Epithelial cells and neutrophils	Powerful natural antibiotics with wide antimicrobial activity
Enzymes (e.g. eosinophil peroxidase and major basic protein)	Eosinophils	Destruction of multicellular pathogens
Histamine	Basophils and mast cells	Dilates local blood vessels, changes permeability of capillaries in inflamed area
Interferons (a type of cytokine)	Virus infected cells	Induce resistance to viral infection in surrounding cells, enhance phagocytosis of apoptotic cells
Interleukins (a type of cytokine)	Macrophages, neutrophils and other leukocytes	Many different functions, usually with pro-inflammatory effects Some trigger further release of histamine by mast cells and reset body thermostat to a higher temperature
Intracellular enzymes and reactive oxygen molecules	Lysosomes	Destroy pathogens after engulfment by phagocytes
Resolvins	Epithelial cells and leukocytes	Switch off movement of leukocytes to the site of inflammation, reverse vasodilation, reduce permeability of fine blood vessels

QUESTION SET 11.6

Remembering

1 Complement proteins are found in the blood in an inactive form. Identify what activates them.
2 Describe three effects that follow activation of complement.
3 Identify the source of interferon.
4 List five epithelial surfaces that secrete defensins.
5 Identify the types of pathogens against which defensins are effective.

Understanding

6 Describe two beneficial effects of interferons.
7 Discuss what is involved in the resolution of inflammation and why this is important for limiting tissue damage at sites of infection.

Plant defence strategies

Plants are prone to the ravages of parasites, pests and disease. They are subject to attack by a huge array of mites, insects, roundworms, fungi, bacteria and viruses (Figure 12.16), yet the plants usually survive. They too 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 all people, because plants are a vital component of our ecosystems. We depend on plants for food, as well as valuable materials including wood and a range of plastics, textiles, medicines, dyes, inks and industrial chemicals.

Figure 11.17 ▼
Leaves of: a) a healthy tobacco plant, *Nicotiana sylvestris*, and b) a plant infected with tobacco mosaic virus

Alamy/Joshua Carpenter

Nature Picture Library/Visuals Unlimited

Barriers preventing entry of pathogens

Plants possess physical and chemical barriers 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 that prevent invasion by pathogens include the thick bark of stems and a thick and waxy cuticle (leaf surface) (Figure 11.18). 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, as they offer an entry point. Many plants have hairs that guard these openings, or have sunken stomata in the leaf that make access difficult.

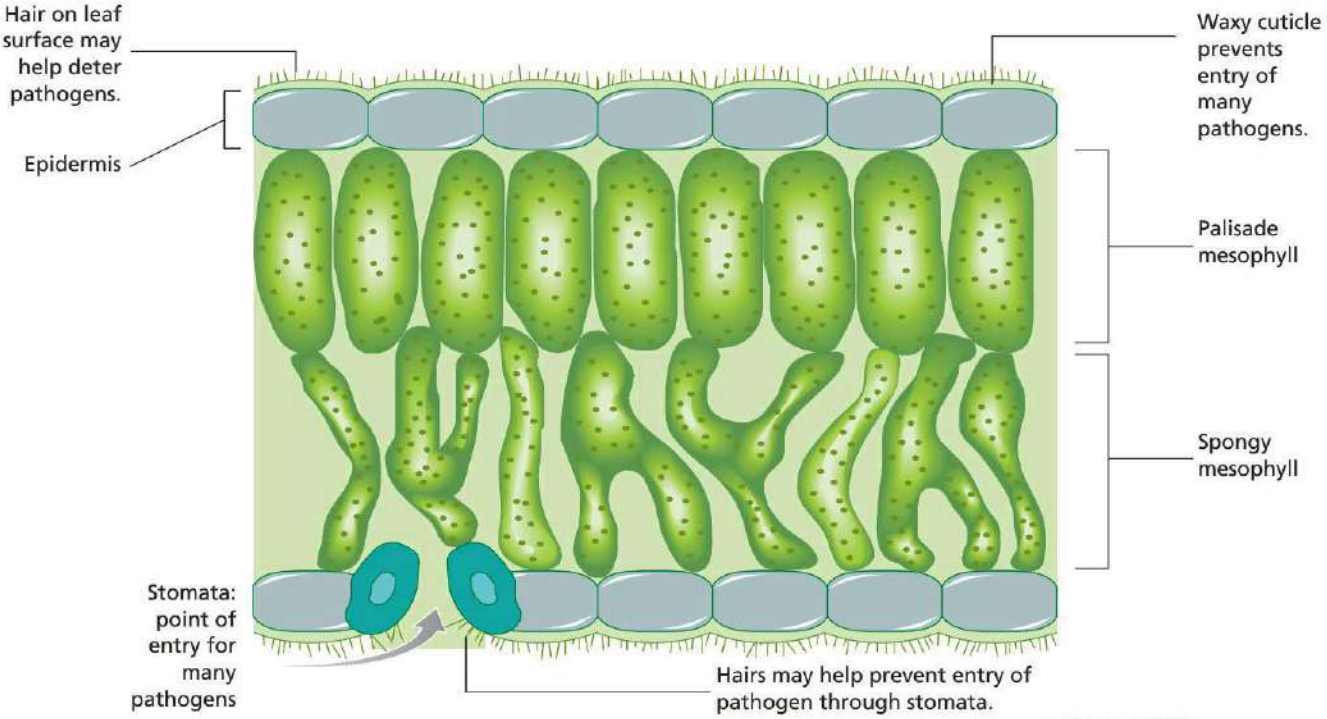
Chemical barriers

The first line of defence in plants includes chemicals that inhibit the growth and development of pathogens. Some of these substances are released into the environment; for example, by asparagus plants and marigolds. The chemicals they secrete into the soil are toxic to nematodes, making them good **companion plants** for tomatoes, which are commonly attacked by these parasitic roundworms. Other substances remain in the plant ready to stop invaders. These include wetting agents that destroy fungal cell membranes, and phenols and other compounds on leaf surfaces that discourage herbivore feeding and inhibit many potential pathogens.

Humans have come to appreciate and rely on some of the substances that plants produce to defend themselves from attack. Many of our spices, seasonings, condiments and perfumes are made using essential plant oils that function as insect toxins and protection against fungal or bacterial attack. The bitter-tasting tannins that give tea its characteristic colour and taste are

widespread throughout the plant kingdom and are toxic to insects. Caffeine, the alkaloid found in plants such as coffee, tea and cocoa, is toxic to both insects and fungi, while pyrethrins are esters produced by chrysanthemum plants that act as insect neurotoxins.

Plants use physical and chemical barriers to prevent invasion by pathogens.



▲ **Figure 11.18**
A cross-section of a typical dicotyledon leaf showing some barriers to pathogens found in plants

WOW

Spiky leaves leave the bedbugs floored

Bedbugs are common all over the world and it is almost impossible to get rid of them. Now, thanks to a combination of traditional herbal treatment and nanoscience, this annoying insect may have lost the battle.

In rural parts of the Balkans, the leaves of the red kidney bean, *Phaseolis vulgaris*, are scattered on the floor next to the bed, snagging the parasites as they move around. Scientists used scanning electron microscopy and found that the sharp, hook-like hairs (trichomes) of the bean pierce the bedbugs' legs, immobilising them. If we can copy the leaves we could make a durable bedbug barrier that can be used on any surface.

As another part of the first line of defence, plants contain small, stable peptides that are able to inhibit the development of fungi, as well as bacteria, viruses and insects. Because of their similarity to the defensins of insects and mammals, they have been termed plant defensins, and 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, regulation of growth and heavy metal tolerance. While many defensins accumulate during normal plant development, some are produced in response to attack by pathogens while others are induced by environmental stress such as drought, salt and cold. As they have been shown to inhibit the human cancer cell cycle, plant defensins may potentially be used to treat human diseases.

CLASS ACTIVITY: A-MAIZING PLANT DISEASE GAME

Your challenge is to grow a healthy, productive maize crop while maximising your profit.

EXPERIMENT 11.2

BIOCHEMICAL WARFARE!

Antibiotics are killer molecules produced by bacteria and fungi to defend themselves from other microbes. People now use these antibiotics to treat disease, but not all antibiotics are effective against all bacteria. To enable selection of the best treatment for a given disease, we need to know which bacteria are susceptible to which antibiotics. As you saw in Experiment 11.1, if bacteria are grown on agar plates, they produce a bacterial 'lawn', a cloudy film of millions of bacterial colonies. When paper discs containing antibiotics (**mast rings**) are placed on the agar before the bacteria have had a chance to grow, they produce clear areas where they have killed the bacteria.

Aim

To observe the effectiveness of a variety of antibiotics against two species of bacteria

Materials

Class requires:

- broth cultures of *Escherichia coli* and *Staphylococcus epidermidis*
- incubator set to 25°C
- lab coats
- safety glasses
- gloves

Each group requires:

- two nutrient agar plates
- two antibiotic mast rings
- two sterile 5mL pipettes
- forceps
- glass spreader
- Bunsen burner
- sticky tape
- ruler
- disinfectant solution; for example, bleach

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
Bacteria may cause disease; assume them to be pathogenic.	Wear lab coats, gloves and safety glasses; wash hands thoroughly at the end. Decontaminate benches before and after activity. Flood spills with bleach. Avoid contamination through touching your hands to your mouth or face.
Micro-organisms will grow on the agar plates.	Do not open plates once they are securely taped. Dispose of plates appropriately in autoclave or pressure cooker.
Bleach may leave a corrosive residue and discolour clothes.	Wear lab coats and gloves. Wipe the bleach off the bench after decontamination.

Procedure

1. Before starting, ensure the bench is swabbed down with bleach to minimise contamination.
2. Label base of plates with date, name of group and type of bacteria.
3. Remove 0.1mL of *E. coli* culture with the pipette, lift the lid off the labelled plate and transfer the bacteria to the surface of the agar. Spread with a sterile glass spreader.
4. Either replace the lid quickly and spread the liquid evenly by swirling, or spread the liquid evenly with the glass spreader, then replace the lid. Leave on bench for 2 minutes to allow bacteria to penetrate agar.
5. Repeat steps 3 and 4 using *S. epidermis* culture on the second plate.
6. Sterilise the forceps in the flame of the Bunsen burner, allow to cool and then use them to place a mast ring on the surface of each of the plates. Each lobe of the mast ring is impregnated with a different antibiotic, as shown by the code on the packet.

7 Replace the lid and seal both plates with sticky tape and incubate at 25°C for 24 hours.
8 Ensure the bench is swabbed down with bleach and wash hands thoroughly.
9 The next day, observe for the presence or absence of growth near the discs.
10 Carefully avoiding contamination, measure the diameter of zone of inhibition: the clear area around each disc. This shows the degree of sensitivity of the bacteria to each antibiotic.

Results

Copy and complete the table, adding as many rows as needed.

	Diameter of zone of inhibition (mm)	
Antibiotic	***Escherichia coli***	***Staphylococcus epidermidis***

Analysis of results

Describe the trend of your results, ensuring that you compare the effects of each antibiotic on the two different species of bacteria.

Discussion

1 Which antibiotic would be most suitable to treat an infection by *Staphylococcus epidermidis*?
2 Which antibiotic would you use if you were unsure of the pathogen in an infection? Explain your answer.
3 Explain why a control was not used in this experiment. If you were asked to use a control, what would you set up?
4 State four variables that you kept constant in this experiment and describe how you controlled them.
5 Explain the benefit of pooling the data from different class groups and finding average areas of inhibition for each antibiotic.
6 Why have antibiotics become a less effective treatment for infection in recent years?

Reactions to invasion

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 possess 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.

Stimulation of plant immune responses by pathogens

Plants recognise invaders in much the same way as the cells of animals. The broad molecular patterns commonly shared by pathogens (such as flagellin, glycoproteins, lipopolysaccharides and chitin) are recognised as PAMPs. These molecules activate PRRs, which in turn trigger a number of actions that attempt to destroy the invaders. DAMPs, such as breakdown products of plant cell walls, can also stimulate the innate defence responses of plants.

Plant responses to the detection of invaders

Once invaders have been detected, part of the chemical attack involves the synthesis of a toxic cocktail of antimicrobial compounds that includes defensins and **phytoalexins**. Since their discovery, more than 350 phytoalexins have been found in more than 100 plant species from 30 families of plants. They 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 the inhibition of phytoalexin biosynthesis. Such plants show an increased susceptibility to infection and are extensively colonised by pathogens.

Another chemical response to invasion is the production of a burst of highly reactive oxygen molecules, like that produced by neutrophils as discussed earlier. These substances have a direct antimicrobial action, and are also highly toxic to plant cells, causing rapid and localised programmed cell death at the site of pathogen invasion. This has the effect of producing a physical barrier around the area of infection, which keeps the pathogen isolated from the rest of the plant. Similarities between programmed cell death in plants and apoptosis in animal cells suggest that cell suicide is an ancient defence response conserved through many millions of years of evolution.

Several other mechanisms serve to help stop the spread of infection through the plant. In an option not open to animals, parts of some plants are treated as disposable, with the plant shedding infected leaves and branches. 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.

After the initial reaction to invasion, plant tissues may become highly resistant to a broad range of pathogens for an extended period of time. This is called **systemic acquired resistance** (SAR). It occurs because when a pathogen attacks a plant, a signal travels through the vascular system to activate synthesis of antimicrobial proteins in distant tissues. This brings about a heightened state of readiness in which the whole plant, not just the part initially attacked, is prepared in case of further invasion. SAR is effective against a broad spectrum 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 causing poor 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.

Like animals, plants use PRRs to detect PAMPs on invading pathogens. They mount a rapid innate immune response to the invasion of pathogens.

QUESTION SET 11.7

Remembering

1 Provide three reasons that explain why it is important for us to understand plant disease and defence.
2 Describe five physical adaptations that prevent the entry of pathogens into plants.
3 Outline the effects of phytoalexins on invaders.
4 Summarise the chemical defences of plants in a suitable table, including their names and the ways in which they act.
5 Describe the interactions of a companion plant with a crop plant.

Understanding

6 Describe the mechanism by which defensins kill invading micro-organisms.
7 Draw a flow chart to summarise plant responses to invasion by a pathogen.

CHAPTER SUMMARY

- Chemical, physical and biological barriers to invasion form the first line of defence.
- Innate immune defences detect and respond to any invader, regardless of its type.
- Vertebrates have adaptive immune responses that develop as a result of exposure to a specific pathogen.
- The lymphatic system comprises the lymphoid organs, the lymph vessels and the lymph.
- Many different cells with specific structures and functions play an important part in innate immune responses.
- Both plants and animals use PRRs to recognise the broad molecular patterns commonly shared by various types of pathogens.
- The key weapon of the innate immune response is inflammation.
- Inflammation involves the detection of a foreign invader and subsequent activation of cells of the immune system, particularly phagocytes.
- The complement cascade can result in opsonisation of a pathogen, enhancing its uptake and destruction, and formation of the MAC in the pathogen's cell membrane, causing cell death.
- Cytokines are secreted by activated cells during infection and stimulate local inflammatory responses, including recruitment of cells of the immune system to sites of infection and the enhancement of their activities.
- Phagocytes are an essential component of the innate immune system, clearing pathogens and apoptotic cells.
- The innate immune system stimulates the adaptive immune system and the recognition of PAMPs or DAMPs is essential for adaptive responses.
- The defences of plants have many features in common with the innate immune defences of animals.
- A plant's chemical and physical barriers are adaptations to prevent the entry of pathogens.
- Entry of a pathogen into a plant stimulates innate immune responses.
- After infection by a pathogen, plants may develop a systemic acquired resistance that protects them from a wide variety of pathogens.

CHAPTER GLOSSARY

adaptive immune response an immune response that is acquired; after an initial response to a pathogen, the immune system creates a 'memory' that leads to an enhanced response to subsequent encounters with the same pathogen

antibiotic naturally produced or synthetic compounds that are toxic to bacteria

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

antiseptic a substance that kills or inhibits the growth of micro-organisms on external surfaces of living things

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cell by various enzymes including caspases

basophil a circulating leukocyte that secretes histamines when damaged

chemokine a molecule that induces chemotaxis; some cytokines are chemokines

chemotaxis the movement of an organism or cell along a chemical concentration gradient either towards (positive chemotaxis) or away from (negative chemotaxis) the chemical stimulus

cilia slender hair-like structures projecting from the cell surface, which beat against fluid outside the cell

companion plant a plant that is grown together with another plant because one species improves the growth of the other

complement system a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

cytokines small signalling molecules that coordinate inflammation and immune responses, and that leukocytes use to communicate with one another; includes interleukins and interferons

damage- or danger-associated molecular pattern (DAMP) a body (or plant) component that is released during tissue damage, such as internal cellular components that stimulate innate immune responses

defensin a small antimicrobial peptide secreted by virtually all plants and animals

disinfectant a substance that destroys micro-organisms and their spores but is too strong to be used directly on skin

eosinophil a leukocyte that secretes powerful enzymes capable of rupturing multicellular pathogens

epithelial cells cells that line the inner and outer surfaces of body and body cavities; bound together in sheets of tissue called epithelia

epitope a small part of a larger molecule that binds to a receptor site; examples are B cell receptors and T cell receptors

fever increased body temperature

granulocyte a leukocyte containing intracellular granules

histamine a chemical released by mast cells and basophils that increases blood flow and the permeability of capillaries

immune having resistance to infection by a specific pathogen

immune system a complex network of cells, tissues and organs in the body that detects differences between 'self' and foreign organisms, and mounts an immune response

inflammation an innate response to infection or damage that causes swelling, pain and redness

innate immune response 'innate' means not learned; as applied to the innate immune response, one that is not specific and does not have 'memory'

interferon a class of glycoprotein cytokines 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 a larger group of cellular messenger molecules called cytokines, which are cell signalling molecules

interstitial fluid a fluid that lies in the spaces between cells; also known as tissue fluid

keratin a strong, stable structural protein found in skin, hair, horn and nails

keratinised a process by which keratin is deposited in skin cells; the surface becomes tough and waterproof

leukocyte the general term for white blood cell

lymph a colourless fluid that originates from the extracellular (tissue) fluid

lymph node an immunologic organ in which antigens are trapped or delivered by phagocytes for initiation of an adaptive response

lymphatic system part of the immune system; a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and in removing foreign matter

lymphocyte a type of leukocyte involved in adaptive immune responses

lysis the process of a cell bursting (verb: to lyse)

lysozyme an antibacterial enzyme found in tears, saliva and other body fluids

macrophage a large white blood cell in tissues that phagocytoses pathogens

mast cell located in the tissues; when activated, releases granules containing histamine

mast ring a ring of paper with small circular 'offshoots' impregnated with different antibiotics

microflora a community of micro-organisms, including fungi and bacteria that live in or on another living organism

monocyte a white blood cell that circulates in the blood and matures into a macrophage when it moves from the blood into the tissues

mucus membrane a mucus-secreting membrane that lines the respiratory, excretory and reproductive tracts

natural killer (NK) cell a circulating leukocyte that kills body cells infected with a virus or transformed by cancer

necrosis unprogrammed cell death that stimulates inflammation

neutrophil a phagocytic leukocyte found in the blood and tissues

NOD-like receptor (NLR) a type of pattern recognition receptor (PRR); intracellular sensors of PAMPs and DAMPs

'non-self' describes agents (e.g. cells, organisms, substances) that are not recognised by the immune system as being part of the organism itself; they are foreign

non-specific when the response is the same regardless of the type of pathogen

opsonisation a process in which a pathogen is coated with antibodies and marked for ingestion and destruction by phagocytes

pathogen-associated molecular pattern (PAMP) a broad molecular pattern commonly shared by a number of pathogens

pattern recognition receptor (PRR) a cell receptor that recognises molecular patterns commonly shared by a number of pathogens; includes nod-like receptors and toll-like receptors

phagocyte a cell that is capable of phagocytosis; includes macrophages and neutrophils

phagocytosis the bulk transport of solids into a cell inside a vesicle

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

phytoalexin a chemical produced by plants under attack

platelet a cell fragment found in the blood that helps blood to clot

primary lymphoid organs the bone marrow and thymus; responsible for the production and maturation of immune cells

resolvin a mediator that reduces and resolves inflammation

secondary lymphoid organ an organ that provides an environment for the initiation of the immune response

'self' describes agents (e.g. cells, organisms, substances) that are recognised by the immune system of an organism as being part of that organism; the immune system coexists with all cells in the body without attacking them because cells carry marker molecules that identify them as belonging to 'self'

sterile inflammation inflammation resulting from detection of DAMPs released during tissue injury

systemic acquired resistance a plant's reaction to invasion by a pathogen that leads to long-term resistance to a broad range of pathogens; 'systemic' refers to the whole body

tissue fluid the fluid surrounding the tissue cells; it was originally blood plasma

toll-like receptor (TLR) a pattern recognition receptor in membranes that responds to PAMPs and DAMPs

vasodilation dilation (widening) of blood vessels, particularly arterioles

CHAPTER REVIEW QUESTIONS

Remembering

1 Identify the groups of organisms that have:
   - **a** innate immune responses.
   - **b** adaptive immune responses.
2 Identify the type of change (physical or chemical) that PRRs detect in host tissues after a pathogen has entered.
3 Outline the roles of the primary and secondary lymphoid organs.
4 Outline the role of the bone marrow in the defence system.
5 Outline the advantage of the keratinisation of skin cells.

Understanding

6 As defensins are peptides, 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.
7 Describe the events that follow the activation of complement.
8 There is no pump in the lymphatic system, yet lymph moves along lymph vessels. Describe how this occurs.
9 Explain what is meant when we say the body can discriminate between 'self' and 'non-self'.

Applying

10 Cigarette smoke has been shown to decrease ciliary beat frequency and reduce the number of ciliated cells in the airway epithelium. Predict the effect of smoking on the body's defences.
11 People who have had their spleen removed live successfully without any great burden of disease. By considering the role of the spleen, suggest how this is possible.
12 A person needs clotting proteins in their blood to form a blood clot; normally, the liver uses vitamin K to make these proteins. Vitamin K is obtained from many foods, especially green vegetables. Warfarin is a drug that reduces the liver's ability to use vitamin K to make these blood clotting proteins.
   - **a** Warfarin has been called a blood thinner. Create an argument that either refutes or supports this name.
   - **b** Predict the effect on a person of a diet very low in green vegetables
13 The disease elephantiasis was described in Chapter 10. It is caused by adult worms becoming lodged in the lymph nodes. Explain why this would cause the legs to become enlarged as shown in Figure 10.22 (page 300).
14 Both an infection in your foot and a sprained ankle will 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

15 Use a table to compare and contrast the actions of lysosomes and lysozyme.
16 Draw a flow chart to show the movement of plasma from the time it leaves the blood at the tissues to its return to the blood via the lymphatic system.

17 The life cycle of *Trypanosoma brucei* is described in Chapter 10 (page 300). The parasite has a number of intermediate stages in both the tsetse fly and the human hosts. Explain, in terms of detection and response by the human immune system, why this is an advantage to the parasite.

18 Compare and contrast the actions of defensins in plants and animals.

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 Menthol is the major compound in peppermint oil. It chemically triggers the cold-sensitive receptors in our skin. Capsaicin is the chemical responsible for the spiciness of hot chillies. Create a scenario that allows you to predict the role of these substances in the plants' defences.

22 Your task is to design an experiment to distinguish between three samples of bacteria. You will do this by using fluorescent labelling techniques to observe macrophages engulfing the bacteria in a Petri dish.

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 labeled phagolysosomes.

Reflecting

23 Consider the observation that plant defensins have been shown to inhibit the growth of human cancer cells. Reflect on this relationship by discussing the evolutionary history of eukaryotes and the conservation of useful molecules over time.

CHAPTER 12 ADAPTIVE RESPONSES

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

Science Understanding

- In vertebrates, adaptive responses to specific antigens include the production of humoral immunity through the production of antibodies by B lymphocytes, and the provision of cell-mediated immunity by T lymphocytes; in both cases memory cells are produced that confirm long-term immunity to the specific antigen (ACSBL122)
- In vertebrates, immunity may be passive (for example, antibodies gained via the placenta or via antibody serum injection) or active (for example, acquired through actions of the immune system as a result of natural exposure to a pathogen or through the use of vaccines) (ACSBL123)

Figure 12.1 ▶
David Vetter was raised from birth in a sterile isolator unit designed by NASA to protect him from pathogens.

Courtesy of Baylor College of Medicine

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 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 David 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.

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.

SCID and other ways in which dysfunction of the immune system can cause disease are discussed on page 356.

Immunity with memory

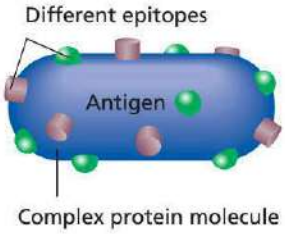
In Chapter 11 you learned about the cells and actions of the **immune system**. **Innate immune responses** are non-specific, meaning that they do not distinguish between one type of pathogen and another. These responses are also characterised by the fact that they are non-adaptive. That is, the innate immune system does not have 'memory' and responds in a similar fashion every time a particular pathogen invades the body's territory.

In addition to the protection provided by innate immunity, vertebrates have a further line of defence known as the **adaptive immune response**. The cells and processes of this response differ from those previously described because they are specific. This means that the cells can detect and distinguish between different types of invaders, attacking only those that contain the specific molecular pattern matching the receptors on their surface. The adaptive immune response is also characterised by having memory; this allows the immune system to mount an enhanced defence against a pathogen that infects the host for a second time. This memory is the reason that people who suffer from chickenpox as a child are not able to catch the disease again. It is also the scientific basis for immunisation.

The adaptive immune response differs from the innate immune response because it has specific recognition of antigens and displays memory.

Telling friend from foe

Lymphocytes are the key cells of the adaptive immune system and there are two major types: B and T lymphocytes. B and T lymphocytes (or B and T cells) look so similar that scientists cannot tell them apart under the microscope; special tests that measure surface proteins are required distinguish between them. **B lymphocytes (B cells)** are responsible for the destruction of pathogens by producing proteins that bind to them, known as **antibodies**. Destroying virally infected and cancerous cells is the major role of **cytotoxic T (T_C or killer T) lymphocytes** (**cytotoxic T cells**). **Helper T (T_H) lymphocytes** (**helper T cells**) and **regulatory T (T_{reg}) lymphocytes** (**regulatory T cells**) assist the other lymphocytes in performing their roles. We will explore how this occurs later in the chapter, but first it is important to understand how lymphocytes tell friend from foe.



▲ **Figure 12.2**
The distinction between an antigen and an epitope.

For the immune system to function properly, it is important that cells of the immune system are 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 markers are expressed according to the information coded in genes. The group of genes that determine 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 individual. It is as if each cell of an individual'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.

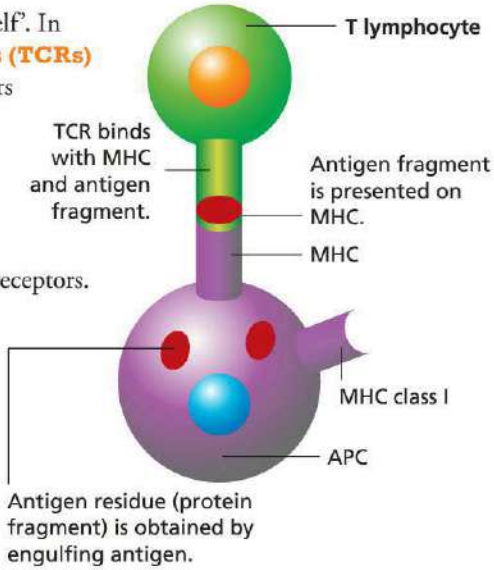
Recall epitopes and antigens from Chapter 11.

Antigen receptors

Lymphocytes have surface receptors that are used to distinguish 'self' from 'non-self'. In this section we will explore how **B cell receptors (BCRs)** and **T cell receptors (TCRs)** allow lymphocytes to identify foreign antigens. B cell receptors and T cell receptors recognise and bind to small regions of antigens that are known as **epitopes**. The binding of an antigen to a lymphocyte receptor is similar to that of a substrate binding to an enzyme. Again, the emphasis is on the molecules having 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. When antibodies are bound to the surface of B lymphocytes they act as the B cell receptors. Antibodies also serve as effector molecules when secreted by B lymphocytes.

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 12.3**
T cells must be activated to respond. TCRs will only recognise antigens presented by MHC markers.

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 identify 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. Dendritic cells are specialist antigen presenting cells that are named for small finger like projections, or dendrites, that are on the surface of the cell.

MHC proteins contain a deep groove that is capable of holding 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 that sit inside the groove. The MHC protein (bound to a peptide) travels to the cell surface where T cell receptors can then bind to the MHC–antigen complex.

MHC class I and MHC class II differ in the type of antigen that they can present (see Figure 12.4). 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

You have already learned about the roles of macrophages in Chapter 11 on page 316.

You learned about how cells of the innate immune system use pattern recognition receptors to recognise pathogens in Chapter 11, page 316.

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 it recognises 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.

MHC class II molecules are only found on antigen presenting cells and are used to present extracellular antigens. Antigen presenting cells phagocytose pathogens following recognition by pattern recognition receptors, and then break them down in lysosomes. Antigen presenting cells then travel to the lymph nodes to present these antigens to T cells. Peptides that are derived from these pathogens are presented on MHC class II proteins. Antigen presenting cells are usually macrophages and dendritic cells but B cells can also present antigens this way.

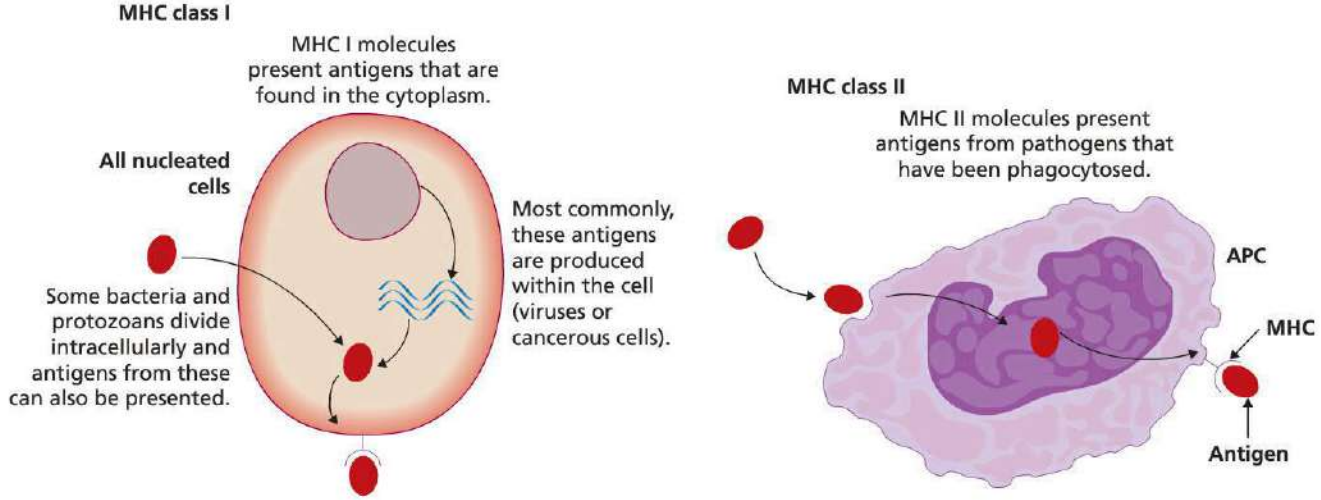


Figure 12.4 ▲ MHC I and MHC II proteins are cell-surface structures that present pieces of antigen to T lymphocytes.

Receptor diversity

Each B or T lymphocyte carries a large number of identical copies of a receptor protein, which will bind to a single, specific antigen. There are so many different receptors that around 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.

How can the genome encode for this number of different receptors? In fact, it doesn't. 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!

Avoiding self-recognition

The random generation of receptors also results in some receptors that will bind to 'self' molecules. Both T and B cells are produced from stem cells in the bone marrow. B lymphocytes remain within the bone marrow to mature, while T lymphocytes travel to the thymus to undergo further development. As B and T lymphocytes develop, those clones that carry receptors for molecules that already exist in the body are either inactivated or they self-destruct by apoptosis. This process provides the capacity of the immune system 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. However, sometimes the clones that react against 'self' molecules are not completely eliminated and the immune system will attack the body. This can result in autoimmune diseases, which are discussed further on page 357.

Case study

Peter C Doherty and Rolf Zinkernagel

How the immune system recognises and eliminates cells modified by viral infection or cancer had eluded scientists for many years. The mechanism was discovered by Australian scientist Peter C Doherty and his Swiss colleague, Rolf Zinkernagel. This discovery led to the awarding of the Nobel Prize in Physiology or Medicine in 1996.

When organs are transplanted from one person to another, the immune system mounts a very aggressive response. 'We knew that this occurs when transplant molecules on the surface of cells in the donor organ differ to those of the recipient,' Professor Doherty explains. Although scientists had several models and theories, it was not understood why this transplant system had evolved and what it was for. Doherty and Zinkernagel showed that the transplant molecules, the MHC proteins, actually play a role in the detection of virally infected cells. 'When a cell becomes infected … a short sequence (peptide) from the virus binds to MHC proteins in the cytoplasm, which are then transported to the cell surface. Recirculating killer (or cytotoxic) T cells that are programmed to recognise such peptide–MHC complexes then see the infected cell as foreign'. The infected cell is then destroyed. Professor Doherty explains that this new model replaced previous theories, 'changed our understanding of the cell-mediated half of the adaptive immune system', and has implications for the understanding and treatment of autoimmune disease and cancer.

Newspix/Shannon Morris

▲ **Figure 12.5** Professor Peter Doherty pictured in 2005 at Melbourne University.

Professor Doherty reflects that the fact living organisms have evolved means that biological systems, including MHC recognition by cytotoxic T lymphocytes, do not always follow what might be the simplest path. He likens these systems to a house that has been renovated and repurposed innumerable times: 'A grand designer might well do it differently! Evolution is the only way that anything in biology makes sense'.

The biggest change in science over Professor Doherty's career has been the technological advances that are shaping the face of biological research. 'In many ways we are asking the same questions,' Professor Doherty explains, 'but technology has provided us with different ways of trying to answer those questions.' As technology continues to develop, Professor Doherty predicts that treatment of protein folding diseases, such as Alzheimer's disease, will be an important challenge for the next generations of biologists.

Today, Professor Doherty remains heavily involved in research, with his work focusing on how the immune system responds to influenza infection.

