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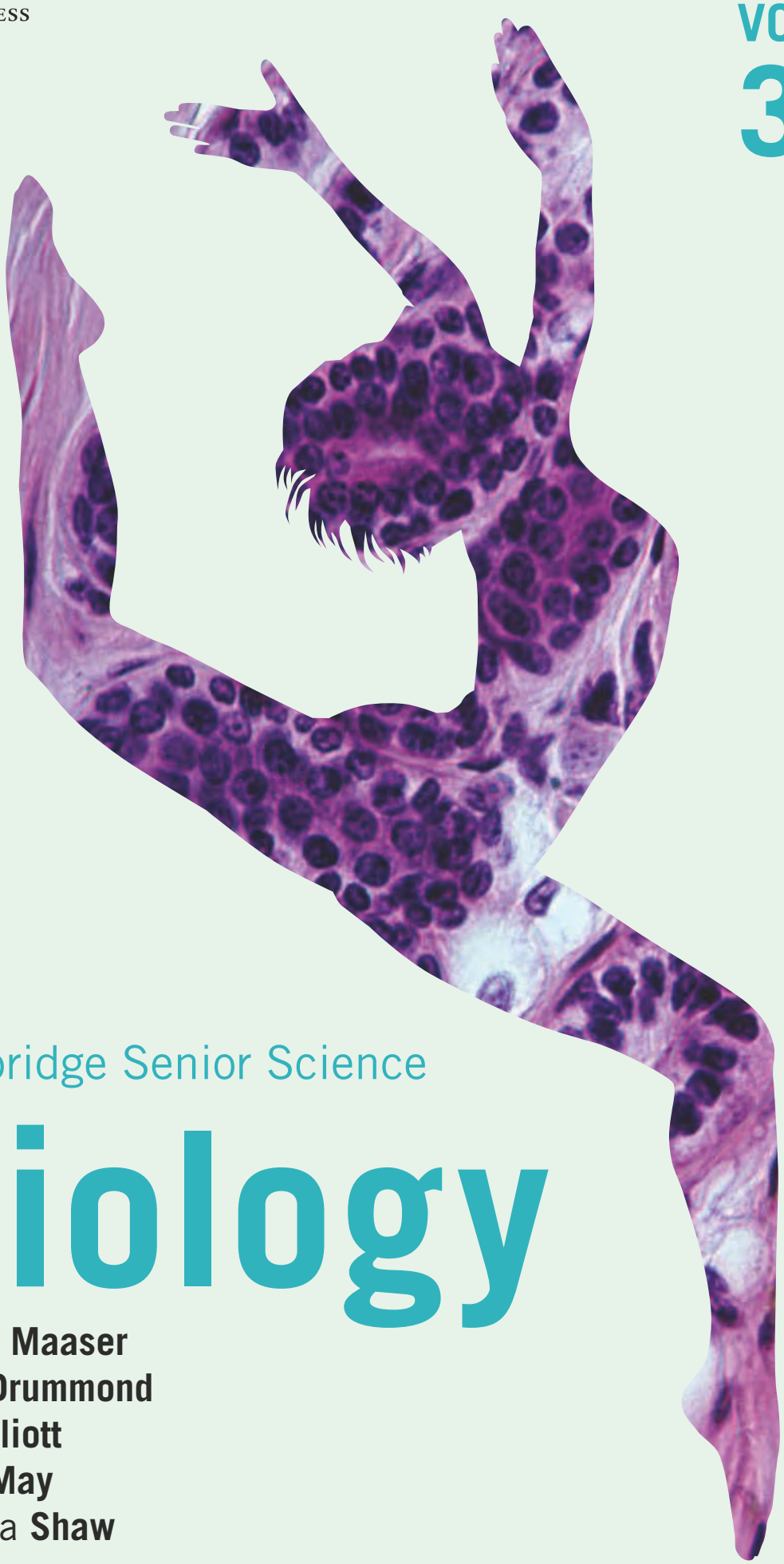
3&4



Cambridge Senior Science

Biology

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Please be aware that this publication may contain images of Aboriginal and Torres Strait Islander peoples now deceased. Several variations of Aboriginal and Torres Strait Islander terms and spellings may also appear; no disrespect is intended. Please note that the terms 'Indigenous Australians' and 'Aboriginal and Torres Strait Islander peoples' and 'First Australians' may be used interchangeably in this publication.

About the authors

Simon Maaser is Lead Author of the VCE Biology team. He has held roles as both Head of Science and Director of Curriculum across various schools. He has also been involved in leading VCAA Biology Exam assessing and Study Design reviews.

Brett Drummond is a science communicator and co-founder of MStranslate, an organisation that communicates research summaries on multiple sclerosis. He has been a private tutor for VCE Biology and Chemistry.

Ben Elliott has been a Years 7–10 Science Co-ordinator and a VCE Biology teacher for a number of years. He also taught Science and A-Level Biology in the UK.

Kylie May has taught VCE Biology for the last 14 years. She has held the positions of Head of Science and Head of Biology at various schools throughout her career. She has also been a VCAA assessor for Biology and completed studies with the Harvard Graduate School of Education.

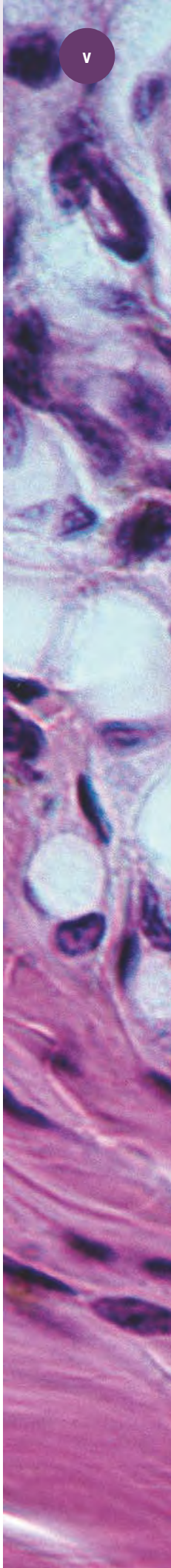
Victoria Shaw has been committed to sharing her love for science with Year 7–12 students for the past 21 years. She was Head of Science at an independent school for several years and has been an assessor for both the VCAA and IBO.

Contents

UNIT 4

How does life change and respond to challenges?

Answers are available in the Interactive Textbook and the teacher resources.



Overview: How to use this resource

This overview guides you through all the components of the **print and PDF textbooks**, the **Interactive Textbook (ITB)**, and the teacher resources in the **Online Teaching Suite (OTS)**. Examples are shown from the Units 1&2 resource. Users of the award-winning *Cambridge Science 7–10 for the Victorian Curriculum* will recognise some similarities with this senior science resource, including the hosting of the digital material on the Edjin platform, which was developed from *Cambridge HOTmaths* and is already being used successfully by thousands of teachers and students across Victoria

Print book features

Learning intentions

In the Curriculum table at the start of each chapter, the Study Design dot points are translated into Learning Intentions, describing what students should be able to do by the end of the chapter:

Black text indicates the portion of the dot point covered by the section shown in the second column

White text indicates the portion of the dot point covered by other sections

Curriculum

From Unit 1 Area of Study 1 Outcome 1
Cellular structure and function

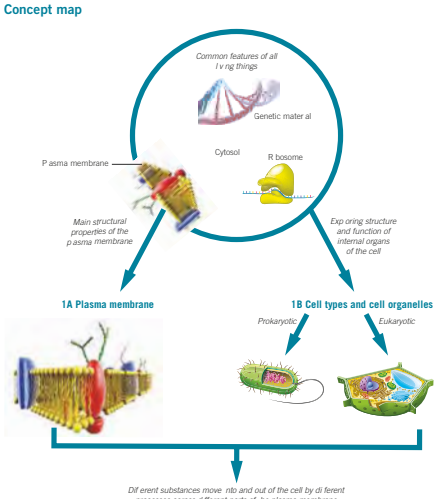
Study Design	Learning intentions – at the end of this chapter I will be able to:
• Cells as the basic structural feature of life on Earth, including the distinction between prokaryotic and eukaryotic cells	**1A Plasma membrane** **1A.1** Recall the four common factors for all organisms **1A.2** Outline the function of the four common factors for an organism

Learning intentions are turned into Success Criteria (achievement standards) at the end of the chapter and are assessed in the Chapter review and tracked in the Checklists

Relevant Study Design dot points are repeated at the start of each section in the chapter, and an overall curriculum grid is provided in the teacher resources.

Concept maps

Concept maps display each chapter's structure with annotations emphasising interconnectedness, providing a great memory aid. The versions in the ITB are hyperlinked and offer an alternative way of navigating through the course. An overall concept map of Units 3&4 is also provided after this overview.



Links

The interconnectedness of topics in Biology is demonstrated through links between sections, displayed in the margins. In the ITB, these are hyperlinks that provide an alternative way of navigating through the course.

Comparison of plant and animal cells

Plant cells and animal cells have many organelles in common, but there are some differences (Figure 1B–6).

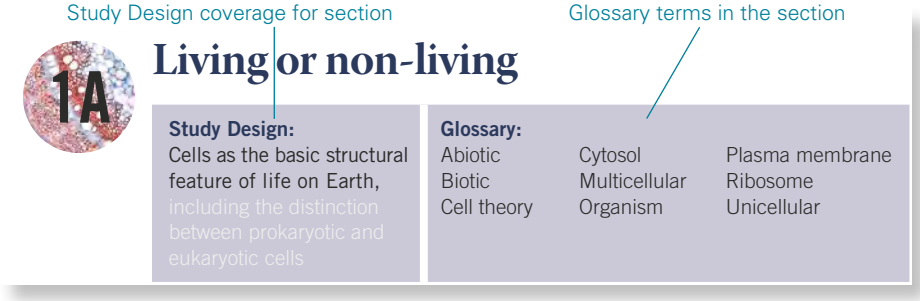
A misconception is that plant cells only perform photosynthesis and do not respire. In fact, plant cells perform both photosynthesis and cellular respiration, and so a plant cell has both mitochondria and chloroplasts. Not all the cells in a plant photosynthesise (for example, root cells do not photosynthesise), but they do all have a cell wall. In contrast, animal cells do not have any chloroplasts, and do not photosynthesise.

LINK 5A PHOTOSYNTHESIS

LINK 5C CELLULAR RESPIRATION

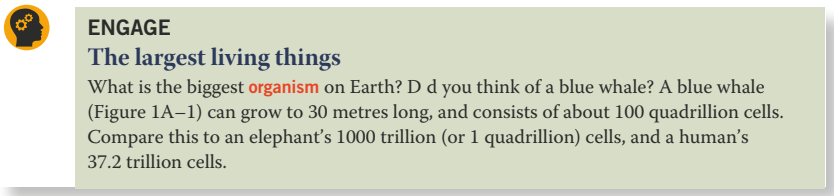
Chapter sections

Chapters are divided into numbered sections each with a consistent set of features.



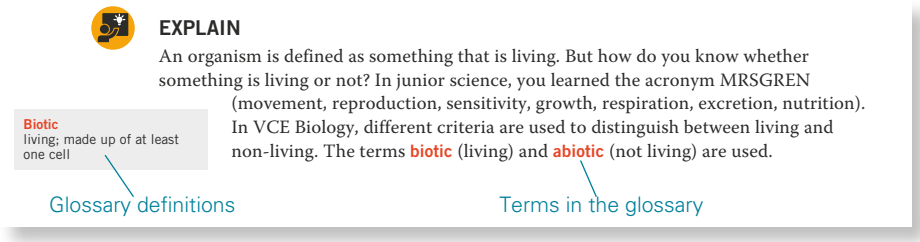
Engage

At the start of each section, these boxes provide points of interest for the topic emphasising its place in Biology. This material, though not assessable, can be used as examples of applications.



Explain

This icon marks the start of essential content that is assessed.

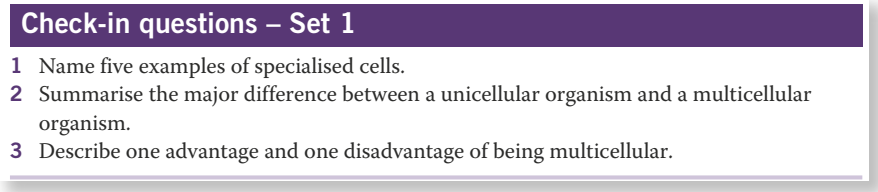


Glossary

Scientific terms are highlighted in the text, definitions are given in the margin of the print and PDF textbooks, or on mouseover in the ITB, and the terms are listed at the start of each chapter and section.

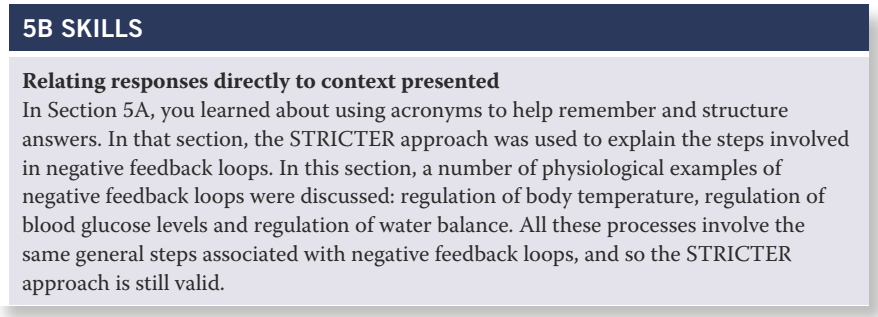
Check-in questions

Each section in the chapter has one or more sets of check-in questions, for formative assessment. Full answers are provided in the digital resources.



Skills

Skills boxes in every section provide advice and guidance on how to answer and prepare for questions, especially in examinations. The ITB has video versions of these guided by experienced teachers which provide extra comments and an alternative medium of delivery.



Charts, diagrams and tables

Detailed charts integrating text and diagrams, and illustrated tables, feature throughout the print books. In the ITB, most of these are available as animated slide-show presentations for students to use, with copies for teachers to display on data projector or whiteboard.

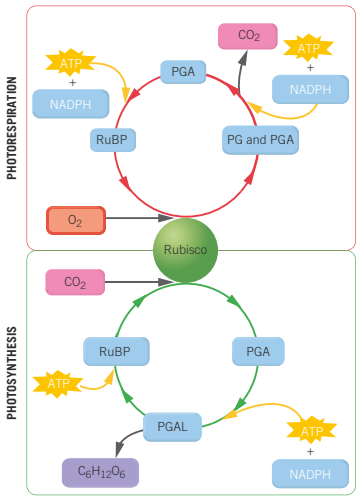


Figure 5B–6 A comparison of the Calvin-Benson cycle in photosynthesis (when Rubisco binds to CO_2) and photorespiration (when Rubisco binds to O_2)

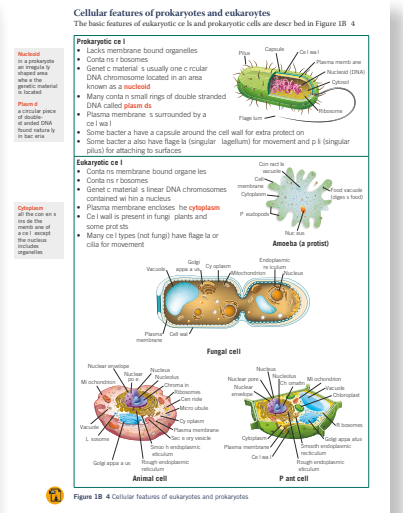


Figure 1B 4 Cellular features of eukaryotes and prokaryotes

Section questions

Summative assessment is provided at the end of each section, again with full answers provided in the digital resources.

Section 5B questions

1 What two hormones are involved in the maintenance of blood glucose homeostasis? Explain the action of both of these in the circumstances when they are required.
2 What property of proteins makes body temperature homeostasis particularly important?

Chapter reviews

Summaries: Students are encouraged to make their own set of summary notes, to help them assimilate the material. Model summaries are provided in the teacher resources, to be given to those who need help. Creating summaries can also be turned into an assessment task, with the models serving as the answer.

Checklists and **Success criteria:** The learning intentions from the front of the chapter are listed again in the form of success criteria linked to the **multiple-choice** and **short-answer questions** that follow. The checklists are printable from the ITB, and students can tick off their achievement manually. If they do the questions in the ITB, they are ticked automatically when the questions are marked.

Chapter 2 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
2A.1	List the differences between DNA and RNA	7 ☐
2A.2	Draw schematic diagrams of a nucleotide, a single strand and a double strand of DNA	12 ☐

Multiple-choice questions

1 What is the correct RNA sequence that would be copied from the following DNA template sequence: CGA AGT TTA ATT CGC AGT?
A CGA AGT TTA ATT CGC AGT
B GCT TCA AAT TAA GCG TCA
C GCU UCA AAT TAA GCG UCA
D GCU UCA AAU UAA GCG TCU

2 Which of the following is a step involved in RNA processing?

6 The molecule that is the intermediate step between DNA and protein is
A amino acid.
B rRNA.
C mRNA.
D tRNA.

7 One of the features that distinguishes between DNA and RNA is the
A phosphate group.
B nitrogenous base cytosine.

Short-answer questions

11 The *lac* operon contains the genetic code for the proteins necessary to break down lactose, the sugar found in milk. Three coding genes make up the lac operon – *lacZ*, *lacY* and *lacA*. For this question, use the *trp* operon as a model but note that it functions and responds to lactose in the **opposite** way that the *trp* operon does for tryptophan.

a What is an operon? (1 mark)

b Draw a representative diagram of the gene structure for the *lac* operon, including all the key elements. (2 marks)

Unit revision exercises

Each Unit has a revision exercise in the print book, with both multiple-choice and short-answer questions.

Special content

- **Section 11B Migration of modern humans** includes the migration of Aboriginal and Torres Strait Islander peoples and their connection to Country and Place.

Connection to Country

A population's interaction with their environment can also be referred to as **Connection to Country**. This was (and still is) an important way of life for Indigenous populations in the Pacific region, specifically that of Aboriginal and Torres Strait Islanders.

Connection to Country the relationship between people and their indigenous land or environment

It is understood that Aboriginal Australians have the longest unbroken record of ancient art. For their ancestors, creating art would have been a way of forming a greater connection between each other, and also a way to define their unique identity among other populations.

The transmission of this information and key cultural practices within and between generations would not have been possible without an increase in brain volume and the development of key regions of the brain. Therefore, the use of language, in both a verbal and an artistic sense, is a key component of Aboriginal and Torres Strait Islanders' way of life. Current generations have continued to inherit this cultural understanding and the practices of their ancestors, continuing to add to this record.

Figure 11B–5 An example of artwork found in Quinkan country, Queensland, featuring images of humans, dingoes and eels. This work has been dated to approximately 15 000 years ago.

Consider how the ancestors of these Indigenous populations would have used the key developments in hominin evolution (outlined in Table 11B–2) to enhance their survival and way of life.

- Also featured is the 2017 return of Mungo Man to the traditional owners of the Country where the remains were found.

In 2003, geologists uncovered 460 footprints in a clay pan around the lake. This was the largest collection to date globally of fossilised footprints in a single find, and some of the oldest – dating estimated the to be from 20 000 years ago.

Today, the Paakantji, Mutthi Mutthi and Ngyimpaa people continue to maintain a close connection to the now World Heritage-listed Willandra Lakes, which include Lake Mungo. An important Connection to Country for Aboriginal Australians and part of their communal history was the return of the remains of Mungo Lady (in 1992) and Mungo Man (in 2017)

Figure 11B–7 The return of Mungo Man in 2017 to the Paakantji, Mutthi Mutthi and Ngyimpaa people (left) was marked with a traditional Aboriginal ceremony (right).

- **Chapter 12 Scientific investigations** repeats modelling of logbook development for students' own practical investigations, with detailed examples, from Chapter 6 in the Units 1&2 resource. Some new notes and questions are included.

- **Chapter 1 Key background knowledge required for Units 3&4** is a copy of material on cells from the beginning of the Units 1&2 resource. It is provided for revision and also for students studying Units 3 and 4 without having done 1&2.

VIDEO 6A–1 LOGBOOK EXAMPLE

Title the research question under investigation; includes information about what is being tested

Introduction a detailed but succinct explanation of the reason for undertaking an investigation; includes key biological concepts, aim and hypothesis

Logbook

Title

1 What temperature do enzymes work at?

2 What is the optimal temperature for the enzyme catalase to break down hydrogen peroxide into oxygen and water?

Introduction

1 Enzymes are essential to help reactions proceed more efficiently, in order to sustain life. They are made of proteins, which are coded for by an organism's DNA, and they are specific to the substance they act on.

2 The enzyme catalase reacts with the substrate hydrogen peroxide (H_2O_2), breaking it down into water and oxygen (products) in a catabolic reaction. Catalase is primarily found in the liver and is important in protecting the organism from damage caused by hydrogen peroxide, which is constantly produced by mammals.

Notes

The title should include reference to the variables being changed (independent variable) and measured (dependent variable), along with enough detail for the reader to decide whether they want to continue reading.

In the introduction (to a poster) it can also be appropriate to present a labelled diagram of the concept/idea being investigated.

Interactive Textbook features

The digital version of the textbook is hosted on the Edjin platform, offering easy navigation, excellent on-screen display and multimedia assets, as well as auto-marking of multiple-choice questions, and workspaces for other questions with self-assessment and confidence rating tools. The different kinds of digital assets are listed below and are accessed by:

- Printable **Worksheets** with extra questions and activities (and content in some cases) are provided for most chapters, marked by an icon in the margin, as shown on the right.

WORKSHEET 1A–1 LIVING OR NON-LIVING?

- **Videos** are provided for all chapters, and are of two kinds: **concept videos** demonstrate or illustrate important theory, while **skills and example videos** feature experienced teachers working through the textbook's skills and example boxes, providing extra explanation and guidance. Some videos are provided in the print pages as QR codes for immediate access and review.

VIDEO 3A–1 DIFFERENT TYPES OF RESTRICTION ENDONUCLEASES

- **Animated slide-show presentations** (in PowerPoint Show format) are provided of many charts, diagrams and tables, as marked by an icon in the margin as shown at right, enabling them to be explored interactively.
- **Answers** (worked solutions) to questions are provided in pop-up windows next to the questions, or in printable PDFs for use with the print textbook.
- In the Units 1&2 resource, **prior knowledge** can be tested with an auto-marked quiz with questions from the Year 9 and 10 *Cambridge Science for the Victorian Curriculum*.

Online Teaching Suite features (teacher resources)

The OTS provides Edjin's learning management system, which allows teachers to set tasks, track progress and scores, prepare reports on individuals and the class, and give students feedback. The assets include:

- **Curriculum Grid** and **teaching programs**
- Editable and printable **Chapter tests** with answers
- **Checklists** with linkage to the success criteria for the chapter question sets and tests
- A **question bank** and test generator, with answers
- **Practice exams** and **assessment tasks**, with answers
- Editable versions of **Worksheets** in the Interactive Textbook, and answers to them
- Editable versions of the PowerPoint files in the Interactive Textbook
- Downloadable, editable and printable **practicals**
- Editable and printable chapter **summaries** (model answers for the chapter summary activity)
- **Teacher notes** on selected content with additional theory explanation and suggestions for further activities and resources
- **Curated links** to internet resources such as videos and interactives.

Exam generator

The Online Teaching Suite includes a comprehensive bank of exam style and actual VCAA exam questions to create custom trial exams to target topics that students are having difficulty with. Features include:

- Filtering by question-type, topic and degree of difficulty
- Answers provided to teachers
- VCAA marking scheme
- Multiple-choice questions will be auto-marked if completed online
- Tests can be downloaded and used in class or for revision.

Acknowledgements

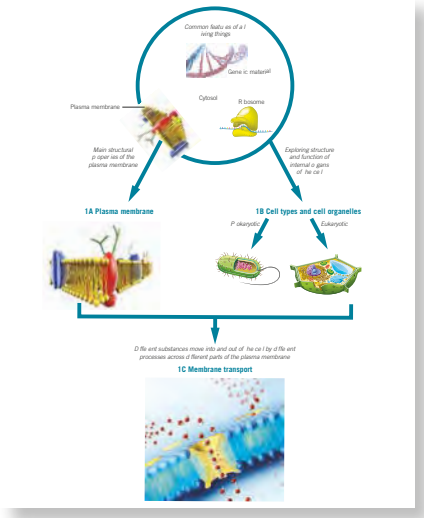
The authors and publishers wish to thank the following people for help and advice in preparing this resource:

- Dr Kaye Price AM for advice on Aboriginal and Torres Strait Islander knowledge and perspectives
- Joe Sambono, Curriculum Specialist Aboriginal and Torres Strait Islander Priority and Science Curriculum, Australian Curriculum, Assessment and Reporting Authority, for providing links to resources and references
- Harry Leather and Jan Leather, authors of *Cambridge Checkpoints VCE Biology Units 3&4* for advising on aspects of the resource.

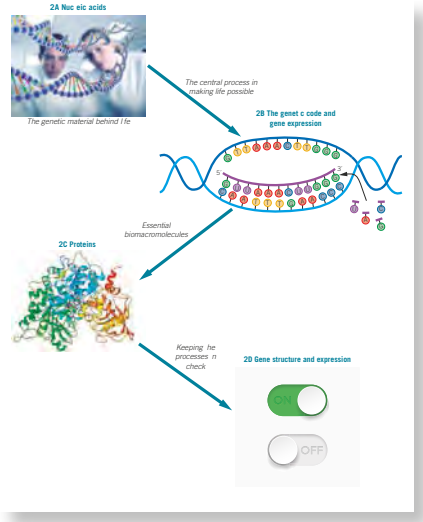
Concept maps for Units 3&4

This spread displays the concept maps for topics Chapters 1–11. (Chapter 12 Scientific investigations is not included as it covers skill development rather than topics.) Access the digital version of this concept map in the ITB to zoom in on the details and click on hyperlinks to explore the interconnections of the topics.

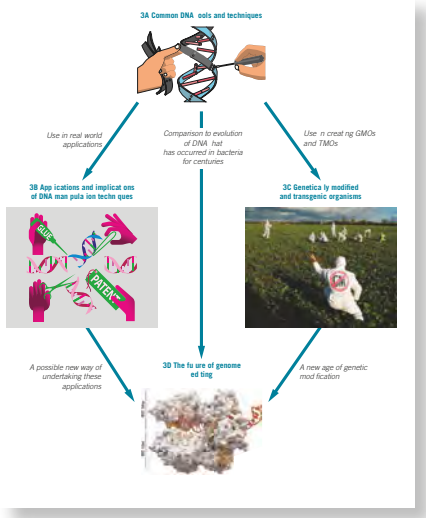
Chapter 1 Key background knowledge required for Units 3&4



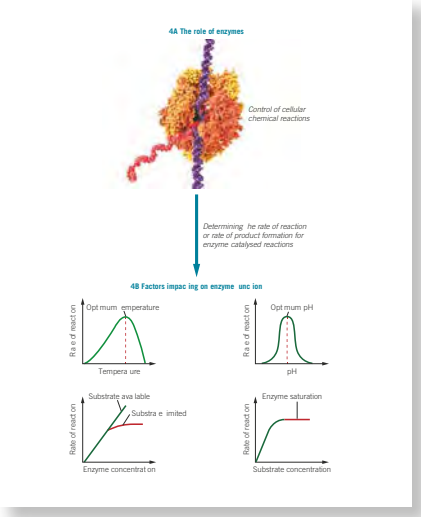
Chapter 2 From DNA to proteins



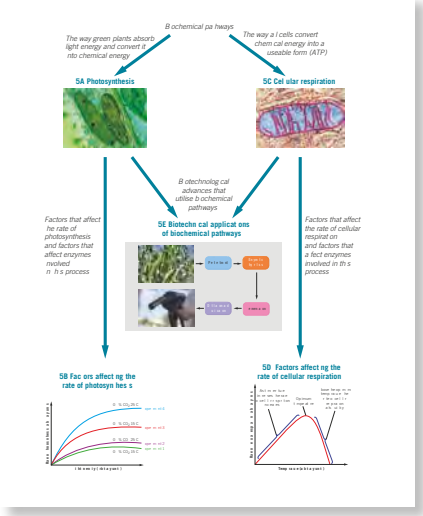
Chapter 3 DNA manipulation techniques and their applications



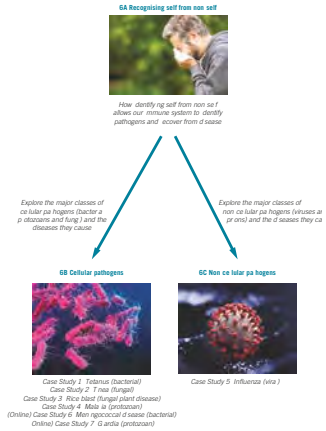
Chapter 4 Enzymes



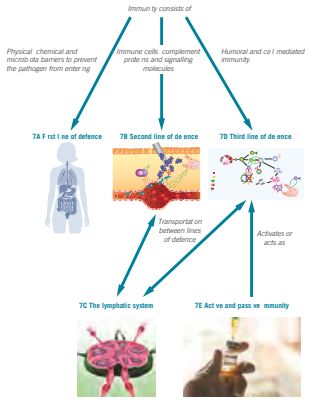
Chapter 5 Biochemical pathways: photosynthesis and cellular respiration



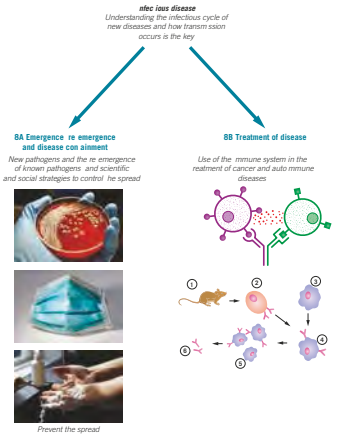
Chapter 6 Foreign invaders: self versus non-self



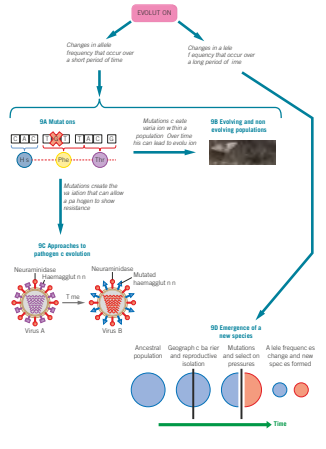
Chapter 7 Immunity: lines of defence



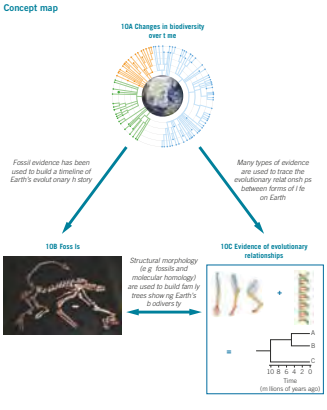
Chapter 8 Emergence and treatment of new diseases



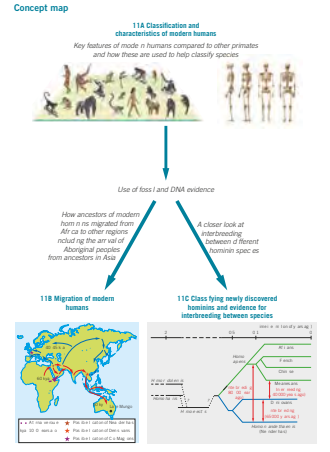
Chapter 9 Evolution: genetic changes in populations over time



Chapter 10 Evolution over time



Chapter 11 Human evolution



UNIT 3

HOW DO CELLS MAINTAIN LIFE?

CHAPTER 1

KEY BACKGROUND KNOWLEDGE REQUIRED FOR UNITS 3&4

Introduction

For Units 3 & 4 Biology, it is not a requirement that you have completed Units 1 and/or 2. However, some key concepts in Units 1 & 2 are fundamental to understanding the processes studied in the Units 3 & 4 course. Even if you have done Units 1 & 2, you can use this chapter to revise those key concepts.

This chapter examines cells as the basic structural feature of life on Earth. Prokaryotes (such as bacteria) and eukaryotes (such as plants and animals) are the two main cell types, and being able to distinguish between them is an important skill. You will understand that all cells have four common features – genetic material, plasma membrane, cytosol and ribosomes – and you will explore the structure and function of the main types of specialised organelles.

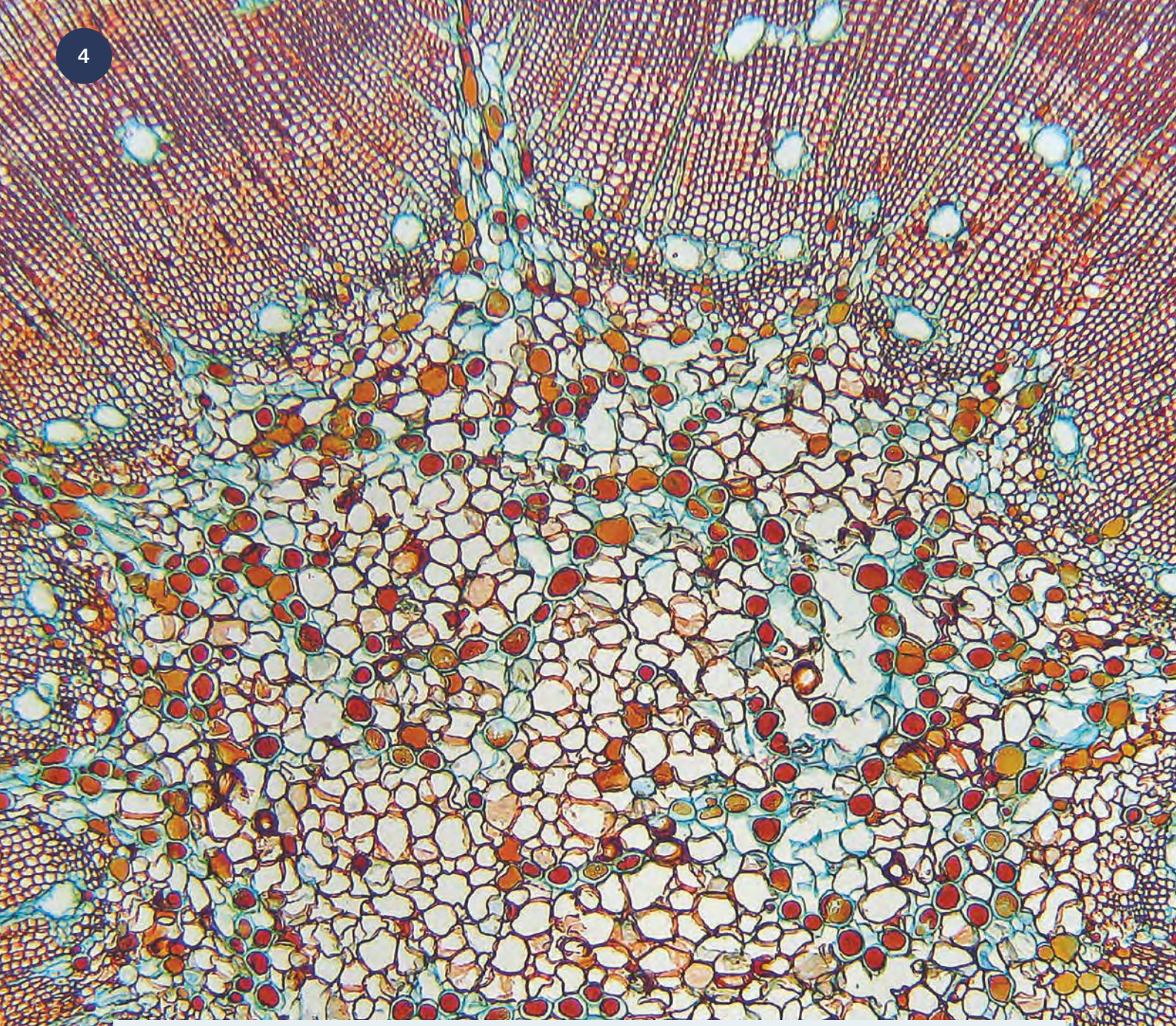
A considerable portion of this chapter focuses closely on the structure and function of the plasma membrane and how it contributes to the functioning of the cell by regulating the movement of substances into and out of the cell. These substances are crucial to processes that will be explored throughout this book, including protein synthesis, photosynthesis, cellular respiration, and immunity.