Questions

1. Summarise the discovery made by Peter Doherty and Rolf Zinkernagel that led to the awarding of the Nobel Prize.
2. Professor Doherty talks about how technology has changed the way that research in his field is conducted. Give an example, reflecting on your studies over the year, of a discovery or technique that was only possible because of a technological development before it.
3. The theory developed by Peter Doherty and Rolf Zinkernagel was based on ideas from previous research and theories. This is the case for most scientific discoveries; however, a Nobel Prize cannot be shared by more than three people. Do you think Nobel Prizes are the best way to reward scientific discoveries? Justify your response.
4. MHC proteins were originally discovered for their role in causing the rejection of transplanted tissue. Suggest why scientists, including Professor Doherty and Rolf Zinkernagel, continued to search for another function of these molecules. (Hint: Think about the selective pressures that would have led to the evolution of MHC proteins.)

PETER C DOHERTY

Read more about Peter Doherty's life on the Nobel Prize website.

Pathogen-associated molecular patterns and damage- or danger-associated molecular patterns were discussed in Chapter 11.

The interaction between antigen presenting cells and T cells is another mechanism for preventing responses against 'self' antigens. A T cell that recognises a complementary antigen on a MHC protein must interact with the antigen presenting cell presenting that antigen. If that antigen presenting cell has recognised a pathogen-associated molecular pattern or a damage- or danger-associated molecular pattern, 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 interferons. Without this 'danger signal', a T cell will not respond if it recognises a peptide bound to an MHC protein. T cells can only recognise an antigen if it is loaded on to an MHC protein, which means they must interact with an antigen presenting cell. This provides an additional safeguard that prevents T cells from mounting an immune response against the body's own cells and tissues.

WOW

The thymus: a protein showroom

T cells undergo development in the thymus, where self-reactive clones are destroyed or inactivated. Different tissues in the body express different proteins. So, how do T cells encounter all of the body's proteins when this 'education' occurs only in the thymus?

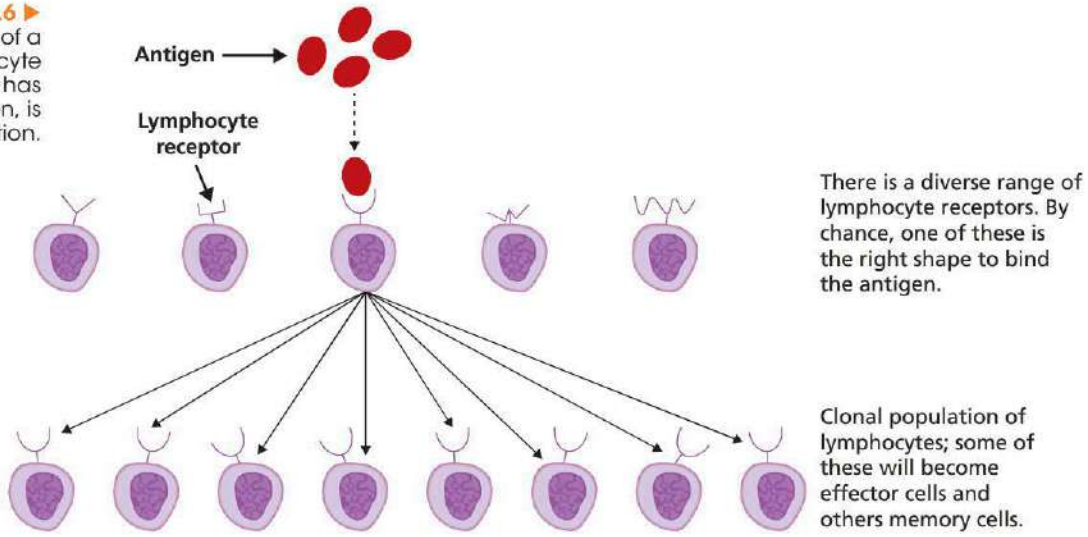
A group of cells in the thymus expresses a wide range of proteins that are usually found elsewhere in the body. These proteins are not expressed to perform their normal function, but rather so that T cells can develop self-tolerance. In effect, these cells serve as a 'showroom' of the proteins that the body is capable of producing. Any T cell bearing a T cell receptor that recognises a peptide presented in the thymus is deleted from the collection. This is critical in preventing the development of autoimmune disease.

Clonal selection

B and T lymphocytes originate as stem cells, a process that starts when we are embryos. During their development, each lymphocyte divides rapidly to produce identical cells that are clones of it, all with the same lymphocyte receptor. 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 are programmed to 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 amazing response in the cell, causing it to divide rapidly, forming two types of cells: effector and memory cells (Figure 12.6).

Figure 12.6 ▶ The rapid division of a particular lymphocyte clone, once it has bound to an antigen, is termed clonal selection.



Random genetic rearrangements allow for a diverse range of lymphocyte receptors to be generated. Clonal selection is responsible for the proliferation of lymphocyte clones that have bound to antigens.

Thus, the antigen itself selects which of the millions of different B or T cell clones becomes active. Australian Sir Macfarlane Burnet played a key role in developing this theory, known as **clonal selection**. He was awarded a Nobel Prize for his contribution to the field of immunology in the 1950s.

CLONAL SELECTION

Watch the video outlining the role of clonal selection in generating immune responses.

WOW

The naming of B cells: a fortunate coincidence

Where do the names of B and T cells come from? It is a coincidence that the 'b' in B cells is also the first letter of bone marrow. B cells were actually named after the bursa of Fabricius, an organ found only in birds and which is necessary for B cell development in birds. It wasn't until years after the discovery of B cells that researchers discovered that the equivalent process in mammals occurs in the bone marrow.

QUESTION SET 12.1

Remembering

1 List the different types of lymphocytes that make up the adaptive immune system.
2 Name the type of molecule that lymphocyte receptors are composed of.
3 Define the term 'self tolerance'.

Understanding

4 Distinguish between the innate and adaptive immune responses.
5 a Draw a diagram to show how B cells bind to antigens and another to show how T cells bind to antigens.
  b Describe how antigen–lymphocyte binding is like a lock and key.
6 Imagine that you are a newspaper journalist writing an article about Sir Macfarlane Burnet's contributions to immunology. Write a brief paragraph using simple language that explains the principle of clonal selection to the general public.

Applying

7 Azathioprine is a drug that blocks the production of purine nucleotides (adenine and guanine) in lymphocytes. Predict the effect that azathioprine would have on the process of clonal selection.

Humoral immunity

The **humoral** immune response is brought about by B cells, which produce an amazing array of different antibodies that attack foreign antigens. (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, also known as **immunoglobulins (Ig)**, consist of four polypeptide chains (two heavy chains and two light chains) arranged in the shape of a Y (Figure 12.7). The antibodies are identical except for a region at the two ends of the Y, where there are two identical binding sites that match, or are complementary to, a particular antigen. 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.

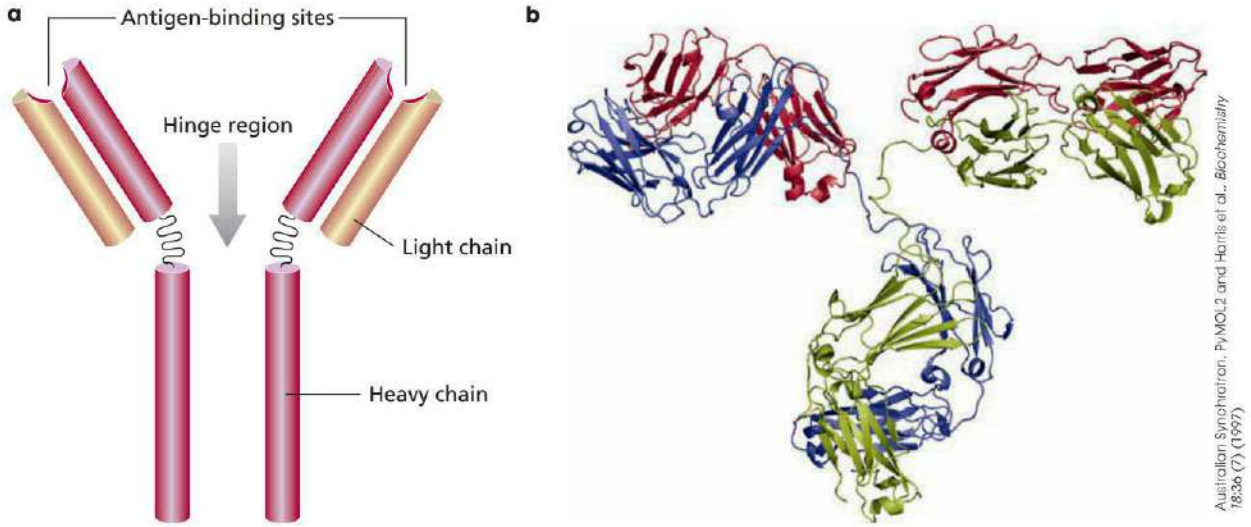


Figure 12.7 ▲
a) The Y-shaped structure of an antibody. The hinge region gives antibodies great flexibility to improve binding to the antigen. b) A ribbon diagram representation of crystal structure '1IGT' – an antibody of the IgG family produced by plasma B cells

Antibodies, once bound to an antigen, can lead to the destruction of pathogens in four ways, all of which may occur simultaneously (Figure 12.8). First, antibodies that are bound to antigens are potent activators of the complement cascade, **complement activation**. The second way is that bound antibodies are able to attract phagocytes, effectively 'tagging' pathogens for phagocytosis and destruction, a process known as **opsonisation**. 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** and is the third way that antibodies can act. Finally, the binding of antibodies can also cause **agglutination** of pathogens meaning that they become stuck together in an antibody–pathogen net. In other words, the pathogens are immobile and not able to spread. Being clumped together in one spot also makes them more susceptible to destruction by phagocytosis.

See page 325 in Chapter 11 for details about the complement cascade and how complement, like antibodies, can also result in opsonisation.

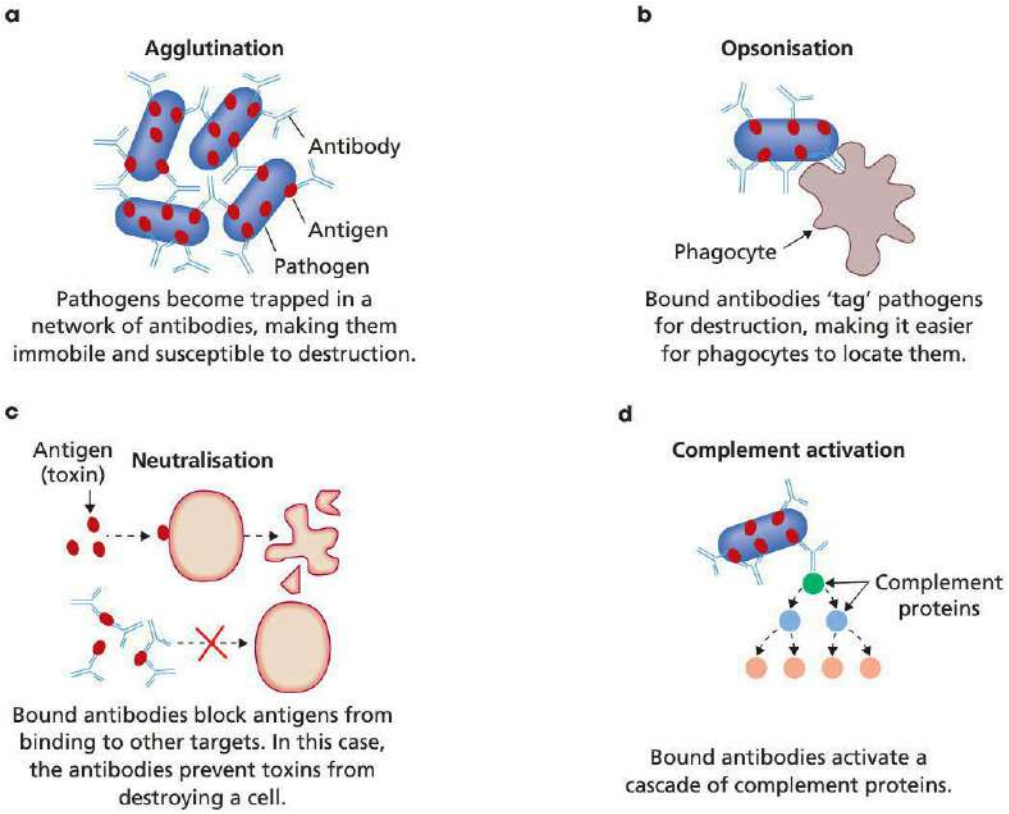


Figure 12.8 ▶
Antibodies can cause the destruction of pathogens in four ways: agglutination, opsonisation, neutralisation and complement activation.

Isotypes

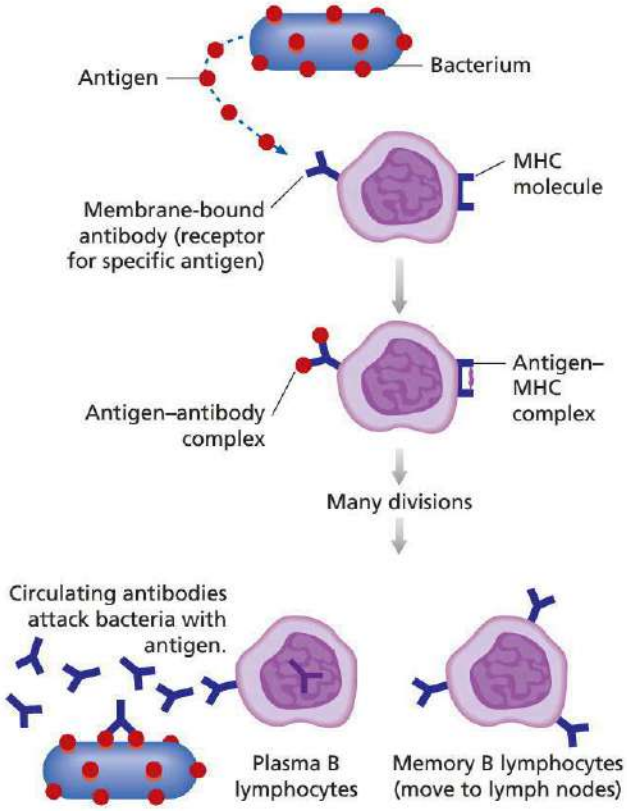
Antibodies occur in several different classes, known as **isotypes**. Each isotype has a different heavy chain that allows them to have different functions.

- IgM antibodies are the first to be secreted in an infection. They cause agglutination of cells bearing antigens, causing them to form large clumps that are more easily eliminated by phagocytes. IgM antibodies are also embedded in the phospholipid bilayer, with the two arms sticking out, exposing the antigen-binding sites. These membrane-mounted antibodies act as the B cell receptors.
- IgG antibodies are produced by effector and memory B cells that have 'matured' after encountering their specific antigen. IgG is responsible for activating complement proteins in the blood and can neutralise toxins directly. Interestingly, this is the type of antibody most commonly passed between mother and baby, either through the placenta before birth or in breast milk later. On a memory B cell, the B cell receptor is usually an IgG antibody instead of an IgM antibody.
- IgA antibodies neutralise pathogens in the respiratory, digestive and reproductive tracts.
- IgE antibodies are important in protection against parasites. It is this type of antibody that also causes allergic reactions to non-pathogenic agents.
- IgD antibodies are found bound to the plasma membrane as the B cell receptor, on B cells that also produce IgM. IgD is not secreted into the circulation.

B lymphocytes and antibody production

In order to produce antibodies, a B cell needs to be activated by an antigen. When a pathogen enters the body it will encounter a large number of B cells. The B cell receptors (antibodies) on the surface of that B cell may or may not be able to bind to antigens on the pathogen. Once an antigen binds to a B cell receptor, the B cell becomes activated and starts rapidly dividing to produce effector and memory B cells (Figure 12.9). The B cell clones that have been activated will then be present in much greater numbers than others (clonal expansion). This division occurs most effectively with the assistance of T_H cells that have been activated by the same antigen. T_H cells assist by producing **cytokines** that promote B cell division and antibody production.

Further information about the activation and roles of T_H cells is on page 354.



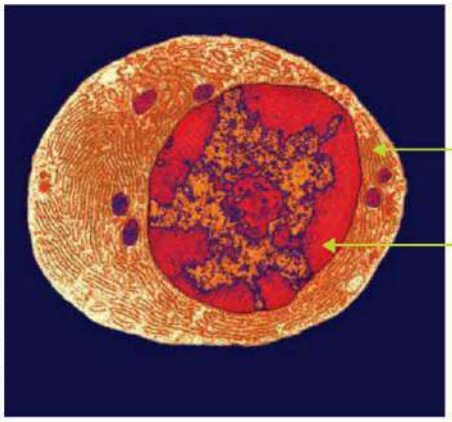
An immature B lymphocyte presents its specific antibody mounted on its membrane.

The immature B lymphocyte encounters the specific antigen recognised by its antibody. The antigen and antibody bond and form an antigen–antibody complex. This is assisted by cytokines and contact-dependent signalling provided by T_H lymphocytes that recognise the antigen–MHC complex.

A series of reactions occur and the activated B lymphocyte divides many times. This is assisted by cytokines from T_H lymphocytes.

Some of the activated B lymphocyte clones become plasma cells that produce and secrete large amounts of antibody molecules that help to destroy the pathogen. Others can become memory B cells.

◀ Figure 12.9 Example of an antibody-mediated immune response to a bacterial pathogen, summarising the steps of B lymphocyte activation.



Corbis/Dr Donald Fawcett

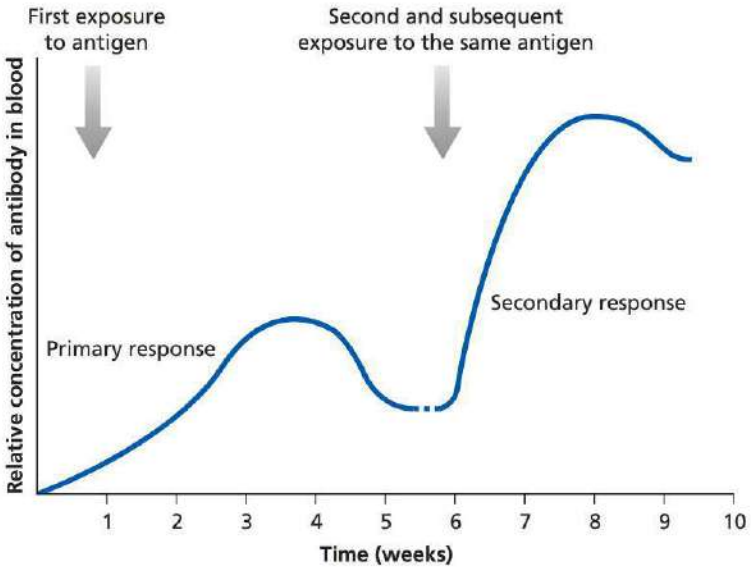
Figure 12.10 ▲ Transmission electron micrograph of a plasma cell. There is extensive rough endoplasmic reticulum to allow for the production of antibodies.

The effector B cells are known as **plasma cells**. Plasma cells have differentiated to become highly specialised for antibody production (Figure 12.10) secreting up to 10 000 molecules of a specific antibody per second into the body fluids. 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 a number of different antigens if they are closely related and have similar structures, such as the smallpox and cowpox viruses.

But what of the memory B lymphocytes? These cells persist within the body in fairly large numbers for some months or even years, not secreting antibodies but still displaying them mounted on their plasma membrane. In this way, they are ready to ambush the same pathogen quickly should it reinvade the body of the host. Once activated by the revisiting antigen, the B memory cells divide and rapidly produce large quantities of antibody, destroying the pathogen before any symptoms of its presence arise.

Figure 12.11 shows the speed of antibody production following initial and subsequent exposure to an antigen. Imagine that the antigen in this diagram is from the varicella zoster virus, which causes chickenpox. When first exposed to the virus the body produces antibodies, but there is a delay before enough are produced to fight off the virus. This is why people develop symptoms of chickenpox when first exposed to the virus. When the same person is later exposed to the virus again, memory cells allow them to produce antibodies much more rapidly. You can see in this figure that the **secondary response** is of a greater magnitude than the **primary response** to that same antigen. This is why most people only experience chickenpox once in their lives. After an initial infection and bout of the disease, they become immune to future infections.

Figure 12.11 ▶ Graph showing antibody levels after an initial (primary) infection by an antigen and after a second exposure to the same antigen



WOW

Naturally occurring antibodies?

Red blood cells are coated with antigens that can be important for blood transfusions. You are probably already familiar with the ABO system. People form antibodies against the antigens that they do not have on their red blood cells. As such, a person with type A blood would have anti-B antibodies and could not have a transfusion from somebody with type B or AB blood. The interesting thing is that these antibodies are found in the bloodstream even when people have never been exposed to the antigen.

How can 'naturally occurring' antibodies exist? In this example, scientists think that these antibodies develop in response to epitopes that are structurally similar to the A and B antigens. When two unrelated substances are structurally similar it is known as molecular mimicry. Anti-A and anti-B antibodies are so widespread it is likely that the molecular mimic is found on something commonly encountered, such as bacteria living in the gut.

QUESTION SET 12.2

Remembering

1 Identify what type of compound an antibody is.
2 Draw a diagram of an antibody, labelling where antigens bind.
3 List four ways that antibodies can act to fight off a pathogen.

Understanding

4 Plasma B lymphocytes possess an extensive rough endoplasmic reticulum, many Golgi apparatuses and many mitochondria. Relate the structure of plasma cells to their function.

Vaccination

Unfortunately, it takes time for the adaptive immune system to locate the invader and mount a defence if that pathogen has never been encountered before. If it is a second infection, memory B and T lymphocytes circulate and lie in wait in the lymph nodes, ready to be activated quickly to destroy the pathogen before symptoms of the disease arise. How, therefore, can we take advantage of this 'immune memory' so that memory cells exist before the first invasion? **Vaccines** have been developed that work by preparing the immune system for the pathogens it may encounter.

A vaccine contains antigens from a pathogen that stimulate the immune system to form memory B or T cells. These memory cells then wait in the lymph nodes ready to detect and respond to the real pathogen with these antigens as soon as it invades.

Substances called **adjuvants** are usually added to vaccines along with the antigen. These adjuvants activate the innate immune system, ensuring that B and T cells receive the assistance from antigen presenting cells they need to respond to the antigen. In other words, a vaccination gives an organism the experience of a particular pathogen's antigens without the host actually developing symptoms of the disease itself. This means that if the pathogen is encountered, the immune system is able to mount a secondary response (Figure 12.11).

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 rapidly activated if that antigen is encountered again. This kind of immunity 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.

Active immunity is contrasted with **passive immunity** when antibodies are provided from an external source (Table 12.1). This will provide protection from the pathogen, but only for as long as those antibodies last. As there are no memory cells, if the person encounters the pathogen again, they will not be immune.

Table 12.1 Examples of active and passive immunity

	Active immunity	Passive immunity
Naturally occurring	Exposure to a pathogen	Transfer of antibodies from mother to foetus via the placenta Transfer of antibodies from mother to baby via breast milk
Artificial	Vaccination	Anti-venom Antibodies against particular pathogens (for example, rabies) Mix of antibodies for immunodeficiency

Passive immunity occurs naturally when antibodies pass from a mother to her foetus via the placenta and during breastfeeding. These antibodies are essential for protecting the young baby from pathogens soon after it is born. A baby is most vulnerable to infection two to three months

after birth, as its own immune system is not yet fully developed and the antibodies it received from its mother have disappeared.

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 somebody who has been exposed to a pathogen. Rabies is a viral disease that is spread in the saliva of infected animals. Untreated, it is a disease that 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 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 very susceptible to infections. A condition such as this where the immune system doesn't function properly is called an **immunodeficiency**. A way of treating this type of immunodeficiency is to the give the patient a mix of antibodies taken from healthy donors. These will provide protection for only a short period of time and so these patients will need to have antibody infusions every month or so.

The protection provided by antibodies may be passive or active. Only active immunity provides long-term protection against pathogens.

Refer to the case study in Chapter 9 (page 267) for the role Australian scientists have in working to reduce the number of snakebite deaths in Asia.

Antibodies and snakebite

Despite having many of the world's most dangerous snakes, the number of deaths in Australia from snakebite remains very low. In part this is 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.

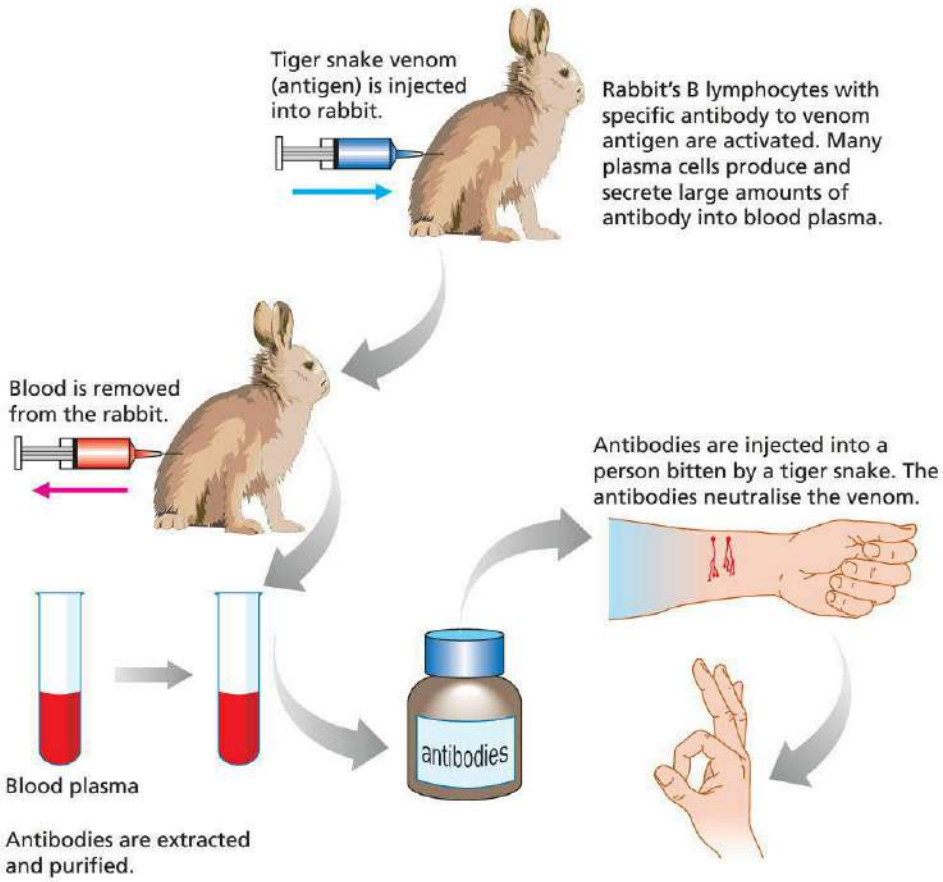


Figure 12.12 ▶ Anti-venom for a tiger snakebite can be produced by collecting antibodies from a rabbit that has been injected with small amounts of venom.

One way to prepare a pure sample is to initially inject the specific antigen into a host, such as a rabbit or horse. This induces the animal to produce antibodies, which are secreted into their bloodstream. These are then extracted for use (Figure 12.12). This process is quite costly and time consuming, and the purification of the sample is difficult.

Scientific literacy: World's first plastic antibody works in mice

Antibodies made entirely from plastic have saved the lives of mice injected with bee venom – the first time such a strategy has worked in live animals.

Researchers developing the antibodies say it is the first step towards customised antibodies for a host of other medical applications, from treating people who have been poisoned to combating infection.

Natural antibodies are made by the body's immune system to lock onto a specific antigen. Likewise, the plastic antibodies contain cavities moulded in exactly the right shape to capture target molecules, in this case, melittin – the active agent in bee venom.

Kenneth Shea of the University of California at Irvine led the team which made melittin antibodies through a process called molecular imprinting. They used a catalyst to stimulate polymers to form around molecules of bee venom, then dissolved away the venom itself, leaving empty cavities with the exact shape to trap melittin.

Shea injected these tiny plastic nanoparticles into mice 20 seconds after they'd been injected with bee venom; 60 per cent survived whereas all the untreated mice died. The plastic antibodies were then destroyed by the liver.

'We conclude that imprinted polymer nanoparticles efficiently capture melittin in the bloodstream,' say Shea and his colleagues in the paper.

'We see this as a very significant paper, and the first demonstration in living things of these materials, effectively using them as a drug,' says Mike Whitcombe, whose lab at Cranfield University in the UK develops imprinted polymers and runs a database of the polymers available.

Philipp Holliger of the Laboratory of Molecular Biology in Cambridge, UK, said that the plastic antibodies do perform some of the functions of natural antibodies – capturing toxins and sending them to the liver for destruction. 'These properties should make them attractive alternatives to antibodies in antidote anti-toxin treatments,' he says.

However, Holliger doubts whether they could perform other important functions of natural antibodies, such as priming the body's immune system to fight future infections. Unlike natural antibodies, they are not equipped to communicate with other cells and components of the immune system.

Coghlan, A. (2010) 'World's first plastic antibody works in mice', *New Scientist*, 11 June.

Questions

1. Would these plastic antibodies be considered a form of passive or active immunity? Explain your response.
2. Explain, with reference to the structure of antibodies, why these plastic antibodies are unable to perform certain roles that natural antibodies perform.
3. The manufacturing process of plastic antibodies means that there is considerable variation in the shape that the polymer forms around the antigen. Predict whether you think the mice would have developed an adaptive immune response against the plastic antibodies. Justify your answer.
4. Philipp Holliger suggests that plastic antibodies could be used as an alternative to conventional antidotes. Does this article provide sufficient evidence that these plastic antibodies are safe to be trialled in humans? Justify your response. If not, what other experiments do you think need to be performed first?

Antibodies in the lab

Another way to produce antibodies is to culture B cells in a laboratory and collect the antibodies they produce. The problem here is that B cells, like most mammalian cells, do not live very long in culture 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 an antibody of interest with cells extracted from a plasma cell **tumour**, creating what is called a **hybridoma**. The hybridoma has the ability to produce antibodies coupled with the property of tumour cells to divide repeatedly

Alamy/Ronald Karpilo

Figure 12.13 ▲ A pregnancy test uses antibodies against the hormone β-HCG, which is only present in urine if a woman is pregnant. The photo shows an example when β-HCG is not present.

(they 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** as they are produced by clones of the same hybrid cell and are thus identical. Hybridomas have revolutionised the production of antibodies.

Antibodies produced this way have a variety of commercial and scientific uses. Due to their ability to bind to a particular antigen, monoclonal antibodies can be used to detect whether a substance is present and, if so, to measure it. Urine pregnancy tests are a commercial use of antibody technology (Figure 12.13). These tests work by detecting a hormone, β-HCG, which is only present in the urine if a woman is pregnant. The tests contain antibodies that bind to β-HCG. The antibodies have an enzyme attached that causes a colour change if bound. This colour change appears as a strip indicating a positive test. Antibodies have also being produced to treat some diseases, including cancer.
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 immune destruction.

EXPERIMENT 12.1

A SNAKE SLEUTH SANDWICH

Imagine that you are on a bushwalk and feel a sharp pain on your ankle. You look down to see a bite mark and a snake moving rapidly away. You were not able to identify the snake but know that there are a number of different anti-venoms used for different snakes. How will the doctor know which anti-venom to give?

Many of the world's most venomous snakes are found in Australia, and it is important to have a system to identify them. Doctors can use a snake venom detection kit (made by CSL Limited) to test venom from the bite site and determine the type of snake, allowing the correct anti-venom to be administered. The basis of this kit is a technique known as enzyme linked immunosorbent assay (ELISA), which uses antibodies that bind to the venom. In this experiment you will learn how ELISAs are performed and how to interpret the results of this test. Note that you will not be required to perform the actual test yourself as the results are provided.

Aim

To determine the type of snake from a venom swab taken from a victim's bite wound (snake A) and how much venom has made it into the bloodstream

Procedure

Part A: Determining the type of snake

1 Take a strip of plastic with seven wells (small containers). Each well contains two types of antibodies: a primary antibody (which is firmly stuck to the bottom of the well) and a conjugate antibody (which is free-floating). Table 12.2 shows what the antibodies are designed to bind to.

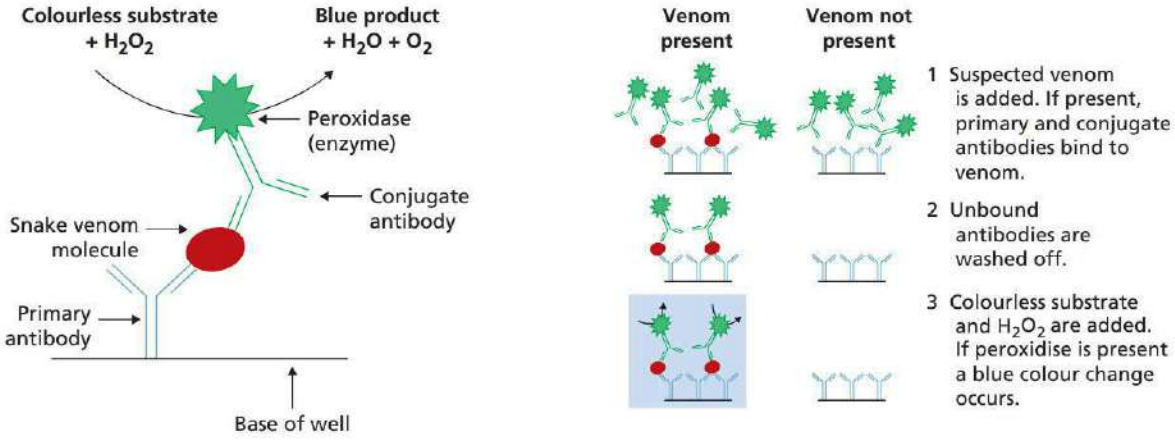
Table 12.2 Antibody composition of the wells used

	Test wells					Control wells	
	1	2	3	4	5	6	7
Primary antibody binds to:	Tiger snake venom	Brown snake venom	Black snake venom	Death Adder venom	Taipan venom	nothing	conjugate antibody
Conjugate antibody binds to:						Tiger snake venom	primary antibody

2 Add a small amount of the sample (a swab of venom from the bite wound) to each well. If venom is present that the antibodies can bind to, the venom will bind to the primary antibody and the conjugate antibody (like an antibody sandwich).