Curriculum

From Unit 1 Area of Study 1 Outcome 1
Cellular structure and function

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Cells as the basic structural feature of life on Earth, including the distinction between prokaryotic and eukaryotic cells	**1A Plasma membrane** **1A.1** Recall the four common factors for all organisms **1A.2** Outline the function of the four common factors for an organism

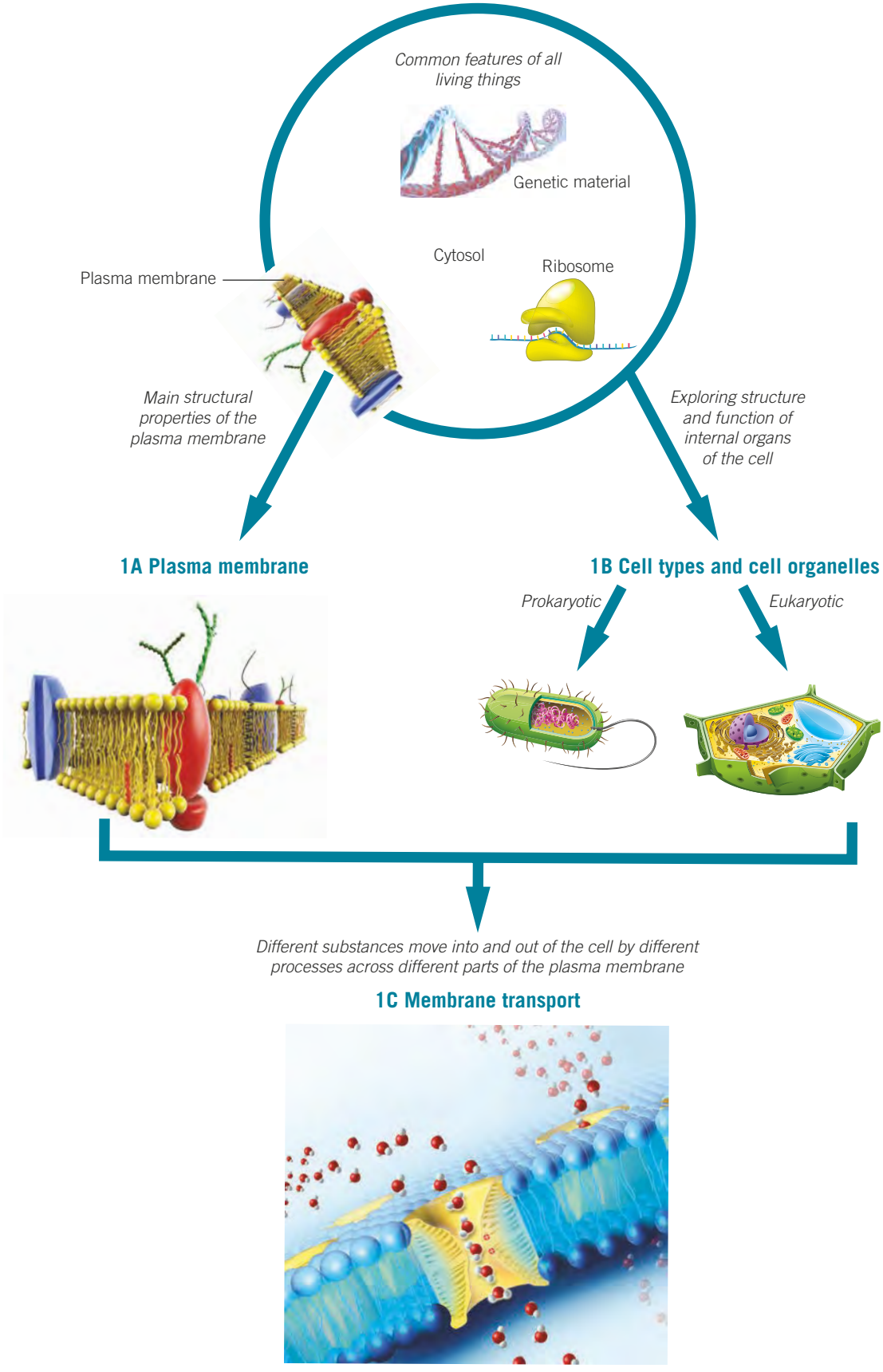
Study Design	Learning intentions – at the end of this chapter I will be able to:
• The structure and function of the plasma membrane in the passage of water, hydrophilic substances via osmosis, facilitated diffusion and active transport	**1A.3** Recall and define the following terms: fluid mosaic model, hydrophilic, hydrophobic, plasma membrane, semi-permeable membrane **1A.4** Recall and define the function of different components of the plasma membrane **1A.5** Draw and identify the different components of the plasma membrane **1A.6** Use evidence from a diagram to explain the structure of the plasma membrane
• Cells as the basic structural feature of life on Earth, including the distinction between prokaryotic and eukaryotic cells	**1B Cell types and cell organelles** **1B.1** Recall and define the following terms: eukaryote, nucleoid, prokaryote
• The structure and specialisation of plant and animal cell organelles for distinct functions, including chloroplasts and mitochondria	**1B.2** Recall and define the following terms: cell wall, chloroplast, cilia, flagella, Golgi apparatus, lysosome, mitochondrion, nucleus, ribosome, rough endoplasmic reticulum, smooth endoplasmic reticulum, vacuole, vesicle **1B.3** Identify whether a cell is a eukaryote (including animal or plant) or a prokaryote, based on its cellular features (organelles) **1B.4** Draw animal, plant and bacterial cells, including the identification of organelles **1B.5** Compare the structure of plant and animal cells **1B.6** Explain what organelles would be present in different cell types so that the cell can perform its particular function
• The structure and function of the plasma membrane in the passage of water, hydrophilic substances via osmosis, facilitated diffusion and active transport	**1C Membrane transport** **1C.1** Give reasons why substances need to move through the plasma membrane **1C.2** Define semi-permeable, hydrophilic and hydrophobic **1C.3** Summarise the modes of transport used by substances crossing the plasma membrane, including the nature of substances moving, whether energy is required, and the component of the membrane involved in the transport **1C.4** Define tonicity



Glossary

Active transport
ATP (adenosine triphosphate)
Bulk transport
Carrier protein
Cell theory
Cell wall
Channel protein
Chloroplast
Cilia
Concentration gradient
Cytoplasm
Cytosol
Endocytosis
Eukaryote
Exocytosis
Facilitated diffusion
Flagella
Fluid mosaic model
Golgi apparatus
Hydrophilic
Hydrophobic
Lipophilic
Lipophobic
Lysosome
Mitochondrion
Nucleoid
Nucleus
Organelle
Osmosis
Passive transport
Permeable
Phagocytosis
Pinocytosis
Plasma membrane
Polar
Prokaryote
Protein mediated transport
Ribosome
Rough endoplasmic reticulum
Semi-permeable membrane
Simple diffusion
Smooth endoplasmic reticulum
Tonicity
Vacuole
Vesicle
Vesicle mediated transport

Concept map



See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

Plasma membrane

Study Design (from Unit 1):

- Cells as the basic structural feature of life on Earth, including the distinction between prokaryotic and eukaryotic cells
- The structure and function of the plasma membrane in the passage of water, hydrophilic substances via osmosis, facilitated diffusion and active transport

Glossary:

Cell theory
Cytosol
Fluid mosaic model
Hydrophilic
Hydrophobic
Plasma membrane
Ribosome
Semi-permeable membrane

ENGAGE

Separating internal and external

The *plasma membrane* (also known as the *cell membrane*) acts as a boundary between the internal environment of a cell and its external environment. Figures 1A–1 and 1A–2 illustrate how the cell membrane helps to determine the two different environments. Without a plasma membrane a cell would not exist, because the survival of the cell depends on how substances are exchanged between the internal and external environments. Remember that a plasma membrane is one of four common features of all cell types.

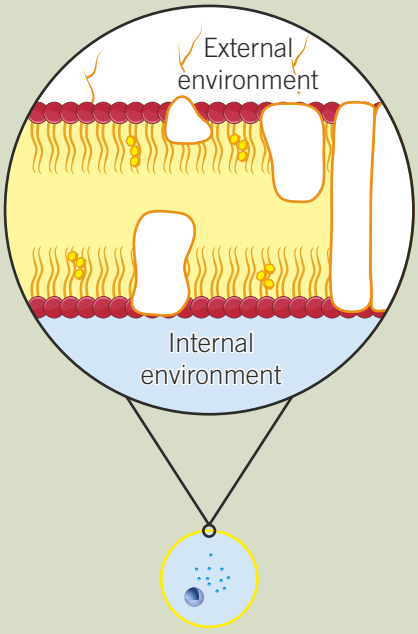


Figure 1A–1 Zoomed-in diagram of a cell membrane, showing the internal and external environments

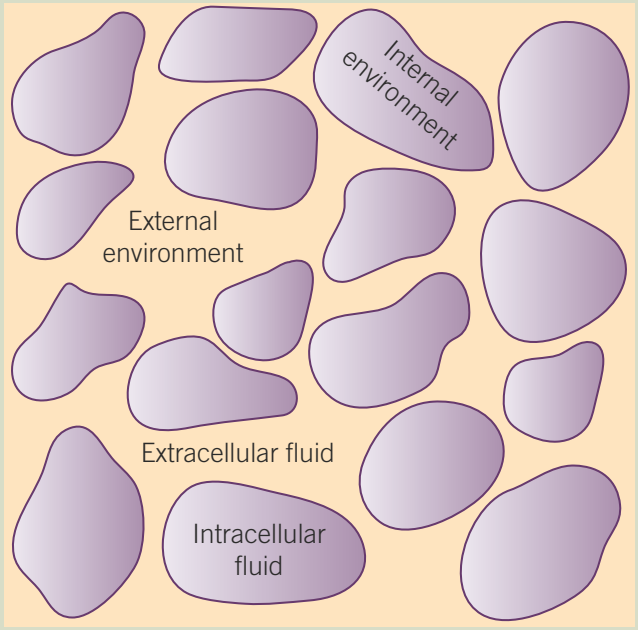
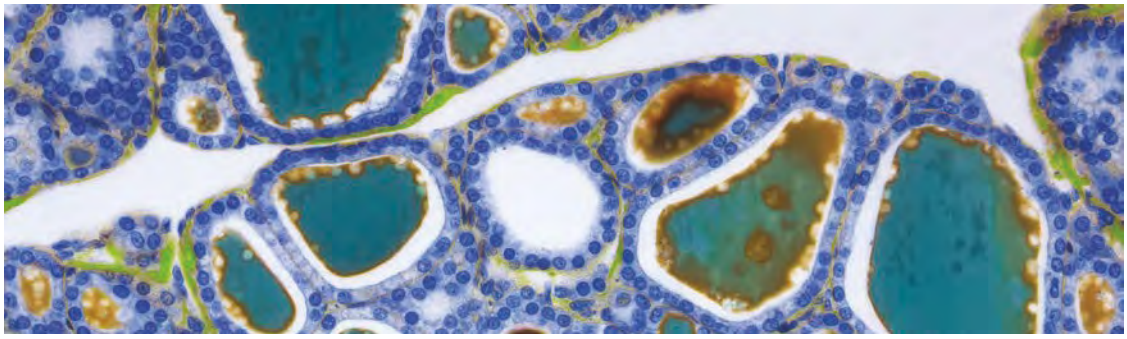


Figure 1A–2 Simplified diagram of several cells, showing the internal or intracellular (within each cell) and external or extracellular (between cells) environments



EXPLAIN

Cell theory

Before we begin discussing the importance of the plasma membrane, it is important to know that the cell is the basic unit of life, and every cell comes from a pre-existing cell. This is known as **cell theory**. The cells of plants, animals, bacteria and amoebas all have the following four common factors (Figure 1A–3):

- genetic material
- cytosol
- ribosomes
- a plasma membrane.

These are summarised in Table 1A–1.

Cell theory the theory that living things are made up of at least one cell, and that these cells are the basic unit of life and came from pre-existing cells

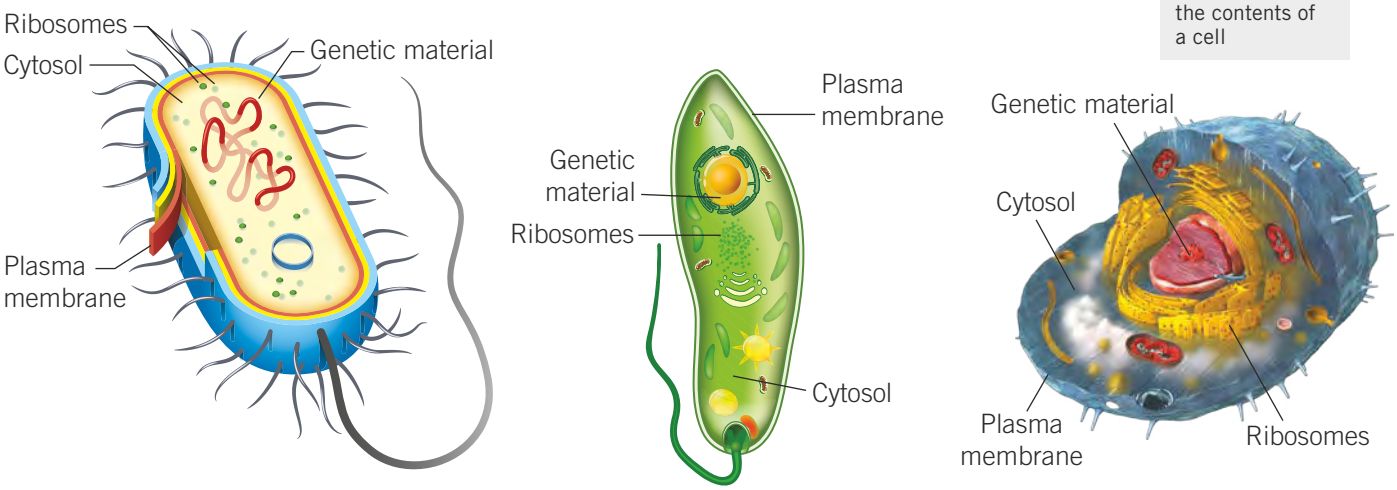
Cytosol the liquid inside a cell, between the organelles (doesn't include the organelles)

Ribosome a non-membrane-bound organelle involved in synthesis of proteins

Plasma membrane a membrane made up of two layers (known as a bilayer) of phospholipids that encloses the contents of a cell

Table 1A–1 Common factors for all organisms

Feature	Function
Genetic material	Contains hereditary information, containing genes that code for proteins
Cytosol	The liquid inside cells. Consists of 80% water, salts and organic molecules. The site of many cellular reactions
Ribosomes	Site of protein synthesis
Plasma membrane	Separates the interior and exterior environments, selecting what enters and leaves the cell



Bacterium (prokaryote) **Protist (eukaryote)** **Animal cell (eukaryote)**

Figure 1A–3 Different types of cells all contain four common factors.

Check-in questions – Set 1

1 Recall and state the function of the four common features of all cells.
2 Recall the cell theory.

VIDEO 1A–1 CELL TYPES AND THEIR FOUR COMMON FACTORS

Semi-permeable membrane
a membrane that only lets certain substances cross it; also called partially permeable, differentially permeable or selectively permeable

Structure and function of the plasma membrane

The *structure* of the plasma membrane relates to its *function* and the type of cell it is in. It is a **semi-permeable membrane** that allows substances to be transported between the internal and external environments of the cell. It consists of a phospholipid bilayer, proteins, carbohydrates and cholesterol.

The functions of the plasma membrane include but are not limited to:

- recognising other cells when forming tissue, or determining whether cells are foreign during an immune response
- communicating with other cells
- selectively controlling which substances are transported into or out of the cell.

6A RECOGNISING SELF FROM NON-SELF LINK

1C MEMBRANE TRANSPORT LINK

The process of transporting substances across the plasma membrane is explored in detail in Section 1C.

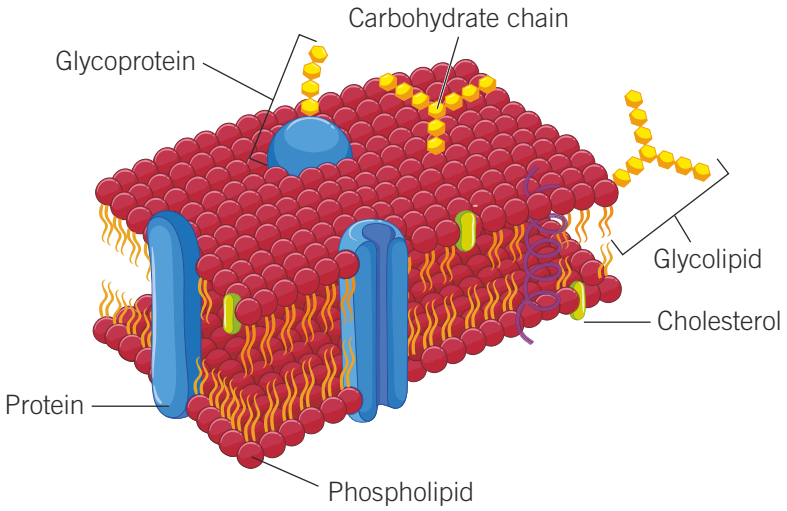


Figure 1A–4 Structure of the plasma membrane

Hydrophobic
water-repelling; does not dissolve readily in water

Hydrophilic
water-attracting or water-soluble; dissolves readily in water

The phospholipid bilayer is arranged with the **hydrophobic** (water-repelling) tails facing inwards and the **hydrophilic** (water-attracting) heads on the outsides of the membrane, towards the watery internal and external environments. Because the plasma membrane is made up of phospholipids, it is able to selectively allow different substances to enter and leave the cell. Water-soluble (hydrophilic) substances need the assistance of a protein channel to cross the phospholipid part of the membrane. Lipid-soluble (hydrophobic) substances are able to move across the phospholipids of the plasma membrane.

WORKSHEET 1A–1 STRUCTURE AND FUNCTION OF THE PLASMA MEMBRANE DOC

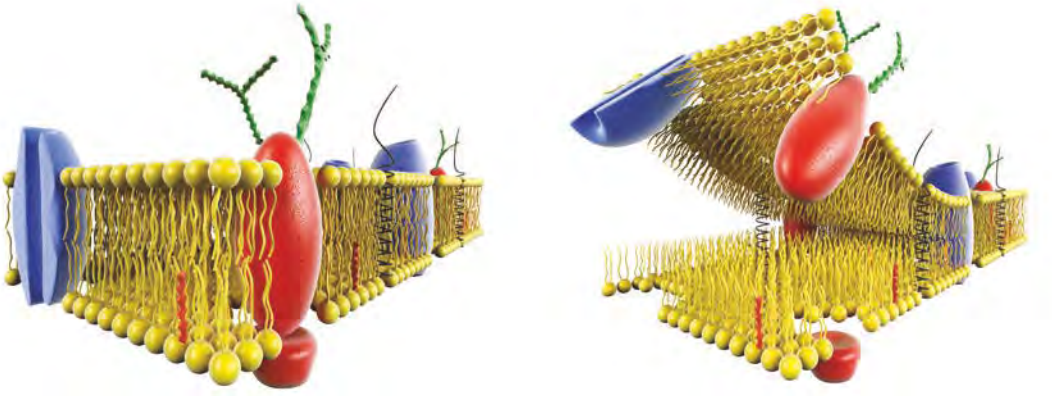
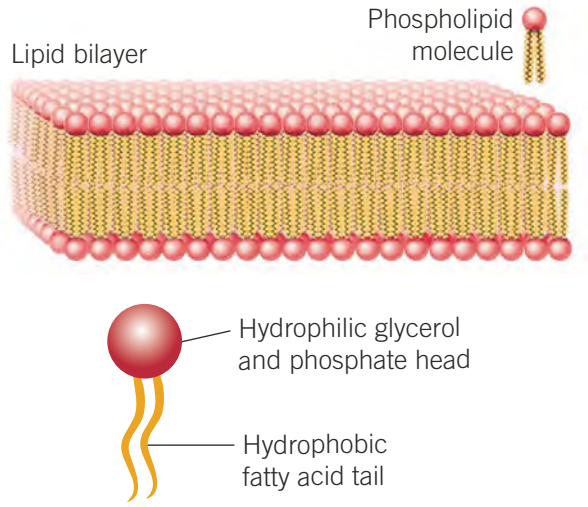
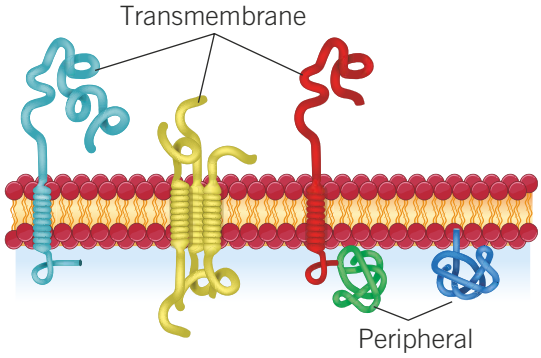
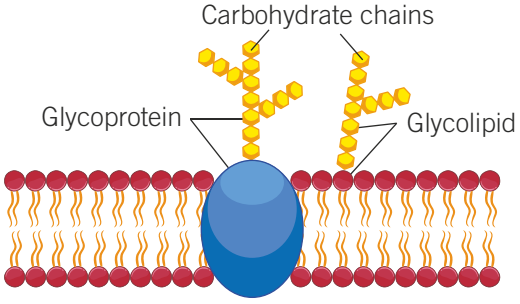
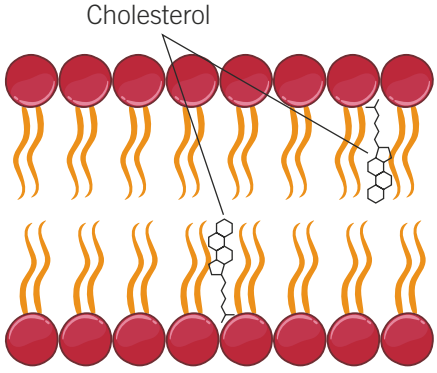


Figure 1A–5 Plasma membrane with the same components as Figure 1A-4, shown intact (left) and with the two layers of phospholipids separated (right)

Table 1A–2 Components of the plasma membrane

Component	Diagram	Description
Phospholipids		Phospholipids are arranged in two layers, with their hydrophilic (water-attracting) glycerol and phosphate heads facing outwards, towards both the intra- and extra-cellular environments. The hydrophobic (water-repelling) fatty acid tails face inwards, towards each other.
Proteins		Proteins play a variety of roles including transport, signalling and cell-to-cell recognition. When they span the length of the membrane, they are called transmembrane proteins. When present on only one side of the membrane, they are called peripheral proteins.
Carbohydrates		Carbohydrates play a role in adhesion between cells and in cell recognition. When a carbohydrate is attached to a protein, it is called a glycoprotein. When a carbohydrate is attached to the head of a phospholipid, it is called a glycolipid.
Cholesterol		Cholesterol helps to increase the stability of the plasma membrane without affecting the membrane's fluidity (ability to move). It also increases membrane permeability.

Check-in questions – Set 2

VIDEO 1A–2 THE PLASMA MEMBRANE

1 What does the plasma membrane define for the cell?
2 List the key components of plasma membrane.
3 Which part of the plasma membrane is hydrophilic?

Fluid mosaic model

Fluid mosaic model a model that represents the plasma membrane as a combination (mosaic) of phospholipids, proteins, cholesterol and carbohydrates that gives the membrane its fluid nature

A plasma membrane is made up of different types (a mosaic) of molecules. It is also fluid – that is, the phospholipids and proteins are able to move around within each layer. Referring to plasma membranes in this way is known as the **fluid mosaic model** (see Figure 1A–5 on the previous page).

Recall that a phospholipid consists of a head and two tails, and the tails consist of fatty acids. If these fatty acids are saturated, they have only one bond between each carbon atom, and this makes them pack together tightly. If there are unsaturated fatty acids in the tails, they have double bonds between some carbon atoms and this makes them pack together less tightly. So more unsaturated fatty acids in the tails of the phospholipids increases the fluidity of the plasma membrane (Figure 1A–6).

1C MEMBRANE TRANSPORT LINK

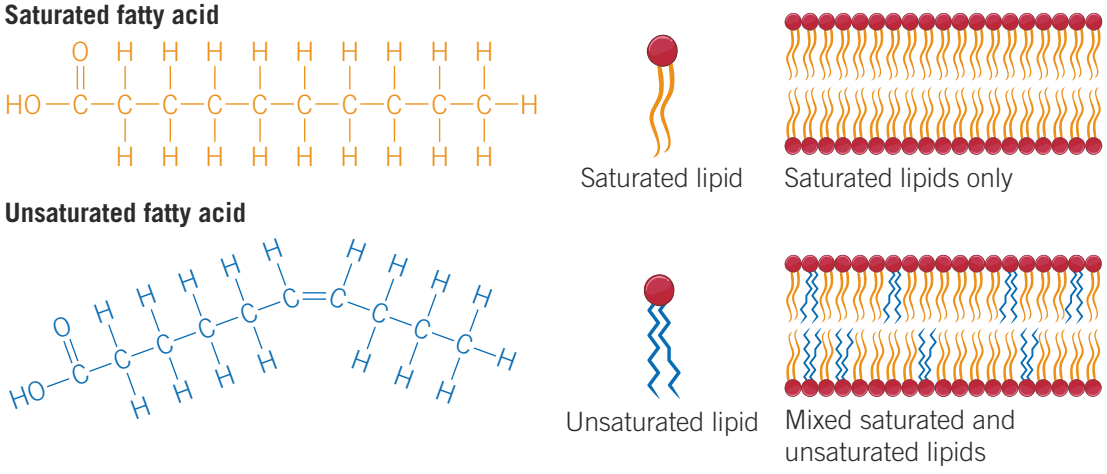


Figure 1A–6 Double bonds between carbon atoms in unsaturated fatty acids cause the carbon chain to kink. The presence of unsaturated lipids in the bilayer increases the fluidity (elasticity) of the plasma membrane.

1A SKILLS

VIDEO 1A–3 SKILLS: IDENTIFYING AND EXPLAINING

The difference between 'identify' and 'explain'

The ability to identify the components of a plasma membrane is a key skill. It is important that you become familiar with a variety of representations of the plasma membrane, as different diagrams can be used during an assessment.

Figure 1A–7 is a representation of a plasma membrane. If the question asks you to *identify* some or all of the components, be succinct and specific. If the question asks you to *explain*, this is the time to elaborate on your answer.

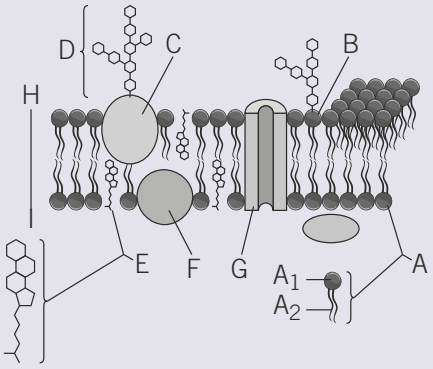


Figure 1A–7 Unlabelled diagram of a plasma membrane. You should be able to identify all the components shown.

For example:

Question: Identify components A and E from the diagram of the plasma membrane (Figure 1A–7).

Answer: A= phospholipid, E= cholesterol

This sample response has done exactly what the question required, without elaboration.

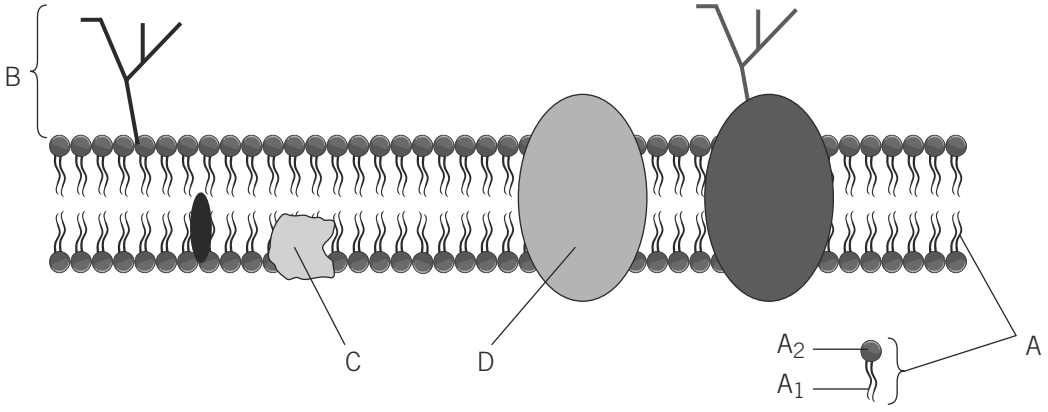
Question: Explain how you know whether a component of a plasma membrane is a glycoprotein or a glycolipid.

Answer: A glycoprotein consists of a carbohydrate chain attached to a protein molecule, as indicated by C in the diagram. A glycolipid, by comparison, consists of a carbohydrate chain attached to a phospholipid, as indicated by B in the diagram.

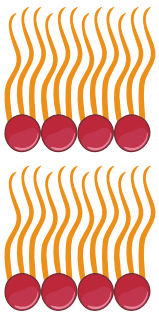
This answer gives an elaborated response that demonstrates the ability to differentiate between the two components of the plasma membrane. To ensure that the answer is clear, a comparative term is included ('by comparison'). The answer also *incorporates specific evidence from the diagram* to further demonstrate the correct understanding of the difference between the two components of the plasma membrane.

Section 1A questions

1 Phospholipids are a component of the plasma membrane. Recall the other three components.
2 State the functions of the three components you identified in Question 1.
3 Identify the labelled components in this diagram.



4 Outline the orientation of phospholipids in a plasma membrane.
5 Explain why phospholipids have their particular orientation in a plasma membrane.
6 Identify the errors in the diagram of a plasma membrane shown on the right.



Cell types and cell organelles

Study Design (from Unit 1):

- Cells as the basic structural feature of life on Earth, including the distinction between prokaryotic and eukaryotic cells
- The structure and specialisation of plant and animal cell organelles for distinct functions, including chloroplasts and mitochondria

Glossary:

Cell wall
Chloroplast
Cilia
Cytoplasm
Eukaryote
Flagella
Golgi apparatus
Lysosome
Mitochondrion
Nucleoid
Nucleus
Organelle
Plasmid
Prokaryote
Rough endoplasmic reticulum
Smooth endoplasmic reticulum
Vacuole
Vesicle

ENGAGE

Bacteria

Fossil records reveal that bacteria were the first type of organism on Earth, 3.5 billion years ago. Bacteria are now the most prevalent organism on Earth – an estimated 5 million trillion trillion (that is, a 5 with 30 zeros after it). This means there are more bacteria on Earth than stars in the universe. At any one time, you can find up to 10^{10} bacteria in your mouth and 10^{14} bacteria in your gut. Of all the bacteria, 85% are considered 'good' bacteria and 15% 'bad' bacteria. Even though the number of bacteria in your body outnumbers the number of cells in your body by about 10 to 1, bacteria contribute only 1–3% of your body mass. Bacteria are able to live in extreme environments, such as volcanic hot springs and hydrothermal vents.

6B CELLULAR PATHOGENS LINK

Figure 1B–1 A volcanic hot spring, Sunset Lake, Yellowstone National Park, USA. The colours are created by thermophilic bacteria growing in the hot water.

EXPLAIN

Benefits of compartmentalisation within cells

A cell can be thought of as a 'reaction factory', a place where many molecules (reactants) interact to release new products. For a successful reaction, the molecules need to move into and around the cell at the correct rate. They also need to be in the correct concentration for specific chemical reactions to occur.

A eukaryotic cell is relatively large, making it more difficult for chemical reactions to occur efficiently within it. This problem is overcome by having smaller compartments within the cell. Organelles are membrane-bound compartments within a cell that perform specific functions. Each organelle (with the exception of ribosomes) has a plasma membrane. This membrane enables the organelle to maintain the concentrations of molecules at

levels that allow the reactants to interact with each other at optimal rates. The compartmentalisation that occurs with organelles also allows various chemical reactions to occur simultaneously in different places without interfering with each other (Figure 1B–3).

Prokaryotes and eukaryotes

Organisms can be classified into one of two groups: prokaryotes and eukaryotes (see Figure 1B–2). A **prokaryote** is generally a single-celled organism that does not contain membrane-bound organelles. Examples of prokaryotic organisms are bacteria and archaea.

Eukaryotes can be single-celled or multicellular organisms. Eukaryotic cells contain membrane-bound organelles and are usually much larger than prokaryotic cells. Examples of eukaryotic organisms are protists, fungi, plants and animals.

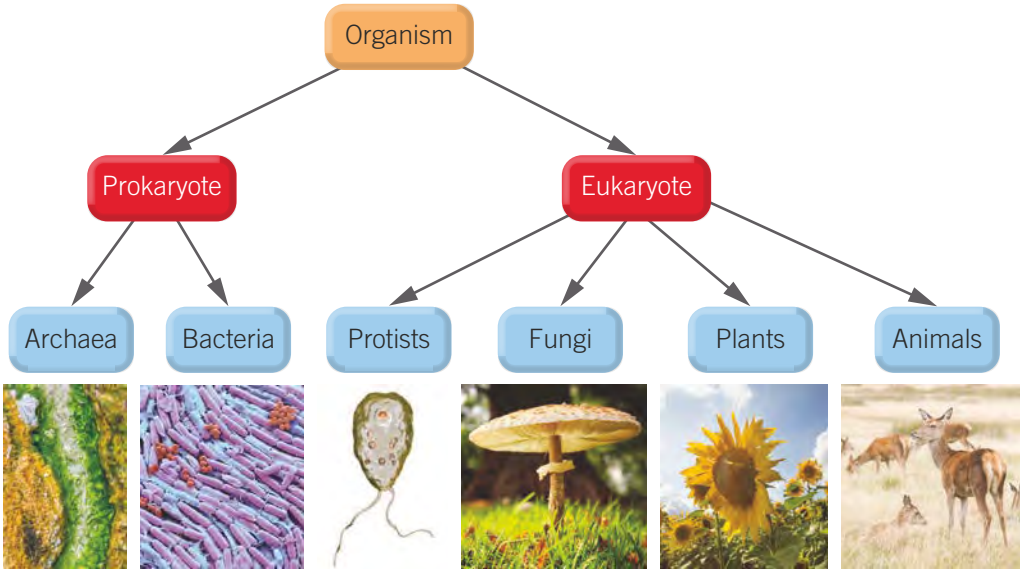
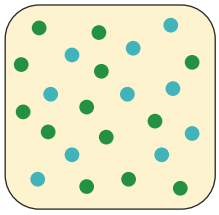
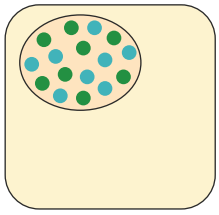


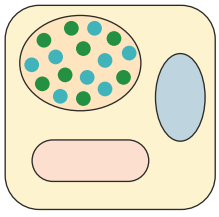
Figure 1B–2 Organisms can be classified as prokaryotes or eukaryotes. This diagram also shows another way of classifying them: into kingdoms (blue boxes).



Reactant molecules spread out in a large compartment.



If the reactant molecules are restricted to a smaller space by being enclosed in an organelle, they are more likely to bump into each other and react.



More organelles allow other specialised reactions to take place without being affected by the presence of other reactants.

Figure 1B–3 The benefits of compartmentalisation within a cell

Check-in questions – Set 1

1 Define eukaryote and prokaryote.
2 Which kingdoms consist of multicellular organisms?
3 Explain whether the organism in the image is a prokaryote or a eukaryote.

Prokaryote
a single-celled organism that does not have membrane-bound organelles; includes bacteria and archaea

Eukaryote
a single-celled or multicellular organism whose cells include membrane-bound organelles; includes protists, fungi, plants and animals

Cellular features of prokaryotes and eukaroytes

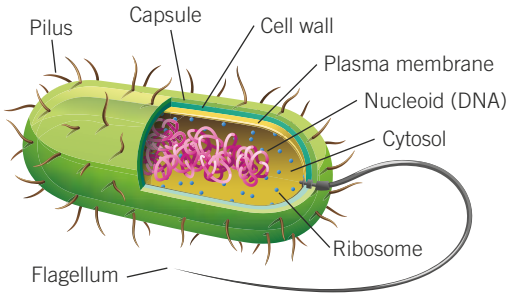
The basic features of eukaryotic cells and prokaryotic cells are described in Figure 1B–4.

Nucleoid
in a prokaryote, an irregularly shaped area where the genetic material is located

Plasmid
a circular piece of double-stranded DNA found naturally in bacteria

Prokaryotic cell

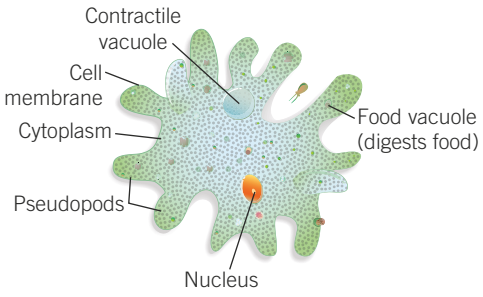
- Lacks membrane-bound organelles
- Contains ribosomes
- Genetic material is usually one circular DNA chromosome located in an area known as a **nucleoid**
- Many contain small rings of double-stranded DNA called **plasmids**
- Plasma membrane is surrounded by a cell wall
- Some bacteria have a capsule around the cell wall for extra protection
- Some bacteria also have flagella (singular: flagellum) for movement and pili (singular: pilus) for attaching to surfaces



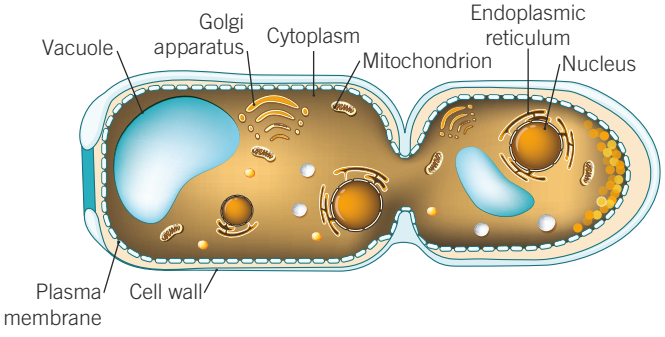
Cytoplasm
all the contents inside the membrane of a cell, except the nucleus; includes organelles

Eukaryotic cell

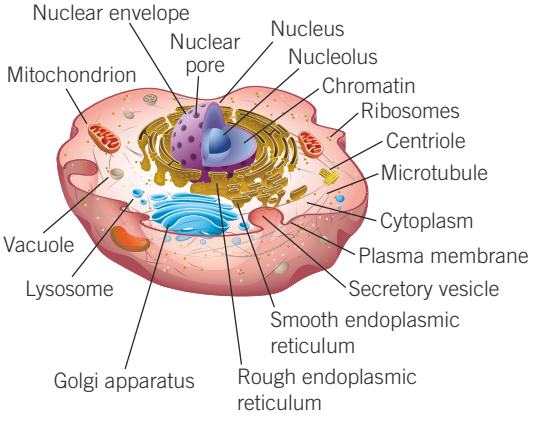
- Contains membrane-bound organelles
- Contains ribosomes
- Genetic material is linear DNA chromosomes contained within a nucleus
- Plasma membrane encloses the **cytoplasm**
- Cell wall is present in fungi, plants and some protists
- Many cell types (not fungi) have flagella or cilia for movement



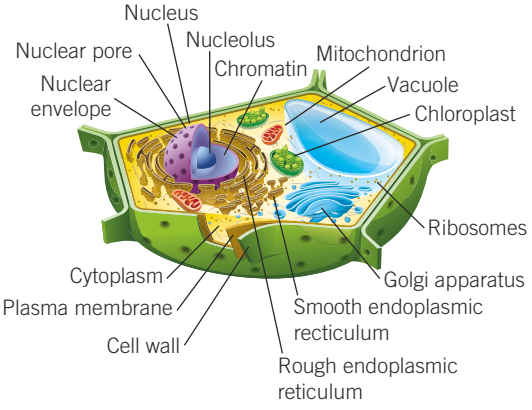
Amoeba (a protist)



Fungal cell



Animal cell



Plant cell

Figure 1B–4 Cellular features of eukaryotes and prokaryotes

Check-in questions – Set 2

1 What is a plasmid?
2 What is the difference between the DNA structure of a prokaryote and that of a eukaryote?
3 Which kingdom's cells contain a cell wall?
4 Which type of cell is larger: prokaryote or eukaryote?