3 Wash the wells several times to remove any antibodies that are not bound to the base.

4 The next step is to add a colourless substrate and hydrogen peroxide to each well. The conjugate antibody has an enzyme (peroxidase) stuck on, or conjugated to, the end of it. If the peroxidase is present, it will catalyse the conversion of the colourless substrate and hydrogen peroxide to a blue product plus water and oxygen. Figure 12.14 summarises these steps.



▲ **Figure 12.14** ELISA uses antibodies against an antigen (in this case venom) to determine if the antigen is present.

Part B: Determining the concentration of venom in the bloodstream

The same technique can be used to determine the concentration of venom because it correlates with the intensity of blue colour produced. A machine called a colorimeter measures the intensity of the colour produced. It does this by shining light of a wavelength that will be absorbed by the blue product through the sample and measuring how much is absorbed. The degree of intensity of colour is called the optical density (OD).

5 Take a new set of nine wells that all contain primary and conjugate antibodies against snake A venom.

6 Place a small amount of blood from the victim in one well and the bite sample in another. In the remaining seven wells, place solutions containing known concentrations of snake A venom (standards) as per Table 12.4.

7 Repeat steps 3 and 4.

8 Measure the OD in each of the wells using a colorimeter.

Results

Part A: Determining the type of snake

The colour of each well is shown in Table 12.3.

Table 12.3 Results from the snake venom ELISA to determine snake A

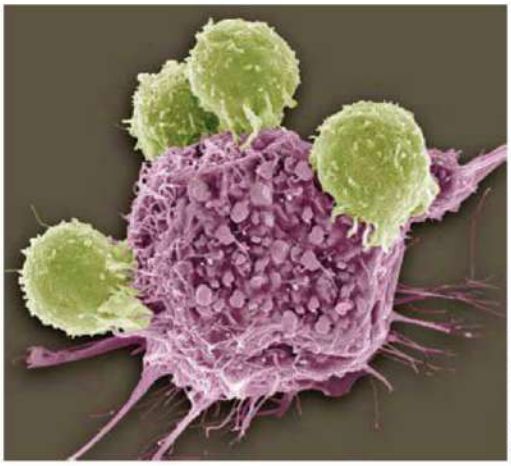
	Test wells					Control wells	
	1	2	3	4	5	6	7
Colour	Very pale blue	Clear	Bright blue	Clear	Clear	Clear	Bright blue

Part B: Determining the concentration of venom in the bloodstream

Table 12.4 Results of ELISA to determine concentration of venom from snake A in blood

	Standards							Blood sample	Bite sample
Venom concentration (ng/mL)	5	25	75	150	300	600	1200	X	Y
OD	0.01	0.04	0.13	0.23	0.54	0.99	1.87	0.18	1.67

Cell-mediated immunity



SPL/Steve Gschmeissner

▲ **Figure 12.16** Scanning electron micrograph showing T_C lymphocytes attacking a cancer cell

Cell-mediated immunity involves the direct killing of virally infected and cancerous cells by T_C lymphocytes. Like B lymphocytes, they are able to distinguish 'self' from 'non-self' due to special membrane-bound receptors, T cell receptors, that interact with antigens. You have already learned that T lymphocytes 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.

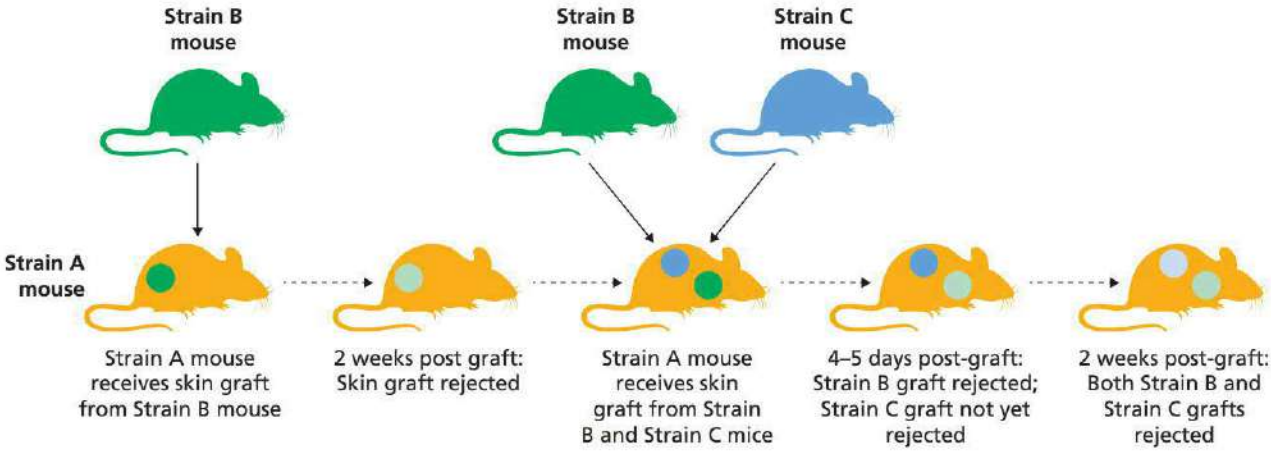
Some viruses have evolved mechanisms to stop or reduce the expression of MHC class I on infected cells. This prevents T_C cells from recognising these cells as virally infected, allowing the virus to evade destruction and continue to divide. As a result of natural selection, natural killer cells have evolved to destroy cells that have low levels of MHC class I on the surface. This is a clear example of how selective pressures from the immune system can lead to changes in pathogens and vice versa.

Like B cells, activated T_C cells (with the help of T_H cell cytokines) divide many times to form an army of clones. Some of these clones become effector cells, while others remain as memory cells and migrate in the lymph and through lymph nodes where they can be activated quickly upon a second encounter with the same pathogen.

T_C cells are amazing killers; they can eliminate infected body cells or tumour cells by releasing powerful cytotoxins when they 'touch' a cell that carries an unrecognised antigen (Figure 12.16). These include chemicals called perforin and granzymes that work together to induce apoptosis in the target 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, it will be rejected after around 14 days. If that same mouse later receives a second graft from the same donor mouse, the rejection only takes 4–5 days (Figure 12.17). This is because memory T_C cells formed after the first graft respond more rapidly when they encounter the foreign graft a second time.

▼ **Figure 12.17** Graft-rejection experiment demonstrating that cell-mediated immunity displays memory.



As indispensable as these cells are to our immune system, they can also cause problems for patients requiring organ transplants. These cells are the primary cause of transplant tissue rejection as 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.

Analysis of results

1 Based on the information in Table 12.3, determine the type of snake that snake A is.

2 Plot each of the standard values in Table 12.4 on a set of axes. Remember to choose an appropriate scale and add labels, a title and a line of best fit.

3 Describe the relationship between venom concentration and OD.

4 Use this graph to interpolate the concentration of venom in the blood sample (X) and bite sample (Y).

Discussion

1 Explain the function of each of the following components of the kit.
   - **a** Primary antibody
   - **b** Conjugate antibody
   - **c** Colourless substrate

2 The snake venom detection kit uses two different controls.
   - **a** Identify which is the positive control and which is the negative control.
   - **b** Suggest the role of each control within the kit.
   - **c** Draw a diagram to show how the positive control well works.

3 In Table 12.3 you can see that well 1 has turned a very pale blue. Suggest a possible explanation, in terms of the structure of antibodies, as to why this has occurred.

4 If, instead of 1.67, the OD of the bite sample had been 2.61, would it still be valid to use the same graph to determine the concentration? Justify your answer.

5 Predict a possible outcome if the wells were not washed thoroughly before the addition of H_2O_2. Explain and justify your prediction.

6 You can see that antibody technology is critical for the diagnosis as well as the treatment of snakebites.
   - **a** Describe one way that the antibodies used in the ELISAs could have been produced.
   - **b** Explain why different snake species bites require different anti-venoms.
   - **c** If a patient who has been given anti-venom is bitten by the same type of snake a year later, would they still be protected? Explain why or why not.

QUESTION SET 12.3

Remembering

1 List three different commercial uses for antibodies.

2 List two examples of passive immunity and two examples of active immunity.

Understanding

3 Figure 12.15 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.

4 Outline why passive immunity only lasts about 28 days.

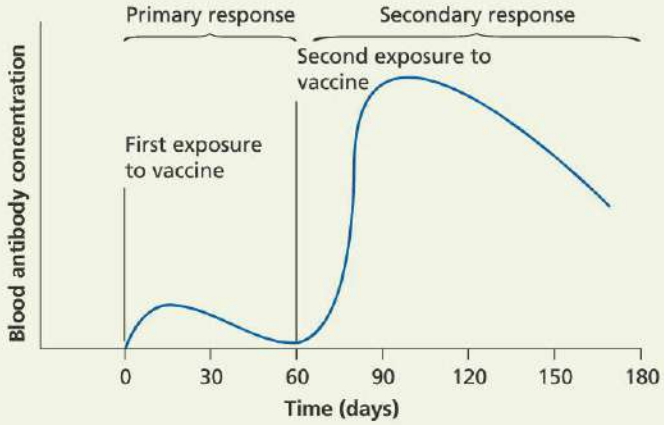


Figure 12.15 ▲ Response to vaccination against tetanus

Helper T and regulatory T lymphocytes

As the name suggests, helper T cells (T_H cells) assist other cells of the immune system. They do this by secreting chemicals (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 and memory cells. Cytokines can also stimulate macrophages to engulf invading cells more readily.

CYTOTOXIC T CELLS IN ACTION

Watch the video of a T_C cell (green, then yellow) recognising and destroying a cancerous cell (whose nucleus is blue).

T cells can only recognise antigen presented on an MHC protein: MHC class I for T_C cells and MHC class II for T_H cells.

The importance of T_H cells is illustrated by the human immunodeficiency virus (HIV). This pathogen is deadly because it leads to the destruction of T_H cells. HIV is discussed later in this chapter on page 358.

Another type of T cell, called regulatory T cells (T_{reg} cells) play an important role in modulating the action of lymphocytes. They may enhance or suppress the actions of other lymphocytes. T_{reg} cells are also capable of suppressing the action of phagocytes. In this way, they help prevent the immune system overreacting to a stimulus. T_{reg} deficiency causes a very severe autoimmune disease resulting from overactive lymphocytes.

QUESTION SET 12.4

Remembering

1 Copy and complete the following table, indicating with a tick or cross whether the following statements are true or false for T_H cells and T_C cells.

	T_H cells	T_C cells
Recognises antigens presented by MHC class I molecules		
Undergoes clonal selection once an antigen has bound		
Destroys cells by producing cytotoxins		

2 Describe the role of T_{reg} cells.

Understanding

3 The adaptive immune system is often described as having memory. Explain what this means, using T_C cells as an example.

Linking the parts of the immune system

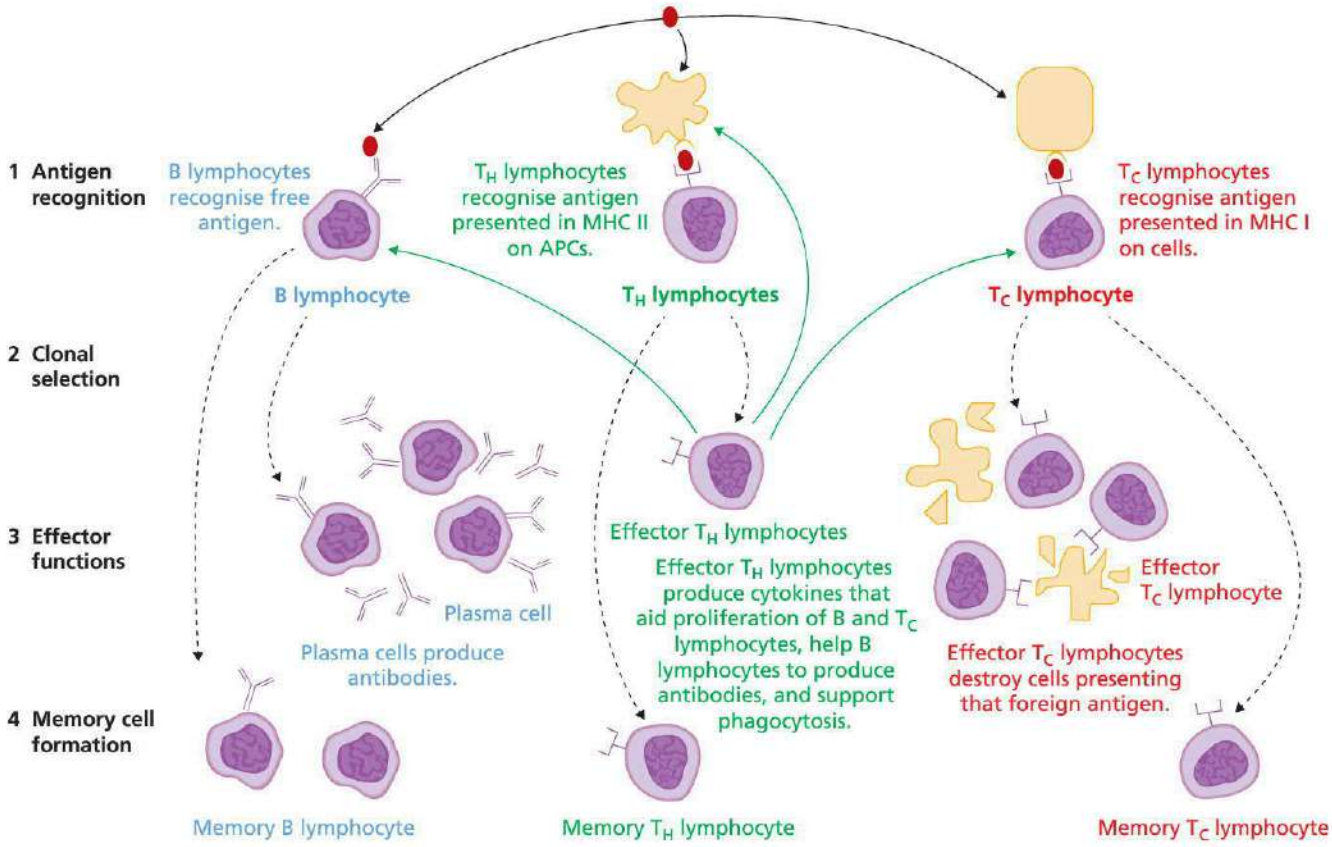
The immune system is a complex network of cells that rely on one another in order to function properly.

B and T lymphocytes all share a number of features that are summarised in Figure 12.18. They both have a system for generating a diverse range of receptors for different antigens and rely on clonal selection to allow for proliferation of relevant clones. Both B and T cells form effector and memory cells. Importantly, specific recognition of antigens and the ability to exhibit memory distinguishes the adaptive immune functions of lymphocytes from the cells of the innate immune system.

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 summarises 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 antigen presenting cells that have recognised a pathogen-associated molecular pattern or a damage- or danger-associated molecular pattern.
- 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 12.18 Summary of the actions and functions of the cells of the adaptive immune system



WOW

The lamprey: an analogous immune system

Until recently, it was believed that the adaptive nature of the immune system was unique to jawed vertebrates. Evidence now suggests that lampreys, a group of jawless vertebrates, have evolved a separate adaptive immune system that is analogous to ours.

Lampreys have two distinct populations of lymphocytes that have different receptors. Like B and T cells, the DNA in these lymphocytes undergoes recombination to form a range of receptors. These receptors are known as variable lymphocyte receptors (VLRs) of two types, A and B. VLRB receptors are secreted by activated lymphocytes, – much like antibodies. VLRA is expressed on the surface of the other group of lymphocytes, which behave like T cells.

Given the complexity of the adaptive immune system, it is quite extraordinary these two similar systems appear to have evolved separately!


Alamy/blickwinkel

▲ Figure 12.19 The lamprey has an adaptive immune system that has some similarities to ours – but appears to have evolved independently.

ACTIVITY 12.1

ADAPTIVE IMMUNE ANALOGIES

Aim

To develop a set of analogies for different parts of the immune system

You will need

- pen
- paper

What to do

1 Working with a partner, brainstorm an analogy for each of the parts of the immune system listed below. Be creative and try to think outside the box! The following is an example of such an analogy for 'vaccine': 'A vaccine is like a trial exam. In a trial exam, exposure to questions trains a student to perform better on the real exam. Similarly, exposure to an antigen in a vaccine trains the body to respond more rapidly and effectively to the real antigen.'
   - Antibody
   - APC
   - T_H cell
   - T_C cell
   - MHC class I molecule
   - MHC class II molecule
   - Phagocyte
   - Vaccine
   - Plasma cell
   - Cytokine
   - Lymphocyte receptor

2 Combine into groups of three of four and discuss your lists. To what degree does each analogy work? Are there limitations? Decide among the group on the analogy that best fits each term.

3 Present your group's list to the rest of the class. Perhaps you could vote and have a prize for the best analogy.

What did you discover?

1 Reflect on whether these analogies have helped your understanding of the adaptive immune system. Make a list of things that have become clearer as a result of this exercise.

QUESTION SET 12.5

Remembering

1 List three ways that the innate and adaptive immune systems communicate.

Understanding

2 Explain how T_H cells aid multiple other cells of the immune system in fighting off invaders.

When the immune system malfunctions

Our immune systems have evolved to provide protection against a wide variety of pathogens. While effective at performing this job, the immune system can also malfunction and cause illness. This may be because the body mistakes a usually harmless substance or its own cells for 'non-self' and launches an attack. The immune system can also fail to defend the body when it needs to, resulting in catastrophic infection. In this section we will explore what happens when the immune system misfires.

Allergy

The incidence of asthma and hay fever is on the increase in Australia. These disorders are forms of allergies – exaggerated immune responses to the antigens of normally harmless substances that may be found on some pollens, foods, cosmetics, drugs or animals. An allergic response involves the production of antibodies against harmless substances. These antibodies then bind to mast cells and basophils, triggering the release of chemicals including histamine, which causes inflammation.

Symptoms include inflammation and excessive mucus production. In most cases, allergies are simply annoying but they can be life-threatening if they result in **anaphylactic shock**, in which inflammatory responses race through the body, leading to constriction of the lung airways and loss of fluid from leaky capillaries into body tissues. This latter response 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.

Autoimmunity

Our immune system is carefully tuned to remove or suppress any B or T cells that may respond to antigens in our own body. But sometimes this system of self-recognition fails and the immune system starts to react against its own tissues. In other words, it recognises 'self' as 'non-self'. This gives rise to what is called an autoimmune disease. There are many different types of autoimmune diseases and almost any part of the body can be affected, but symptoms commonly involve the skin, kidneys and joints. What effects the autoimmune disease has on the body depends on which 'self' antigen (known as an autoantigen) the body is reacting to.

A striking example is Graves' disease, a type of hyperthyroidism. It is caused by antibodies that bind to the thyroid-stimulating hormone (TSH) receptor on thyroid cells. TSH is responsible for stimulating thyroid hormone production within these cells. Interestingly, the binding of these antibodies has the same effect and causes extremely high levels of thyroid hormones. Thyroid hormone levels are usually maintained in a narrow range, but because this is an abnormal stimulus the normal feedback mechanism cannot compensate. The high thyroid hormone levels can cause weight loss, diarrhoea, a tremor and anxiety. Graves' disease can also cause inflammation around the eyes, causing them to bulge out of their sockets (Figure 12.20).

Corbis

▲ **Figure 12.20** Graves' disease is a form of hyperthyroidism caused by antibodies that bind to the TSH receptor. It can cause bulging of the eyes.

Immunodeficiency

In some people, the immune response is not sufficient to fight off pathogens that are usually easily defeated. Conditions where a defective immune system renders someone vulnerable to infection are known as immunodeficiency. Immunodeficiency may be caused by genetic defects or acquired later in life.

SCID is an inherited condition that results in a severely reduced or totally absent army of B and T lymphocytes. People with SCID may develop infections

that generally have little effect on most of the human population. The main way of treating SCID is with a bone marrow transplant, which provides the patient with a new immune system. Scientists and doctors are also trialling forms of gene therapy to try to replace the faulty gene.

Immunodeficiency may also be acquired, as in the case of HIV. This viral infection results in a severe form of immunodeficiency known as acquired immunodeficiency syndrome (AIDS). More than 30 million people are infected worldwide and this number is steadily increasing. HIV is a major cause of mortality in parts of Sub-Saharan Africa where infection rates are extremely high.

HIV generally targets T_H cells by binding to specific receptors on these cells and injecting its RNA. The T_H cell is then stimulated to produce more viral particles, which bud from the host cell's own plasma membrane, ready to infect other T_H cells (Figure 12.21). The virus spreads through the immune system, slowly depleting the army of T_H cells, eventually causing AIDS.

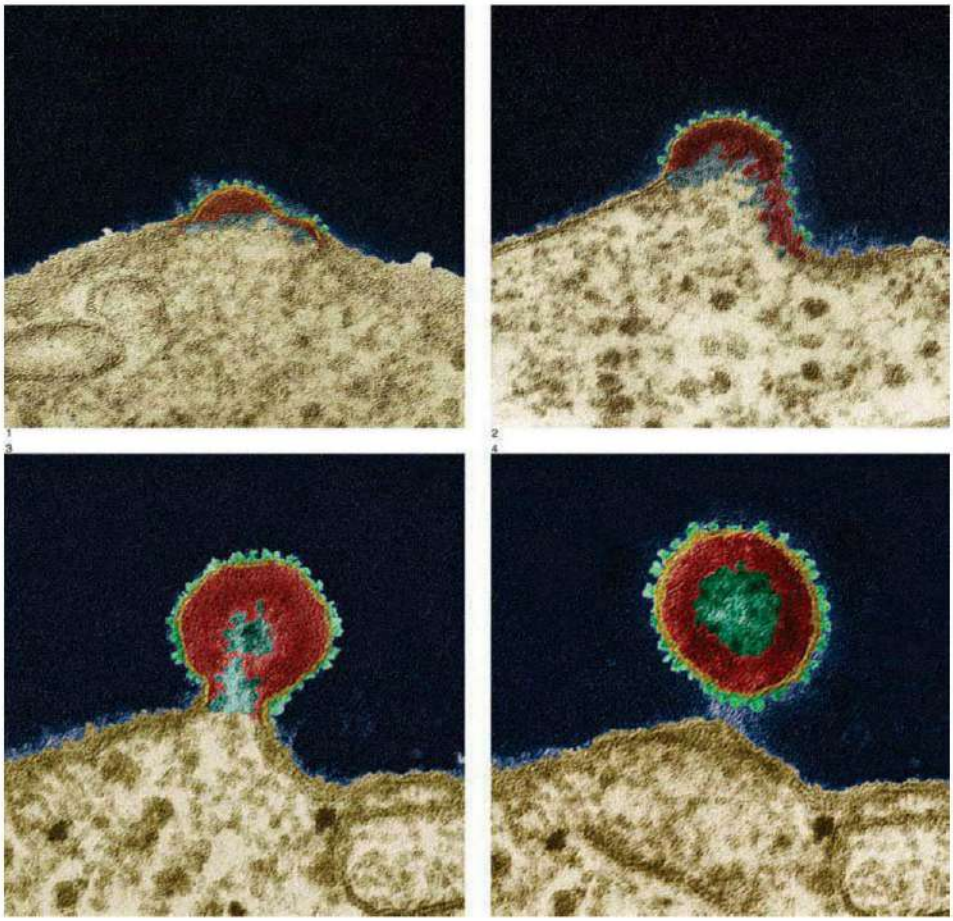


Figure 12.21 ▶ Transmission electron micrograph of HIV budding out of a T_H lymphocyte

WOW

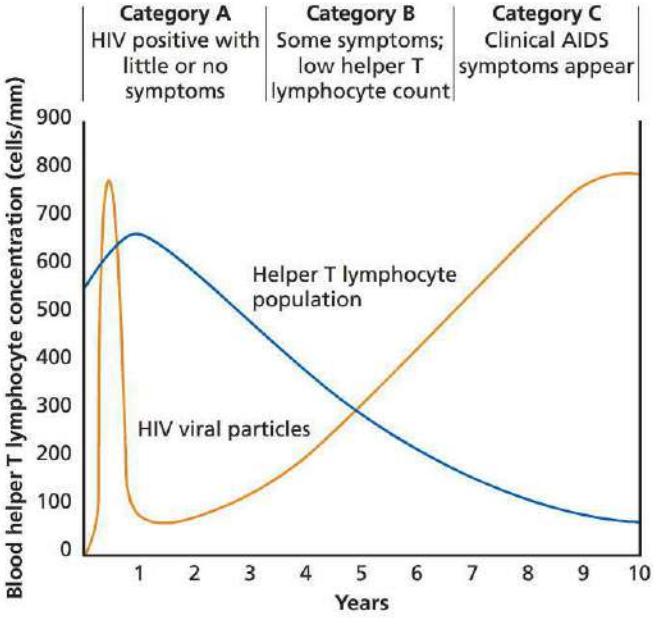
A new immune system?

A bone marrow transplant can be used to treat patients with severe immunodeficiency.

The patient is given high levels of chemotherapy designed to destroy their bone marrow. The next step is harvesting bone marrow or stem cells from the blood of a donor. The donor may or may not be related to the patient, but needs to have matching MHC proteins. The donor cells are then infused into the patient. The cells settle in the bone marrow and begin to divide.

By replacing the bone marrow stem cells, the procedure replaces all of the cells of the immune system. As such, the patient will now be able to produce functional lymphocytes and fight infections. Sometimes this new immune system recognises the body as 'non-self' and mounts an immune attack, called graft-versus-host disease. This is the opposite of the immune system rejecting a transplanted organ – the transplanted immune system is rejecting the body!

Early on during HIV infection the immune system is capable of producing a response against the virus. However, as more and more T_H cells become disarmed, the effectiveness of humoral and cell-mediated responses decreases and the number of viral particles present increases (Figure 12.22).



◀ **Figure 12.22**
Stages of infection by HIV

After several years, an infected individual becomes prone to multiple infections from pathogens that would be relatively harmless under normal conditions. Many sufferers die from simple yeast or bacterial infections, pneumonia or unusual tumours and cancers.

As yet there is no cure for HIV infection. Drugs have been developed to reduce the spread of the virus in the host, although they do not destroy it. These drugs can prevent viral replication and the development of AIDS. The discovery of effective drugs has significantly improved the lifespan of people living with HIV. Despite this, financial and logistic barriers mean that these drugs remain inaccessible to many people infected worldwide.

Advances in microbiology, genetics and molecular biology may produce more effective treatments and possibly a cure. The best defence at the moment is prevention of transmission. HIV is found in human body fluids and spreads directly, primarily through sexual contact, and through entry into blood by sharing of syringes and transfusion of contaminated blood. It can also be transmitted to infants during pregnancy and during birth. Knowing the method of transmission assists control.

QUESTION SET 12.6

Remembering

1 List three ways that dysfunction of the immune system can cause disease, giving specific examples.

2 Identify whether the following statements are true or false.

- a Immunodeficiency can be hereditary or acquired.
- b An autoimmune disease is one where the immune system attacks the body's own cells.
- c People born without B cells have a fully functioning immune system.
- d An allergy is caused by the body's immune response to a 'self' antigen.

Understanding

3 Explain how the body's ability to distinguish between 'self' and 'non-self' is important in the development of autoimmune diseases.

4 Explain how HIV infection impacts on the immune system.

CHAPTER SUMMARY

- The adaptive immune system is able to recognise a wide range of antigens and respond specifically to different antigens. It also displays memory and is therefore able to mount a stronger and more rapid response when it encounters an antigen for the second time. These two features distinguish it from the innate immune system.
- Each T and B cell undergoes genetic recombination so that it expresses a unique antigen receptor. The diversity of receptors produced means that, by chance, there will be a lymphocyte that can bind to almost any antigen.
- The ability to distinguish between 'self' and 'non-self' antigens is critical to the functioning of the adaptive immune system and prevention of autoimmune disease.
- During development, lymphocytes which produce receptors that can bind to 'self' antigens are inactivated or destroyed. This leads to self-tolerance.
- The binding of an antigen causes a lymphocyte to rapidly divide to produce effector and memory cells; a process known as clonal selection.
- There are three major groups of lymphocytes, which are summarised in Table 12.5.

Table 12.5 Three major groups of lymphocytes: B cells, T_H cells and T_C cells.

	B lymphocytes	Helper T (T_H) lymphocytes	Cytotoxic T (T_C) lymphocytes
Development of self-tolerance	Occurs in bone marrow	Occurs in thymus	Occurs in thymus
Receptors	BCR (antibody)	TCR	TCR
Antigen recognition	Recognises antigens not presented in MHC	Recognises antigen in MHC class II	Recognises antigen in MHC class I
Undergo clonal selection	Yes	Yes	Yes
Effector functions	Plasma cells produce antibodies	Production of cytokines to aid B cell, T_C cell and macrophage functions	Induction of apoptosis in target cells
Formation of memory cells	Yes	Yes	Yes

- Antibodies produced by B cells and their derivative effector cells, plasma cells, can lead to the destruction of pathogens in several ways: agglutination, opsonisation, neutralisation and complement activation.
- Commercially produced antibodies can be used as anti-venom, to provide protection against infection, to treat certain diseases and to capture, detect and measure substances.
- Cell-mediated immunity involves the destruction of virally infected or cancerous cells by T_C cells.
- Dysfunction of the immune system can result in several diseases. Autoimmune diseases occur when the immune system recognises 'self' antigens as 'non-self'. Immunodeficiency conditions occur when the immune system cannot successfully fight off pathogens. They may be genetic or acquired (such as HIV).

CHAPTER GLOSSARY

active immunity the immunity formed by stimulation of the immune system with an antigen and the generation of effector and memory cells; it is contrasted with passive immunity

adaptive immune response an immune response that is acquired; after an initial response to a pathogen, the immune system creates a 'memory' that leads to an enhanced response to subsequent encounters with the same pathogen

adjuvant a substance added into a vaccine along with an antigen to improve the immune response to that antigen

agglutination when antigens or pathogens become stuck together because of antibody binding

anaphylactic shock a severe form of allergic reaction that causes widespread swelling, including of the face and neck, which can make breathing difficult

antibody a Y-shaped protein produced by plasma cells that binds to a specific antigen; also called immunoglobulin

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

antigen presenting cell (APC) a cell that processes antigens of pathogens after phagocytosis and presents them to T cells in MHC class II; macrophages and dendritic cells

B cell receptor (BCR) a surface-bound antibody that serves as a receptor so that B cells are able to detect antigens

B lymphocyte/cell a class of lymphocytes; once activated, they are characterised by the production of antibodies

clonal selection the process in which lymphocytes that have bound to an antigen rapidly divide and become more numerous than other clones

complement activation bound antibodies activate complement proteins

cytokines small signalling molecules that coordinate inflammation and immune responses, and that leukocytes use to communicate with one another; includes interleukins and interferons

cytotoxic T (T_C or killer T) lymphocyte/cell a class of lymphocytes that destroys virally infected or cancerous cells by secreting proteins that cause apoptosis

dendritic cell a type of antigen presenting cell

epitope a small part of a larger molecule that binds to a receptor site; examples are B cell receptors and T cell receptors

helper T (T_H) lymphocyte/cell a class of lymphocytes that aids T_C cells, B cells and macrophages by secreting cytokines

humoral an immune response brought about by antibodies that circulate freely in the bloodstream and can lead to the destruction of pathogens

hybridoma created by fusing a B cell clone with cells from a plasma cell tumour; produces monoclonal antibodies and divides repeatedly

immune system a complex network of cells, tissues and organs in the body that detects differences between 'self' and foreign organisms, and mounts an immune response

immunodeficiency a state in which the immune system does not function properly, leaving a person susceptible to infections the immune system could normally fight off

immunoglobulin (Ig) see *antibody*

innate immune response 'innate' means not learned; as applied to the innate immune response, one that is not specific and does not have 'memory'

isotype a subtype of immunoglobulin; each isotype (IgG, IgM, IgA, IgE and IgD) performs a different function

major histocompatibility complex (MHC) protein markers found on the cell surface that are important in recognising 'self' from 'non-self'; there are two classes: MHC class I is found on all cells and MHC class II is found only on antigen presenting cells

MHC restriction refers to the fact that T cells can only recognise antigens that are presented in MHC proteins

monoclonal antibody the antibodies produced by a hybridoma; they are identical to the antibodies produced by the original cell

neutralisation the process by which antibodies prevent toxins from acting; that is, by binding to them and blocking them from binding to anything else

opsonisation a process in which a pathogen is coated with antibodies and marked for ingestion and destruction by phagocytes

passive immunity immunity characterised by the transfer of antibodies from one individual to another; this type of immunity does not show memory

plasma cell an effector B cell that has differentiated to become highly specialised for producing antibodies

primary response the response generated when an antigen is encountered for the first time; contrasted with the secondary response

regulatory T (T_{reg}) lymphocyte/cell a class of lymphocytes that helps to regulate the immune response

secondary response the response generated when the body encounters a pathogen it has previously generated an immune response to; occurs more rapidly and is of greater magnitude than the primary response

self-tolerance the deletion or inactivation of lymphocyte clones that can bind to 'self' antigens;

as a result, means that no immune response can be mounted against these antigens

T cell receptor (TCR) a protein receptor found on the surface of T cells; binds to antigens presented on MHC proteins

tumour an abnormal growth of tissue

vaccine an injected solution of antigens or pathogens that is designed to elicit a primary response and promote the formation of memory cells

CHAPTER REVIEW QUESTIONS

Remembering

1 Identify two functions of the MHC proteins.

2 Recall where T cells undergo their development. Repeat for B cells.

3 The ability to distinguish between 'self' and 'non-self' antigens is crucial to the functioning of the immune system.

- a Define what is meant by the term 'self antigen'.
- b Outline how the lymphocytes learn to distinguish between 'self' and 'non-self' antigens.
- c Name the problem that can occur if the immune system responds to 'self' antigens.

4 Draw a diagram comparing the amount of antibody produced in response to an antigen after the first and second exposures.

5 List the different ways that antibody binding can lead to the destruction of pathogens.

6 a Describe the role of T_H cells.

- b Identify how they are able to perform this function.

Understanding

7 Describe what is meant by the term 'clonal selection', using B cells as an example.

8 Millions of different antibodies are able to be made by our B cells, even though our genome has only around 30 000 genes. Explain how this is able to occur.

9 Passive immunity does not display memory. Present an argument as to why this is the case.

10 Draw a diagram to illustrate one way that antibodies can be produced for commercial uses.

11 Explain how vaccinations work to prevent infection, giving a specific example.

Applying

12 Liver, heart and kidney transplants are now fairly common procedures in many hospitals. However, recipients of these transplants face the problem of rejection of these organs.

- a Explain why the immune system rejects these organs.

Transplant patients are usually prescribed immunosuppressant drugs to prevent transplant rejection. Many immunosuppressant drugs work by interfering with DNA synthesis.

- b Suggest a negative effect that these drugs may have on the health of the patient.
- c Explain how a drug that interferes with DNA synthesis can prevent transplant rejection.
- d 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.

13 Australia has many venomous snakes. One species, commonly called the 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. 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.
- c Explain how the alpha-neurotoxin prevents the acetylcholine from working.

Fortunately, there is an anti-venom available to people who have been bitten by a death adder. If the anti-venom is injected quickly enough, it 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.

- d Name the substances the horse would produce in order to counteract the snake venom in its body.

e Name the cells in the horse that would be responsible for the formation of this substance.

f Explain why small amounts of venom are injected into the horse over a long period of time.

g Outline the steps involved in the formation of these substances.

h After 10–12 months blood is extracted from the horse and the plasma can be injected into snakebite victims. Identify what term is given to the use of horse plasma as a treatment for snakebite.

i Explain how this is effective in treating the snakebite victim.

14 Monoclonal antibodies are commonly manufactured using a hybridoma. A hybridoma is made from fusing a B cell to a plasma-cell tumour cell.

a Describe two situations in which monoclonal antibodies may help save a person's life.

b Patients with plasma-cell tumours have an abnormally high level of protein in their blood (called a paraprotein), which can 'clog' the kidneys and cause kidney failure. Predict what type of protein this paraprotein consists of.

c Explain what property of tumour cells makes them useful for fusing to B cells for monoclonal antibody production.

15 Immune thrombocytopaenic purpure (ITP) is an autoimmune disease where the platelet counts of those affected drops extremely low. These 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.

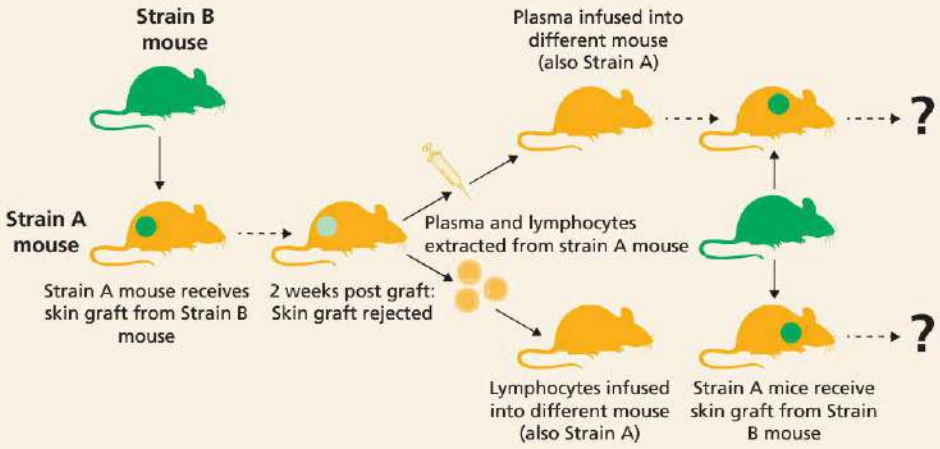
Analysing

16 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.

17 Compare and contrast the MHC class I and MHC class II molecules.

18 Distinguish between the immune response that leads to an allergy and the response that leads to an autoimmune disease.

19 Figure 12.23 is another graft-rejection experiment that builds upon the one you have previously seen in Figure 12.17 on page 353. 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.



◀ **Figure 12.23** Experimental setup to see if immune memory 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.

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.

20 A mutation in a single gene found 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 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.
- **c** Miriam is planning to start a family but her father had XLA and she is concerned she could pass the condition onto her children. Use your knowledge of genetics to calculate the risk of Miriam's children developing XLA. Does it make a difference whether Miriam has a boy or girl?

Evaluating

21 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 fits.

22 Provide an argument for or against the following statement: 'The importance of antibodies as technological tools has almost come to surpass that of their original function.'

Creating

23 Draw a diagram that shows all the different defences encountered by an antigen when it enters the body. Be sure to indicate how these different defences communicate.

24 Consider the workings of the immune system. Synthesise your knowledge of biology and immunology to create a presentation discussing some of the different challenges medical researchers face when tackling diseases such as cancer, influenza and multiple sclerosis.

25 Now that you have completed your studies of the innate and adaptive immune systems, prepare a brief summary that distinguishes between the two. You may like to use a table or Venn diagram.

26 'Humans have evolved to not generate lymphocyte receptors that can bind to self-antigens.' Do you agree with this statement? Justify your response.

Reflecting

27 This chapter has included a number of diagrams to show the steps of an adaptive immune response. Reflect on whether you prefer to use these or the descriptions in the text to understand the concepts presented. Apply the best strategy for your own study, and develop useful ways to summarise and synthesise everything you need to know about immunity.

28 Describe how learning about some of the commercial applications of antibody technology has impacted upon your understanding of how antibody structure relates to its function.

CHAPTER 13
PUBLIC HEALTH

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

Science Understanding

- Transmission and spread of disease is facilitated by regional and global movement of organisms (ACSBL124)
- The spread of a specific disease involves a wide range of interrelated factors (for example, persistence of the pathogen within hosts, the transmission mechanism, the proportion of the population that are immune or have been immunised, and the mobility of individuals of the affected population); analysis of these factors can enable prediction of the potential for an outbreak, as well as evaluation of strategies to control the spread of disease (ACSBL125)

Science Photo Library/National Library of Medicine

Figure 13.1 ▶
Signs such as these were used during the Spanish influenza outbreak of 1918–19 to try to prevent the spread of the disease.

As the battles of World War I drew to a close, many nations faced another major threat. The 1918–19 Spanish influenza **outbreak** spread rapidly throughout the world, killing up to 100 million people, many more people than the war itself. Despite intensive efforts to stop the disease spreading to our shores, the Spanish influenza outbreak killed around 12 000 Australians. One of the most striking things about this outbreak was that Spanish influenza killed healthy, young people along with the frail.

The reason this outbreak was so devastating is likely to be multifactorial. Undoubtedly, the fact that populations were exhausted and poorly nourished as a result of the war increased susceptibility to infection. The transportation of soldiers around the world facilitated the rapid spread of the disease. War had also disrupted normal healthcare programs, leaving countries unprepared to respond. Additionally, the strain of influenza that became known as Spanish influenza was deadlier than regular seasonal influenza. Even people with strong immune systems were susceptible to it.

Spanish influenza provides a strong example of how factors relating to the **host**, the pathogen and the environment all contribute to the spread of disease. In this chapter we will explore the factors that impact on disease transmission, how disease spread is monitored and how outbreaks are managed.

How disease spreads

The rate of disease transmission is not constant and varies with different diseases, populations and time. However, there are several recognisable patterns of disease transmission as shown in Figure 13.2. An **endemic** disease is one that occurs at a relatively constant rate within a population. In contrast, diseases that are uncommon and occur irregularly are said to occur **sporadically** within a population. **Epidemics** occur when there is an increase in the number of **cases** of a disease within a population to above what is considered normal. The terms 'outbreak' and 'epidemic' can be used interchangeably, although epidemics tend to refer to larger or more serious incidents. Sometimes an epidemic may spread across multiple continents or throughout the world, in which case it is referred to as a **pandemic**.

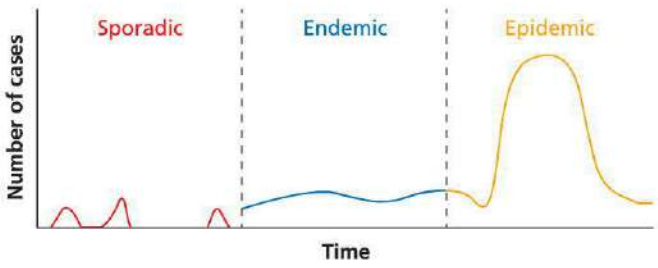


Figure 13.2 ▶
Within a population, a disease may occur sporadically, at an endemic level or, occasionally, as an epidemic.

Understanding the factors that contribute to the spread of disease within a population is an important first step in managing disease outbreaks. Interventions can then be designed to target these factors and prevent further spread of disease. Factors that impact on transmission include characteristics of the disease itself (such as the mechanism of transmission), environmental factors (such as climate) and characteristics of the population infected (such as levels of immunisation). These factors interact to determine the extent of a disease spread at a particular place and time. Factors affecting disease transmission may be modified by humans unintentionally, or intentionally as disease control measures.

The transmission of disease is affected by a wide range of interrelated factors.

Pathogen factors that affect disease transmission

The mechanism of transmission of a disease strongly impacts on the ability of a pathogen to spread within a particular population. Chapter 10 discusses different ways that pathogens can spread from one individual to another. Some of these transmission methods restrict the spread of disease to particular climates or geographic areas. As an example, 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**. Malaria is found only in areas of South America, Africa and Asia that are near to the equator. This is because the spread of malaria is restricted to geographic areas where the Anopheles mosquito can live. Influenza, in contrast, is able to spread in a variety of populations throughout the world because its mechanism (droplet transmission) is not dependent on a vector or specific environmental conditions.

You have already learnt about the lifecycle of the malaria parasite Plasmodium *on page 289 in Chapter 10.*

Some modes of transmission mean that infections are more likely to spread in certain groups within the one population. In such groups, behaviours or risk factors that promote the spread of infection are more common than in other members of the population. 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, one group at a high risk of acquiring the infection is those who share needles and syringes to inject drugs. Historically, another group at risk of acquiring hepatitis C infection was patients who require regular transfusions of blood products, such as those with haemophilia. Prior to 1990, blood donated for transfusion was not screened for viruses and cases of hepatitis C transmission occurred when patients received transfusions from infected individuals. Nowadays, extensive screening means that the risk of acquiring infections this way is very rare.

The transmission of disease is also influenced by a pathogen's **infectivity**: the ability of a pathogen to spread from one host to another host. Diseases with a high infectivity, such as influenza, are readily able to spread through a population. It is important to understand that the infectivity of a pathogen is distinct from its **virulence**: the capacity of a pathogen to cause severe disease within its host. Rabies, for example, 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.

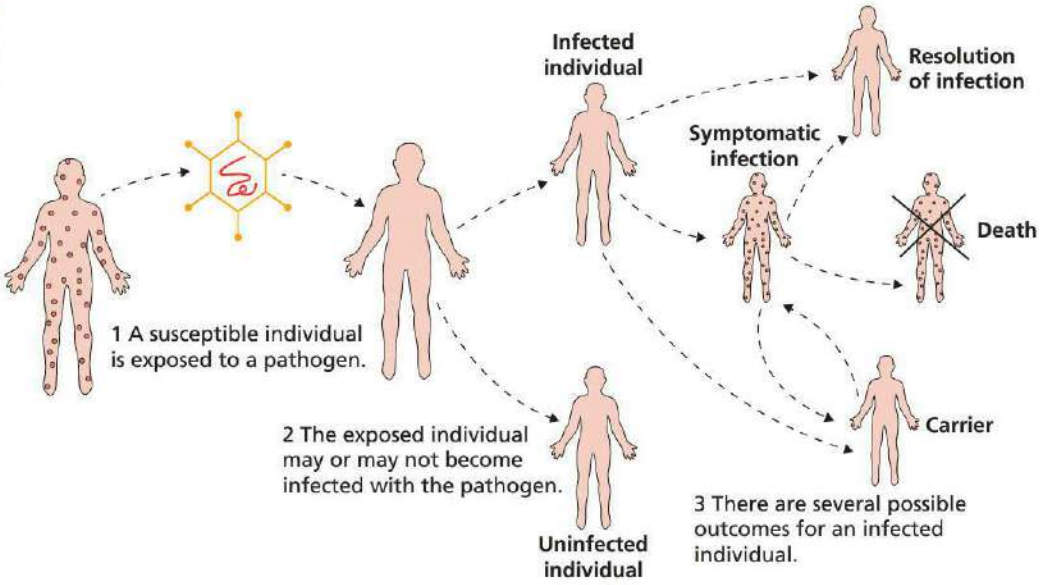
Once an individual has been infected with a pathogen, there are several possible outcomes from the infection (Figure 13.3). An infected host may be able to transmit the infection at several of these stages, including before the development of symptoms and when symptomatic. The different stages of infection are known as the **natural history** of an infection and can vary with each pathogen. It can influence how an infection spreads within a population.

Finally, the persistence of a pathogen within its **definitive host** or **intermediate hosts** can also contribute to the spread of the disease. Some pathogens may persist in asymptomatic **carriers** who are still capable of transmitting infection to others. Several viruses fall into this category. In some other diseases, such as tuberculosis (TB), the causative pathogen can survive within its host for long periods of time before causing symptomatic infection. TB is caused by *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 and not contagious. In roughly 10%

The life cycle of different pathogens, including the roles of hosts and intermediate hosts, was discussed in more detail in Chapter 10 on page 289.

of those infected, the disease can reactivate years or even decades later and cause symptoms. A period of latent infection like this may be advantageous for a pathogen, allowing for its spread within a population over longer periods of time or into new populations as individuals move.

Figure 13.3 ▶ Exposure to a pathogen can result in several different outcomes.



WOW

Typhoid Mary

At the beginning of the 20th century, a number of outbreaks of the diarrhoeal disease typhoid were traced back to food prepared by a single cook, Mary Mallon, in New York City. An asymptomatic carrier of the bacterium *Salmonella typhi*, Mallon was unaffected by typhoid but was still able to pass on the disease.

Mallon, infamously known as 'Typhoid Mary', was held in **quarantine** for a period of three years on North Brother Island to prevent spread of the disease. After agreeing to no longer work as a cook, Mallon was released back into the community.

Mary changed her surname to Brown and resumed work as a cook. When further outbreaks of typhoid were traced back to her, she was held in quarantine for the rest of her life: more than 20 years. Nowadays, treatment with antibiotics, rather than imprisonment, is used to manage asymptomatic typhoid carriers.

Environmental factors that affect disease transmission

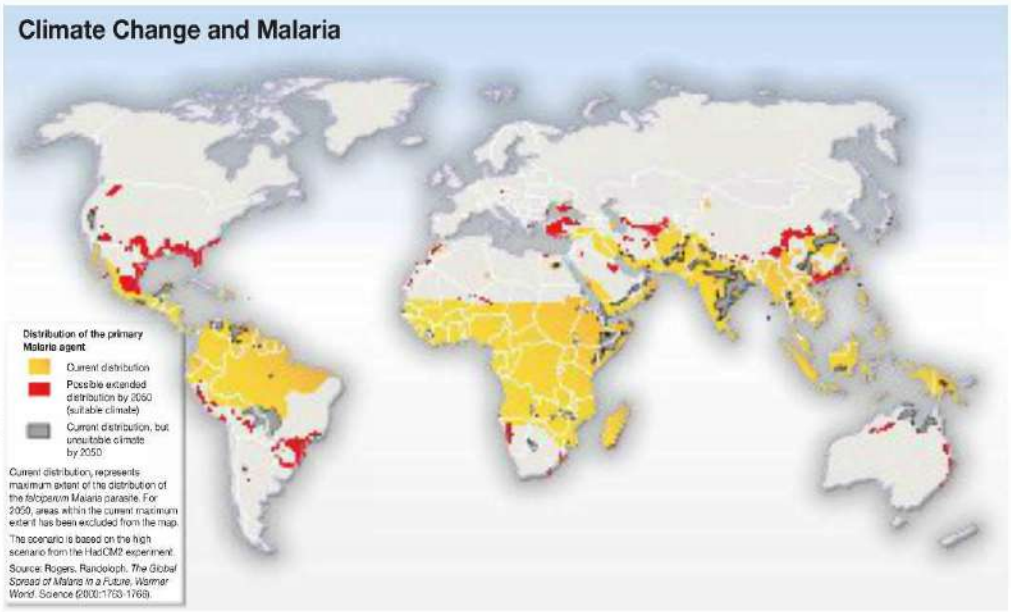
A wide variety of environmental factors, including infrastructure and climate, can affect the spread of disease. Efficient infrastructure, such as water supply, roads and sewerage systems, can have a profound impact on disease transmission.

The spread of TB following the fragmentation of the Union of Soviet Socialist Republics (USSR) provides an example of how infrastructure breakdown can result in the spread of infectious disease. Effective treatment of TB can prevent the spread of disease but requires therapy with multiple drugs over a long period of time. This in turn requires collaboration between drug manufacturers, supply networks, public health officials and healthcare workers. Following the breakdown of the USSR, previously well-coordinated treatment programs became fragmented and countries of the former USSR could no longer ensure regular, constant supply of anti-tubercular medications. The fact that many patients received partial, but not complete, courses of treatment has 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 can result in 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 upon human health in several ways. Models are used to make predictions about the possible spread of disease under new conditions. Creating models helps to predict changes within the already complex set of factors 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 13.4 shows the predicted changes in the distribution of malaria as a result of climate change. You can read more about predictive models and their limitations on page 385.



UNEP/GRID-Arendal http://www.grida.no/graphicslib/detail/climate-change-and-malaria-scenario-for-2050_bffe#

◀ **Figure 13.4**
Map showing the predicted change in distribution by 2050 of *Plasmodium falciparum* malaria, based on modelling data

Extreme climate events, such as tsunamis, floods and droughts, can also promote the spread of disease. In these situations, displacement of populations and/or the breakdown of usual 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. 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.

▼ **Figure 13.5**
An increase in diarrhoeal illness in Pakistan was observed following extensive flooding in 2010.

Getty Images/Arif Ali

How hosts can affect disease transmission

It is not only the characteristics of a disease and the environment that impact upon the spread of disease; characteristics of the infected populations are also important. Risk of exposure may be due to behaviours, as in the example of blood-borne viruses, but may also vary with age, sex or socioeconomic status. Once exposed to a pathogen some groups, such as the elderly or chronically ill, may be more susceptible to contracting infections, as their immune system may be less able to combat the pathogen.

There may also be differences in the proportion of the population that is immune to a disease, which will also impact on disease spread. The introduction of pathogens into previously unexposed populations can be particularly devastating as the disease spreads rapidly because so many individuals are susceptible. Reducing the number of susceptible individuals to a given disease is the basis for immunisation, which will be discussed on page 375.

The spread of a disease within a population is also dependent upon population density. In areas where large numbers of people live in close proximity to one another there may be more opportunities for diseases to spread between individuals.

WOW

Handwashing saves lives

Each year, almost 200 000 hospital-acquired infections occur in Australian hospitals. In 1840s Vienna, Dr Ignaz Semmelweis proved that the unhygienic practices of his medical staff caused septic infections and death in 13% of women after childbirth. Almost two centuries later, hospital staff worldwide are still being told to wash their hands to reduce disease transmission between patients. Handwashing campaigns in Australia have raised hand hygiene compliance in hospitals from less than 50% to more than 75% in recent years.

ACTIVITY 13.1

MODELLING DISEASE SPREAD

Models are tools employed by **epidemiologists** to predict the impact of different factors on disease spread. The disease lab simulator allows you to modify various disease characteristics (infectivity, **mortality** rate and duration of infection), population density and vaccination status, and observe their impact on the spread of disease.

DISEASE LAB

Use the interactive lab simulator to investigate the spread of disease.

Aim

To explore the impact of several variables on disease transmission

What to do

1. Access the weblink and perform your own investigation. (The first page of the the weblink provides a link to the simulator and outlines how to use it.) Write up your investigation using the standard scientific report format, from Aim to Conclusion (see Chapter 14).
2. The risk of a pandemic spreading to Australia is very real. Using what you have learnt in this activity, discuss how this risk is different now compared to 100 years ago and identify the pathogen, host and environmental factors that contribute to this risk.

Movement of individuals can facilitate disease transmission

Humans can have a substantial impact on the transmission of disease. This may be intentional, through measures including quarantine and vaccination, or unintentional, by way of agriculture, urbanisation or transportation. As an example, the building of dams or irrigation networks in malaria-prone regions may promote infection through the creation of new breeding sites, as a result of increases in humidity or other downstream impacts on the local ecosystem.

The movement of individuals and populations can facilitate the spread of disease. This is because individuals carrying disease are able to infect other individuals in the areas they are travelling to, allowing diseases to spread faster and over larger geographical areas than they otherwise could.

The movement of diseases can occur when carriers or infected individuals travel into other populations that have never been exposed to them. This is most clearly observed when populations that have been geographically isolated come into contact with one another, and it can be devastating if the previously unexposed individuals in these populations have no immunity. For example, the arrival of Europeans in Australia was associated with the introduction of several new diseases into populations of Indigenous Australians. Infections such as smallpox, measles, influenza and typhoid were introduced, and caused significant **morbidity** and mortality for the Indigenous population that had never before encountered these pathogens.

The nature of travel, however, has changed significantly since the boat voyages of the 18th century. The advent of air travel means that people are now able to travel large distances quickly and frequently. Increasing mobility means that emerging infectious diseases now have the potential to spread worldwide at alarming rates. The 2003 severe acute respiratory syndrome (SARS) epidemic is a striking example of how air travel can facilitate the rapid transmission of infectious diseases.

On page 376, you will learn more about how the immunity of most individuals in a population can protect others from infection.

Getty Images/Christian Keenan

◀ Figure 13.6
Residents of Hong Kong used facial masks to try to protect themselves from SARS during the 2003 epidemic.

In late 2002 and early 2003, reports of a new respiratory illness that would later be named SARS began to emerge in China. Infected individuals developed a variety of symptoms including very high temperatures and a cough or difficulty breathing. Almost 1 in 10 of those infected died as a result. The pathogen causing SARS was identified as a type of coronavirus. Figure 13.7 demonstrates how the disease was able to spread so rapidly. Epidemiologists determined that the disease was initially carried by a doctor from Guangdong province in China to Hong Kong and spread among other guests staying on the same floor of the doctor's Hong Kong hotel. These guests then flew to various destinations including Toronto, Hanoi, Bangkok and Singapore, rapidly spreading the disease.

While the impact was substantial, SARS did not become as widespread as was initially feared and the epidemic was over within a few months. It did, however, demonstrate that air travel has the potential to facilitate the spread of disease, making pandemics more likely. This has important implications for the control of infectious diseases. When emerging infections have the possibility to involve multiple countries and regions, collaboration between scientists worldwide is extremely important. Furthermore, public health authorities need to work together to develop coordinated responses to prevent the spread of potential pandemics. In the wake of SARS, public health authorities such as the World Health Organization (WHO) have been able to review and improve global systems for responding to new infectious diseases.

The 2011 thriller movie Contagion portrays the response of international bodies such as WHO to the spread of a new viral pandemic. The unfolding of the fictional pandemic bears several similarities to the SARS outbreak in 2003.

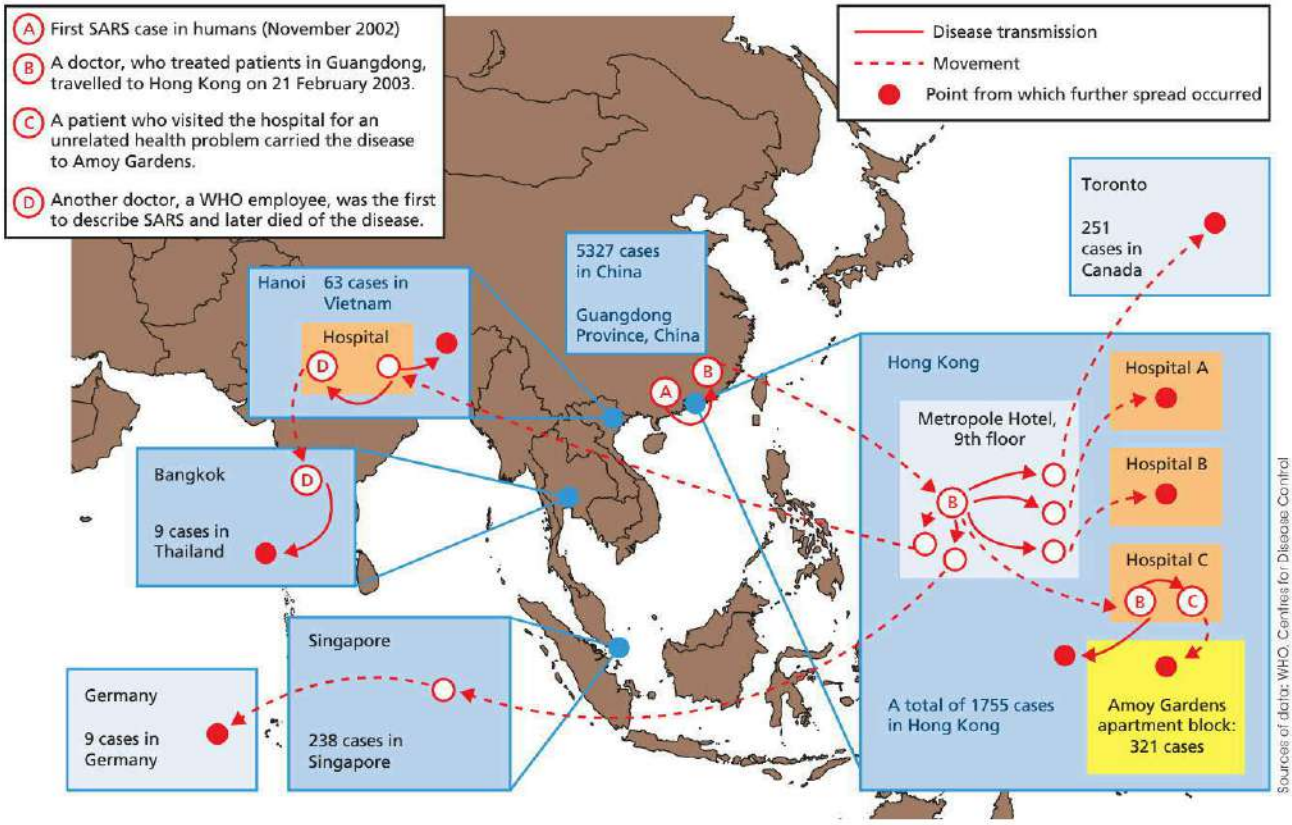


Figure 13.7 ▲
A map showing the initial spread of SARS infection. The rapid worldwide spread was facilitated by infected individuals travelling by plane.

The mobility of infected individuals and carriers allows for the rapid spread of disease within and between populations.

The geographic spread of infections due to the movement of infected individuals is not limited to human diseases; many plant and animal diseases have made their way to our shores. As recently as 2010, a fungal disease known as myrtle rust was detected in Australia for the first time. Myrtle rust disrupts the growth of young leaves on plants and can cause plant death. It is capable of infecting many native Australian plants including eucalypts, ti-trees and bottle brushes. The fungus causing myrtle rust, *Uredo rangelii*, originated in South America. Although it is not known exactly how or when the disease first entered Australia, it is possible that fungal spores were carried in undetected on imported goods or plants.

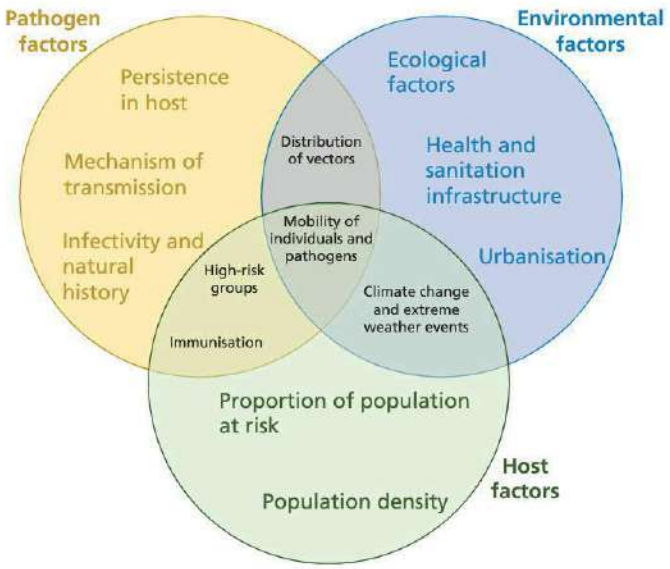


Figure 13.8 ▼
The transmission of disease is affected by a wide range of factors, many of which are interrelated.

Factors of disease transmission are interrelated

You can see that 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, makes understanding the transmission of a particular disease difficult. Figure 13.8 summarises the concepts already discussed and shows how some of these are connected. It is important to keep in mind that factors other than those discussed may impact on disease transmission; as an epidemiologist it is important to keep an open mind!

QUESTION SET 13.1

Remembering

1 Recall the term that best describes the frequency of occurrence of disease for each of the following.
   a In India, TB occurs at a high but relatively constant rate within the population.
   b In 2009 a new strain of influenza (H1N1) spread rapidly across the globe.
   c An unusually high number of cases of listeriosis was noted by public health authorities.
2 List the three types of factors that may affect the spread of a disease, providing an example for each.

Understanding

3 Explain how persisting for a long time within a host may be a selective advantage to a pathogen.
4 Explain how climate change could affect the distribution of dengue fever.

Preventing the spread of disease

A key aim of scientists who study disease transmission is to be able to design interventions to halt disease spread. The implementation of several interventions, such as hand hygiene, vaccination programs and quarantine measures, has made an enormous impact on our control of communicable diseases. In this section we will explore the ongoing public health role of these interventions in preventing the spread of disease.

WOW

Snake or parasite as the symbol of medicine?

A single snake wrapped around a staff is the symbol of the ancient Greek god of medicine and healing, Asclepius. Today, the rod of Asclepius remains a symbol of the medical profession and is used in the logos of a number of organisations including the Australian Medical Association, Medicalert, the World Health Organization and the British Medical Association.

The symbol is thought to represent the guinea worm (*Dranunculus medinensis*). Adult guinea worms migrate through the body to the hands or feet of an infected individual where they emerge from the skin, causing an intense burning sensation.

The treatment of guinea worm involves extracting the worm from where it emerges by wrapping it around a stick and slowly winding it out. This extraction can take days or weeks and, interestingly, is the same method that was used by ancient civilisations.

Shutterstock.com/nwawritto

▲ **Figure 13.9**
The rod of Asclepius is used in many logos associated with the medical profession around the world. It resembles a guinea worm (*Dranunculus medinensis*) being wound around a stick.

Hand hygiene

Prior to the mid-19th century, the transmission of infection was not well understood. In hospitals, surgeons did not wash their hands and rates of death 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, had read of Louis Pasteur's theory that micro-organisms cause disease, and he 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 asepsis 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 healthcare. The mouthwash product Listerine is named after Joseph Lister.

Regular handwashing can prevent individuals from contracting infections, particularly those that are spread by faecal–oral or direct contact routes. On a global scale, handwashing can significantly reduce the mortality from certain infections, such as diarrhoeal illnesses.

Good handwashing practices are also important in preventing the spread of infections in hospitals. Infections spread by healthcare workers are known as **nosocomial infections** and can be a cause of significant morbidity and mortality. Of particular concern is the potential for spread of antibiotic-resistant bacteria between patients. Effective and frequent handwashing is a key strategy for preventing these infections.

EXPERIMENT 13.1

THE EFFICACY OF ALCOHOL-BASED ANTISEPSIS

Alcohol-based hand rubs are widely used in hospitals as an alternative to frequent handwashing with soap and water. To use the hand rub, you simply squirt a small amount into the palm of your hand and rub your hands together so that the liquid covers your hands. The alcohol rapidly evaporates leaving your hands dry. In this experiment, you will compare the efficacy of alcohol-based hand rubs with that of traditional handwashing.

HOW TO HANDRUB? HOW TO HANDWASH?

WHO guidelines for handwashing and using alcohol-based hand rubs are available here.

Aim

To determine whether alcohol-based hand rubs or handwashing with soap and water is more effective in reducing bacterial load on hands

Materials

- alcohol-based hand rub
- liquid soap (not antibacterial handwash)
- sink with water
- paper towel
- sterile agar plates (two per student)
- clear tape or Parafilm
- Hand Hygiene Australia or WHO guidelines for proper handwashing and use of hand rub

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
Agar plates may culture dangerous bacteria.	Take care not to open agar plates once they have been incubated. Autoclave used plates for safe disposal.
Liquid soap or alcohol-based hand rub may be irritating to people with sensitive skin.	If you know you cannot use one of these products, inform your teacher or arrange to use the alternative one.

Method

1. You will conduct this experiment in pairs. One person will use an alcohol-based hand rub and the other will use soap and water to wash their hands. Form a hypothesis before beginning.
2. Label your agar plates on the underside with your name, the date and your treatment. Label one plate 'before washing' and the other 'after washing'.
3. Remove the lid from the plate labelled 'before washing' and press the palm of one hand down firmly on the agar, covering as much of the plate as possible. Replace the lid.
4. Following the guidelines, wash your hands using either the alcohol-based hand rub or soap and water.
5. Without touching anything, repeat step 3 using the opposite hand on the plate labelled 'after washing'.

6 Place the plates upside down (agar layer on top), seal with clear tape or Parafilm and incubate at 25°C for 24 hours.

Results

1 Count the number of colonies on each agar plate before and after handwashing. Record your results in a table similar to Table 13.1. Combine all class data to increase sample size.

Table 13.1 Results of experiment comparing alcohol based hand rub to soap and water

Pair	Alcohol-based hand rub			Soap and water		
	Number of colonies before washing	Number of colonies after washing	Percentage reduction in number of colonies	Number of colonies before washing	Number of colonies after washing	Percentage reduction in number of colonies
1						
2						
...						
Mean						
SD						

Analysis of results

1 Calculate the percentage reduction in number of colonies for each treatment.

2 Calculate the mean percentage reduction in number of colonies for each treatment and the standard deviation (SD). You can calculate SD using a scientific calculator.

Discussion

1 Compare the mean percentage reduction between the two treatments. Is there any difference? Do your results support your hypothesis?

2 Explain what the SD you have calculated for each treatment represents. If the SD was high, suggest why this could be the case.

3 Identify some potential sources of error in this experimental design.

4 Explain why you have calculated the percentage reduction in number of colonies, rather than comparing the number of colonies remaining for each treatment.

5 Do you think that it would be better to use the same hand or the opposite hand for the control plate? Justify your response.

6 In hospitals, it is not just the ability of the treatment to reduce the number of bacteria on hands that influences the transmission of infection. Make a list of other factors that might influence whether alcohol-based hand rubs or soap and water are more effective in reducing nosocomial infections.

7 Design an experiment to test these two treatments in the hospital environment. Ensure that you list appropriate control(s) and what outcomes you will measure.

Immunisation

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 a large number of infectious diseases including hepatitis B, pertussis, measles, tetanus 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 13.10 shows the rates of infection of *Haemophilus influenzae* and meningococcal C after the introduction of vaccines against these pathogens.