Organelles in plant and animal cells

Table 1B–1 identifies the types of **organelles** found in plants and animals, and their structure and function.

Organelle
a compartment within a cell that performs specific functions

Table 1B–1 The structure and function of organelles in plant and animal cells

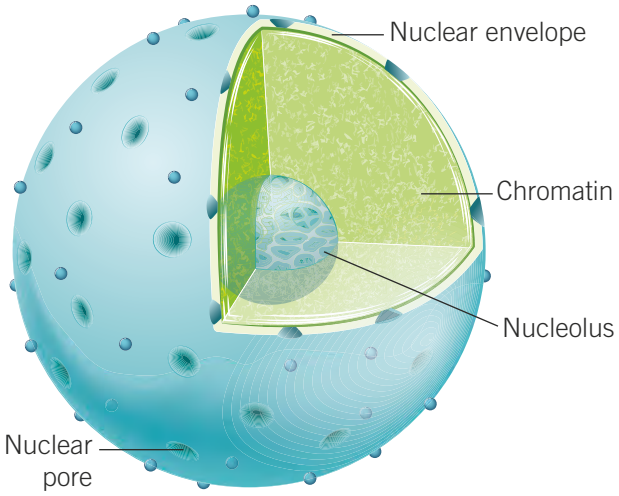
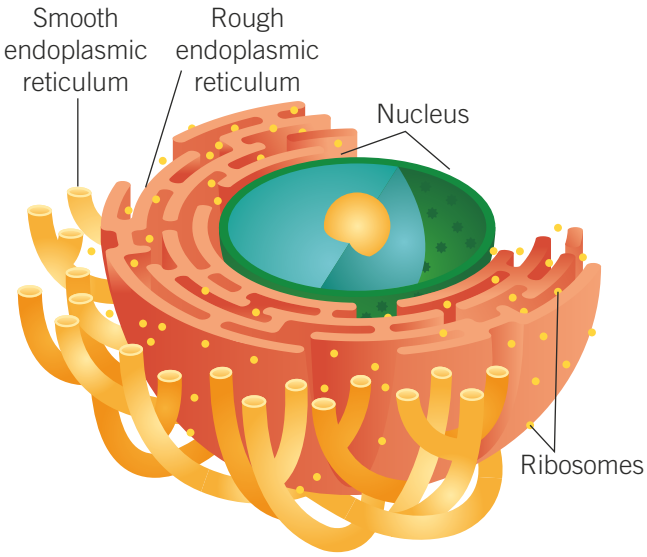
Organelle	Structure	Function
Nucleus a double-membrane-bound organelle that contains genetic material (DNA, RNA) 	Enclosed in a double-layered nuclear membrane Nucleolus inside contains RNA Nuclear pores allow a type of RNA (messenger RNA) to leave the nucleus following transcription LINK 2B THE GENETIC CODE AND GENE EXPRESSION	Controls the cell activities. Contains DNA, which codes for proteins. This DNA is passed onto daughter cells in mitosis and meiosis.
Endoplasmic reticulum (smooth and **rough)** **Smooth endoplasmic reticulum** an organelle that synthesises and transports lipids **Rough endoplasmic reticulum** an organelle that transports proteins in vesicles to the Golgi apparatus 	Membrane bound with a system of tubules Rough ER is studded with ribosomes Smooth ER has no ribosomes	Smooth ER: synthesis and transport of lipids Rough ER: transports proteins in vesicles to the Golgi apparatus

Table 1B–1 *Continued*

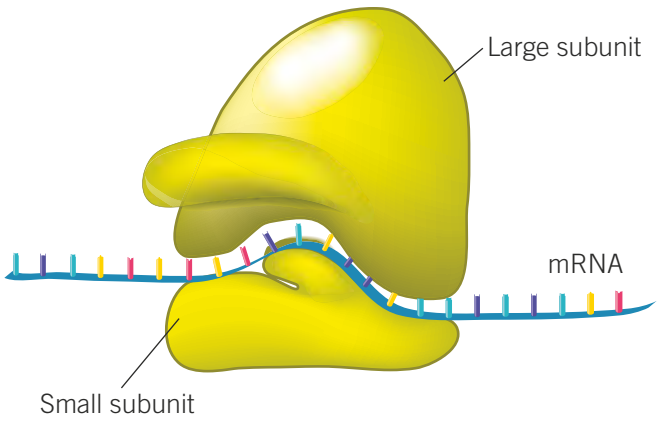
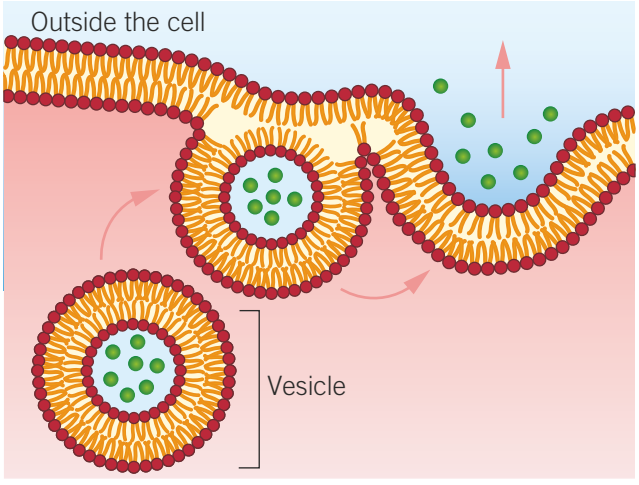
Organelle	Structure	Function
Ribosome  	A non-membrane-bound organelle, made up of protein and ribosomal RNA	Synthesis of proteins, a process known as translation Ribosomes in the cytosol make proteins for use inside the cell, whereas ribosomes on the rough ER make proteins for use outside the cell LINK **2B** THE GENETIC CODE AND GENE EXPRESSION
Golgi apparatus an organelle consisting of layers that modifies and packages proteins  	Layers of membrane-bound stacks	Modifies and packages proteins into secretory vesicles for exporting from the cell
Vesicle an organelle that transports materials between organelles and within the cell  	Membrane-bound sac	Transport of materials (e.g. proteins) between organelles and within the cell Also used for exporting molecules from the cell (exocytosis) or bringing molecules into the cell (endocytosis, see Figure 1C-12) LINK **7B** SECOND LINE OF DEFENCE

Table 1B–1 *Continued*

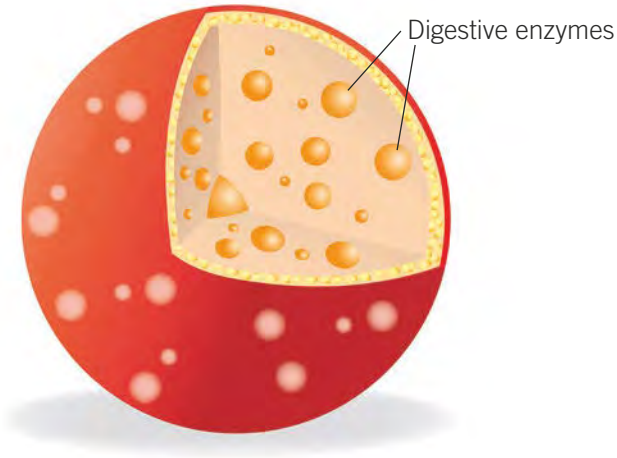
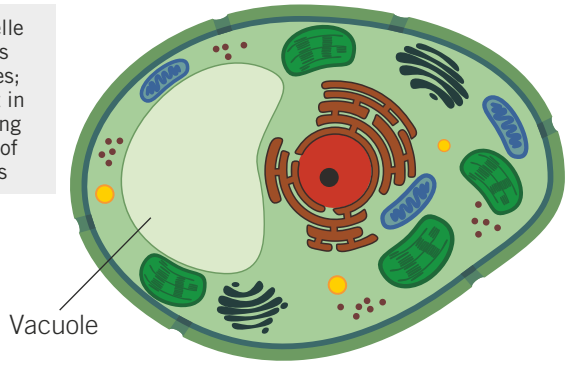
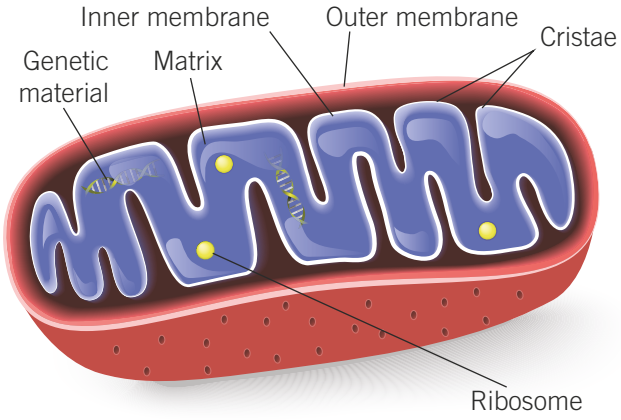
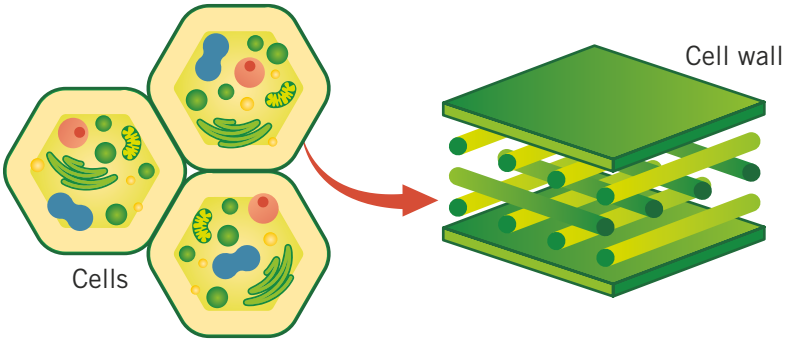
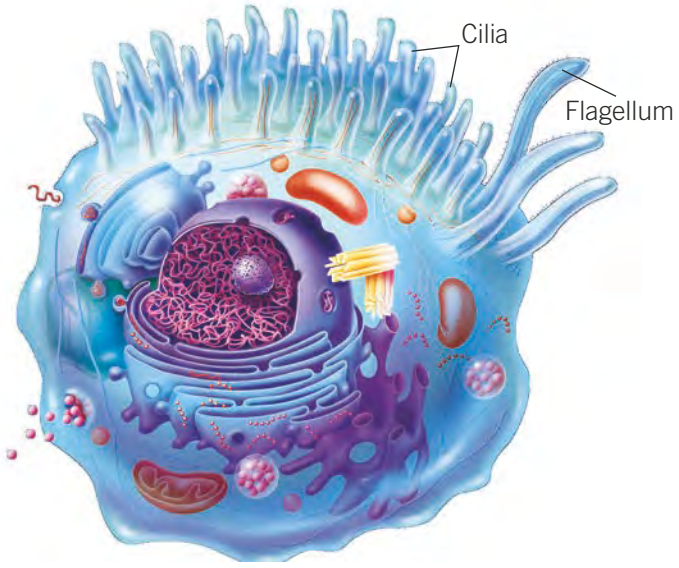
Organelle	Structure	Function
Lysosome an organelle containing enzymes that break down foreign matter or materials no longer required 	Membrane-bound sacs containing digestive enzymes	Break down materials no longer required, or foreign matter LINK 7B SECOND LINE OF DEFENCE
Vacuole an organelle that stores substances; important in maintaining structure of plant cells 	Membrane (tonoplast)-bound sac	Storage of substances (e.g. water and ions) Vacuoles are larger in plants than in animals and are important in maintaining plant structure
Mitochondrion an organelle where respiration occurs, releasing energy (ATP) 	Has a double membrane, with the inner membrane folded to form cristae, with matrix between	Site of stages 2 and 3 of aerobic cellular respiration, which releases usable energy in the form of ATP molecules LINK 5C CELLULAR RESPIRATION

Table 1B–1 *Continued*

Organelle	Structure	Function
Chloroplast (plants) an organelle where photosynthesis occurs; contains chlorophyll 	Double membrane comprising grana (stacks of membrane discs called thylakoids) and stroma (fluid). Grana contain chlorophyll.	Site of photosynthesis, which converts carbon dioxide and water, with the assistance of light, to glucose and oxygen Animal cells do not have chloroplasts. LINK 5A PHOTOSYNTHESIS
Cell wall (plants) a structure only in plants that surrounds the cell and provides support and protection 	Surrounds the cell, lies outside the plasma membrane Contains cellulose (This covers plant cell walls, but fungal cells also have a cell wall, made of chitin. Cell walls made of other substances are also found in bacteria, archaea and some protista.)	Provides cellular structure and protection Animal cells do not have a cell wall
Cilia and **flagella** **Cilia** short microtubules projecting from a cell that move to provide motility (movement of the cell) or movement of fluid **Flagella** long microtubules projecting from a cell that move to provide motility (movement of the cell) or movement of fluid 	Microtubule projections from the cell. Cilia are generally shorter, flagella longer.	Provide motility (movement of the cell) or movement of fluid

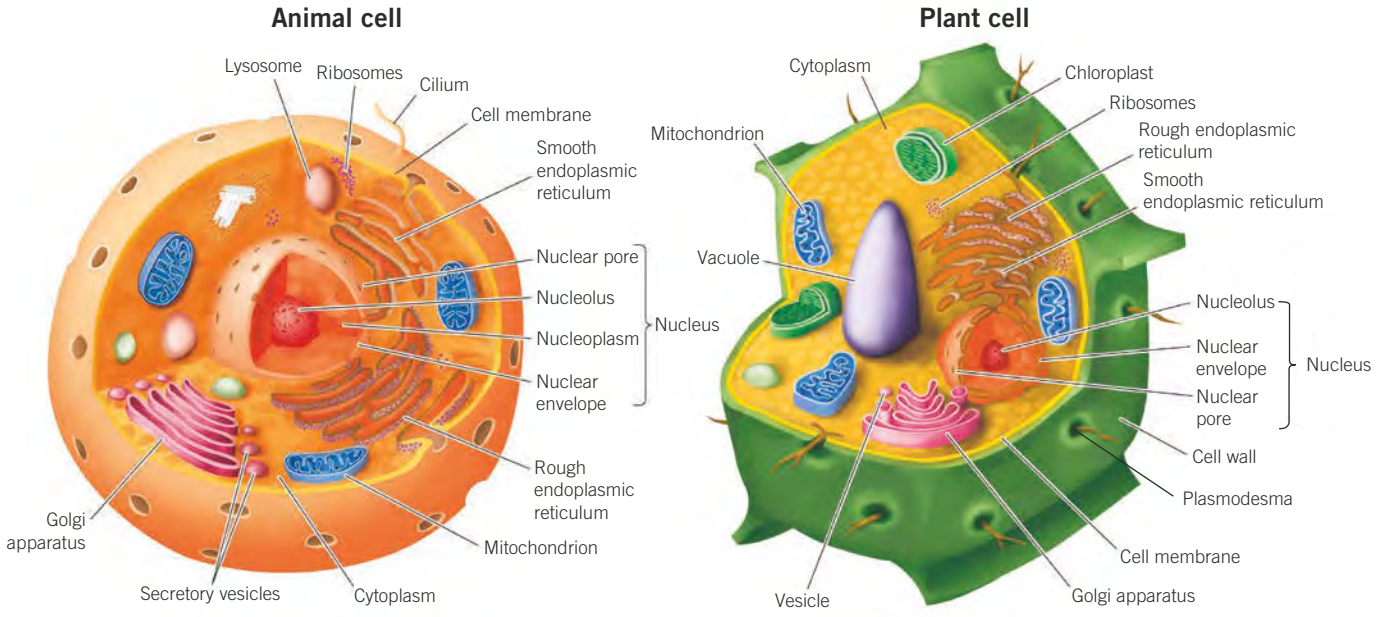


Figure 1B–5 Generalised animal cell and plant cell, with all organelles labelled

It is important to realise that cells do not all have the same types or number of organelles. The types and numbers of organelles present in a cell are specific to the type of tissue in which the cell is found. For example, a sperm cell requires a great deal of energy, so you would expect to find a large number of mitochondria in it, as mitochondria provide energy for the cell. The secretory cells that line the stomach are part of the digestive system, and hence they require many Golgi apparatus to enable them to release enzymes that aid in the breakdown of food molecules.

WORKSHEET 1B–1 CELLS AND THEIR ORGANELLES

WORKSHEET 1B–2 MODELLING CELLS

Check-in questions – Set 3

1 What is an organelle?
2 Provide an advantage of having organelles, for a eukaryotic cell.
3 Define compartmentalisation.
4 What is the function of ribosomes?

Comparison of plant and animal cells

Plant cells and animal cells have many organelles in common, but there are some differences (Figure 1B–6).

A misconception is that plant cells only perform photosynthesis and do not respire. In fact, plant cells perform both photosynthesis and cellular respiration, and so a plant cell has both mitochondria and chloroplasts. Not all the cells in a plant photosynthesise (for example, root cells do not photosynthesise), but they do all have a cell wall. In contrast, animal cells do not have any chloroplasts, and do not photosynthesise.

LINK **5A** PHOTOSYNTHESIS

LINK **5C** CELLULAR RESPIRATION

VIDEO 1B–1 ORGANELLES IN PLANT AND ANIMAL CELLS

Two further key differences between plant cells and animal cells:

- *Cell wall* – plant cells have a cell wall to aid with structural support for the whole plant, whereas animal cells do not. Animals have other features, such as exoskeletons or bones, to provide support.
- *Permanent vacuole* – plant cells have a permanent large vacuole for the storage of water, minerals and ions. The vacuole also assists with the structural support of the cell through turgor pressure (pressure exerted by water). Animal cells do have vacuoles but they are small and are not permanently present, and they do not provide structural support to the cell.