Immunisation programs also have the potential to eradicate diseases by making spread impossible. Smallpox, the first disease for which a vaccine was created, was also the first disease

Figure 13.10 ▶
Rates of *Haemophilus influenzae* type B and meningococcal infection since the introduction of vaccines against these organisms.

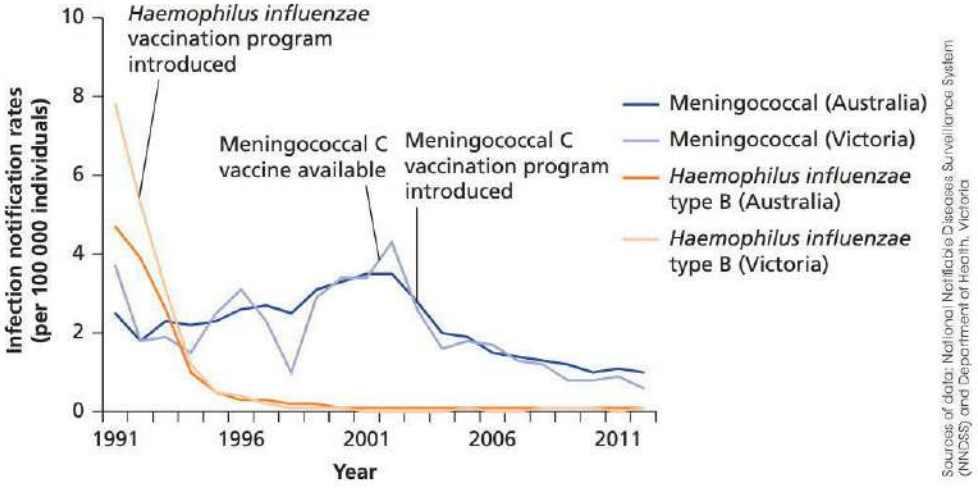
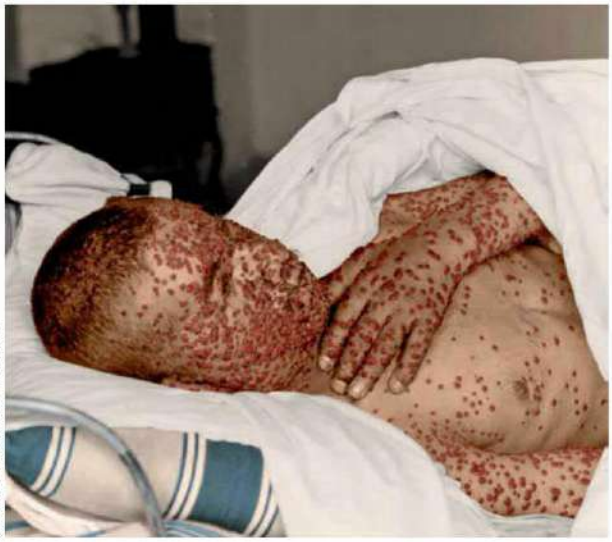


Figure 13.11 ▼
A child infected with smallpox. Vaccination efforts meant that the disease was declared to have been eliminated in 1980.


Science Source/Robin Treadwell

to be eradicated through vaccination. The viral infection, which causes characteristic skin lesions and has a high mortality rate, had been known to infect humans for thousands of years. A coordinated global strategy to eliminate the disease involved mass vaccination as well as targeted vaccination of those who lived near known epidemic areas. In 1980 WHO declared that smallpox had successfully been eradicated worldwide. Similar attempts to eradicate other diseases such as polio have not yet succeeded.

Not all individuals within a population need to be vaccinated for spread of a disease to be prevented. If a large enough proportion of the population is immune to a disease, there are too few susceptible individuals to sustain disease spread. This effect is known as **herd immunity**. Figure 13.12 demonstrates why this is the case. Imagine that infected individuals (orange) are only able to spread the disease with those they come into contact with. When there are enough immune individuals (blue), the chance of an infected individual coming into contact with a susceptible individual (black) is so low that the disease cannot spread. For herd immunity to prevent the spread of disease, a high proportion of the population needs to be immune. The exact proportion depends on the virulence and infectivity of a particular disease. Some individuals have health conditions that mean that they cannot be immunised and rely on herd immunity for protection from infection.

GLOBAL POLIO ERADICATION INITIATIVE

Learn more about the coordinated worldwide effort to eradicate polio.

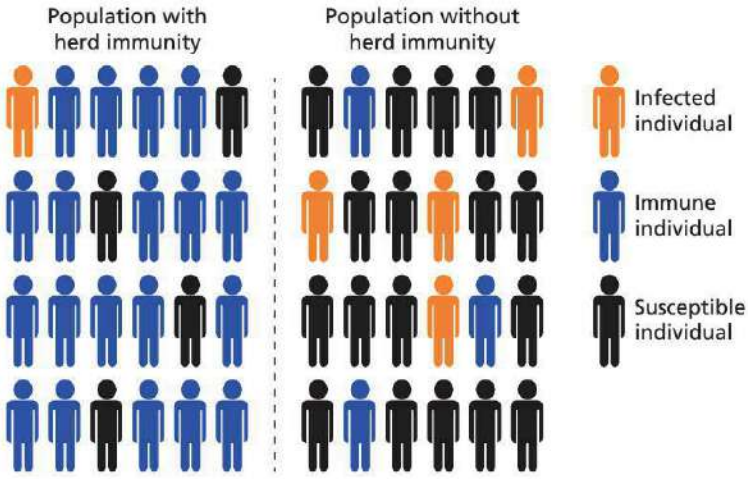


Figure 13.12 ▶
Herd immunity occurs when a large enough proportion of a population is immune to a disease. Disease spread cannot occur as there are too few susceptible individuals.

There are also groups that 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) and serious reactions occur very rarely. Overall, vaccines are far safer than the diseases they protect against.

One of the conditions erroneously linked to vaccination is autism. A small, unsubstantiated report was released in 1998 suggesting that the measles, mumps, rubella (MMR) vaccine could cause autism. It was later discredited and further research has not shown any link. Despite the lack of scientific evidence, vaccination rates dropped substantially and measles infections rose following the publication of this report.

In some cases, levels of immunity within the population have dropped so low that herd immunity is no longer sustained. In 2013, the occurrence of several measles outbreaks in the United Kingdom were linked to low vaccination rates following the MMR scaremongering. The risk of potentially devastating infectious diseases re-emerging is substantial if high vaccination rates are not maintained. In attempts to combat this risk, several states in Australia have legislation requiring that children must be vaccinated before they can be enrolled in childcare or school.

Quarantine

You have already seen how the mobility of individuals can facilitate the rapid spread of infections, such as SARS, around the world (see page 371). Quarantine is a practice used to stop individuals who have been exposed to infectious diseases from carrying that disease into healthy populations. It is used to counter the threat of spreading disease via the movement of infected individuals. Exposed individuals are kept from entering a healthy population until the incubation period of that disease has passed, proving that they are not infected.

The practice was first used during the 14th century to stop the spread of the bubonic plague. Ships arriving into Venetian ports were made to anchor just outside the port for 40 days before passengers could disembark. It is from the Italian word 'quaranta' (meaning 40) that the modern term 'quarantine' derives.

From the mid-19th century, individuals arriving on ships to Australia from ports where certain infections were present were subject to quarantine procedures. Luggage was fumigated and non-immune individuals were detained at quarantine stations, such as at Point Nepean in Victoria. People who did contract the disease were given appropriate treatment.

Quarantine is currently used in this way to prevent the spread of animal pathogens. When people import pet cats or dogs into Australia from overseas the animal is held at a quarantine facility for at least 30 days. Quarantine measures to protect human health can still occur. Captains of planes and ships carrying passengers are required to report passengers displaying symptoms of certain infections. In exceptional circumstances intensified quarantine measures may be implemented at airports to try to prevent the spread of disease by air travel. For example, in 2009 during the H1N1 influenza pandemic, thermal imaging cameras were used at airports to try to detect people with a fever who might have the disease.

Goods brought into Australia on passenger planes and commercial shipments are also inspected for high risk items such as meat or plant products. These products can then be stopped from entering the country. Australia has particularly strict quarantine laws because of the potentially devastating impact of imported pathogens on our unique flora and fauna. As an island, protecting our borders from imported pathogens and pests is easier than in many other parts of the world.

Public health interventions such as handwashing, immunisation and quarantine are highly effective in preventing the spread of disease.

WOW

Microbes from the Moon?

One of the concerns with sending humans to the Moon was the potential risk of contamination by unknown lunar pathogens. Just in case, astronauts Neil Armstrong, Michael Collins and Edwin 'Buzz' Aldrin were held in quarantine for 3 weeks on their return from the Moon. Upon landing, the crew members were housed in a sealed, mobile quarantine facility. Crew members from the following Apollo missions were also subjected to quarantine on return.

Alamy/NASA Photo

▲ **Figure 13.13** US President Richard Nixon visits the Apollo 11 crew in a mobile quarantine facility following their return from the Moon.

Case study

Constantly evolving: monitoring and managing the threat of influenza

While some vaccines provide lifelong protection against a disease, the rapid evolution of influenza viruses means that influenza vaccines may provide protection for only about 12 months. Professor Anne Kelso is the Director of the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne, the organisation responsible for monitoring changes in influenza viruses and advising what strains we should vaccinate against.

Professor Kelso explains that the rapid evolution of influenza viruses means that new vaccines regularly need to be developed. 'As influenza viruses pass from one person to the next ... they need to mutate to avoid the antibodies that people have made against earlier versions of influenza viruses. Human immunity is the Darwinian selection pressure for flu viruses to keep changing and it's also the reason that we need to update influenza vaccines every year or so in order that they're matched as closely as possible to the viruses that are likely to be circulating in the next season.'

The Centre is sent samples of influenza virus from patients all over the Asia–Pacific region. 'We're not diagnosing influenza for patient care, but rather comparing influenza viruses that are circulating in humans around the region for their antigenic and genetic properties,' says Professor Kelso. This information is then used to inform the updating of influenza vaccines. 'We, and four other WHO laboratories like ours around the world, meet twice a year with WHO in Geneva and help them develop recommendations on which influenza strains should be included in influenza vaccines for the northern and southern hemispheres.' The process is a strong example of international collaboration, with laboratories in 111 countries providing samples.

The development of influenza vaccines involves extensive collaboration between different countries as well as the vaccine industry. To monitor circulating viral strains, the Centre relies on countries to provide influenza samples for analysis. The samples 'don't have to come to our lab but they need to come to one of the WHO Collaborating Centres for Influenza so that they get into the global monitoring system,' says Professor Kelso.

The Centre also provides vaccine manufacturers with the actual viruses that are used in vaccine production. 'We've often had strains from Brisbane or Melbourne or Perth in vaccines used in Europe and North America, as well as Australia,' says Professor Kelso. She also explains that recommendations and virus samples are made freely available to vaccine manufacturers worldwide. 'Through WHO, we work equally with all of them. It is very important that we are not giving an advantage to one company over another.

'There's a remarkable amount of sharing of information, even of vaccine viruses, between companies as well as between WHO labs and companies,' says Professor Kelso. This free sharing of information and samples is not always seen in science. Professor Kelso comments, 'It really is in the global public interest that there be this sort of open sharing.'

Another important role of the Centre is to monitor changes in influenza viruses that may be of public health concern. 'If we see a rise in resistance to antiviral drugs like Tamiflu or Relenza then this is important advice that we can give to public health authorities,' says Professor Kelso. 'Of course, what we're most looking out for is something that's actually not a previous human influenza virus but which might be a new flu virus that has jumped from animals and could potentially cause a pandemic.' Professor Kelso says that if a worrying influenza strain is isolated, 'I will be in direct contact with the Chief Medical Officer or with the relevant people in the Department of Health and Aging to say we've found something we think they should know about'.

Professor Kelso encourages those with an interest in science to 'take a leap into the unknown' and believes that the unpredictability of science makes this a particularly appealing field. 'You're always being exposed to new things, you're always part of this rapidly moving world of new knowledge.'

Courtesy of Anne Kelso

Figure 13.14 ▲
Professor Anne Kelso, Director of the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne.

Questions

1. List the roles of the WHO Collaborating Centre for Reference and Research on Influenza.
2. Draw a flow chart that shows the different parties involved in vaccine development, using arrows to show how information or technology is shared.
3. Explain why this kind of collaboration is particularly important for rapidly evolving pathogens, such as influenza.

Scientific literacy: This mite be the bees' worst enemy

Bees, those small insects that collect nectar and pollen and make honey and wax, are in precipitous decline: populations in the United States and Britain, for example, have halved over the past 25 years.

Their biggest threat is from the evil-sounding *Varroa destructor*, an oval-shaped, reddish-brown mite that sucks the blood from bees and transmits virulent diseases, such as deformed-wing virus. The pinhead-sized bloodsuckers have decimated bee populations worldwide, including in neighbouring New Zealand and Papua New Guinea, but have not arrived yet in Australia.

'If they enter this country, the mites will completely wipe out our wild honeybees, which means crop growers will lose their largest and free source of pollination, worth more than $1 billion a year,' says bee pathologist Denis Anderson of CSIRO Ecosystem Sciences in Canberra.

The mites will also reduce the number of managed honeybee colonies, he explains. 'This means keepers will pay more for scarce paid pollination services – costs that would flow through to consumers.' In addition, most of Australia's horticultural and agricultural crops, worth billions of dollars, rely on bees for pollination.

Australia's national port surveillance program, although currently inadequate to deal with the threat, is being strengthened. Surveillance for honeybees and bee pests and parasites that are exotic to Australia forms part of the National Sentinel Hive Program, which is coordinated by Plant Health Australia.

The program, established in 2000, has been growing steadily, Dr Anderson says. 'It operates on the premise that most of the important exotic pests and parasites will enter Australia on live honeybees from another country – particularly through bee swarms arriving on vessels at our sea ports,' he explains. 'If a swarm was carrying exotic parasites, such as the *Varroa* mite, those parasites would spread to colonies near the port, and then on to colonies further away.'

The National Sentinel Hive Program places special hives at sea ports around Australia and monitors them every 2 months for signs of exotic pests and parasites that may have arrived in a bee swarm from overseas.

The program's success depends on how many hives are at each port and the number of ports targeted. 'The more of each, the higher the chance of success,' he says. 'At present, only a few hives are based at a few strategic ports – just three hives cover Melbourne and Geelong, for example – and there are not enough funds to expand the current program.'

This is why the state government has set up Bee Force, a pilot project to improve Victoria's capacity to detect incursions of exotic bee pests. Now being trialled in Melbourne and Geelong, the project involves local amateur beekeepers who run sentinel hives.

'This encourages community involvement and expands the number of sentinel hives that can be used for surveillance, thus keeping costs to manageable levels,' Dr Anderson says. 'If the trial Bee Force program proves successful, it could be extended to other port areas.'

Victoria's Department of Environment and Primary Industries has trained a honeybee quarantine response team of 90 beekeepers, including hobby and commercial beekeepers, who may be called on to assist in an emergency.

The early detection of the *Varroa* mite, combined with effective surveillance, it is argued, may increase the chance of eradicating the parasite once it enters the country. This has never been achieved before.

'Since it switched host (from the Asian honeybee to European honeybee), the mite has spread throughout the world,' Dr Anderson says. 'Let's ensure we do everything possible to keep the parasite out of Australia for as long as we possibly can.'

COMBATING *VARROA DESTRUCTOR*

In this video Dr Denis Anderson expands on some of the other strategies being used to combat *Varroa destructor*.

Spinks, P. (2012) 'This mite be the bees' worst enemy', *The Age* online, 26 June.

Questions

1 List at least three industries that could be affected were the *Varroa* mite to spread into Australia.

2 Australia is now the only country in the world with a honey industry that is free of the *Varroa* mite. Spread to New Zealand and Hawaii only occurred relatively recently.
   - **a** What characteristic of these three countries allowed them to remain mite-free for so long?
   - **b** Explain how strategies to prevent the introduction of *Varroa destructor* into Australia utilise this characteristic.

3 Explain what a sentinel hive is and how these are being used to prevent *Varroa* mite spread in Australia.

4 Brainstorm some other strategies that could be used to prevent the introduction of *Varroa destructor* into Australia.

QUESTION SET 13.2

Remembering

1 Name the type of infection that is spread between patients by healthcare workers.
2 Define 'quarantine'.
3 List three ways that quarantine is used in Australia today.

Understanding

4 Mike's daughter Angela has a severe allergy to one of the components of the measles vaccine and is unable to have the vaccination. Mike is concerned about the risk of her contracting measles. Explain how herd immunity will provide Angela with some protection against measles.
5 Australia has much stricter quarantine laws than many other countries. With reference to our biodiversity, suggest why this is the case.

BIOSECURITY IN AUSTRALIA

Learn more about quarantine strategies used to prevent the introduction of plant and animal diseases into Australia.

CHOLERA AND THE THAMES

Learn more about cholera outbreaks in London and worldwide.

Outbreaks and public health

In September 1854, a deadly outbreak of cholera struck Soho in London. Cholera was commonly believed at the time to be spread by a miasma, a noxious vapour thought to cause diseases. John Snow, a physician working in London, spent time talking to residents of the area and observed that almost all of the cases of cholera occurred in people who lived close to and used a single water pump. Importantly, he also showed that people who lived close to the pump and who did not contract the disease used different water sources. The map shown in Figure 13.15 represents the map made by Snow and shows cholera cases (in red) clustered around a water pump on Broad Street. Armed with this data, Snow convinced the local authorities to disable the pump and prevented the further spread of disease.

John Snow (renowned as the father of modern epidemiology) had proposed several years prior that cholera was spread via contaminated water, rather than by a miasma. At the time, it was not known that infectious diseases were caused by micro-organisms. Despite Snow's findings, this theory was not given widespread credit until almost 30 years later when the bacterium *Vibrio cholerae* was found to be the causative agent of cholera. It is spread via the faecal–oral route and is often transmitted in water that has been contaminated with sewerage. Supporting this theory, John Snow also identified that the outbreak likely started with a baby, Frances Lewis, whose soiled nappies were washed in water that most probably leaked into the pump's water supply.

WOW

The Broad Street pump: a symbol of John Snow's legacy

The Broad Street pump in London has become a symbol used to celebrate John Snow's life and scientific contributions. At the site of the original pump on Broad Street (now known as Broadwick Street) is the John Snow pub, used by the John Snow Society as a regular meeting point. On the street outside you can also find a replica of the pump.

The John Snow Society organises the annual Pumphandle Lecture, given by a prominent public health scientist. At the lecture, a ceremony is conducted in which the handle of a replica pump is removed and then put back in place, as a reminder of the continuing challenges to public health.

Wellcome Library, London

Figure 13.15
A reproduction of John Snow's map showing cases of cholera in red and pumps marked as Xs. The cluster of cholera cases around the Broad Street pump strongly suggests a link between the water and the disease.

Monitoring disease activity

In order to define and control disease outbreaks, public health authorities need to know when and where particular infections are occurring. In Australia, the number of cases of a particular disease is monitored by health authorities in each state. On a global level the monitoring of diseases is conducted by WHO, the organisation that coordinates global responses to outbreaks that pose widespread threats.

A widely used method of monitoring disease activity is through the notification of public authorities when individuals are diagnosed. In Australia, the list of **notifiable diseases** contains a diverse mix of more than 70 conditions including chickenpox, syphilis, rabies and influenza. A doctor who diagnoses one of these conditions must report the case to the relevant state health authority. Outbreaks or cases of unusual diseases can then be investigated.

There are a number of limitations to data collected this way. Not all patients who are infected with a disease will seek healthcare and of those that do, not all will receive a diagnosis. Infections can also be under-reported and this may be more of a problem in certain populations or diseases. Together, this means that reported data are likely to be lower than the actual number of cases. There can also be delays between the onset of symptoms, diagnosis and reporting, which can limit the ability of public health authorities to respond quickly to epidemics.

NATIONAL SURVEILLANCE

Visit the Department of Health's website to view the current list of notifiable diseases in Australia.

GOOGLE FLU TRENDS

Visit Google Flu Trends for up-to-date data on influenza-related searches.

In recent years, researchers have been exploring alternative ways of conducting disease surveillance. The widespread use of the Internet and social media provides a novel data source from which information about the frequency of different diseases can be extracted. Data from Facebook, Twitter and mobile phones have been used to monitor disease activity. Google has developed a program that tracks how frequently people use the search engine to look up influenza-like illnesses. Figure 13.16 compares data obtained by this method with traditional reporting data. You can see that the spikes in Google search activity correspond with the peak influenza season.

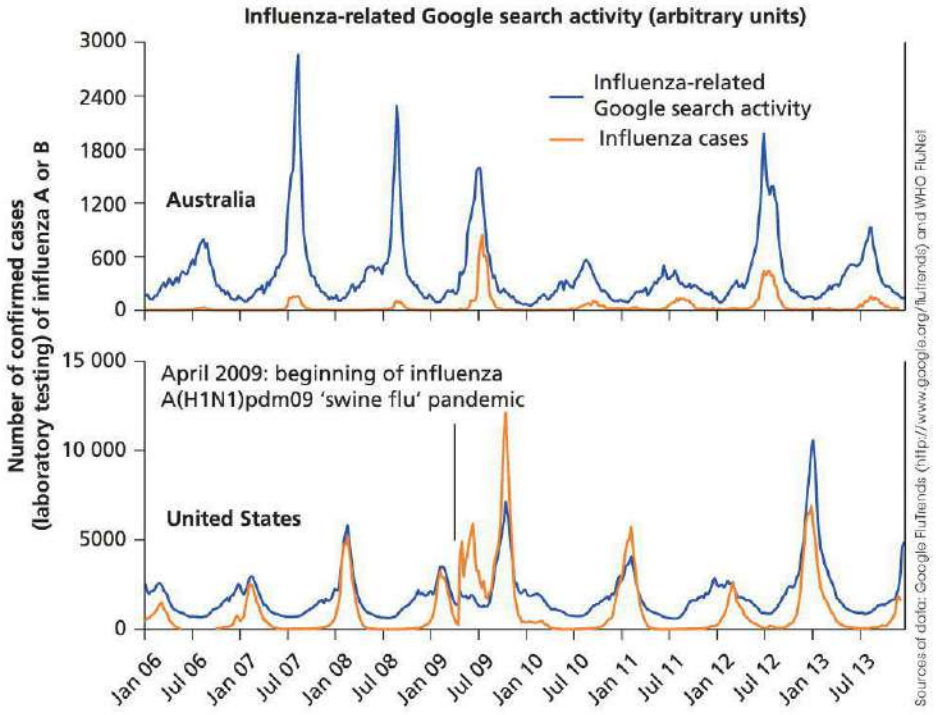


Figure 13.16 ▶ Patterns of influenza infection and Google search activity related to flu-like illness in the United States and Australia

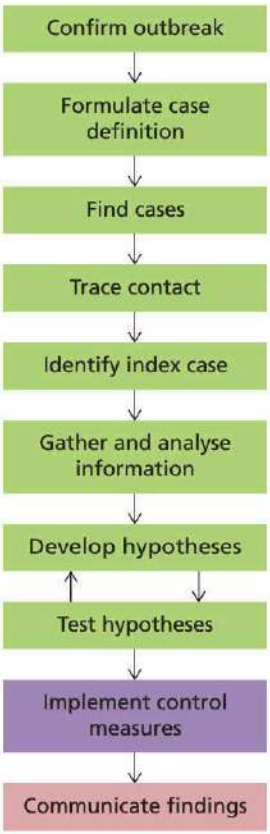


Figure 13.17 ▲ The steps involved in investigating a disease outbreak. Communicating findings and implementing control measures usually happen throughout the investigation.

These digital disease surveillance mechanisms have the advantage of providing information to public health authorities in real time. The quality of data, however, is limited by how effective algorithms are in determining whether or not a tweet or search is actually about an illness. Furthermore, high Internet activity about an illness does not always correspond to high disease activity as it can be skewed by other events, such as the illness of a celebrity.

Due to its limitations, digital disease surveillance is not likely to replace traditional reporting methods. It does, however, provide epidemiologists with an additional tool that complements these methods.

Managing an outbreak

In this section we will discuss the steps taken by investigators when faced with a new disease outbreak (summarised in Figure 13.17), using a gastroenteritis outbreak as an example. Similar steps apply whether the outbreak is a small, localised occurrence or a pandemic.

When an outbreak is suspected, the first step is to confirm that the reported cases do, in fact, meet the definition of an outbreak. This involves confirming the number and diagnosis of known cases and comparing this with background levels of the disease.

Once this has been done, investigators can formulate a **case definition** of which cases are considered to be part of the outbreak. Case definitions include not only the type of illness but also the place and time. Such definitions can change and be refined as the investigation progresses and investigators understand more about the disease. In 2007 in Port Macquarie,

health authorities were alerted to a potential outbreak of gastroenteritis among people who had eaten at a particular restaurant. In this example cases were defined as those 'who attended the restaurant for dinner and subsequently developed diarrhoea plus one more additional symptom of gastroenteritis (nausea, vomiting, abdominal pain, fever or lethargy)'.

The next few steps (finding cases, gathering information and developing hypotheses) are easiest if the mechanism of transmission of a disease is already known. For example, a gastroenteritis outbreak might be caused by several pathogens that are spread by the faecal–oral route and as such, foodborne sources will be the focus of the investigation. When the mechanism is not known, investigators have to consider a wider range of potential cases and disease sources.

Investigators then need to find people affected by the outbreak. Not all of those who are ill will have sought medical care, or been reported to investigators. As such, disease surveillance (or passive case finding) generally does not locate all individuals affected. Investigators in disease outbreaks perform active case finding, where they try to track down infected individuals. An important component of this is **contact tracing**, whereby people who may have infected, or been infected by, known cases are tracked down. The type of contacts sought will vary with the mechanism of transmission. For example, if the disease is sexually transmitted, only sexual contacts of the infected individual will be contacted by investigators. On the other hand, for an airborne disease such as TB, investigators will contact all people who have been in close proximity with the case. In some cases, investigators may focus on people who have been exposed to the same potential sources, even if they didn't have direct contact with an infected individual. The latter method was performed in the Port Macquarie example where all of those who had dined at the restaurant were contacted and asked about symptoms. As part of case finding, investigators may be able to identify the **index case**, or the case that started the outbreak. In the Soho cholera outbreak (see page 380), John Snow was able to identify Frances Lewis as the likely index case.

Investigators will then gather information from cases. Initially, this will involve in-depth interviews to explore any potential sources of infection. These interviews will include asking about usual activities, sick contacts, recent meals and travel. The aim of these interviews is to generate a hypothesis about how the outbreak is spreading. Investigators may visually represent data on maps (as John Snow did; see Figure 13.15) or graphs to help generate hypotheses.

Once a hypothesis has been generated the investigators can search for evidence to support or refute that hypothesis. This evidence might include further interviewing of cases, site inspections and environmental sampling (such as testing water or food for pathogens). In some cases, sufficient data may have already been collected and testing the hypothesis involves analysing that data.

An outbreak investigation is an important aspect of controlling a disease outbreak. The investigation involves a series of steps that aim to determine what has caused the outbreak.

Investigators in the Port Macquarie outbreak determined that 19 people had contracted gastroenteritis after eating at a restaurant on the same night. One of the patients was tested and found to be infected with norovirus, a common viral cause of gastroenteritis. Investigators collected detailed information about what each person ate, as shown in Table 13.2. You will notice that all of those who contracted the infection, and none of those who didn't, ate oysters for their entrée. No evidence of the virus was found in the kitchen or in other batches at the oyster farm, so the investigators concluded the outbreak was likely caused by a single infected batch of oysters.

The final steps of an outbreak investigation are to implement measures to control the spread and to communicate the findings. In practice, both of these steps may take place while the other steps are taking place. The likely source of the outbreak in the Port Macquarie investigation was a single batch of oysters. As such, no further control measures needed to be implemented.

SOLVE THE OUTBREAK

The Centres for Disease Control and Prevention's Solve the Outbreak app allows you to be the epidemiologist in a series of cases.

Table 13.2 Study of foods eaten at a restaurant in Port Macquarie, NSW, showing items eaten by those who did and did not contract gastroenteritis following a dinner function

	Customers who ate the food		Customers who did not eat the food	
	Contracted infection	Total	Contracted infection	Total
Entrée				
Oysters	19	34	0	19
Prawns	17	37	2	16
Lettuce garnish	10	15	9	38
Cocktail sauce	11	20	8	33
Chicken skewers	1	16	18	37
Main				
Leg ham	17	40	2	13
Lamb	15	42	4	11
Beef	11	41	8	12
Chicken	12	37	7	16
Cucumber and tomato salad	5	7	14	46
Dessert				
Pavlova	6	9	13	44
Toffee pudding	0	6	19	47
Apple strudel	3	6	16	47
Fruit salad	3	8	16	45
Cream	11	25	8	28

Source: Huppatz, C., Munoch, S.A., Worgan, T., Merritt, T.D. et al. (2008) 'A norovirus outbreak associated with consumption of NSW oysters: implications for quality assurance systems', *Communicable Diseases Intelligence, 32*(1), pp. 88–91.

123rf/Peter Titmuss

Figure 13.18 ▶ An investigation into a gastroenteritis outbreak in Port Macquarie revealed the likely source was a batch of oysters contaminated with norovirus.

ACTIVITY 13.2

OUTBREAK MANAGEMENT IN AUSTRALIA

Aim

To learn about the control of disease outbreaks in Australia through the examination of a recent case

You will need

- a computer with Internet access
- a large sheet of poster paper
- markers and pens

What to do

1. Choose one of the following outbreaks from recent Australian history.
   - 2000 Legionnaires' disease outbreak in Melbourne
   - 2012–13 Listeria outbreak
   - 2008–09 Dengue fever outbreak in Cairns
   - 2011 Hendra virus outbreak
2. Perform an Internet search about the disease and outbreak, aiming to find the following information.
   - Characteristics of the disease (for example, the type of pathogen, symptoms, mortality, incubation period and mode of transmission)
   - Size and impact of the outbreak
   - Outcomes of the epidemiological investigation (for example, was a source found?)
   - Control measures used by public health authorities
3. Summarise the information on the poster paper. You could use a flow chart or timeline to show how events unfolded.
4. Using your poster, explain to a classmate what you have found.
5. What sources of information did you choose to use? Explain why you chose these sources and how you know that they are reliable.

What did you discover?

1. Outline the process of investigating an outbreak, using your case as an example.
2. Explain, using your case as an example, how the mode of transmission of a disease can direct an outbreak investigation.

Predicting the spread of disease

Mathematical models that can predict the spread of disease are important tools in the control of outbreaks. Such models can be used to explore the likely effects of newly emerging pathogens and changes in environmental conditions. They can also be used to design and predict the effects of potential public health interventions.

In order to make these predictions, mathematical models include several assumptions about the way that different variables behave. The accuracy of these models is dependent on these assumptions being met. You have already seen how a large number of factors can impact on disease transmission. In order for a model to have good predictive ability, the design of the model needs to reflect this complexity. The use of mathematical models to predict disease spread involves close collaboration between mathematicians and biologists.

QUESTION SET 13.3

Remembering

1 Outline the steps in performing an outbreak investigation.

2 Explain what a notifiable disease is, giving an example.

3 Describe what the job of an epidemiologist entails.

Understanding

4 Explain how mathematical modelling can aid epidemiologists in controlling disease spread.

Applying

5 Legionnaire's disease is a bacterial lung infection caused by *Legionella* spp. The bacterium breeds in aquatic environments and is transmitted to humans when they inhale contaminated water vapour. Outbreaks of Legionnaire's disease are usually linked to a particular source, such as a spa, a hot water system or an air conditioner. Suppose that the local health department has received reports of four cases of Legionnaire's disease occurring in the same suburb over 3 days.

a Imagine that you are the epidemiologist leading the investigation. Draw a flow chart showing the steps you would take. Be specific about what you would do for each step.

Based on detailed interviews you produce the following data (Table 13.3).

Table 13.3 Epidemiological evidence gathered from Legionnaire's outbreak investigation

Cases	1	2	3	4
Lives	Apartment block A	Apartment block B	Apartment block A	Private home
Works	Store manager at local shopping centre	Builder working on carpark extension at shopping centre	School teacher	Retired
Other places visited in last week	Bank Swimming pool Movie cinema	Apartment block A Shopping centre	Shopping centre Zoo Hospital	Hospital Swimming pool Shopping centre

b Predict where these individuals may have contracted the infection. Justify your response.

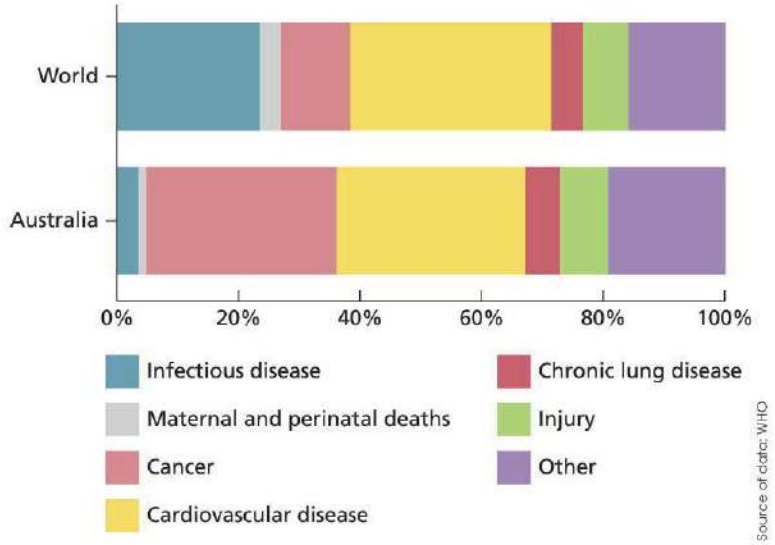
c Explain how you would confirm this.

Epidemiology and non-infectious disease

Despite improvements in prevention and treatment, infectious diseases are still a major cause of deaths worldwide. However, the burden of disease varies significantly from country to country. Figure 13.19 compares causes of death in Australia with the world as a whole. You can see that infectious diseases cause a much smaller proportion of deaths in Australia than the worldwide average. There are many reasons for this including widespread access to healthcare, good sanitation, clean drinking water and good nutrition. A lack of access to these basic services is a substantial contributor to disease burden and a target for public health interventions.

This figure shows that cardiovascular disease, cancer and chronic lung disease are major causes of death around the world. These non-communicable diseases share several risk factors including unhealthy diet, lack of exercise and smoking.

The principles of epidemiology can also be applied to non-communicable diseases such as cancer or cardiovascular disease. Epidemiology may be used to analyse for possible causes of disease or test the efficacy of public health interventions.



◀ **Figure 13.19**
Age-standardised cause of death in Australia and worldwide (2008)

QUESTION SET 13.4

Remembering

1 List the differences seen in Figure 13.19 between Australia and the rest of the world.

Applying

2 Asbestos is a silicate mineral that was widely used in construction because of its excellent heat retardant and insulating properties. The small asbestos particles become easily airborne and can be inhaled. It is now known that asbestos inhalation can cause a severe lung disease known as asbestosis and a type of lung cancer known as mesothelioma. A link between asbestos exposure and lung disease was first suggested when it was noticed that asbestos mining towns had particularly high rates of lung disease.

- **a** Imagine you are heading up an investigation into asbestos and lung disease. Explain how you would apply the steps of conducting an outbreak investigation to this situation.
- **b** The development of lung disease or cancer from asbestos can take many, many years. Explain why this might make an outbreak investigation more difficult than the food poisoning example discussed on page 383.

CHAPTER SUMMARY

- The transmission of infectious disease is affected by characteristics of each individual disease, environment and affected population. Characteristics include pathogen infectivity, persistence in host, population density and host movements.
- Human activities can have substantial impacts on the transmission of disease. These impacts may be intended (as in the case of public health measures) or unintended.
- It can be challenging to understand the transmission of a particular disease because there are so many factors that impact on this process.
- Movement of individuals can facilitate the spread of pathogens into new populations. Air travel has increased the ease with which individuals can move between populations and can allow for rapid spread of disease.
- Hand hygiene, immunisation and quarantine are examples of measures introduced to prevent the spread of infection.

- Immunisation is an important tool in preventing the spread of disease to individuals and throughout a population (through herd immunity).
- Quarantine is a measure used to prevent the spread of disease into healthy populations. In Australia, the emphasis of quarantine policy has changed over time from human diseases to plant and animal pathogens that threaten our unique wildlife.
- Systems for monitoring the spread of disease are needed so that public health interventions can be well-targeted. The most common form of disease monitoring involves reporting of notifiable diseases.
- Management of an outbreak of a disease requires an investigation to discover the causative factors.
- Principles of epidemiology can also be applied to non-communicable diseases, which are a cause of substantial disease burden in Australia and worldwide.

CHAPTER GLOSSARY

carrier usually used in reference to disease; a healthy, heterozygous organism carrying an allele for a recessive phenotype; the organism may transmit the recessive allele and resulting phenotype to its offspring or to others

case an individual who is infected with an infectious disease

case definition a definition that includes a particular disease, time and place and is used to help identify individuals affected by a disease outbreak

contact tracing a process for identifying potential cases; recent contacts of an infected individual are contacted and screened for the infection

definitive host a host in which the adult phase of a parasite produces gametes

endemic broadly, common to a particular area; specifically, a pathogen that is prevalent at a constant rate within a population

epidemic an increase in the occurrence of a particular disease above the baseline level for that population; tends to refer to larger, more serious events than the term outbreak

epidemiologist a scientist who studies the causes and effects of diseases at a population level

herd immunity refers to the phenomenon that once a particular proportion of a population is immune to a disease, susceptible individuals are also better protected from the disease

host an organism that is infected by a pathogen

index case the initial case of a contagious disease

infectivity the ability of a pathogen to spread from one host and infect another host

intermediate host an organism in which a pathogen or parasite undergoes development and spends a small proportion of its life cycle

morbidity the impact of a disease within a population, measured by the number of people affected by that disease

mortality the impact of a disease within a population, measured by the number of deaths caused by that disease

natural history the course that a disease would be expected to follow if left untreated

nosocomial infection an infection that is spread in a healthcare setting

notifiable disease a disease that, if diagnosed, is required to be reported to public health authorities

outbreak an increase in the occurrence of a particular disease above the baseline level for that population; see also *epidemic*

pandemic an epidemic that has spread across multiple continents or worldwide

quarantine the enforced isolation of individuals at risk of carrying disease to prevent the spread of that disease into healthy populations

sporadically refers to a disease that occurs infrequently and irregularly within a population

vector a living organism that transmits pathogens from one host to another; a vehicle used to transfer DNA sequences from one organism to another

virulence refers to the ability of a pathogen to cause severe disease within its host

CHAPTER REVIEW QUESTIONS

Remembering

1 List two ways in which climate change could impact upon disease transmission.
2 Define 'asymptomatic carrier'.
3 Describe the impact of mobility on disease transmission:
   a in the context of island-based wildlife.
   b in the context of emerging human viruses.

4 Using an example, describe 'herd immunity'.

5 Outline how hand hygiene can prevent the transmission of disease.

Understanding

6 Explain what is meant by: 'Polio is only endemic in a few countries, including Pakistan, but sporadic cases occasionally occur elsewhere.'

7 Some people are unable to receive vaccinations because of a medical condition. Explain why the vaccination of healthy individuals is important for those who cannot receive vaccinations.

8 Explain why it is important to collect data about disease rates even when levels are fairly stable.

9 Give an example of a population, environmental or disease characteristic that can impact upon the spread of:

- a malaria.
- b TB.
- c norovirus.

10 Define 'case definition' and explain why this concept is important.

11 Provide an example where several factors that impact on disease transmission are interrelated.

Applying

12 TB is caused by a bacterium that can lie latent in the body for many years. When it reactivates it can cause serious infections of any part of the body, but most commonly the lung. When diagnosed, patients with reactivated TB are treated for 2 weeks in hospital isolation before continuing treatment at home. All visitors must wear masks to enter the isolation unit.

- a Explain why patients are initially treated in isolation.
- b Is this a type of quarantine? Explain why or why not.
- c Public health authorities will contact and test other people in the household of somebody diagnosed with TB. Name this process.

13 Malaria is a disease that is a significant cause of mortality worldwide.

- a Summarise the life cycle of the malaria parasite, *Plasmodium*.
- b Explain how this life cycle affects the distribution of this disease.

There are several conditions caused by abnormal alleles of the gene that codes for haemoglobin. One of these, the sickle cell allele (*h*), causes red blood cells produced to be abnormal shapes. For patients that are homozygous (*hh*) for this allele, these abnormally shaped red blood cells cause a serious disease known as sickle cell anaemia. Heterozygotes who also have one normal allele (*Hh*), however, are usually unaffected. Heterozygotes are also more resistant to infection with malaria.

- c In areas where malaria is common, which genotype would provide an individual with the biggest selective advantage: *HH*, *Hh* or *hh*? Justify your response.
- d Sickle cell anaemia is much more common in parts of the world near the equator. Linking this to your knowledge of evolution, explain why this is the case.

14 Influenza is a viral infection that is most common in winter. It is spread from person to person by airborne droplets produced when sneezing or coughing. The rapid evolution of the virus means that individuals who have been exposed to one strain may not have immunity against others.

- a Suggest some disease, population and environmental characteristics that may explain why influenza is most common in winter.
- b Explain how handwashing can be used to prevent the spread of influenza.
- c Explain why herd immunity is not able to provide effective protection against influenza.

Analysing

15 Draw a table that compares disease surveillance and predictive modelling (including the type of information and when each technique is useful).

16 Based on your knowledge of the lifecycle of the parasite *Schistosoma* spp. (discussed on page 298 in Chapter 10) identify a number of disease, population and environmental factors that could affect the spread of this disease.

Evaluating

17 During the SARS outbreak in 2003 (see Figure 13.7, page 372) many people were held in quarantine. Do you think that quarantine is a violation of peoples' rights? Justify your response.

18 'The human impacts on the transmission of disease are mostly negative.' Provide an argument for or against this statement.

19 Disease monitoring, whether by notification or analysis of social media, involves information about individuals being used without asking their permission. Do you think this is justified? Provide a reasoned argument for your answer.

Creating

20 Choose one of the diseases that has been discussed in this chapter and research the transmission of this disease. Create a diagram that shows how the host, pathogen and environmental factors can impact on the spread of this disease.

21 Synthesise your knowledge of pathogens, immune responses and infectious diseases, and discuss the complex interactions involved in controlling disease spread in the modern world. Assess where you think the biggest challenges lie for infectious disease control in the 21st century.

Reflecting

22 You will have been familiar with the concepts of quarantine, handwashing and immunisation before starting this chapter. Make a list of ways that your understanding has changed as a result of completing this section.

23 Reflect on your personal encounters with infectious diseases (such as the common cold or influenza). Explain how this experience relates to what you have learned in this section.

CHAPTER 14 SCIENTIFIC INVESTIGATIONS

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

Science Inquiry Skills

- Identify, research and construct questions for investigation; propose hypotheses; and predict possible outcomes (ACSBL061 and ACSBL096)
- Design investigations, including the procedure/s to be followed, the materials required, and the type and amount of primary and/or secondary data to be collected; conduct risk assessments; and consider research ethics, including animal ethics (ACSBL062) and including the rights of living organisms (ACSBL097)
- Conduct investigations, including the use of probabilities to predict inheritance patterns, real or virtual gel electrophoresis, and population simulations to predict population changes, safely, competently and methodically for the collection of valid and reliable data (ACSBL063)
- Conduct investigations, including using models of homeostasis and disease transmission, safely, competently and methodically for valid and reliable collection of data (ACSBL098)
- Represent data in meaningful and useful ways, including the use of mean, median, range and probability; organise and analyse data to identify trends, patterns and relationships; discuss the ways in which measurement error, instrumental accuracy, the nature of the procedure and the sample size may influence uncertainty and limitations in data; and select, synthesise and use evidence to make and justify conclusions (ACSBL064 and ACSBL099)
- Interpret a range of scientific and media texts, and evaluate models, processes, claims and conclusions by considering the quality of available evidence, including interpreting confidence intervals in secondary data; and use reasoning to construct scientific arguments (ACSBL065 and ACSBL100)
- Select, construct and use appropriate representations, including models of DNA replication, transcription and translation, Punnett squares and probability models of expression of a specific gene in a population, to communicate conceptual understanding, solve problems and make predictions (ACSBL066)
- Select, construct and use appropriate representations, including diagrams and flow charts, to communicate conceptual understanding, solve problems and make predictions (ACSBL101)
- Communicate to specific audiences and for specific purposes using appropriate language, nomenclature, genres and modes, including scientific reports (ACSBL067 and ACSBL102)

Performing investigations is your chance to experience what doing science is really like. Science is about finding things out through observation and experiment, which is what doing investigations is all about. This is why investigations are central to science, *and* why they are so much fun.

Sometimes an important advance in science begins with a casual observation or a lucky accident. For example, after hearing from milkmaids that people who contracted cowpox (a relatively innocuous disease picked up after working with cattle) were protected from deadly smallpox, the British physician Edward Jenner effectively kick-started the science of vaccination. Jenner used samples from open cowpox sores on a dairymaid's hands to inoculate a young boy and protect him against smallpox. However, it would be another 50 years and a lot of carefully planned research before scientists truly began to understand the biological basis for immunity. This sort of lucky accident may begin a new field of research, but it then proceeds by carefully planned investigation.

Scientific investigations can take years to complete and may involve collaboration among many scientists. They may require access to special equipment in Australia or overseas. They may cost a lot of money, sometimes millions of dollars, to complete. Hence scientists invest time in *planning* investigations before they begin. When scientists apply for grants to carry out investigations they need to show that they have carefully planned what they will do and how any money provided will be spent. Good planning is crucial to the success of the investigation.

Scientists then make careful *measurements and observations* and record their *results*. They *keep records* of all their experiments. This is a legal requirement. Typically, experimental results need to be kept for 5–7 years. There are also requirements on how and where data is stored.

Once data is collected it needs to be *analysed*. There are various ways this is done, but in the biological and biomedical sciences it typically involves constructing graphs. Once a relationship is established graphically, a mathematical relationship can be derived.

Finally, the results of the investigation must be *communicated*. Usually this involves publishing a scientific paper either in a journal or conference proceedings. It often includes presenting the results in talks or posters at conferences. If the result is funded by a grant then a research report must be submitted. If the results are really exciting, then the scientists may write a media release. However the results are communicated, this step must happen for the investigation to be completed.

Planning your investigation

There are many things to consider when planning an investigation. You need to think about how much time you will have inside and outside class. You also need to think about the space and equipment you will need and where you will go if you want to make measurements or observations outside.

You may be working in a group or on your own. Most scientists work in groups. If you can choose who you work with, think about this carefully. It is not always best to work with friends. Think about working with people who have skills that are different from your own.

Finally, and probably the first thing that most students think about, is the topic of the research. You will need to come up with a **research question** or **hypothesis**.

Choosing a research question

Obviously, it is a good idea to investigate something that you find interesting. If you are working in a group try to find something that is interesting to everyone in the group.

A good way to start is by 'brainstorming' for ideas. This works whether you are working on your own or in a group. Write down as many ideas as you can think of. Don't be critical at this stage. Get everyone in the group to contribute and accept all contributions uncritically. Write down every idea.

Alamy/Jim West

◀ **Figure 14.1**
Brainstorm as many ideas as you can in your group.

After you have run out of ideas, it is time to start being critical. Decide which questions or ideas are the most interesting. Think about which of these it is actually possible to investigate given the time and equipment available. Make a shortlist, but keep the long list too for the moment. Once you have your shortlist it is time to start refining your ideas.

Researching and refining your question

The next step is to find out what is already known about the ideas on your list. Use the Internet, your text books and the library to find out. Make sure you *keep a record* of the information that you find as well as the *sources*. You should start a **logbook** at this stage. You can write in **references**, or attach printouts to your logbook. This can save you a lot of time later on! Many research students forget to do this when they first start reading about their topic and then have to search all over again.

Good record keeping is important in scientific research, and it begins at this stage of the investigation.

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. Textbooks, and websites from universities and government research agencies are usually very reliable. Publications and web pages from professional associations, such as the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australian Academy of Science and equivalent international organisations are also good sources. Blogs and homepages of other students are not usually reliable, although they are useful to give you ideas. Websites that are trying to sell you something should also be treated sceptically. Talk to your teacher about sources of information as well. They will be able to tell you if a website is reliable, and suggest sites that they know are suitable.

You may find examples of similar investigations to the one you are thinking of. It is a good idea to look at these, so you can learn from the experience other researchers. However, in general, it is better not to try to replicate someone else's investigation exactly. If you do decide to replicate someone else's investigation then you need to acknowledge and carefully reference their work. See the section on referencing below. If you do not do so, it is **plagiarism**. This is a very serious form of academic misconduct. Talk to your teacher about how original your research needs to be, and how closely it can be based on someone else's work. It is much better to do this at the start than to be accused of cheating later on!

Finally, talk to your teacher about your ideas. They will be able to tell you whether your ideas are likely to be possible given the equipment available. They may have had students with similar ideas in the past and can make suggestions.

After you have researched your questions and ideas, you will hopefully be able to narrow the shortlist down to the one question that you want to tackle. If none of the questions or ideas look possible (or still interesting), then you need to go back to the long list.

CSIRO

This site contains useful information on all fields of scientific research, including resources for students and teachers.

AUSTRALIAN ACADEMY OF SCIENCE

This is a useful resource for up-to-date science news.

AUSTRALIAN INSTITUTE OF BIOLOGY

This is another useful resource that aims to promote education and research in Biology.

Proposing a research question or hypothesis

Once you have decided on what you will investigate, you need to turn it into a research question or a hypothesis. Make sure you check with your teacher what sort of investigation or project you are supposed to be doing.

A research question is one that can be answered by performing experiments or making observations. A hypothesis is a prediction of the results of an experiment, which can be tested by performing experiments or making observations.

You may also be able to do a 'design, build and test' project. These are described later.

Research questions

A research question may be of the form 'What effect does a new fertiliser have on root growth?' The aim of your research is to then answer the question. It is important that you frame the question carefully. It needs to be specific enough that it guides the design of the investigation. A specific question rather than a vague one will make the design of your investigation much easier. Asking 'Does the new fertiliser increase root growth more than standard fertiliser?' tells you what you will be varying and what you will be measuring. It also gives a criterion for judging whether you have answered the question.

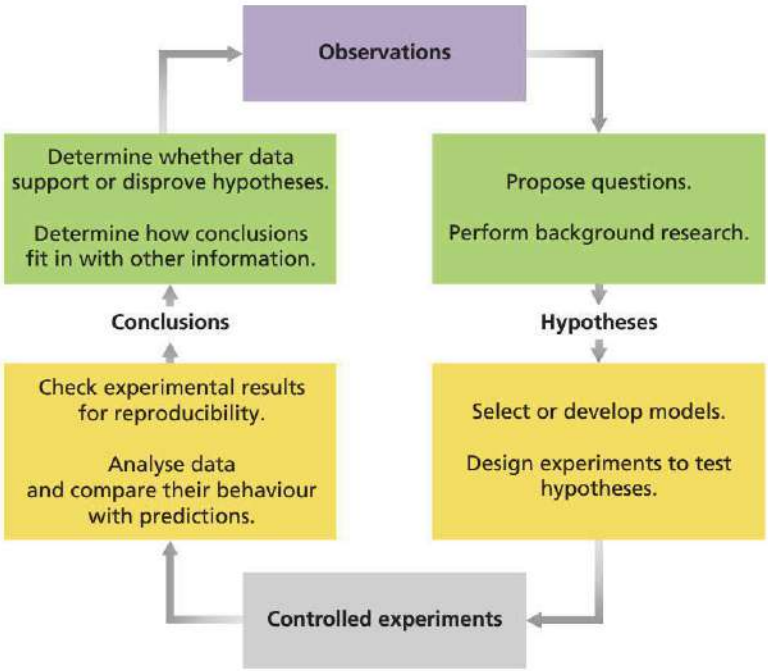


Figure 14.2 ▲
The scientific method

Asking 'How can we make roots grow the best?' 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. 'Best' is a vague term. What you mean by 'best' may not be what someone else means.

A good research question identifies the **variables** that will be investigated. Usually you will have one **dependent variable** and one **independent** or **controlled variable**. For a lengthy investigation you may have two or more independent variables. Variables are discussed in more detail later.

Finally, a good research question should be answerable with the time and equipment available.

Hypotheses

A hypothesis is a tentative explanation or prediction, not yet confirmed by experiment, such as 'The new fertiliser makes roots grow longer in two weeks than standard fertiliser'. A hypothesis is often based on some existing **model** or **theory**. It is a prediction of what will happen in a specific situation based on that model.

A hypothesis should give you a prediction that you can test by performing an experiment. This means it should at least be **falsifiable**. A good hypothesis should be able to be disproved. However, you will *not* generally be able to claim that you have proved your hypothesis.

If your experiments 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*. Hence an aim for an experiment should not start 'To prove ...', as it is not possible to actually prove a hypothesis, only to disprove it.

If your experimental results disagree, then you may have disproved your hypothesis. This is *not* a bad thing! Often the most interesting discoveries in science start when a hypothesis based on an existing model is disproved. This means that the model it was based upon is either not a good model, or does not apply to the particular situation. You could then try to work out why the model does not apply, or try to formulate a better model. What to do when your hypothesis is not supported is discussed further in the analysis section.

In summary, a good research question is a question that is specific and can be answered by performing experiments and making measurements. A good hypothesis is a statement that predicts the results of an experiment and can be tested using measurements.

Even if your question or hypothesis meets these criteria, do not be surprised if you change or modify it during the course of your investigation. In scientific research, the question you set out to answer is often only a starting point for more questions.

Designing your investigation

Once you have a specific research question or hypothesis, you need to design your investigation. It is fun to start making measurements or observations immediately, but it is also important to spend time learning how to use the equipment, and experimenting to find the best way to set up your investigation. You may also discover that you need different or more equipment. This may save you time later on.

It is also important not to get distracted and forget the purpose of your investigation. At the end of the process, you need good data that answers your question or tests your hypothesis. Having a plan ensures that you make 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?
- What materials and equipment will you need?
- When and where will you collect the data?
- If you are working in a group, who will collect the data?
- Who will be responsible for record keeping?
- How will the data be analysed?

The data that you collect will always include **secondary data**, and will usually include **primary data**. Secondary data is data that has been collected by someone else.

You will already have collected some secondary data when you investigated your research topic to formulate your question or hypothesis. You will probably want to collect more secondary data. If your topic is not one for which you can collect primary data, then you will need to rely on secondary data. Remember that when you collect secondary data it is important to use reliable, reputable sources.

Primary data is data that you collect yourself. You can collect data by performing experiments or making observations in the field. You should be able to measure parameters that are relevant to the biological question being asked (e.g. rate of cell division, body temperature, enzyme activity, size of population). You will have had practice at measuring some or all of these things already. You need to decide which variables you will measure and which variables you will control. Consider which variables you can control, and which you cannot.

Consider how you will analyse the data. Will you need access to specific software such as a graphing or statistics package? If so, make sure that you know how to use it. If you are using software to draw graphs then you need to know how to produce a **scatter graph** and fit a **line of best fit** and add **uncertainty bars**. Note that a line of best fit is *not* the same as joining the dots. You should *never join the dots*, even though this is often the default setting in spreadsheet software. You should consult a reference guide, the 'help' menu for your software, or ask your teacher. Graphs are discussed in more detail in the analysis section below.

Keep a record of your planning. This should go in your logbook. Writing down what you plan to do, and why, will help you stay focused during the investigation. If you are working in a group, then a record of what each person agrees to do during the investigation can be very important.

Variables and measurements

Anything that can vary in an experiment is a variable. An independent or controlled variable is one whose properties you can control. For example, if you were doing an experiment to measure the activity of an enzyme at different pH, then you would control the pH of the reaction and measure the rate of catalysis of the substrate. In this case the pH is the independent variable and the rate of catalysis, which varies with pH, is the dependent variable.

In the question 'Does the new fertiliser increase root growth more than standard fertiliser?', the type of fertiliser is the independent variable. The dependent variable is the root length (or change in root length). Other independent variables not mentioned in this question include soil composition, amount of water, light and temperature. These should all be kept constant, so they are not variables in the investigation. If it was a long investigation, the air pressure could be a second controlled variable. If you decide to have two independent variables then it is important to keep one constant while you vary the other, if at all possible. Then you take multiple sets of measurements, keeping one variable at a fixed value for each set of data while you vary the other.

When variables have a numerical value, you make **quantitative measurements**. You measure that numerical value in the appropriate units. For example, you may measure root length in centimetres or weight of roots in grams.

Figure 14.3 ▼ Keeping growing conditions as consistent as possible helps to control the independent variables.

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Continuous variables may take any possible value, usually within some range. Length, time and current are continuous. In the root growth example, root length is a continuous variable, as it will likely change over time. A variable that may take only fixed values is called a **discrete variable**. Often these are whole numbers of things that cannot be broken into smaller parts, such as electrons or students. In the root growth example, the number of roots is a discrete variable.

Your measuring equipment will sometimes restrict you to only measuring discrete values. This is always the case with digital equipment. A set of digital scales that measures in grams gives you discrete values. It does not, however, mean that weight itself is a discrete variable. The weight of the roots is a continuous variable, but digital scales will only give you discrete measurements of the weight.

In some investigations you may use **qualitative measurements** or data. For example, a chemical reaction may lead to a colour change. You would usually describe the colour in words, such as 'pink' or 'green', rather than using a number. 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).

Once you have decided on the variables you will be measuring, you will be able to identify the equipment and other resources you will need.

Identifying the resources required

If you are going to collect primary data, make a list of all the equipment that you need. Consider how precise the measurements will need to 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 a root growth experiment, you may need to measure the dry weight of the roots, which means finding a consistent way to dry them.

Consider who will supply the materials and how much they might cost. Scientists generally have tight budgets that they have to work within. Also, the equipment you plan to use must be safe. Will you need special protective equipment, such as lab coats, safety glasses or gloves? There is a section on risk assessment below. Make sure that you include any safety equipment needed in your equipment list.

When you have your list, talk to your teacher about what equipment is available. You might find that you need to modify your question or hypothesis at this stage.

Consider where you will perform your experiments or observations. Can you use normal classroom space, or do you need to be outside? If you are outside, what provisions can be made for ensuring 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.

Planning the experimental procedure

The most common problem that students have when doing research is time management. It is important to plan to have enough time to perform the experiments, *and* to analyse them, *and* to report on them. You also need to allow time to learn how to use the equipment if you have not used it before.

In any investigation you will need to collect reliable and precise data. You cannot do this if you do not know how to use the equipment. Always ask if you are unsure. Reading the user manual is also a good idea. It will usually specify the precision of the device, and let you know of any potential safety risks.

Whenever possible you should make repeat measurements, so allow time for this. This allows you to check that your measurements are **valid**. Valid results are affected only by a single independent variable. If the results are similar each time, then your results are likely to be valid. If a result is not **reproducible**, it is probably not a valid result. A result is reproducible if you make exactly the same measurement more than once and get the same result, within the limits of experimental uncertainty. If a result is not reproducible, then a variable other than the one you are controlling is affecting its value. If this is the case, you need to determine what this other variable is, and control it if possible.

Think about how you can minimise uncertainties. Minimising uncertainty is not just about using the most precise equipment you can find, it is also about clever experimental technique. Important discoveries are possible using simple equipment and techniques. For example, Gregor Mendel defined his laws of Mendelian inheritance through the careful, meticulous cultivation of pea plants.

Sometimes experiments simply don't work or can't be done for some reason such as equipment failure or unforseen variables. For example, root growth will be affected if the plants contract a disease during the experiment. Try to think of all the things that could go wrong. If possible, come up with backup plans. Allowing plenty of time helps with this, as does starting your experiments as soon as possible.

Make sure you allow time for analysis. Ideally, 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. If you identify an outlier while you still have access to equipment and space, you can check the

measurement and make sure that you didn't make a mistake or that the experiment hasn't been compromised by an uncontrolled variable.

After you have analysed your results, you need to write your report or communicate your findings in some other form. You need to plan ahead how this will be done. If you are working in a group, who will write which part of the report, and when? Who will proofread it? Who will be responsible for making sure all the parts fit together?

You may find a timeline useful. A timeline helps keep you on track, and reminds everyone of their responsibilities. If you are working in a group, get everyone to agree on it.

You can use the following table as a template.

Date and place	What will be done	By whom	Outcomes

Risk assessment

You may be required to complete a risk assessment before you begin your investigation. Even if this is not a requirement, it is a good idea to think about it. You need to think about three things.

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

A 'risk matrix', such as Table 14.1, can be used to assess the severity of a risk associated with an investigation. The consequences are listed across the top, from negligible to catastrophic. Negligible may be getting clothes dirty or a very minor injury such as a scratch. Marginal might be a bruise from falling off a bike, or a broken branch in a tree. Severe could be a more substantial injury or a broken window. Catastrophic would be a death or the release of a toxin into the environment. In general, you need to ensure that your investigation is low risk. You can use a risk matrix either for individual identified risks, or for the investigation overall. If there are multiple experiments, then you would use a risk matrix for each one.

Table 14.1 Matrix for assessing for severity of risk

Consequences→ Likelihood↓	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 what the possible risks are, you need to think about what you will do about them. What will you do to minimise them, and what will you do to deal with the consequences if something does happen? This may be as simple as 'Always wear a lab coat, gloves and safety glasses.' You can use a risk assessment table similar to the one shown.

What are the risks in doing this experiment?	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.

Safe use and disposal of biological material

When dealing with many biological materials, it is important to be aware of safe handling and disposal. For example, when growing known or unknown microbes on agar plates, it is important to use safe sterile techniques (discussed below) and to wear lab coat, gloves, safety glasses and, if required, face masks. Treat all microbes on agar plates as potentially pathogenic and **autoclave** used plates before disposing.

Ethics

Ethics in research can be controversial. More than one scientist has lost their job for unethical research behaviour. Being ethical in your research has two aspects. The first is about being honest as a scientist. This means recording data accurately, and not ignoring, hiding or changing any data that doesn't 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 and permission. Put simply, it is showing integrity or 'doing the right thing'. A good rule is that if you wouldn't want someone to know what you are doing, you probably shouldn't be doing it. It is no different from behaving ethically in any other area of your life.

The other aspect to ethics is treating animals, other people and the environment with care and respect. If your investigation will be using humans then you need to make sure you do not harm them, either physically or psychologically. If you are working with animals, then you need to make a strong case for any investigation that harms or could potentially harm them. When scientists want to use humans or animals in their research, they need to be able to show that the benefits to the environment, other animals or humans significantly outweigh the negative effects on the animals or humans used.

The use and welfare of animals for the purposes of research is legislated by State and Federal laws and respect for all animals (vertebrate and invertebrate) used in research is of the utmost importance. When using animals for research, scientist must adhere to the '3Rs'. These are:

- *replacement* of animal research with other types of research where possible
- *reduction* of the number of animals used in research
- *refinement* of experimental techniques to minimise pain and distress.

The National Institute of Health and Medical Research (NHMRC) has guidelines on the ethical use of humans and animals in experimentation.

If you are planning to collect live specimens in the field, be aware of specific State laws that may pertain to native species (including plants).

▼ **Figure 14.4**
The use of animals for research purposes is governed by State and Federal laws.

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Experimental technique

Once you have planned the experimental procedure, taking into account the minimisation of uncertainty, the risks involved and ethical considerations, it is time to begin the experiment.

In biology, a number of specialised techniques exist to ensure that the data collected is as reliable as possible. For example, in many laboratories, it is essential to use the **aseptic technique** to ensure that only the desired organisms grow in culture. Outside the lab, biological parameters can vary widely within populations and it is important to sample the population carefully to get an accurate representation of the population at large.

NHMRC

You can find NHMRC human and animal ethics guidelines here.

Aseptic technique

When dealing with cell culture (plant, animal and microbial), it is important to practice good aseptic (sterile) technique. This may mean working in a laminar flow hood – an enclosed workspace that prevents contamination of biological samples by maintaining positive pressure using filtered, sterile air. If a laminar flow hood is not available, a small sterile space can be

created using a Bunsen burner, whose flame creates an updraft and kills airborne contaminants in the surrounding air.

Working in a sterile space must be combined with careful handling of all biological material and equipment and regular decontamination, typically with a solution of 80% ethanol.

Alternatively, when preparing microbial cultures on agar, utensils used to transfer the microbes may first be held over a flame to sterilise them, then cooled in a sterile environment before use. Holding the Petri dish upside down as much as possible minimises the opportunity for airborne contaminants to land on the agar.

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Figure 14.5 ▲
The use of a laminar flow hood can prevent contamination of biological samples.

Corbis/Jonathan Blair

Figure 14.6 ▲
Tagging captured sea turtles, then performing recapture, helps estimate their population size from those sampled.

Population sampling

Taking accurate measurements in the field relies on measuring a large enough, representative sample of the population. Various standardised techniques have been developed to accurately sample populations and ecosystems. Different techniques are suitable for different types of studies and the method of sampling a population is an important component of the experimental design. For example, the **capture–mark–recapture** method is a powerful technique for estimating a population size, but relies on the captured sample redistributing equally throughout the population and being recaptured at the same frequency as individuals that had not been captured. It is important that such limitations be considered when planning your experiment.

Collecting your data

Once your experiment is underway, it is time to start collecting data. This is usually the fun part of any investigation. Don't forget you have a question to answer or a hypothesis to test! To do this, you need to make sure that you think carefully about what you do and keep good records.

Record keeping

You will need to keep a record of what you do during your investigation. You do this is in a logbook.

Scientists keep a logbook for each project that they work on. It is a record of what they did, why they did it, and what they found out. A logbook is a legal document for a working scientist. If someone's work is called into question, or there are disputes over patents or ownership of data, then the logbook acts as important evidence. Every entry in a scientists logbook is dated, records are kept in indelible form (pen, *not pencil*), and entries may even be signed. Scientists' logbooks include details of experiments such as methods and results. They include comments and ideas, thoughts about the experiments, and analysis. They frequently include printouts of data, photocopies of relevant information, photos and other items. The logbook is the primary source of information when a scientist writes up their work for publication.

Some scientists keep their research records electronically, but most experimental scientists still keep a hardcopy logbook. There are several advantages to a hardcopy logbook over an electronic one. First, electronic records are easy to make changes to, and it is hard to track what was changed, when and by whom. Second, if you are working in a group, it can be hard to keep track of who has the most recent version of the file/s. Third, files can be easily deleted or corrupted. It takes much more care and discipline to maintain a good electronic logbook than a good hardcopy. Remember that the purpose of a logbook is to record and maintain evidence of what you did. Electronic evidence is not as reliable as a signed hardcopy document.

You should talk to your teacher about what form of logbook records they require you to keep.

If you are working in a group then you will need to decide whether to keep one logbook for the entire group, or one each. If you will all be working in the same places at the same times, then one for the whole group is best. If you will be in different places (for example, doing field observations) then you will need one each. Your teacher may also require each of you to keep your own logbook for assessment, or for authentication purposes.

Your logbook is a detailed record of *what you did* and *what you found out* during your investigation. Make an entry in the logbook *every* time you work on your investigation. At the start of each session you should record the date and the names of all the people with whom you are working at the time.

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.

Include large, clear diagrams of any experimental set-up and include details of equipment used. You can also include photos of experiments.

Record the results of *all* measurements *immediately and directly into your logbook, in pen. Never* record data onto bits of scrap paper instead of your logbook! Results must be recorded in indelible form. This means using a pen. Never write your results in pencil. Never use white-out or scribble over anything in your logbook. If you want to cross something out, just put a line through it. It is also a good idea to make a note explaining why it was crossed out.

Performing experiments

If you have planned carefully and learned how to use the equipment, then hopefully your experiments will go smoothly.

The raw data should always be recorded directly into the logbook unless it is recorded using data loggers connected to a computer. In this case a printout of the data should be attached to the logbook, and the file name and location recorded. Make sure that you measure and record everything you will need for your analysis. For example, if you are investigating root growth, you could measure the amount of fertiliser, the temperature and the starting length of the roots. It is much better to measure something and then discover that you didn't need to, than to start your analysis and realise that you didn't measure something that you do need.

Use appropriate units; for example, centimetres for lengths and grams for weights. If you are going to be collecting multiple data points, it is a good idea to draw a table to record them in. Label the columns in the table with the name and units of the variables. Do not put the units in the table cells. Note that the accuracy of your measurements will often be restricted by the

instruments you use to take them. For example, a ruler may only have markings down to 0.1 cm. Make a note of these restrictions as they may affect the accuracy of your final results, especially if the changes measured are very small.

If you have not made a mistake, then plotting and analysing as you go allows you to spot something interesting early on. You then have a choice between revising your hypothesis or question to follow this new discovery, or continuing with your plan. Many research projects start with one question and end up answering a completely different one. These are often the most fun, because they involve something new and exciting.