1C MEMBRANE TRANSPORT LINK

WORKSHEET 1B–3 COMPARING ANIMAL AND PLANT CELLS

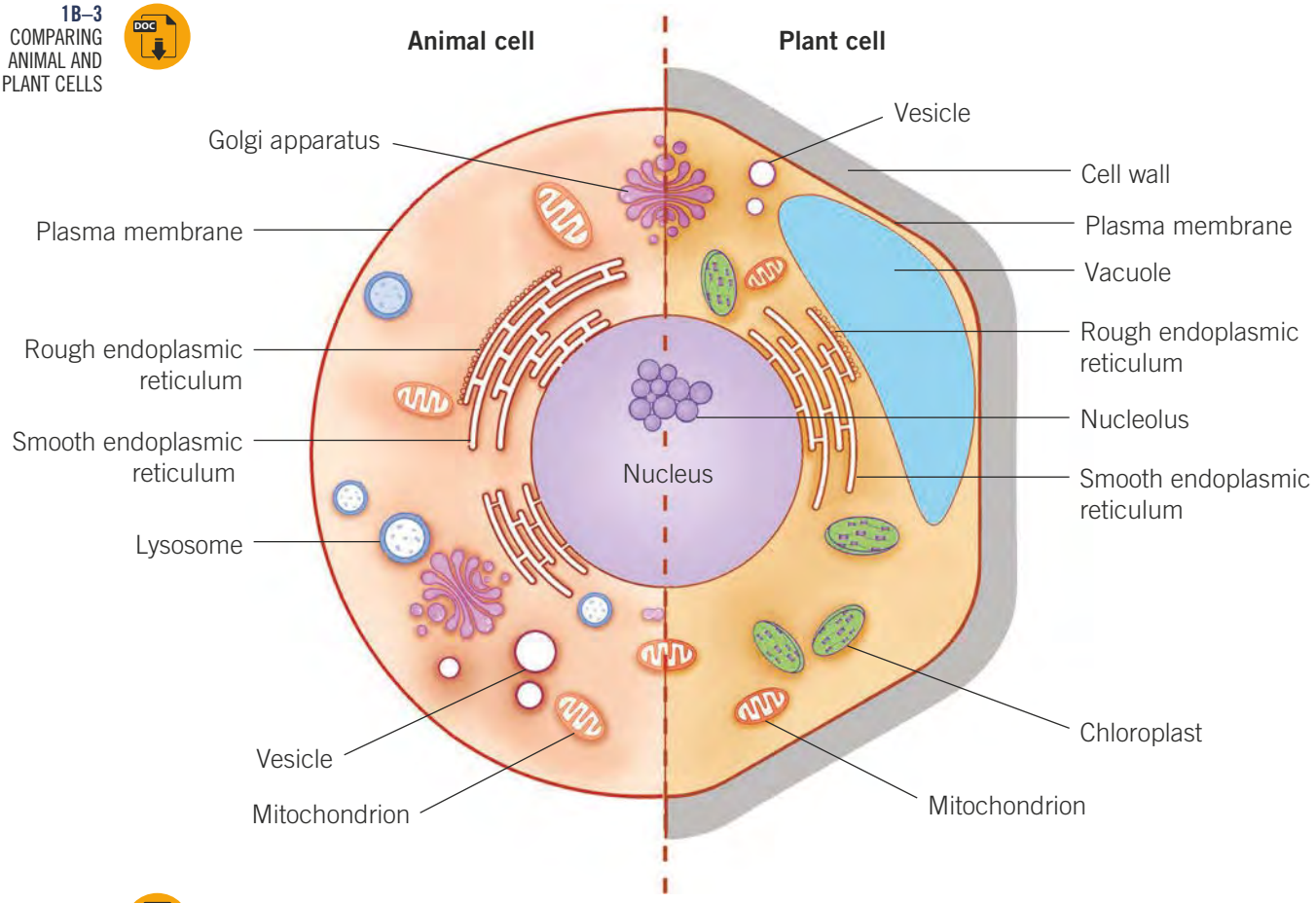


Figure 1B–6 Similarities and differences between an animal cell and a plant cell

1B SKILLS

VIDEO 1B–2 SKILLS: USING DIAGRAMS TO ANSWER QUESTIONS

Using diagrams to answer questions

When providing examples of organisms that are either eukaryotic or prokaryotic, it is important to use cellular features as your justification. A key cellular feature is the presence or absence of membrane-bound organelles. If the cell contains a membrane-bound organelle, then it is a eukaryote.

One type of organism that often causes confusion is protists. A protist is unicellular but it is not a prokaryote. It is important to remember that some unicellular organisms, such as paramecium, contain membrane-bound organelles, as shown in Figure 1B–7. For this reason, paramecium is classified as a eukaryote.

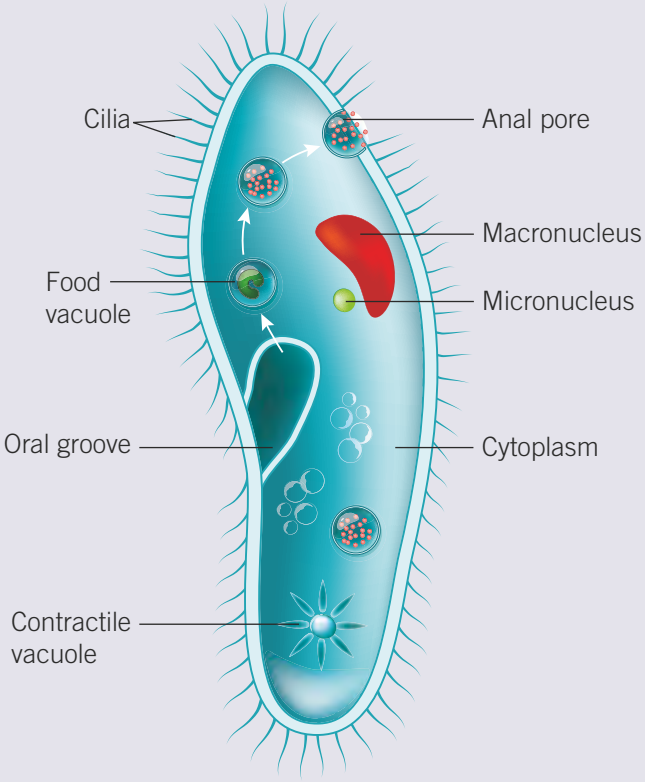


Figure 1B–7 Paramecium is a unicellular organism that has membrane-bound organelles.

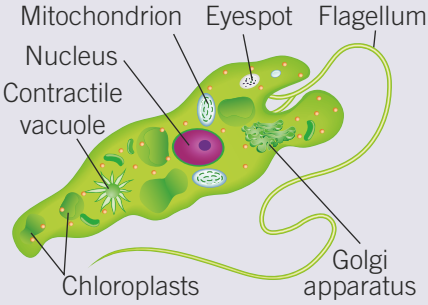
You could be asked to *identify* which of the examples are either a prokaryote or a eukaryote. When a question asks you to identify, there is no need to include a justification or explanation. All you need to do is put the example into a category, or label it as either prokaryote or eukaryote.

For example:

Question: Identify which of the diagrams shows a prokaryote.

A

B



C

Answer: C

As you can see, the answer consists of just the letter associated with the correct diagram. This is in contrast to a question that asks you to *justify* your choice. For example:

Question: Provide a justification for why you have identified your answer as a prokaryote.

Answer: C lacks membrane-bound organelles. The other two diagrams show a nucleus or another membrane-bound organelle. Hence C is a prokaryote.

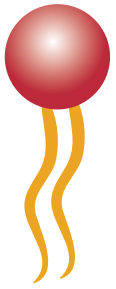
Note that the answer does not repeat the question, and it gives a specific piece of evidence as the reason for the choice. The response also finishes off by coming back to the context of the question, re-stating that the chosen option is a prokaryote.

Creating flashcards

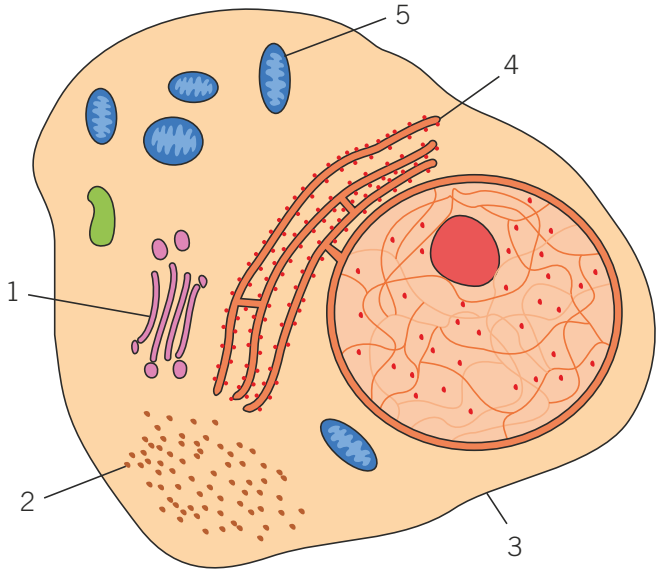
One strategy to help you remember the functions of the organelles is to use the diagrams and definitions from Table 1B–1 to create flash cards. The more you use images associated with terms and definitions, the easier it will be for you to correctly recall the organelle functions.

Section 1B questions

1 Define prokaryote.
2 Provide three examples of eukaryotic organisms.
3 State which kingdoms are eukaryotic.
4 Outline how you would correct the following statement: Prokaryotes only have circular pieces of DNA, whereas eukaryotes only have linear pieces of DNA.
5 Explain why a prokaryote is still an organism.
6 Redraw the image of the phospholipid and annotate it with the following labels: fatty acid tail, glycerol and phosphate head, hydrophilic, hydrophobic.



7 Where in a cell would a phospholipid be found?
8 Using a Venn diagram, identify the similarities and differences between unicellular and multicellular organisms.
9 Describe the function of the cell wall for a plant.
10 Identify each of the numbered structures in the diagram.



11 Define the role of:
   a the mitochondrion b the plasma membrane c organelles.
12 Is a bacterial cell a prokaryote or a eukaryote?
13 Identify what you would *not* find in a bacterial cell.
14 Explain why you would not include the label of 'cytoplasm' in a diagram of a bacterial cell.
15 Compare a eukaryotic cell to a prokaryotic cell.
16 Plant cells perform photosynthesis in order to produce glucose. Explain whether it is reasonable to expect chloroplasts to be found in all cells of a plant.

Membrane transport

Study Design (from Unit 1):
The structure and function of the plasma membrane in the passage of water, hydrophilic substances via osmosis, facilitated diffusion and active transport

Glossary:

Active transport
ATP (adenosine triphosphate)
Bulk transport
Carrier protein
Channel protein
Concentration gradient
Endocytosis
Exocytosis
Facilitated diffusion
Lipophilic
Lipophobic
Osmosis
Passive transport
Permeable
Phagocytosis
Pinocytosis
Polar
Protein-mediated transport
Simple diffusion
Tonicity
Vesicle mediated transport

ENGAGE

Transportation across the plasma membrane

One of the main roles of the plasma membrane is to control the entry and exit of substances between the internal and external environments of the cell. This is essential for the cell's survival. Like us, cells need nutrients so that important cellular processes can occur. For example, they need water (for photosynthesis by plant cells), oxygen (for aerobic cellular respiration) and amino acids (for protein synthesis). At the same time, cells must ensure that unwanted substances or waste do not accumulate, so these substances must be disposed of. Examples are carbon dioxide resulting from cellular respiration, excess water from condensation reactions that occur in the synthesis of biomacromolecules, and nitrogenous waste from proteins (such as urea). All these substances move into and out of the cell through the plasma membrane.

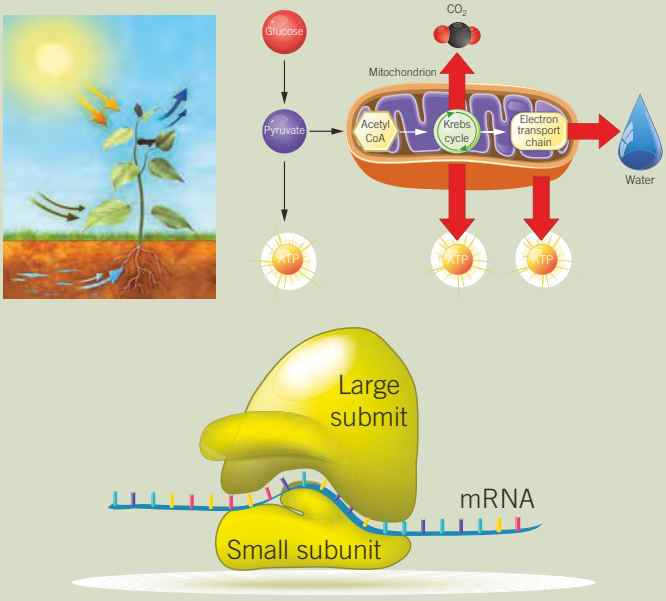


Figure 1C–1 The plasma membrane controls the entry and exit of important substances, like those needed in photosynthesis (top left), cellular respiration (top right) and protein synthesis (bottom).

LINK **5A** PHOTOSYNTHESIS
LINK **5C** CELLULAR RESPIRATION
LINK **2B** THE GENETIC CODE AND GENE EXPRESSION

EXPLAIN

The nature of substances

Polar describes a molecule that has different charged sides ('poles') and dissolves in water, which is also a polar substance

Lipophilic dissolves easily in lipids; also called hydrophobic

Lipophobic does not dissolve readily in lipids; also called hydrophilic

What makes understanding the movement of substances challenging is that they do not all have the same characteristics. The molecules of some substances are large, some are small, some are charged and some uncharged, some are **polar**, some dissolve in water (hydrophilic) and others dissolve in lipids (**lipophilic**).

As you progress through this chapter, you will see that several terms can be used interchangeably. For example, substances that dissolve readily in water (and do not dissolve readily in lipids) can be described as *hydrophilic* or **lipophobic**. In the same way, substances that dissolve readily in lipids (and do not dissolve readily in water) can be described as *lipophilic* or hydrophobic.

NOTE

Hydrophilic comes from *hydro*, which means water, and *philic*, which means loving. *Lipophobic* comes from *lipo*, which means lipids or fats, and *phobic*, which means hating.
Hydrophobic comes from *hydro*, which means water and *phobic*, which means hating. *Lipophilic* comes from *lipo*, which means lipids or fats, and *philic*, which means loving.

The different characteristics of the substances being transported mean that these substances require different ways of transport across the plasma membrane. It is the different components of the plasma membrane that allow this to happen.

Structural elements of the plasma membrane

1A PLASMA MEMBRANE LINK

In Section 1A, the characteristics of the plasma membrane were described. These include the phospholipid bilayer, proteins, carbohydrate chains and cholesterol. Movement through the plasma membrane occurs through two of these structural elements:

- Phospholipid bilayer
  - Lipophilic (hydrophobic) substances move freely through the phospholipid bilayer due to the hydrophobic nature of the fatty acid tails of phospholipids.
  - Water, gases, and other small hydrophobic molecules can diffuse directly across the phospholipid bilayer.
- Protein channels/carriers
  - Polar or hydrophilic substances pass through the channel proteins and carrier proteins.

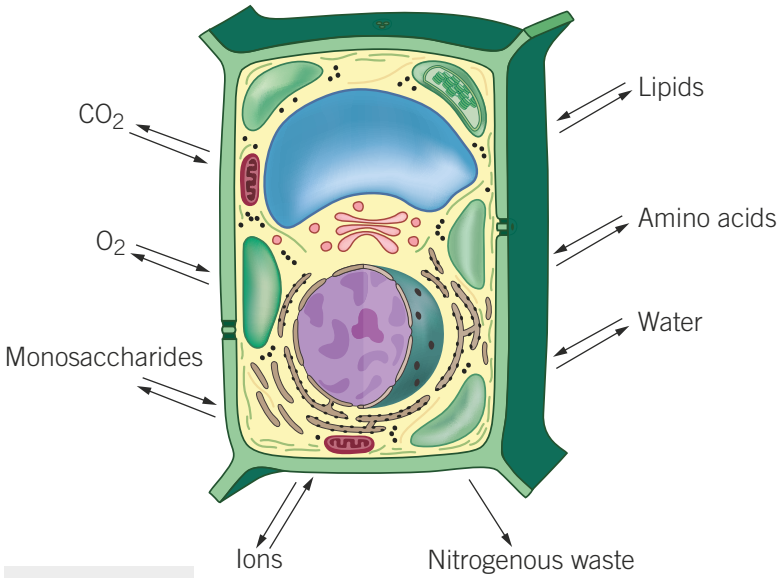


Figure 1C–2 The movement of different substances across the plasma membrane is essential for the survival of the cell.

Permeable allows things to pass through; for example, a semi-permeable membrane only allows some substances to pass through

It is these structural elements that allow the plasma membrane to be selective about what moves from one side of the plasma membrane to the other. Therefore, the plasma membrane is referred to as being semi-permeable, or partially **permeable**, selectively permeable or differentially permeable.

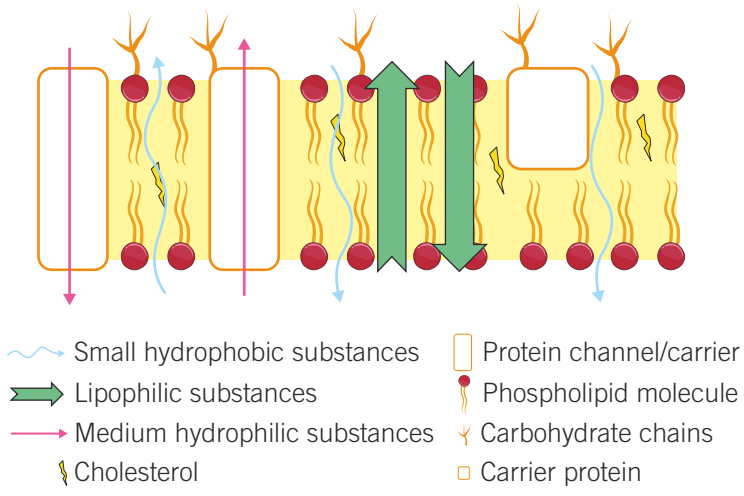


Figure 1C–3 Substances move into and out of a cell through the pores (green arrows), phospholipid bilayer (blue arrows) or protein channels/carriers (red arrows), depending on the nature of the substance.

WORKSHEET 1C–1 MEMBRANES

The movement of substances

The modes of transport of substances across the plasma membrane include simple diffusion, osmosis, facilitated diffusion, active transport and bulk transport.

Biologists classify these forms of transport into two broad categories: **passive transport** and **active transport**. Passive transport does not require energy (in the form of ATP, to be explained later); active transport does require energy. Table 1C–1 summarises the varied nature of substances that a cell needs to move across its plasma membrane in order to survive, the component of the plasma membrane they move through, which mode of transport is used, and whether this mode of transport is active or passive.