Analysing your data

Figure 14.7 ▲ Plan exactly what you will measure to collect your data. Do you want to test how to grow longer roots, or a greater mass of roots? Where do the roots end? Will you use fresh weight or dry weight?

When you have collected all your data you will need to analyse it. Record all your analyses in your logbook. If this is done on a computer, then record the file name and location and attach a printout of the analyses into your book. Many scientists have logbooks that are bulging with printouts.

The first step is organising your data. If you have more than a few data points, it is a good idea to display them in a table. You may have several tables for different experiments. You may also need to do some analysis of the data. For example, you may wish to show the change in root length over the course of the experiment in addition to the lengths at the beginning and end of the experiment.

Plotting graphs is a useful way to begin the analysis of your data. Graphs are a very useful way of representing data so that trends and relationships can be identified. There are many different sorts of graphs that can be used to organise and display data. These are described below. You will usually need to do some calculations with your data to be able to answer your question or test your hypothesis. Remember to keep units on all quantities, so that any derived values have the correct units. You will also need to calculate uncertainties on any derived quantities.

Identifying trends, patterns and relationships

You may be able to see a pattern simply by looking at a list of numbers in a table. However, the most reliable 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. Choose a scale so that your data takes up most of the plot area. This will often mean that the origin is not shown in your graph. Usually there is no reason that it should be.

When you are looking for a relationship between variables, plot a scatter graph. This is a graph showing your data as points. Do not join them up as in a dot-to-dot picture. Usually the independent variable is plotted on the x-axis and the dependent variable goes on the y-axis, unless there is a good reason to do otherwise. For example, if you were measuring root growth in response to temperature, root growth (change in length) would be plotted on the y-axis against temperature on the x-axis.

To determine a relationship 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 is generally considered adequate if the relationship is expected to be linear, but always collect as many as you reasonably can, given the available time. For non-linear relationships you need more data points than this, so collect as many as possible.

DATA POINTS

This website contains some helpful advice on deciding the number of data points.

A good graph to start with is simply a graph of the raw data. You will usually be able to tell by looking whether the graph is linear. If it is, then fit a straight line using a graphing package. You can then use a **linear regression** tool to check how good the straight line fit is. This will give you an R^2 number, which is a measure of 'goodness of fit'. The closer R^2 is to 1 (or –1), the better the fit. If it is not *very* close to 1, then the relationship is not linear.

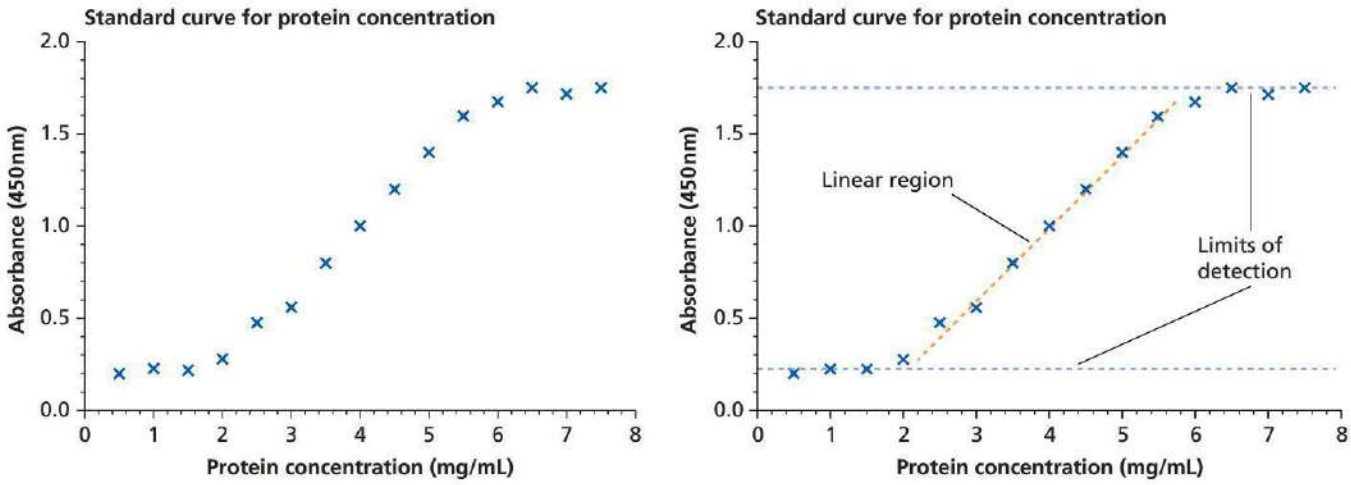
If it is a linear relationship, then finding the equation for the line of best fit may be useful. *Never* force a line of best fit through the origin. Often the intercept gives you useful information. It may even indicate a systematic error, such as a zero error in calibration of your equipment.

When you plot your raw data you may find that one or two points are outliers. These are points that do not fit the pattern of the rest of the data. These points may be mistakes; for example, they may have been incorrectly recorded or a mistake was made during measurement. They may also be telling you something important. For example, if they occur at extreme values of the independent variable then it might be that the behaviour of the system is linear in a certain range only. This is the case for many biological **assays**. You may choose to ignore outliers when fitting a line to your data, but you should be able to justify why.

When you extend a line of best fit beyond your measured points, this is called **extrapolation**. Any data that you read off a graph outside the range of your data points is extrapolated, and should be viewed with caution. You cannot say for sure that the system continues to behave in the same way beyond the bounds of your data.

Reading points, other than data points, from a line of best fit within the region in which you have data is called **interpolation**. You cannot be sure that this is exactly what you would find if you measured that point. However, if your line of best fit really represents the behaviour of the system, then you can use interpolated points in your analysis.

For example, an assay that measures the concentration of protein in a solution involves creation of a standard curve from which the concentration of samples can be interpolated (Figure 14.8). Since the assay has a minimum level of detection (its **sensitivity limit**) and a maximum detection limit above which the assay is saturated, it is not possible to extrapolate outside the linear region of the standard curve. To measure samples whose concentration is higher than the detection limits for the assay, the samples must be diluted so their concentration lies within the range of the assay (the linear region of the standard curve).



▲ **Figure 14.8**
Standard curves can be used to interpolate values, but only within the limits of detection.

In biological experiments, you are often comparing the effects of different independent variables on a single dependent variable. In the root growth example, you are comparing the effect of the new fertiliser with those of the standard fertiliser. In this case, you would typically plot a bar graph of the change in root length against the type of fertiliser used. If the data is taken from several different plants in each treatment group, you would plot the mean plus or minus one **standard deviation** (Figure 14.9). You might then use appropriate statistical analysis to determine whether or not there is a **significant difference** between the treatments.

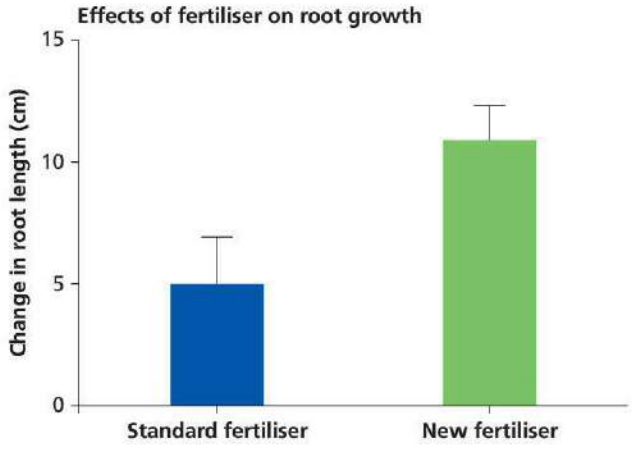


Figure 14.9 ▲ Bar graph of two data sets, with standard deviation

For data of this type, you might, for example, use a **t-test** to test for a significant difference between the two treatments. Graphing software typically has functions for calculating standard deviation and for statistical analysis of the data.

Interpreting your results

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. If you have performed statistical analysis, does this support your hypothesis? For example, if the new fertiliser induces statistically greater root growth than the standard fertiliser, with all other variables being equal, this would support the hypothesis that 'The new fertiliser makes roots grow longer in two weeks than standard fertiliser'. If there was no difference between the two, or if the new fertiliser induced significantly less root growth than the standard fertiliser, then this would argue against the hypothesis.

If your hypothesis is not supported

It is not enough to simply say 'our hypothesis is wrong'. If the hypothesis is wrong, *what* is wrong with it?

It may be that you have used a model that is too simple, or did not take into account all of the other variables. For example, in the root growth experiments, it may be that the new fertiliser works best at a particular temperature, or over a longer time, or in conjunction with certain soil conditions. Or maybe it doesn't work with the type of plant you chose to use. It may be that the experiment was simply too limited to fully test the hypothesis. Thus, you might conclude that further experiments are required to test these other variables.

Before you decide that the model is at fault, however, it is a good idea to check carefully that you have not made any mistakes or ignored any variables.

Think carefully about any factors that you did not take into account but which might have affected your experiment.

Go through your method, results and analysis. Check that your equipment was correctly calibrated, and that you were using it correctly. Check that data is recorded in the correct units, and that units are correctly carried through all calculations during analysis. Check your analysis carefully. If you are working in a group, get another person to repeat the calculations.

It is never good enough to conclude that 'the experiment didn't work'. Either a mistake was made or the model used was not appropriate for the situation. It is your job to work out which.

Communicating your results

If research is not reported on, then no-one else can learn from it. An investigation is not complete until the results have been communicated. Most commonly, a report is written.

Writing reports

A report is a formal and carefully structured account of your research. It is based on the data and analysis in your logbook. However the report is a *summary*. It contains only a small fraction of what appears in the logbook. Your logbook contains all your ideas, rough working and raw data. The report typically contains none of this.

A report consists of several distinct sections, each with a particular purpose. These are:

- Abstract
- Introduction
- Method
- Results and analysis
- Discussion
- Conclusion
- Acknowledgements
- References
- Appendices

Reports are always written in the past tense, because they describe what you have done.

The abstract

The abstract is a very short summary of the entire report. It is the most important part, because often it is the only part that people read. Typically an abstract is between 50 and 200 words long. It appears at the start of the report, but is always the last thing that you write. Try writing just one sentence to summarises each part of your report.

Introduction

The introduction tells the reader why you did the investigation and what your research question or hypothesis is. This is the place to explain why this research is interesting or important.

The introduction also provides any background information needed to be able to understand the rest of the report. This is the place to summarise any existing theories and models. You need to do this to justify your hypothesis. You should also summarise any similar investigations. All of this should be correctly referenced, as described in the section on referencing below.

Method

The method describes what you did. It is not a recipe for someone else to follow.

The method summarises what you measured and how you measured it. It also explains, briefly, why you chose a particular method or technique.

Write your method using sentences, not dot points. Remember that these need to be written in past tense – *it is not a recipe*. You are not commanding anyone to do anything. You are telling people what you did. For example, you would write 'we measured the length' not 'measure the length'.

Include any diagrams, such as circuit diagrams, that are needed to make your method clear. The diagrams in your logbook will usually be rough sketches. The diagrams in your report should be very neat and carefully labelled. Flow charts can be useful to describe any procedures in which a series of steps was followed. Each diagram should have a figure number and you should refer to it in the text of your report. Position the diagram close to where it is referred to in the text. You should take the time to learn how to position figures neatly using your word processor software. When including images taken on a **microscope**, a scale bar and magnification must always be noted.

Results and analysis

The results section is a *summary* of your results. It is usually combined with the analysis section, although they may be kept separate.

Avoid including tables of raw data in your report unless they compare the results of a few different experiments. Wherever possible use a graph instead of a table.

If a table has more than a few rows of data, it is better to represent that data in some other way. Usually this will be a graph.

Think about what sort of graph is appropriate. If you want to show a relationship between two variables then use a scatter plot. Display your data as points with uncertainty bars and clearly label any lines you have fitted to the data. Always make sure you label your axes, including units. Choose an appropriate scale so that the data takes up most of the plot area.

Column and bar charts are useful for comparing two data sets, such as average root length with different types of fertiliser. *Do not* use a column or bar chart to try to show a mathematical relationship between variables.

Figure 14.10 gives examples of the two types of graphs.

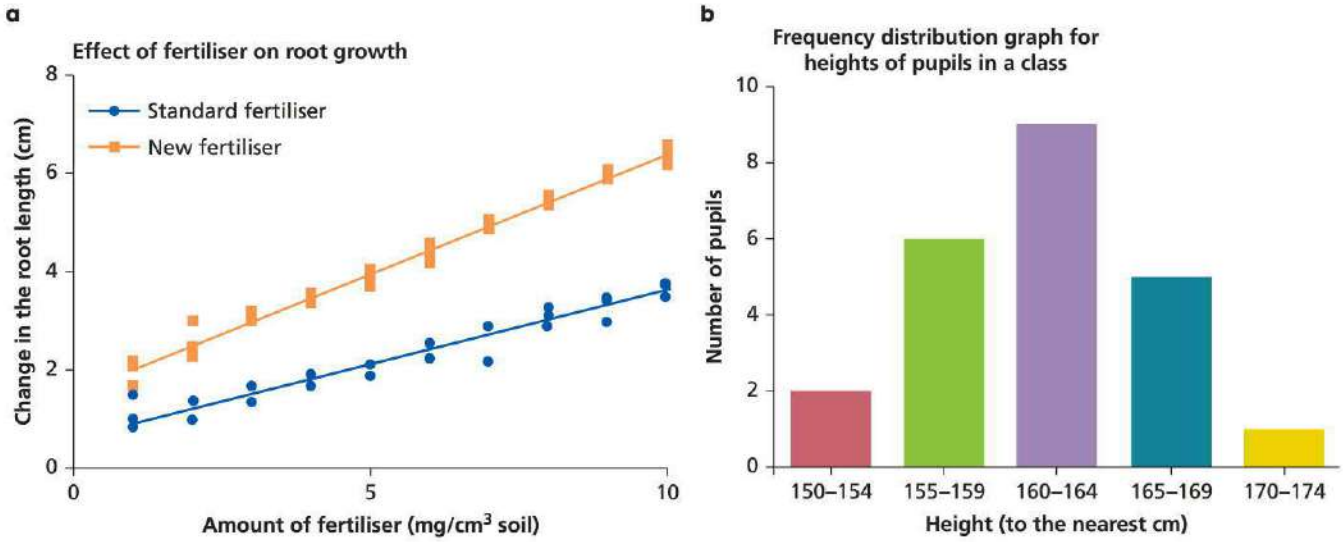


Figure 14.10 ▲ a) A scatter plot with linear regression demonstrating a mathematical relationship; b) A column graph comparing numbers between different groups

Any data and derived results should be given in appropriate units with standard errors of the mean or standard deviation, as appropriate. If you performed calculations or statistical analyses of the data, show the equations or describe the statistics you used. You might want to show one example calculation, but do not show more than one if the procedure used is repeated.

Discussion

The discussion should explain *what your results mean*. If you began with a research question, give the answer to the question here. If you began with a hypothesis, state whether or not your results support your hypothesis. If not, explain why. (You might only be able to say that the model was not suitable for the situation being investigated.)

If there are any implications of your work, such as implications for better agricultural processes or the design of better medicines, put them here.

The discussion is also the place to briefly describe any difficulties that you had and make suggestions for improving the process. Remember that you should never say 'the experiment didn't work' if you didn't get the results you expected. You might choose to make some comments on possible further work that could be done.

Conclusion

The conclusion is a *very* brief summary of the results and their implications. Say what you found out and what it means. A conclusion should only be a few sentences long. It should clearly refer back to your hypothesis or research question.

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 with the analysis. In science, as in other aspects of your life, it is polite to say thank you; however, this is not a compulsory section of a report.

References

A reference list details the sources of all information that were actually used to write the report. Wherever a piece of information or quotation is used in your report it must be referenced *at that point*. This is typically done either by placing a number in brackets at the point [2], or the author and year of publication (Smith, 2014). The reference list is then provided either in a footnote at the end of the page or a single, complete list at the end of the report. Referencing must be done in a consistent style. Check with your teacher what style they prefer. There are several good online guides to referencing.

A reference list is *not* the same as a bibliography. A bibliography is a list of sources that are useful to understanding the research. They may or may not have actually been used by the report authors. You should have a bibliography in your logbook from the planning stage of your investigation. The references will be a subset of these sources.

Appendices

Appendices may be used to provide additional information, such as raw data that is not necessary to understanding the report but which might be of interest to some readers. Your teacher might require you to provide raw data in an appendix. Reports do not always have appendices.

Other ways of communicating your results

You may want to present the results of your investigation in some other way. Scientists communicate their work in many ways. Sometimes a poster is presented or a seminar is given. An article may be written or a website produced. Scientists usually use more than one means, and sometimes several of them, to communicate about a really interesting investigation.

◀ **Figure 14.11**
A poster session is a common way to present scientific findings at a conference.

REFERENCING GUIDE

This guide is designed to help you with referencing your sources for assignments.

REFERENCING i-TUTORIAL

This tutorial will help you understand referencing and show you how to avoid plagiarism.

REPORT WRITING EXAMPLE 1

This online resource guides you through the sections of a typical report.

REPORT WRITING EXAMPLE 2

This online resource will help you write a case study.

Look at examples of articles in the scientific and the popular media, on websites, posters and so on. This will give you an idea of the different styles used in the different modes. Think about the purpose. Is it to inform, to persuade or both? What sort of language is used?

Think about your audience and use appropriate language and style. A poster is not usually as formal as a report. A website may be more or less formal, depending on your audience.

Posters and websites use a lot of images. Images are usually more appealing than words and numbers, but they need to be relevant. Make sure they communicate the information you want them to.

Make sure you keep readability and accessibility in mind if you are creating a poster or website. Posters should use large clear fonts and not have too much text. They should be readable from a few metres away. Fonts also need to be large enough and clear on websites, and digital images should have tags. Refer to the *Website accessibility* weblink for more information on accessibility and web-page design.

However you communicate your work, make sure you know what the message is and who the audience is. Once you have established that, you will be able to let other people know about the interesting things you have discovered in your investigation.

WEBSITE ACCESSIBILITY

The Royal Society for the Blind has information on making websites accessible.

CHAPTER GLOSSARY

aseptic technique the technique of working under sterile conditions to prevent contamination of samples

assay an experimental technique or procedure used to test a specific biological process or effect; for example, an enzyme kinetic assay

autoclave a device used to sterilise equipment, reagents or contaminated waste; autoclaves work by subjecting contents to pressurised steam at 121°C for a set time

capture–mark–recapture an ecological surveying technique used to measure animal populations, in which individual animals are captured, marked and released; after a time, the population is re-sampled and the number of marked animals caught gives an indication of population size

continuous variable a variable that is able to take any value within a range; length, time and temperature are examples of continuous variables

controlled variable the variable that is controlled by the experimenter, so that its values are chosen; also called the independent variable

dependent variable the variable that changes as a result of changes to the independent or controlled variable

discrete variable a variable that may take only certain values; number of individuals, or number of legs on an animal are examples of discrete variables

extrapolation extension beyond the measured range of data to read or construct new data that has not been measured

falsifiable able to be disproved

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

independent variable a variable upon which another variable depends; usually the controlled variable

interpolation to read or construct a new data point that has not been measured but is within the range of measured data

linear regression a statistical tool used to model the dependence of one variable on another

line of best fit the line that most accurately fits the data, usually calculated using linear regression

logbook the record of an experiment or investigation kept by the scientist performing the experiment; it is a legal record of the experiments and their results

model the artificial conceptual or abstract simulation of a real-world process or system, developed by simplifying key steps that produce reliable and consistent agreement as verified by field studies; a model may be mathematical equations, a computer simulation, a physical object, words or other form

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

plagiarism presenting someone else's work, including their words or ideas, as your own

primary data data that you have measured or collected yourself

qualitative measurement a measurement with descriptive or non-numerical results

quantitative measurement a measurement with numerical values

reference the source of a specific piece of information or quotation

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

reproducible giving the same result, within uncertainty limits, when repeated measurements are made

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

scatter graph a graph or plot showing data points, without a line joining the points, and used to demonstrate or determine a mathematic relationship between variables; the axes are defined by the variables

secondary data data or information that has been collected by someone else

sensitivity limit the portion of a curve that is non-linear; data that falls into these non-linear regions cannot be extrapolated

significant difference a difference between data values that is statistically significant; that is, the probability (p) of the difference being due to chance is so small (usually less than 5%) that the result is considered true

standard deviation a measure of the dispersion of a set of data from its mean; expresses the variability of a population or set of data

t-test a statistical test commonly used to analyse differences between two sets of data

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

uncertainty bars bars drawn above and below and/or to left and right of a data point on a graph to indicate the size of the uncertainty in that point

valid 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

GLOSSARY

absolute dating the process of determining the age of rocks and their contained fossils in years on the basis of the physical or chemical properties of materials in the rock

acetylcholine a neurotransmitter in the human nervous system

action potential a brief change in the electrical potential on the surface of a nerve or muscle cell in response to stimulation, which results in the transmission of an electrical impulse

activator a regulatory protein that binds to an enzyme or DNA, causing a change of conformation so that enzymes become active, or activating gene expression

active immunity the immunity formed by stimulation of the immune system with an antigen and the generation of effector and memory cells; it is contrasted with passive immunity

adaptation a developed characteristic that enhances an organism's survival in its natural environment

adaptive evolution changes in populations of organisms that make that population better adapted to its environment over time

adaptive immune response an immune response that is acquired; after an initial response to a pathogen, the immune system creates a 'memory' that leads to an enhanced response to subsequent encounters with the same pathogen

adaptive radiation a process where a lineage of organisms rapidly diversifies into many different forms and taxa with different adaptations; it can be triggered by many factors, such as changes to available resources, or other new challenges or opportunities; this is a type of divergent evolution

adhesion proteins proteins on the surface of cells (e.g. cadherins) that are involved in binding with other cells or to an extracellular matrix in a process called cell adhesion

adjuvant a substance added into a vaccine along with an antigen to improve the immune response to that antigen

aestivation dormancy in some animals during periods of drought

agarose gel gel matrix used for electrophoresis

agglutination when antigens or pathogens become stuck together because of antibody binding

algorithm a method expressed as a list of instructions for calculating a function; the instructions describe a computation from the start through a sequence of steps to the final output

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

allopatric speciation speciation that occurs due to physical or geographic isolation

amine hormone a hormone derived from amino acids; examples include epinephrine, dopamine and thyroxine

amino acid binding site the site of attachment of an amino acid to a tRNA molecule

ammonia a toxic chemical molecule produced as a by-product of protein synthesis

analogous structures features of organisms that have the same function but not the same structure

anaphylactic shock a severe form of allergic reaction that causes widespread swelling, including of the face and neck, which can make breathing difficult

aneuploidy describes a genome that varies from the conventional by the loss or addition of one or just a few chromosomes

annealing in PCR, a process of joining separate strands of DNA together as a result of hydrogen bonds pairing; occurs when the temperature is lowered

antibiotic naturally produced or synthetic compounds that are toxic to bacteria

antibody a Y-shaped protein produced by plasma cells that binds to a specific antigen; also called immunoglobulin

anticodon a sequence of three nucleotide bases on a tRNA molecule that pairs with the complementary bases of an mRNA strand during translation at the ribosome

antigen presenting cell (APC) a cell that processes antigens of pathogens after phagocytosis and presents them to T cells in MH C class II; macrophages and dendritic cells

antigen a large molecule, usually a protein or polysaccharide, that generates an immune response

antiseptic a substance that kills or inhibits the growth of micro-organisms on external surfaces of living things

apoptosis a programmed series of events that lead to cell death as a result of dismantling of the internal contents of the cell by various enzymes including caspases

artificial selection the breeding of plants and animals to produce desirable traits in successive generations; also known as selective breeding

aseptic technique the technique of working under sterile conditions to prevent contamination of samples

asexual reproduction a form of reproduction in which offspring are produced from a single parent

assay an experimental technique or procedure used to test a specific biological process or effect; for example, an enzyme kinetic assay

autoclave a device used to sterilise equipment, reagents or contaminated waste; autoclaves work by subjecting contents to pressurised steam at 121°C for a set time

autocrine hormone a hormone whose target cell is the secretory cell itself or neighbouring cells of the same type

autoimmune disease a condition where the immune system attacks the body's own tissues

autonomic system part of the peripheral nervous system that deals with involuntary control

autosome chromosomes that are the same in both males and females of a species; they do not include sex chromosomes

axon the extension from the cell body that aids the transfer of the electrical impulse along the nerve

B cell receptor (BCR) a surface-bound antibody that serves as a receptor so that B cells are able to detect antigens

B lymphocyte/cell a class of lymphocytes; once activated, they are characterised by the production of antibodies

bacterial capsule a slimy layer sitting outside the cell wall of some species of bacteria

bacteriophage a virus that invades bacteria

basophil a circulating leukocyte that secretes histamines when damaged

behaviour responses and reactions of an organism in particular situations

beneficial mutation a mutation that increases an organism's chances of survival and reproduction

binary fission the division of a cell into two without mitosis; a prokaryotic cell splits to form two daughter cells

binding site a region on a protein, DNA or RNA molecule to which other specif ic molecules and ions bind through forming chemical interactions

binding specificity occurs when the shapes and charges of molecules allow them to selectively recognise and bind to each other

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 form a final product in a cell

biogeography the study of the distribution of living things over a geographical area through geologic time

bioinformatics the science of managing and analysing biological data using advanced computing techniques; it is especially important in genomics research because of the large amount of complex data this research generates

biotechnology the use of living organisms and biological systems and processes for human benefit

bivalent visible bodies in a cell during prophase I of meiosis, which are made up of two homologous chromosomes joined together

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

body fluid any liquid that comes from inside the body

bottleneck effect when a catastrophic event or a period of adverse conditions drastically reduces the size of a population

capture–mark–recapture an ecological surveying method used to measure animal populations, in which individual animals are captured, marked and released; after a time, the population is re-sampled and the number of marked animals caught gives an indication of population size

carrier usually used in reference to disease; a healthy, heterozygous organism carrying an allele for a recessive phenotype; the organism may transmit the recessive allele and resulting phenotype to its offspring or to others

case definition a definition that includes a particular disease, time and place and is used to help identify individuals affected by a disease outbreak

case an individual who is infected with an infectious disease

catabolic reaction a chemical reaction whereby bonds in molecules are broken, releasing energy

cell cycle the sequence of events from one cell division to another

cell differentiation the process by which a less specialised cell develops or matures to have more distinct characteristics and functions

cell metabolism the set of chemical transformations that take place in cells so they can grow and reproduce, maintain structures and respond to their environment

cell plate the structure produced by dividing plant cells where the new cell wall is to be formed

cell signalling a complex system of signal transduction pathways that governs basic cellular processes and coordinates cell actions

cellular process any process that is carried out at the cellular level but is not necessarily restricted to a single cell; for example, cell communication occurs among more than one cell but occurs at the cellular level

cellular response in chemical signalling, any process that results in a change in state or activity of a cell; for example, secretion, movement, gene expression, enzyme activation

centriole a minute rod-shaped body present in many resting cells just outside the nuclear membrane; it doubles before mitosis, moving apart to form the poles of the spindle; usually absent in plants

centromere the waist-like constriction in a chromosome required for the movement of chromosomes during cell division

chemokine a molecule that induces chemotaxis; some cytokines are chemokines

chemoreceptor a sensory cell or organ that detects chemical stimuli

chemotaxis the movement of an organism or cell along a chemical concentration gradient either towards (positive chemotaxis) or away from (negative chemotaxis) the chemical stimulus

chiasmata (singular chiasma) the point of contact between homologous chromosomes during prophase I of meiosis

chitin a polysaccharide that is the main component of fungal cell walls and the exoskeletons of insects and other arthropods

chromatid daughter strands of a duplicated chromosome that are joined together by a centromere

chromatin a complex of proteins and DNA in eukaryotic chromosomes

chromosome a structure composed of DNA and protein that contains along its length linear arrays of genes carrying genetic information; prokaryotes have one circular chromosome whereas eukaryotes have a number of linear chromosomes

cilia slender hair-like structures projecting from the cell surface, which beat against fluid outside the cell

cleavage furrow a shallow, ring-like depression that forms at the cell surface of an animal cell undergoing cytokinesis as contractile microfilaments pull the plasma membrane inward; it defines where the cytoplasm will be cut in two

cleavage division of the cytoplasm in an animal cell

clonal selection the process in which lymphocytes that have bound to an antigen rapidly divide and become more numerous than other clones

cloning vector in cloning, the DNA molecule that is used to carry the cloned piece of DNA

coding region the small part of a DNA strand used as a template for synthesis of an mRNA strand; also known as a gene

codominant a state in which both alleles of a heterozygous individual are fully expressed in the phenotype

codon a series of three adjacent nucleotide bases in mRNA; each codon specifies a particular amino acid to be added to a polypeptide; a stop codon indicates the termination of the polypeptide chain

cognition mental processes relating to sensing, perceiving, thinking, and remembering

common ancestor a species from which other species have evolved

communicable able to be communicated (transmitted) from one organism to another

companion plant a plant that is grown together with another plant because one species improves the growth of the other

comparative dating the process of determining the age of rocks and their contained fossils relative to each other, allowing an estimation of 'oldest to youngest' without assigning an actual age in years

comparative genomics the process of contrasting the entire hereditary information of organisms as encoded in their DNA

complement activation bound antibodies activate complement proteins

complement system a number of small proteins found in the blood that, when activated, promote chemotaxis, cell lysis and phagocytosis

conduction the transfer of heat energy from a relatively hot object to a relatively cool object by direct contact

conformation the shape of a molecule that is determined by the three-dimensional arrangement of its atoms and bonds; important for molecular functioning

conserved (sequences) DNA or protein sequences that are preserved across species

contact tracing a process for identifying potential cases; recent contacts of an infected individual are contacted and screened for the infection

contact-dependent signalling a type of cell signalling system where the signal molecule remains bound to the surface of a cell and will only signal to those cells that come into contact with it

contagious able to be transferred by direct contact

continental drift the relative movement of Earth's continental landmasses that appear to drift or 'float' over Earth's mantle

continuous variable a variable that is able to take any value within a range; length, time and temperature are examples of continuous variables

continuous variation a variation in a characteristic that shows a smooth range of different phenotypes

controlled variable the variable that is controlled by the experimenter, so that its values are chosen; also called the independent variable

convection the transfer of heat by means of the rising currents of warm air or water

convergent evolution a process whereby unrelated organisms evolve similar adaptations in response to their environments

countercurrent a current that follows in the opposite direction to another current

crossing over an event during meiosis in which homologous chromosomes exchange segments with one another

cyclic adenosine monophosphate (cAMP) a second messenger formed from ATP that is responsible for the intracellular mediation of hormonal effects on various cellular processes

cytokines small signalling molecules that coordinate inflammation and immune responses, and that leukocytes use to communicate with one another; includes interleukins and interferons

cytokinesis division of the cytoplasm

cytotoxic T (T_c or killer T) lymphocyte/cell a class of lymphocytes that destroys virally infected or cancerous cells by secreting proteins that cause apoptosis

damage- or danger-associated molecular pattern (DAMP) a body (or plant) component that is released during tissue damage, such as internal cellular components that stimulate innate immune responses

defensin a small antimicrobial peptide secreted by virtually all plants and animals

definitive host a host in which the adult phase of a parasite produces gametes

deleterious mutation a mutation that decreases an organism's chances of survival and reproduction

deletion mutation a mutation in which nucleotide pairs have been lost from a segment of DNA

dendritic cell a type of antigen presenting cell

dependent variable the variable that changes as a result of changes to the independent or controlled variable

descent with modification Darwin's terminology indicating that life today has descended and evolved from common ancestors that were generally different to their modern descendants

dihybrid cross a cross between two organisms that are heterozygous at two gene loci

dihybrid inheritance inheritance of two pairs of contrasting characteristics

diploid (2*n*) describes a cell or organism that has a genome comprising two copies of each chromosome, represented by 2*n*

direct life cycle the life cycle where a parasite completes its development in a single host

directional selection a form of selection that selects against one of two extremes and leads to a change in a trait over time

discontinuous variation a variation in a characteristic that shows two or just a few clearly distinct phenotypes

discrete variable a variable that may take only certain values; number of individuals, or number of legs on an animal are examples of discrete variables

disinfectant a substance that destroys microorganisms and their spores but is too strong to be used directly on skin

disjunction moving apart of homologous chromosomes during anaphase of meiosis

disruptive selection a form of selection that operates in favour of extremes and against intermediate forms

divergent evolution when related species evolve new traits over time, away from the common ancestor, to give rise to new species

DNA (deoxyribonucleic acid) an information molecule that is the universal basis of an organism's genetic material; it contains instructions, written in a chemical code, for the production of proteins by the cell

DNA fingerprinting also called DNA profiling; based on patterns of non-coding, repeating base sequences in the genome

DNA helicase an enzyme that helps the two strands of the DNA double helix unwind and separate

DNA ligase an enzyme used to catalyse the formation of a bond between two pieces of DNA

DNA polymerase an enzyme capable of making exact copies of fragments of DNA

DNA profiling a process that is able to identify natural variations that exist within an individual's genome, by using the polymerase chain reaction and gel electrophoresis

DNA sequencing a process of establishing the nucleotide sequence of a piece of DNA

domain the functional region or portion of a protein

dominant a phenotype that requires only one copy of its allele in an individual to be expressed

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

down-regulate (down-regulation) the process by which a cell decreases the quantity of a cellular component, such as RNA or protein

ductless gland a gland that secretes its product directly into the bloodstream

ectoparasite a parasite that lives on the surface of another organism

ectotherm an animal that depends on a source of external heat

effector an organ, cell or protein that acts in response to a stimulus

embryonic stem cell a pluripotent stem cell derived from an embryo

encyst when organisms produce a covering around themselves and enter a resting stage

endemic broadly, common to a particular area; specifically, a pathogen that is prevalent at a constant rate within a population

endocrine disruptor synthetic or natural chemicals that mimic hormones and in doing so disrupt hormone regulation by interfering with cell signalling pathways

endocrine hormone hormone that is secreted into the bloodstream and can bind to distant target cells

endocrine system the bodily system responsible for the production and secretion of hormones, which are released into the bloodstream to act on specific target cells and organs

endospore tough, dormant structures formed by many bacteria to help them resist unfavourable conditions and disperse to new hosts

endotherm an animal that retains heat generated by metabolic activity

enhancer region regions found in eukaryotic DNA that act as binding sites for some activator proteins

eon a division of geologic time that can be divided into periods, epochs and ages

eosinophil a leukocyte that secretes powerful enzymes capable of rupturing multicellular pathogens

epidemic an increase in the occurrence of a particular disease above the baseline level for that population; tends to refer to larger, more serious events than the term outbreak

epidemiologist a scientist who studies the causes and effects of diseases at a population level

epigenetics the study of chemical modifications to gene function that are not due to DNA sequence changes; DNA methylation is an example

epigenome chemical compounds that modify the genome

epithelial cells cells that line the inner and outer surfaces of body and body cavities; bound together in sheets of tissue called epithelia

epitope a small part of a larger molecule that binds to a receptor site; examples are B cell receptors and T cell receptors

epoch a division of geologic time that is shorter than a period and is marked by one or more significant events

era a division of geologic time comprising periods and epochs

estuary a transitional region where fresh water from a river meets salt water from the sea

ethidium bromide a chemical that binds to double-stranded DNA and fluoresces pink when exposed to ultraviolet light; used to locate DNA in an agarose gel following electrophoresis

euryhaline organisms that can tolerate a wide change in salinity

evaporation the process in which liquid water changes to water vapour through heating

evolution the process of gradual change in the gene pool of a population of organisms that results in new species

exome all the genome's exons

exon the region of DNA or RNA transcript that encodes a protein sequence

exteroceptor a receptor that receives signals from the external environment

extracellular receptor a receptor molecule located in the cell membrane that has a binding site located outside of the cell to which hydrophilic signalling molecules bind

extracellular occurring outside of a cell or cells

extrapolation extension beyond the measured range of data to read or construct new data that has not been measured

falsifiable able to be disproved

feedback inhibition a cellular control mechanism in which an enzyme that catalyses the production of a particular product is inhibited by the product, therefore balancing supply and demand of a product for a cell

feedback mechanism a mechanism in which the output or response affects the input or stimulus

fertilisation the union of male and female gametes

fever increased body temperature

first filial generation (F_1) the first generation of offspring produced from a cross between two homozygous parents (P)

fitness the capacity of an individual to survive and pass on viable offspring

flagellum a whip-like appendage that helps bacteria move

fomite an inanimate object that can be contaminated with a pathogen

fossil preserved remains or traces of an organism

founder effect a 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 that dislocates the translational reading frame

gamete a cell produced in sexual reproduction, which combines at fertilisation; in humans, the gametes are ova and sperm cells; in flowering plants, pollen grains contain male gametes and ova contain a female gamete

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

gene expression the process of information from a gene being transcribed into mRNA and translated into a protein

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 probe a specific short length of singlestranded DNA molecule that can bind specifically to a gene of interest

gene regulation the processes within a cell that control gene expression; it controls what genes are turned on and off, when and where

gene therapy a method of delivering normal and fully functioning genes to individuals who have a mutated defective version of the particular genes

gene a unit of heredity that transmits information from one generation to the next; a segment of DNA that codes for polypeptide

genetic code a system by which each combination of three DNA nucleotides in a gene sequence determines a specific amino acid in the protein

genetic disease a disease arising from mutations inherited from parents

genetic drift a 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 organisms (GMOs) see *transgenic organisms*

genetics the study of the mechanism and patterns of inheritance through the transmission of coded chemical instructions from one generation to the next

genome all of the genetic material contained in an organism or a cell; includes the chromosomes within the nucleus and the DNA in mitochondria and chloroplasts

genomics the study of the genome – how genes interact with each other and the environment and the resultant proteins produced; it requires a knowledge of an organism's entire DNA sequence so studies rely on powerful technologies and bioinformatics

genotype a specific combination of alleles for a particular gene locus belonging to an individual or cell

germ-line gene therapy replacement of a faulty gene within a germ-line cell

germ-line the cell line in eukaryotic organisms from which the gametes are derived

glycogen an important energy-storing polysaccharide

gradualism a theoretical model of the pace of evolution occurring as a steady, slow divergence of lineages at an even speed, irrespective of gaps in the fossil record

granulocyte a leukocyte containing intra-cellular granules

haploid (*n*) describes a cell or organism that has a genome that contains one copy of each chromosome, represented by *n*

helper T (T_H) lymphocyte/cell a class of lymphocytes that aids T_C cells, B cells and macrophages by secreting cytokines

hemizygous a gene that occurs only as a single copy in a diploid organism or cell

herd immunity refers to the phenomenon that once a particular proportion of a population is immune to a disease, susceptible individuals are also better protected from the disease

heredity the study of inheritance; the genetic transmission of characteristics from one generation to another

heterosome non-identical chromosomes pairing up at meiosis (e.g. the XY chromosomes in human males)

heterozygous a genotype with two different alleles for a single gene locus

hibernate a period of dormancy over long periods of cold conditions

histamine a chemical released by mast cells and basophils that increases blood flow and the permeability of capillaries

histone a protein around which DNA winds in eukaryotic cells

homeobox gene a gene that codes for proteins that regulate body formation and patterning in the developing embryo

homeostasis the maintenance of a relatively constant internal environment within small tolerance limits, despite changes in the external environment

homeothermic the ability to maintain a relatively constant internal body temperature

homologous chromosomes a pair of chromosomes that have the same size, shape and genes at the same locations

homologous structures features of organisms that have the same general structure but different functions

homozygous lethal phenotype a phenotype that arises from a homozygous recessive genotype, leading to the premature death of an organism

homozygous a genotype with two identical alleles for a single gene locus

horizontal gene transfer a process by which genetic material from one organism becomes incorporated into the genome of another organism

hormone a chemical messenger secreted directly into the bloodstream, other body fluids, or into adjacent tissues, where they move to their target cells

host an organism that is infected by a pathogen

housekeeping gene a gene that encodes proteins that are required to maintain basic cellular processes

humoral an immune response brought about by antibodies that circulate freely in the bloodstream and can lead to the destruction of pathogens

hybrid offspring from parents from two different species; some hybrids are also fertile and can produce further offspring

hybridoma created by fusing a B cell clone with cells from a plasma cell tumour; produces monoclonal antibodies and divides repeatedly

hydrophilic hormone a hormone that is water soluble and binds to extracellular receptors to initiate a response in that cell; for example, peptide and some amine hormones

hydrophobic hormone a hormone that is water insoluble and binds to intracellular receptors; for example, steroid and thyroid hormones

hyperthermia a state in which the internal temperature rises above the set point

hypertonic describes a solution with a higher solute concentration compared with another solution

hypothermia state in which the internal temperature drops below the set point

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

hypotonic a solution with a lower solute concentration compared with another solution

immune system a complex network of cells, tissues and organs in the body that detects differences between 'self' and foreign organisms, and mounts an immune response

immune having resistance to infection by a specific pathogen

immunodeficiency a state in which the immune system does not function properly, leaving a person susceptible to infections the immune system could normally fight off

immunoglobulin (Ig) see *antibody*

immutable unchanging; the idea (now considered incorrect) that species did not change over time

imprinting an epigenetic process where one allele of a gene is methylated and hence 'silenced'

in vitro when processes or reactions take place in a test tube, culture dish or elsewhere outside a living organism; as compared to *in vivo* where the process takes place in the living organism

incomplete dominance the state in which a heterozygous individual has a phenotype that is intermediate between those of the corresponding homozygous individuals

incubation period the time between infection and the onset of symptoms

independent assortment when alleles of gene pairs redistribute independently into different combinations in gametes during meiosis

independent variable a variable upon which another variable depends; usually the controlled variable

index case the initial case of a contagious disease

infectious an agent that can be transmitted from one organism to another

infectivity the ability of a pathogen to spread from one host and infect another host

inflammation an innate response to infection or damage that causes swelling, pain and redness

inheritable capable of being passed on to the next generation

inheritance genetic acquisition of characteristics by offspring from their parents

inhibitor a substance that slows down or prevents a particular chemical reaction; by binding to proteins, inhibitors change the protein conformation so it no longer performs its job

innate immune response 'innate' means not learned; as applied to the innate immune response, one that is not specific and does not have 'memory'

insertion mutation a mutation in which nucleotide pairs have been added to a segment of DNA

intercellular occurring between cells

interconnecting neuron located in the CNS; transfers signals from sensory neurons to motor neurons

interferon a class of glycoprotein cytokines 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 a larger group of cellular messenger molecules called cytokines, which are cell signalling molecules

intermediate host an organism in which a pathogen or parasite undergoes development and spends a small proportion of its life cycle

interoceptor a receptor that receives signals from the internal environment

interphase the stage between nuclear divisions

interpolation to read or construct a new data point that has not been measured but is within the range of measured data

interstitial fluid a fluid that lies in the spaces between cells; also known as tissue fluid

intracellular receptor a receptor molecule located inside the cell to which hydrophobic chemical signals bind; examples include transcription factors

intracellular occurring within a cell or cells

intraspecific variation differences between individuals of the same species

intron a section of DNA or pre-mRNA that does not encode a protein sequence; an intron is removed ('spliced') from pre-mRNA to form a mature mRNA molecule

ion channel a protein or protein complex that spans the cell membrane, forming a channel to facilitate the movement of ions across the membrane

ion gradient the concentration gradient of ions across a membrane; also referred to as an electrochemical potential

isolating mechanism a mechanism that prevents organisms from mating or producing viable offspring

isotonic a solution with an equal concentration of solutes compared to another fluid

isotope atoms of an element that have the same number of protons but different numbers of neutrons, and therefore different relative atomic masses

isotype a subtype of immunoglobulin; each isotype (IgG, IgM, IgA, IgE and IgD) performs a different function

karyotype a display of the number and appearance of the chromosomes of an organism or cell observed at metaphase

keratin a strong, stable structural protein found in skin, hair, horn and nails

keratinised a process by which keratin is deposited in skin cells; the surface becomes tough and waterproof

leukocyte the general term for white blood cell

line of best fit the line that most accurately fits the data, usually calculated using linear regression

linear regression a statistical tool used to model the dependence of one variable on another

linked genes – or their alleles – that are inherited together more frequently because they are located near each other on the same chromosome

lipopolysaccharide a lipid-sugar compound forming the outer surface of some types of bacteria

locus (plural loci) the position a gene occupies in a chromosome

logbook the record of an experiment or investigation kept by the scientist performing the experiments; it is a legal record of the experiments and their results

lower critical temperature the external temperature at which metabolic activity begins to rise, thereby increasing the output of heat

lymph node an immunologic organ in which antigens are trapped or delivered by phagocytes for initiation of an adaptive response

lymph a colourless fluid that originates from the extracellular (tissue) fluid

lymphatic system part of the immune system; a system of organs (thymus, bone marrow, spleen, lymph nodes, network of vessels) and lymph fluid that are involved in transporting lymphocytes and in removing foreign matter

lymphocyte a type of leukocyte involved in adaptive immune responses

lysis the process of a cell bursting (verb: to lyse)

lysogenic phase part of life cycle of a virus in which the nucleic acid of the virus is integrated into host cell's DNA

lysozyme an antibacterial enzyme found in tears, saliva and other body fluids

lytic phase part of life cycle of a virus in which viral components are replicated and packaged to form new viruses that lyse the host cell

macroevolution the evolution of new groups of organisms comprising many related species through multiple speciation events; includes adaptive radiations

macrophage a large white blood cell in tissues that phagocytoses pathogens

major histocompatibility complex (MHC) protein markers found on the cell surface that are important in recognising 'self' from 'non-self'; there are two classes: MH C class I is found on all cells and MH C class II is found only on antigen presenting cells

mass extinction extinction of many species over a relatively short (geological) period of time

mast cell located in the tissues; when activated, releases granules containing histamine

mast ring a ring of paper with small circular 'offshoots' impregnated with different antibiotics

mechanoreceptor a sensory cell or organ that detects mechanical stimuli such as touch, pressure, vibration or tension

megafauna generally refers to vertebrate species that were significantly larger than other species of the same type

meiosis a two-phase type of cellular division in which the chromosome number of a cell is halved to the haploid number; meiosis is the basis of gamete formation in animals and spore formation in plants

messenger RNA (mRNA) a ribonucleic acid formed in the nucleus during gene transcription, its sequence being complementary to DNA exons; it travels to the cytoplasm, where its information is translated by the ribosomes to add amino acids together to form proteins

metabolism the sum of all chemical reactions occurring within an organism to maintain life

methylated cap a modified guanine nucleotide that has a methyl group and a phosphate group bonded to it, which is added to pre-mRNA at the 5' end; also known as the 5' cap

methylation the attachment of a methyl group to nucleotides or histone proteins

MHC restriction refers to the fact that T cells can only recognise antigens that are presented in MHC proteins

microevolution any change in the gene pool of a single population over a short time

microflora community of micro-organisms, including fungi and bacteria that live in or on another living organism

micro-organism a microscopic organism; for example, bacteria

microRNA (miRNA) a small non-coding segment of RNA that plays a role in regulating gene expression at the post-transcription level

missense mutation a gene mutation that results in one amino acid being replaced by another amino acid in the encoded protein

mitosis a type of nuclear division that maintains the parental number of chromosomes for daughter cells; it is the basis of bodily growth and asexual reproduction in many eukaryotic species

model the artificial conceptual or abstract simulation of a real-world process or system, developed by simplifying key steps that produce reliable and consistent agreement as verified by field studies; a model may be mathematical equations, a computer simulation, a physical object, words or other form

modern synthesis the theory of evolution incorporating our understanding of how traits are inherited

molecular homology the identification of shared biomolecular elements – generally genes – used to test the relationships between organisms, which can demonstrate common ancestry

molecular phylogeny the study of evolutionary relationships using comparative genomics

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 the antibodies produced by a hybridoma; they are identical to the antibodies produced by the original cell

monocyte a white blood cell that circulates in the blood and matures into a macrophage when it moves from the blood into the tissues

monohybrid cross a cross between two organisms that are heterozygous at one gene locus

monoploid (1*n*) a cell or organism that has a functional genome consisting of one copy of each chromosome, represented by 1*n*

monosomy the condition in which somatic cells contain one copy of a particular chromosome

morbidity the impact of a disease within a population, measured by the number of people affected by that disease

morphological species concept to define a species using measurable anatomical criteria and characteristics

mortality the impact of a disease within a population, measured by the number of deaths caused by that disease

motor neuron a nerve that transmits impulses from the central nervous system to effector

mucus membrane a mucus-secreting membrane that lines the respiratory, excretory and reproductive tracts

multipotent a stem cell that is able to differentiate into a limited number of cell types

mutagen an agent capable of inducing mutations

mutant a cell or organism that bears a mutation

mutation rate the number of changes per gene copy in a population over a period of time

mutation a gene or chromosome that 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, sometimes expressed as millions of years before present (myBP), or simply millions of years (my); for example, a fossil dated as being 5 million years old lived 5 mya

myelin sheath the fatty layer surrounding and insulating the axons of many neurons; increases the speed at which electrical impulses travel along the nerve cell

natural history the course that a disease would be expected to follow if left untreated

natural killer (NK) cell a circulating leukocyte that kills body cells infected with a virus or transformed by cancer

natural selection the process where individuals with certain inheritable traits survive and reproduce more successfully than other individuals, leading to evolutionary change in the population

necrosis unprogrammed cell death that stimulates inflammation

negative feedback when a change of variable (stimulus) occurs, a response that reverses the direction of the change

nervous system the network of nerve cells and fibres that transmits nerve impulses to provide communication between parts of the body

neuron a nerve cell

neurotransmitter a chemical substance that carries the action potential across a synaptic cleft

neutral mutation a mutation that has no effect on an organism's chances of survival and reproduction

neutralisation the process by which antibodies can prevent toxins from acting; that is, by binding to them and blocking them from binding to anything else

neutrophil a phagocytic leukocyte found in the blood and tissues

niche an organism's habitat; or a way of life or function of an organism in its environment

nitrogenous bases a structural component of nucloetides, DNA has adenine (A), cytosine (C), guanine (G) and thymine (T); in RNA thymine is replaced with uracil (U)

node the small gap between two myelin cells

NOD-like receptor (NLR) a type of pattern recognition receptor (PRR); intracellular sensors of PAMPs and DAMPs

non-coding DNA all of the DNA sequences within a genome that are not found within RNA-coding exons; examples include introns, promoters and enhancers of genes

non-disjunction the failure of sister chromatids in mitosis or homologs in meiosis to separate and go to opposite poles

non-infectious disease a disease that is not transmitted from one organism to another

'non-self' describes agents (e.g. cells, organisms, substances) that are not recognised by the immune system as being part of the organism itself; they are foreign

nonsense mutation a mutation in which a codon for an amino acid is changed to one that codes for a stop codon, terminating translation

non-specific when the response is the same regardless of the type of pathogen

non-template strand a strand that is complementary to the template strand; it does not guide the synthesis of complementary polynucleotides

nosocomial infection an infection that is spread in a healthcare setting

notifiable disease a disease that, if diagnosed, is required to be reported to public health authorities

nucleoid the region within a prokaryotic cell that contains the genetic material

nucleolus a structure found within the nucleus of a non-dividing cell; a site in which the protein and RNA subunits of ribosomes are assembled

nucleotide the basic building block of nucleic acids (DNA and RNA) linked together by phosphodiester bonds; each nucleotide is made up of a five-carbon sugar, a phosphate group and a nitrogenous base

obligate describes an organism that can survive only in another organism; it is 'obliged' to live there

operator a region of DNA situated around a promoter that interacts with a specific repressor; when bound with a repressor protein, it prevents transcription of the structural gene

opsonisation a process in which a pathogen is coated with antibodies and marked for ingestion and destruction by phagocytes

optimum range the narrow range within the tolerance range an organism has for a particular factor, at which the organism functions best

organelle a specialised part of a cell, with its own specific function; 'little organ'

osmoconformer an organism in which the internal solute concentration changes with the concentration of solutes in the external environment

osmoregulation processes by which internal water and solute concentration are maintained despite fluctuations in the external environment

osmoregulator an organism that has specialised mechanisms for regulating internal water and solute concentrations, despite concentration changes in the external environment.

outbreak an increase in the occurrence of a particular disease above the baseline level for that population; see also *epidemic*

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

pain receptor a sensory cell or organ that detects pain signals

pandemic an epidemic that has spread across multiple continents or worldwide

paracrine hormone a hormone for which the target cell is close to the signal releasing cell, and the hormone is broken down too quickly to be carried to other parts of the body

parasite an organism that lives on or in its host for all or part of its life, causing harm and gaining nutrition from the host

parental generation (P) two individual organisms that represent the start of a breeding experiment; their offspring are the F_1 generation

parthenogenesis the production of offspring from a female gamete without the requirement for fertilisation

partial dominance see *incomplete dominance*

passive immunity immunity characterised by the transfer of antibodies from one individual to another; this type of immunity does not show memory

pathogen a disease-causing agent

pathogen-associated molecular pattern (PAMP) a broad molecular pattern commonly shared by a number of pathogens

pathogenicity the capacity of a pathogen to cause disease

pattern recognition receptor (PRR) a cell receptor that recognises molecular patterns commonly shared by a number of pathogens; includes nod-like receptors and toll-like receptors

peptide bond a bond that forms between two amino acid monomers with the elimination of a water molecule

peptide hormone a hydrophilic hormone composed of a chain of amino acids so it can bind to extracellular receptors on target cells; for example, insulin and ADH

peptidoglycan a protein–carbohydrate compound that forms the cell wall of bacteria

period a division of geologic time; periods and epochs together make up eras

phagocyte a cell that is capable of phagocytosis; includes macrophages and neutrophils

phagocytosis the bulk transport of solids into a cell inside a vesicle

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

phosphorylation the addition of a phosphate group to a protein or other organic molecule

photoreceptor a sensory cell or organ that detects light signals

phototropism a plant's hormonal response to light, whereby auxin accumulates on the darker side of the plant to stimulate cell elongation, bending the plant towards the light to increase its ability to photosynthesise

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 evolutionary relationships that exist between species, often expressed as a tree-like diagram

physiological stress stress caused when an organism experiences conditions outside its tolerance range

phytoalexin a chemical produced by plants under attack

plagiarism presenting someone else's work, including their words or ideas, as your own

plasma cell an effector B cell that has differentiated to become highly specialised for producing antibodies

plasmid a small circular piece of DNA, found in bacteria, which is able to replicate independently of the cell's chromosomes; plasmids carry antibiotic resistance markers

platelet a cell fragment found in the blood that helps blood to clot

pluripotent a stem cell with wide regenerative capacity, able to differentiate into very many different cell types

poikilothermic an organism whose body temperature changes with the temperature of its surroundings

point mutation a mutation that affects a single basepair position within a gene

poly-A tail an untranslated feature of mRNA that enhances its stability; consists of about 100–200 adenine nucleotides added to the 3′ end of pre-mRNA

polygene a gene for which the alleles have a small additive effect on a phenotype; many polygenes together contribute to continuous variation in a phenotype

polygenic inheritance transmission between generations of characteristics that are controlled by polygenes

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

polypeptide the polymer of many amino acids linked by peptide bonds; forms a protein or part of a protein

polyploidy a cell or organism with a genome comprising three or more copies of each chromosome, represented by 3*n*, 4*n*, 5*n*, 6*n* etc.

polyribosome many ribosomes forming into chains along an mRNA strand

population genetics is the study of allele frequencies in populations and how they change over time in response to various evolutionary processes

population a group of individuals of the same species that live in the same area interbreed, producing fertile offspring

positive feedback when a change of variable (stimulus) occurs, a response that changes the variable even more in the same direction

post-reproductive isolating mechanism a mechanism that prevents fertilisation occurring or an embryo developing into viable offspring if fertilisation does occur

pre-mRNA unmodified, 'immature' RNA containing introns and without the 5' methyl cap and 3' poly-A tail

pre-reproductive isolating mechanism a mechanism that prevents organisms from being able to interact to reproduce

primary data data that you have measured or collected yourself

primary lymphoid organs 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

primer a single-stranded DNA molecule that acts as the start of the amplification process

prion a small infectious protein

prokaryote a microscopic single-celled organism with no distinct nuclear membrane and no organelles except ribosomes

promoter a short stretch of DNA usually at the start of the gene to which RNA polymerase can bind and start transcription, specifies the timing and location of gene transcription

prostaglandins autocrine and paracrine hormones made from fatty acids

proteomics the study of the entire protein content expressed by a cell.

punctuated equilibrium a theoretical evolutionary model of an organism's change occurring rapidly and in relatively brief events between longer periods of stasis (or equilibrium) without record in the fossil record

Punnett square a grid used to graphically predict the outcome of a cross or breeding experiment

pure-breeding a line of organisms that always produce offspring with the same phenotype when crossed with each other

qualitative measurement a measurement with descriptive or non-numerical results

quantitative measurement a measurement with numerical values

quarantine the enforced isolation of individuals at risk of carrying disease to prevent the spread of that disease into healthy populations

radiation the transfer of heat from a hot object by infrared waves

receptor a structure that detects or receives a stimulus

recessive a phenotype that requires two copies of its allele in an individual to be expressed

recombinant DNA technology transferring a gene from a cell of a member of one species to the cell of a different species

recombinant plasmid a plasmid with foreign DNA inserted into it

reference the source of a specific piece of information or quotation

regulator gene a gene that codes for the production of a repressor protein that inhibits the action of an operator gene, thereby preventing transcription of a structural gene

regulatory protein a protein that binds DNA to switch on or switch off expression of a gene

regulatory T (T_{reg}) lymphocyte/cell a class of lymphocytes that helps to regulate the immune response

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

repetitive DNA DNA sequences that are present in very many copies in the genome, usually regarded as non-functional DNA

replication fork the junction between the unwound single strands of DNA and the intact double helix during replication

repressor protein a protein coded for by the regulator gene that binds to an operator gene, which inhibits transcription of a structural gene

reproducible giving the same result, within uncertainty limits, when repeated measurements are made

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

resistance describes the extent to which an organism is or is not affected by an agent such as a pathogen or chemical toxin

resolving a mediator that reduces and resolves inflammation

response the result of a stimulus

resting potential the electrical potential difference between the two sides of an unstimulated nerve cell's plasma membrane; when this potential exists, the cell is ready for action

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 specific nucleotide sequence (usually 4–8 bp) that is recognised as a cleaving site for a restriction enzyme

ribosomal RNA (rRNA) a folded molecule of RNA that is formed in the nucleolus of eukaryotic cells, which combines with proteins to form ribosomes

ribosome the site of protein synthesis in all cells; a ribosome consists of two rRNA subunits that lock onto an mRNA molecule; the ribosome moves along mRNA to translate its code and link amino acids, forming a polypeptide

RNA (ribonucleic acid) a molecule consisting of a single strand of nucleotides; it plays an essential role in protein synthesis (as messenger RNA and transfer RNA) and as a structural component of ribosomes

RNA polymerase an enzyme involved with adding RNA nucleotides together

scatter graph a graph or plot showing data points, without a line joining the points, and used to demonstrate or determine a mathematic relationship between variables; the axes are defined by the variables

second filial generation (F_2) offspring of the F_1 generation; the second generation produced from a cross between two homozygous parents (P)

second messenger small molecules that relay a signal from receptors on the cell surface to target molecules inside a cell

secondary data data or information that has been collected by someone else

secondary lymphoid organ an organ that provides an environment for the initiation of the immune response

secondary response the response generated when the body encounters a pathogen it has previously generated an immune response to; occurs more rapidly and is of greater magnitude than the primary response

selection pressures factors that influence the survival of an individual within a population

selective breeding a process by which humans domesticate animals or plants by purposely choosing individuals with the most desirable characteristics as parents for each successive generation of breeding

'self' describes agents (e.g. cells, organisms, substances) that are recognised by the immune system of an organism as being part of that organism; the immune system coexists with all cells in the body without attacking them because cells carry marker molecules that identify them as belonging to 'self'

self-tolerance the deletion or inactivation of lymphocyte clones that can bind to 'self' antigens; as a result, no immune response can be mounted against these antigens

semi-conservative replication the production of two new DNA double helix molecules, each consisting of one parental strand and one daughter strand

sensitivity limit the portion of a curve that is nonlinear; data that falls into these non-linear regions cannot be extrapolated

sensory neuron a nerve that transmits nerve impulses from the receptor towards the central nervous system

sex chromosome chromosomes that affect sexual traits; one sex has homologous sex chromosomes, the other sex has a dissimilar set

sex-linked a gene located on a sex chromosome

sexual dimorphism the situation where males and females of a species have different morphologies, often in shape or size

sexual reproduction a form of reproduction in which offspring are produced from two parents

short tandem repeats (STRs) a short non-coding region of DNA that is repeated many times in the genome of an organism; it is highly variable between individuals and can be used in DNA profiling; ST Rs have a repeat sequence of two to five bases

signal transduction the process by which a cell converts one kind of signal into another; occurs when an extracellular signal binds to and activates a receptor, which, in turn, alters intracellular molecules to bring about a cell response

signalling molecule a chemical involved in transmitting information between cells; hormones and neurotransmitters are signalling molecules

significant difference a difference between data values that is statistically significant; that is, the probability (p) of the difference being due to chance is so small (usually less than 5%) that the result is considered true

silent mutation see *synonymous mutation*

single nucleotide polymorphism (SNP) nucleotide difference that occurs at a given position in the genomes of two or more individuals

SLOSS a term in conservation biology referring to the application of island biogeography theory to the establishment of conservation reserves being either single large or several small reserves

sodium–potassium pump a membrane protein that moves potassium ions into and sodium ions out of a cell, using active transport

solvent a solution that causes a solid substance to dissolve

somatic cell gene therapy gene therapy for a body cell

somatic cell a normal body cell, as compared with a germ-line cell from which a gamete (i.e. sperm or ovum) is derived

somatic system part of the peripheral nervous system associated with voluntary control

somatic a body cell that will not pass its genes onto 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

spontaneous mutation a mutation occurring in the absence of exposure to mutagens

sporadically refers to a disease that occurs infrequently and irregularly within a population

stabilising selection natural selection that tends to advantage organisms similar to their parents; this usually occurs when the environment is very stable and unchanging and selects against extremes of phenotype

standard deviation a measure of the dispersion of a set of data from its mean; expresses the variability of a population or set of data

start codon the first codon of a messenger RNA transcript translated by a ribosome

stem cell an unspecialised cell with the potential to differentiate into many different kinds of cells

sterile inflammation inflammation resulting from detection of DAMP s released during tissue injury

steroid hormones hydrophobic signal molecules found in plants and animals; these are produced from cholesterol, giving them a common chemical structure; examples include oestrogen, testosterone, and cortisone; these signalling molecules are lipophilic so they can slip across the cell membrane and bind to intracellular receptors

sticky end the end of a DNA fragment that is created following cleavage by a restriction enzymecthat cuts DNA at different positions on eachcstrand

stimuli the plural of stimulus

stimulus a signal that causes a response

stop codon the codon that stops the synthesis of a polypeptide chain

subspecies distinct populations of a species, which can interbreed but usually don't due to geographical isolation

substitution mutation a mutation in which a single nucleotide is swapped for another in the original gene sequence

substrate a substance that an enzyme acts on

susceptibility describes the level of response by an organism to a pathogen; that is, its resistance

sympatric speciation speciation that occurs without physical or geographic isolation

symptoms characteristic effects of a pathogen on the body

synapse the point where an axon terminal meets another neuron, a muscle cell or a gland cell, separated by a synaptic cleft

synapsis the pairing of homologous chromosomes

synaptic cleft the space between the presynaptic cell and postsynaptic cell in a synapse, across which neurotransmitters diffuse to transmit a nerve impulse

synonymous 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 'silent' mutation

systemic acquired resistance a plant's reaction to invasion by a pathogen that leads to long-term resistance to a broad range of pathogens; 'systemic' refers to the whole body

T cell receptor (TCR) a protein receptor found on the surface of T cells; binds to antigens (presented on MHC proteins)

target cell a cell that responds to a signalling molecule because it expresses specific receptors for that molecule

temperature danger zone the range of temperatures at which harmful bacteria can grow and reproduce in food

template strand polynucleotide (DNA or RNA) that serves as a guide for making a complementary polynucleotide

test cross a cross using an organism with a recessive phenotype to determine the unknown genotype of an organism with a dominant phenotype

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

thermoreceptor a sensory cell or organ that detects heat or cold

tissue fluid the fluid surrounding the tissue cells; it was originally blood plasma

tolerance range the range within which an organism can function and reproduce

toll-like receptor (TLR) a pattern recognition receptor in membranes that responds to PAMPs and DAMPs

trait a heritable characteristic; phenotype

transcription factors (TFs) regulatory proteins whose function is to activate or to inhibit transcription of DNA by binding to specific DNA sequences

transcription the formation of mRNA by the complementary nucleotide base pairing of the template strand of DNA in the nucleus

transfer RNA (tRNA) an RNA molecule, shaped similar to a clover leaf, that picks up amino acids from the cytoplasm and brings them to the ribosome to match up with specific mRNA codons

transformation the process by which DNA is taken from one organism and inserted into another organism using a plasmid

transgenic organism an organism that has been modified by incorporating into its genome a piece of foreign DNA

translation the joining of amino acids in a specific order, resulting in the formation of a polypeptide, when the information in mRNA is read by ribosomes

transmission the passing of an infectious disease from an infected host to another individual

transposable element a piece of eukaryotic DNA that is capable of cutting itself out of one position in the genome and inserting itself into another position in the genome; also referred to as 'jumping genes'

trisomy the condition in which somatic cells contain three copies of a particular chromosome

t-test a statistical test commonly used to analyse differences between two sets of data

tumour an abnormal growth of tissue

uncertainty bars bars drawn above and below and/or to left and right of a data point on a graph to indicate the size of the uncertainty in that point

upper critical temperature the temperature at which the body's cooling mechanisms fail to keep the body temperature stable and the metabolic rate increases with rise in external temperature

up-regulate (up-regulation) the process by which a cell increases the quantity of a cellular component, such as RNA or protein

urea a less toxic form of nitrogenous waste found in mammals as a result of protein breakdown

uric acid the least toxic form of nitrogenous waste produced by birds and some desert dwelling animals

vaccine an injected solution of antigens or pathogens that is designed to elicit a primary response and promote the formation of memory cells

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

variable nucleotide tandem repeats (VNTRs) a short non-coding region of DNA that is repeated many times in the genome of an organism; it is highly variable between individuals and can be used in DNA profiling; VNTRs have a repeat sequence of more than five bases

variable traits traits that vary in the population due to differences in alleles carried by different individuals

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

vasoconstriction the constriction of blood vessels by the surrounding smooth muscle cells, which increases blood pressure and redirects blood flow away from the constricted vessel

vasodilation dilation (widening) of blood vessels, particularly arterioles

vasopressin an antidiuretic hormone responsible for increased permeability of the distal tubules of the kidney, increasing water reabsorption and reducing urine volume

vector a living organism that transmits pathogens from one host to another; a vehicle used to transfer DNA sequences from one organism to another

vestigial structures structures found in organisms that have lost most, if not all, of their 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 refers to the ability of a pathogen to cause severe disease within its host

virus a non-cellular pathogenic agent with either DNA or RNA that can only reproduce inside a living cell

wildlife corridor a small area of preserved wilderness designed to connect larger reserves; also known as a habitat or green corridor

X-linked recessive when a phenotype is determined by a recessive allele on the X chromosome

X-linked related to a gene located on the X chromosome

Y-linked related to a gene located on the Y chromosome

zoonotic describes a disease that animals pass to humans; infections that are naturally transmitted between vertebrate animals and humans

zygote the first cell of a new individual, which is formed by fusion of a sperm and ovum at fertilisation

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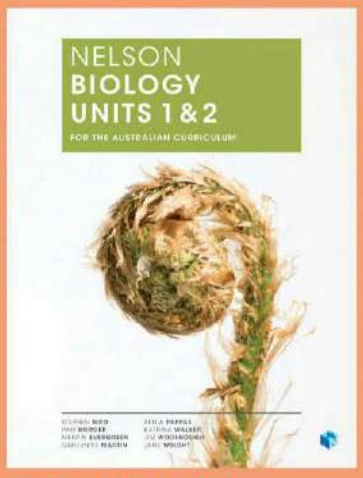

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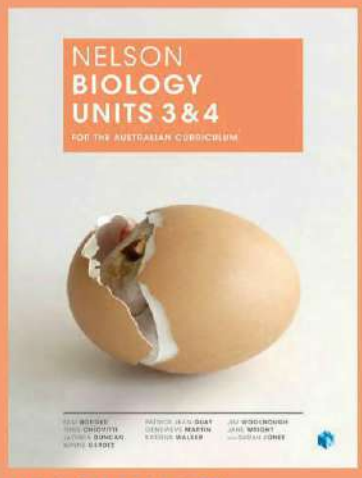

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