Passive transport the net movement of substances from a region of high substance concentration to a region of low substance concentration without the need for energy input; can also occur in non-living systems where there is no cell membrane

Active transport the net movement of substances from a region of low substance concentration to a region of high substance concentration using a protein carrier; requires energy to be input

Table 1C–1 The nature of substances (other than water) a cell needs to move, the component of the plasma membrane they move through, and the type and mode of transport

Nature of substance	Examples	Component of membrane	Type	Mode of transport
Hydrophilic, uncharged	Oxygen Carbon dioxide	Phospholipid bilayer – pores	Simple diffusion	Passive
Hydrophilic, polar	Urea	Phospholipid bilayer – pores	Simple diffusion	Passive
Medium size, lipophilic	Alcohol Lipids/fats/steroids	Phospholipid bilayer	Simple diffusion	Passive
Medium size, hydrophilic	Monosaccharides (glucose) Amino acids	Protein channel/ carrier	Facilitated diffusion Active transport	Passive Active
Ion	Potassium (K^+) Chloride (Cl^-) Sodium (Na^+)	Protein channel/ carrier	Facilitated diffusion Active transport	Passive Active
Large	Proteins Complex carbohydrates	Unable to pass through the plasma membrane	Vesicle-mediated transport (bulk transport)	Active

LINK 2B THE GENETIC CODE AND GENE EXPRESSION

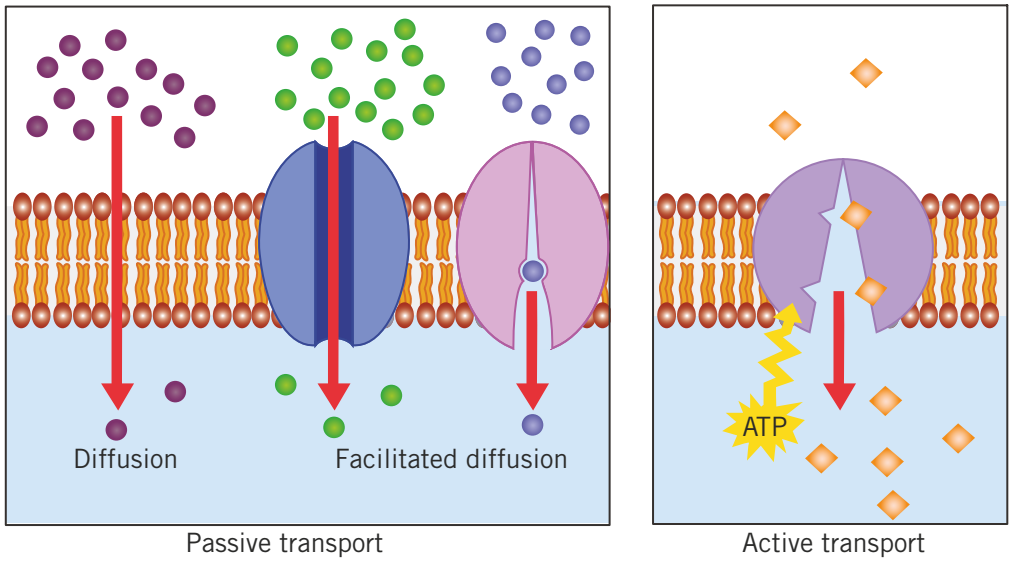


Figure 1C–4 Comparison of passive and active transport. Can you identify the differences?

Check-in questions – Set 1

1 Give examples of substances that are able to move into a cell.
2 Give examples of substances that are moved out of a cell.
3 Define semi-permeable.
4 If a substance is described as 'water soluble', what are some other terms you could use to describe this characteristic?
5 Name the two structural elements involved in movement of substances through the plasma membrane.

VIDEO 1C–1 MODES OF TRANSPORT OF SUBSTANCES ACROSS THE PLASMA MEMBRANE

Passive transport

Simple diffusion

Simple diffusion the net passive movement of a substance from a region of high concentration to a region of low concentration until equilibrium is reached; a form of passive transport, as it does not require energy

The most basic way for substances to move is by **simple diffusion** (Figure 1C–5). Diffusion is a type of passive transport, which means it does not require energy. It occurs naturally in both living and non-living systems. In living systems, diffusion can take place across a plasma membrane, as long as the molecule can pass through the hydrophobic fatty acid tails of the phospholipid bilayer. Examples of substances that move by simple diffusion are oxygen, water, urea, carbon dioxide, alcohol and steroid hormones.

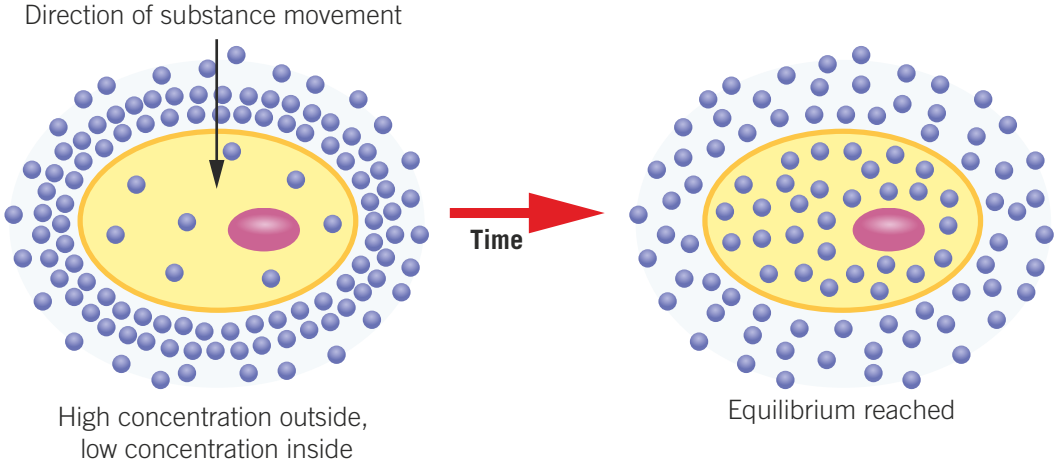


Figure 1C–5 Diffusion: the net passive movement of a substance from a region of high concentration to a region of low concentration across a semi-permeable membrane until equilibrium is reached

Rate of diffusion

The conditions within a cell can affect the rate or speed at which substances diffuse across the plasma membrane. Because diffusion is necessary for a cell to survive, the cell requires optimal conditions so that diffusion is as efficient as possible. Table 1C–2 summarises the main factors that can affect the rate of diffusion.

Table 1C–2 Factors that affect the rate of diffusion

Factor	How diffusion is affected
Size	The smaller the molecules of a substance, the higher the rate of diffusion.
Concentration	The greater the difference in concentration between the two regions (known as a **concentration gradient**), the higher the rate of diffusion.
Temperature	The higher the temperature, the higher the rate of diffusion. When the temperature increases, the vibrations (kinetic energy) of the molecules also increase and so it is easier for them to move.

Concentration gradient the difference between the concentrations of a substance in two regions; if there is a large difference, the concentration gradient is steep

Osmosis

Osmosis is a special case of diffusion: it is the net passive movement of free water from a region of high free water concentration to a region of low free water concentration across a semi-permeable membrane, until equilibrium is reached. Did you notice the difference compared to the definition of diffusion? Osmosis is all about the movement of free water. Just like diffusion (because it is the diffusion of water), osmosis is a passive process that requires no input of energy (ATP) to occur.

VIDEO 1C–2 OSMOSIS

Osmosis the net passive movement of free water from a region of high free water concentration to a region of low free water concentration across a semi-permeable membrane until equilibrium is reached

In Figure 1C–6, there are sugar molecules and water molecules on both sides of the semi-permeable membrane, which runs down the centre. In the left image, note where the lowest and highest concentration of sugar molecules are. However they are too big to pass across the membrane. Also note where the lowest and highest concentration of free water molecules are. Which way will they diffuse (by osmosis) across the membrane? Now compare the left image with the right image. What changes have occurred?

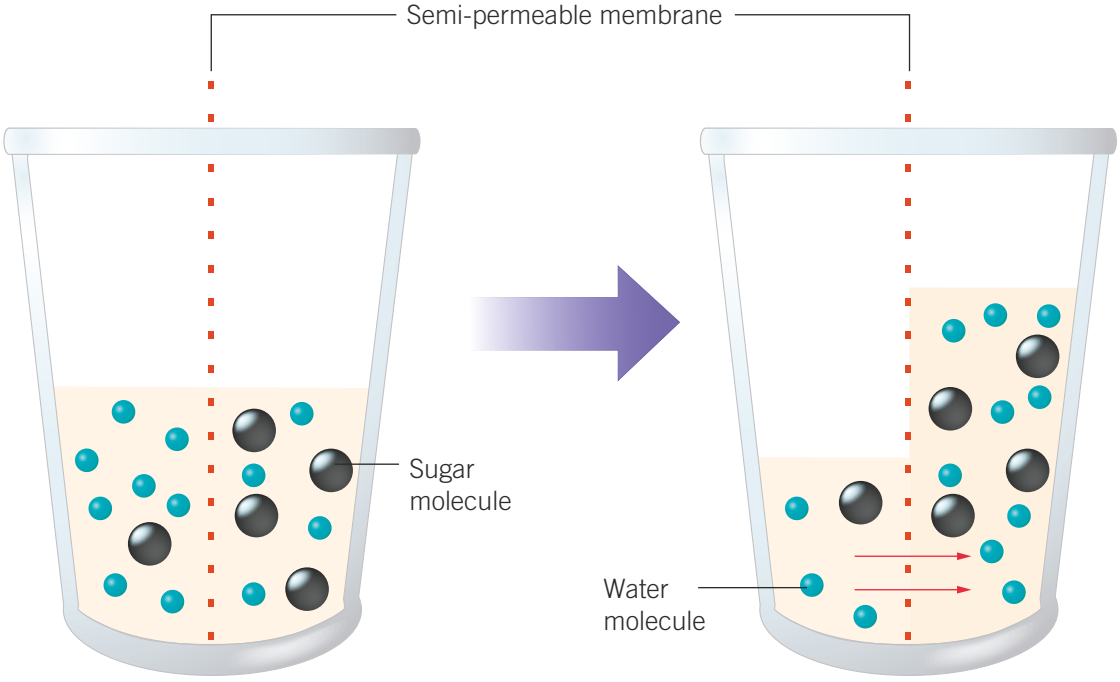


Figure 1C–6 Osmosis: the net passive movement of free water from a region of high free water concentration to a region of low free water concentration across a semi-permeable membrane until equilibrium is reached.

The free water has moved across the semi-permeable membrane from a region of high free water concentration to low free water concentration until evenly distributed on both sides: this is osmosis. Notice that the sugar molecules have not moved, despite there being a difference in the concentration on each side (concentration gradient). This is because the sugar molecules are too large to move through this membrane.

Note that when there is a high concentration of free water molecules in a solution, the solution has a low concentration of solute and is called *dilute*. If there is a low concentration of free water molecules in a solution, there is a comparatively higher concentration of solute and therefore the solution is said to be *concentrated*. In VCE, there are two definitions for osmosis: one refers to free water, as discussed above, and one refers to the solute concentration. In an examination situation, both definitions are equally acceptable as long as you don't get them confused. So the alternative definition for osmosis is:

> *Osmosis* is the net passive movement of free water from a region of low solute concentration to a region of high solute concentration across a semi-permeable membrane until equilibrium is reached.

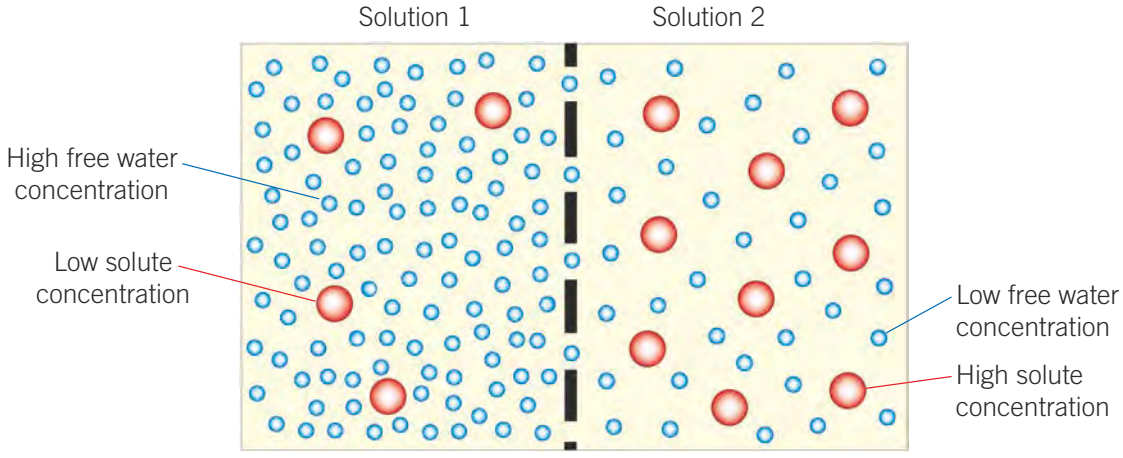


Figure 1C–7 Solutions can be described in two ways: by their water concentration or by their solute concentration.

Check-in questions – Set 2

1 Define osmosis.
2 How could you define osmosis another way?
3 List two similarities and two differences between diffusion and osmosis.

Tonicity how the concentration of solutes dissolved in an extracellular solution determines the direction and rate of osmosis and therefore the volume of a cell

Tonicity

Water is the medium in which many of the chemical processes of cells take place. This is because it is an excellent solvent and can therefore transport a lot of substances (solutes), as water moves easily through the plasma membrane. As a consequence, the water content of the solution outside a cell (the cell's external environment), will affect the conditions inside the cell (the cell's internal environment). This is called **tonicity**. Tonicity means that the concentration of solutes dissolved in an extracellular solution can determine the direction and rate of osmosis, and therefore the volume of a cell.

Tonicity in plant cells

In plant cells, diffusion and osmosis work together to cause carbon dioxide and water (reactants for photosynthesis) to move into the cells, and oxygen and water (products of photosynthesis) to move out of the cells, via the semi-permeable membrane. These substances are continuously entering and exiting through the stomata.

LINK 5A PHOTOSYNTHESIS

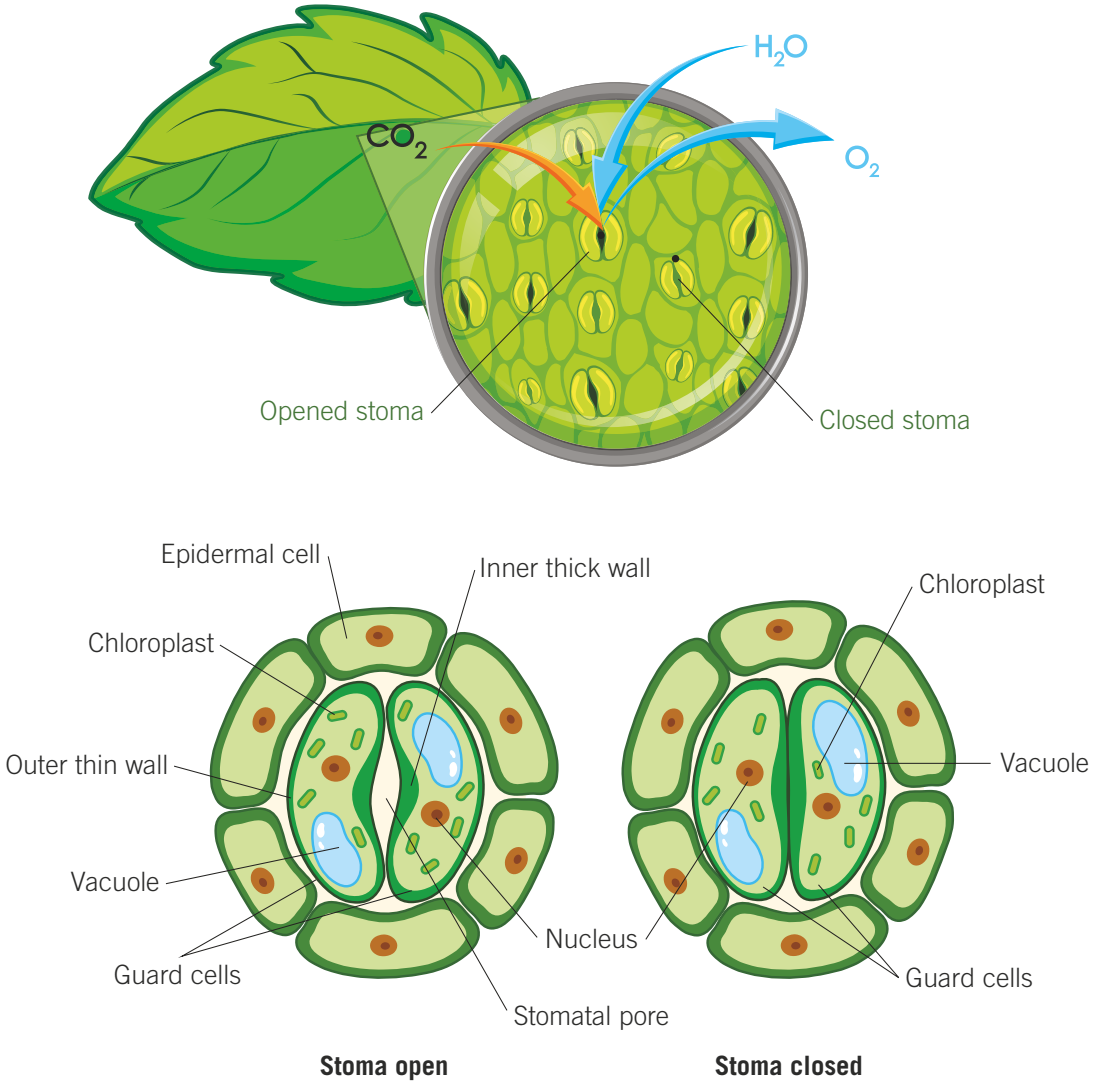


Figure 1C–8 The movement of key inputs (carbon dioxide and water) and outputs (oxygen) through the stomata in plants

Facilitated diffusion

A third type of passive transport is **facilitated diffusion**. As the name implies, this mode of transport is diffusion with assistance from a **carrier protein** or **channel protein**. These are the same protein transport molecules you saw earlier, embedded in the plasma membrane. Again, keep in mind that this process is still diffusion (passive, no energy required, moving from a region of high concentration to a region of low concentration), but it involves

Facilitated diffusion the net passive movement of a particular substance from a region of high concentration to a region of low concentration with the assistance of carrier proteins or channel proteins; also known as protein-mediated transport

Carrier protein a transmembrane protein that binds to a specific substance (e.g. glucose) and changes shape to move that substance across the plasma membrane, releasing it to the other side

Channel protein a transmembrane protein that allows hydrophilic or polar substances to move across the plasma membrane from a region of high concentration to a region of low concentration

Protein-mediated transport when a transmembrane protein assists in the transport of a substance across a plasma membrane; also known as facilitated diffusion

moving hydrophilic or polar substances that cannot diffuse through the phospholipid bilayer portion of the plasma membrane. These hydrophilic substances therefore need help to get across the plasma membrane. Facilitated diffusion is also referred to as **protein-mediated transport**.

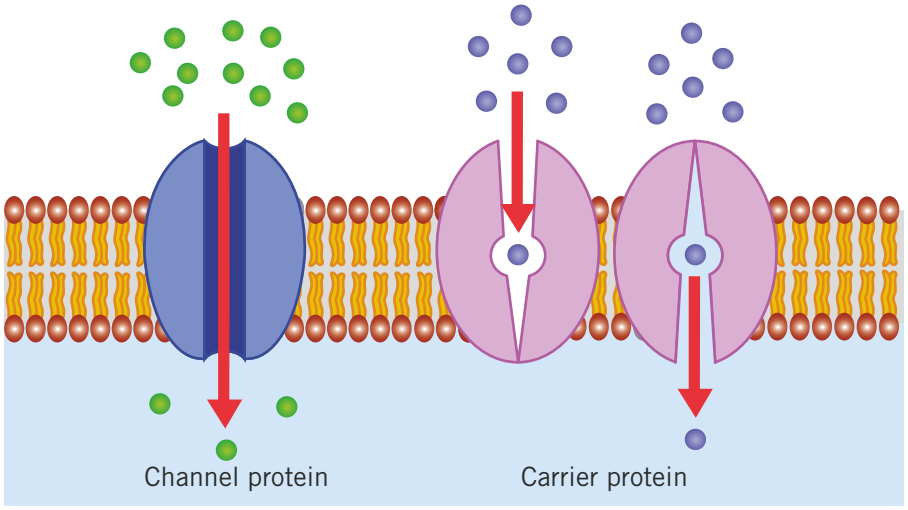


Figure 1C–9 Facilitated diffusion: the net passive movement of particular hydrophilic or polar substances by diffusion across the plasma membrane with the assistance of channel protein molecules (left) or carrier protein molecules (right).

The term 'protein-mediated' means that a carrier protein or channel protein is required for this mode of transport to occur.

- *Carrier proteins* are transmembrane proteins that bind to a particular substance and change shape to move the substance across the membrane to the other side. This is the case with hydrophilic substances such as glucose and amino acids (monomers of proteins). Carrier proteins are specific to the substance they are transporting and sometimes are referred to as being selective, in that some molecules are transported while others are not.
- *Channel proteins* are transmembrane proteins that allow hydrophilic substances to move through the membrane from a region of high concentration to a region of low concentration.

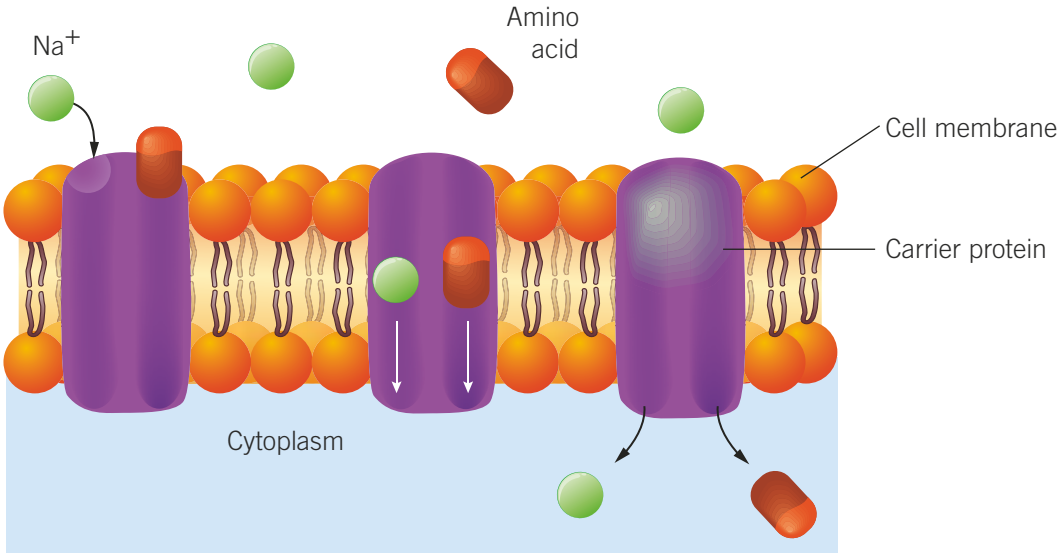


Figure 1C–10 The transport of amino acids by facilitated diffusion across a semi-permeable plasma membrane with the assistance of a carrier protein

Check-in questions – Set 3

1 Define tonicity.
2 Define facilitated diffusion.
3 Name the two types of proteins that may be involved in facilitated diffusion.
4 List the similarities between simple diffusion and facilitated diffusion.
5 List the differences between simple diffusion and facilitated diffusion.

Active transport

Active transport is a process used to move substances that are essential for cellular functioning, such as those involved in maintaining pH balance, regulating cell volume and the uptake of nutrients. Like passive transport, active transport is a selective process for moving substances across a membrane, and it occurs in living cells. However, active transport uses energy, in the form of **ATP (adenosine triphosphate)**, as the substances are moved from a region of low substance concentration to a region of high substance concentration. This is the reverse of the way substances naturally move and become distributed. You will look at ATP and its role as an energy shuttle in more detail in Chapter 5.

ATP (adenosine triphosphate) the main immediate source of chemical energy in a cell, powering most cellular processes; when a phosphate group is removed, energy is released and ADP is formed

Active transport is a protein-mediated process, just like facilitated diffusion. Every plasma membrane contains special protein carriers, also called protein pumps, which use ATP as a fuel for the pump. Some protein carriers transport one substance and some transport two substances simultaneously, but all are specific for certain substances, and in this way they are able to regulate the movement of that substance. This is why active transport is considered to be a selective process. Figure 1C–11 shows the selective nature of the protein carrier and how, during the transport process, the protein carrier changes shape.

LINK 5C CELLULAR RESPIRATION

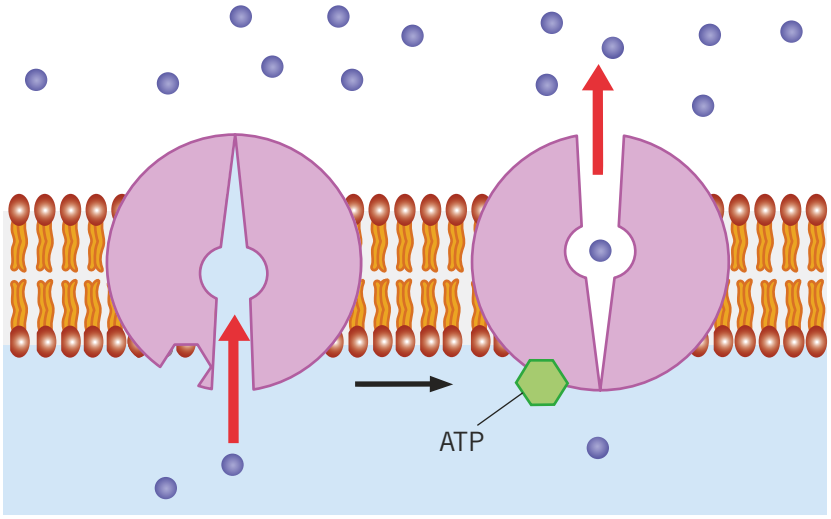


Figure 1C–11 Active transport is a selective process in which substances are moved from a region of low concentration to a region of high concentration. It requires a protein carrier and ATP.

Check-in questions – Set 4

1 Define the following key terms: ATP, carrier protein.
2 List the similarities and differences between active transport and passive transport.
3 List the similarities and differences between active transport and facilitated diffusion.
4 What is the term used to describe protein carriers that are specific for certain substances and regulate the movement of those substances?

Bulk transport the movement of large particles (solid or liquid) through the plasma membrane, requiring the input of energy (ATP)

Vesicle mediated transport the movement of substances through the plasma membrane using membrane-bound structures within the cell

Endocytosis the movement of large particles (or a large quantity of small particles) into the cell without crossing the plasma membrane, using vesicles and ATP

Exocytosis the movement of large particles (or a large quantity of small particles) out of the cell without crossing the plasma membrane, using vesicles and ATP

Bulk transport

If the molecules of a substance are too large (or if there are too many molecules) for any of the forms of transport discussed so far, **bulk transport** is required. Bulk transport is the movement of large particles (or large volumes of liquids) through the plasma membrane, requiring the input of energy (ATP). As the process requires ATP, it is classified as a form of active transport.

Bulk transport operates differently from other transport processes. It is **vesicle mediated transport**, which means it uses membrane-bound structures. This is why cholesterol is such an important component of the plasma membrane – it allows the membrane to be broken and remade as substances enter and leave the cell, avoiding the need for a substance to cross through a particular component of the plasma membrane.

The two main types of bulk transport are **endocytosis** and **exocytosis**. The term 'endocytosis' comes from *endo* meaning 'internal' and *cyte* meaning 'cell'. It is the process in which large molecules (or large quantities of smaller molecules) enter the cell without crossing the membrane. In endocytosis:

- the substance, including fluid, moves closer to the plasma membrane
- a portion of the plasma membrane is invaginated
- the membrane pinches off, forming a membrane-bound vesicle that contains the substance being transported.

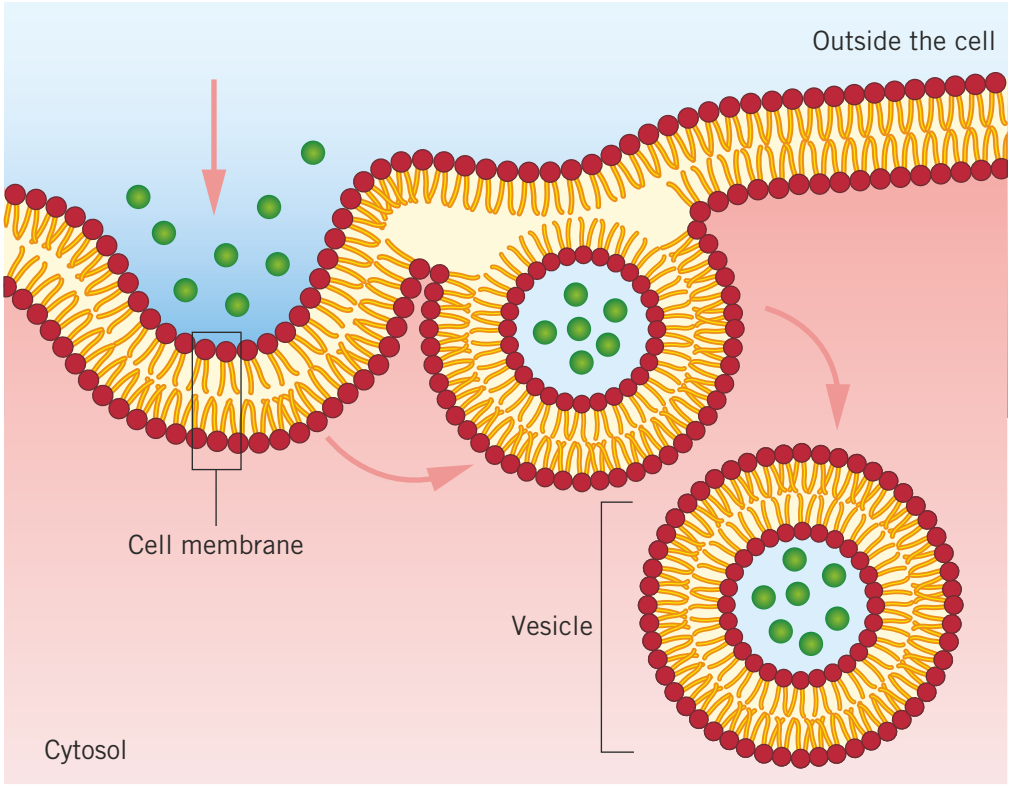


Figure 1C–12 Endocytosis is a type of active transport where large particles are moved into a cell inside vesicles.

If the substance entering the cell is:

- solid (such as a pathogen or food), the process is called **phagocytosis** (*phago* = eating)
- liquid or dissolved, the process is called **pinocytosis** (*pino* = drinking). Pinocytosis occurs in almost all cells, continuously.

Exocytosis is the reverse of endocytosis (*exo* = external). It is the movement of substances out of the cell without crossing the membrane. In exocytosis:

- the substance to be exported (often protein molecules) is enclosed in a vesicle from the Golgi apparatus
- the vesicle moves towards and fuses with the plasma membrane
- the vesicle releases its contents into the extracellular fluid.

Phagocytosis
a type of endocytosis in which a solid substance enters a cell by vesicle mediated transport

Pinocytosis
a type of endocytosis in which a liquid or dissolved substance enters a cell by vesicle mediated transport

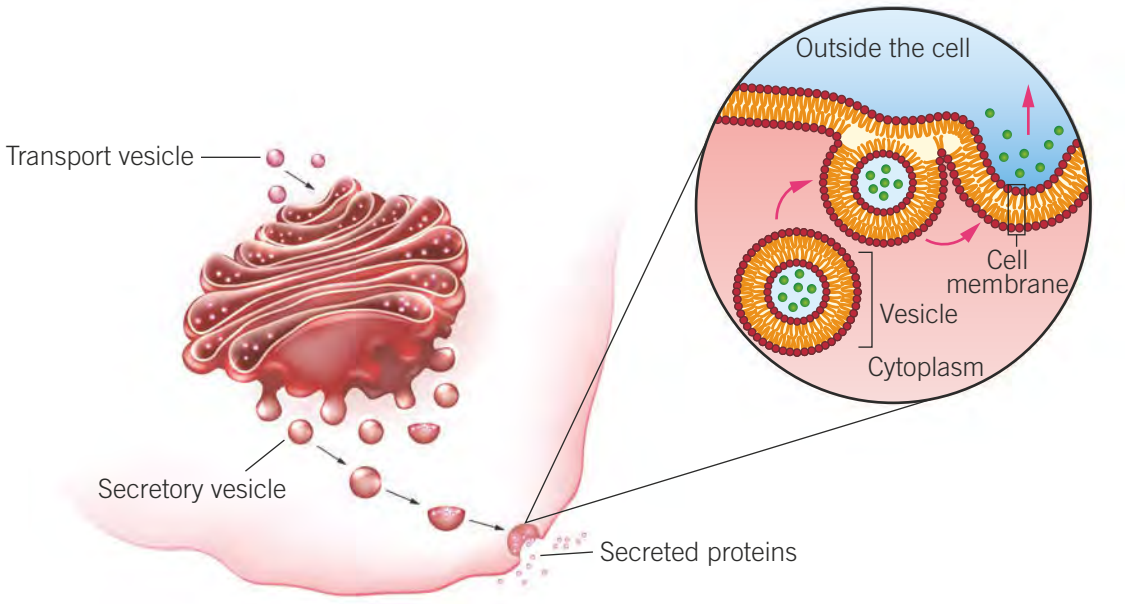


Figure 1C–13 Exocytosis is a type of active transport in which large substances are moved out of a cell using vesicles.

LINK 7B SECOND LINE OF DEFENCE

WORKSHEET 1C–2 MOVEMENT THROUGH MEMBRANES

The focus of Section 2B is protein synthesis, and Section 2C is about proteins and their levels of structure and uses in organisms. When proteins are made by the ribosomes (as was covered earlier in this chapter), they move to the Golgi apparatus for further modification. If that protein is required for use outside the cell, it is packaged into a vesicle for release by exocytosis.

LINK 2B THE GENETIC CODE AND GENE EXPRESSION

LINK 2C PROTEINS

As you progress to Unit 4, you will learn about the role of endocytosis in defending your body against pathogens or disease-causing organisms. Some of your white blood cells have the job of targeting a pathogen, engulfing it, digesting it and destroying it, thus protecting your body from further damage. As the white blood cell is taking in a solid substance, this process is phagocytosis.

LINK 7B SECOND LINE OF DEFENCE

Check-in questions – Set 5

1 Is bulk transport an active or a passive process? Why?
2 What does it mean when we say that bulk transport is vesicle mediated?
3 Define the following key terms: endocytosis, exocytosis.
4 a What is the term that means ‘cell eating’?
  b What is the term that means ‘cell drinking’?

1C SKILLS

VIDEO 1C–3 SKILLS: HOW TO EXPLAIN

How to explain

Command terms are an important component of questions, as they indicate what it is that you need to do to attain full marks. *Explain* is an example of a command term used regularly in exam situations. To explain means to give a detailed account of causes, reasons or mechanisms, or to essentially say why something happened. Consider the following question:

Question: Identical pieces of potato (all of the same mass) were placed in each of four beakers, which contained glucose solution or distilled water (see Figure 1C–14). They were left for 30 minutes. Explain which piece of potato would have the largest loss in mass after 30 minutes. (2 marks)

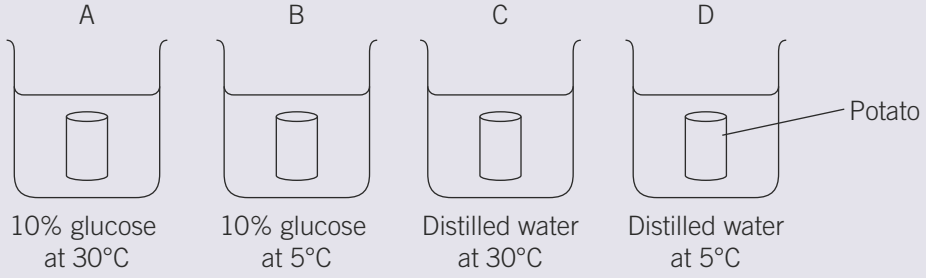


Figure 1C–14 Set-up of a student experiment investigating the loss of mass from potato

Answer: Beaker A.

- Water moves by osmosis from high water concentration to low water concentration along the concentration gradient across a semi-permeable membrane.
- Therefore, for there to be a loss of mass, water must be moving out of the potato, so high water must be inside the cell and low water outside the cell (beaker A or B).
- Osmosis occurs faster at a higher temperature as the molecules have more kinetic energy.
- Therefore, beaker A will show the greatest loss of mass in the 30 minutes.

Notice that this answer is set out in four dot points. This is because you have 2 marks to score and this often equates to needing four points to gain full marks. Examiners don't mark you down in Biology for using dot points in your answer, so if dot points help, use them.

An acronym you might find useful when answering questions is DER, which stands for:

- *Define* – include key and relevant definitions in your answer, for key content words
- *Explain* – give a detailed account of causes, reasons or mechanisms relevant to the question
- *Relate* back to the question – relate your explanation to the information provided in the question so the examiner can clearly see the link.

Here is an example of what this looks like:

Question: A student was carrying out an experiment using an artificial semi-permeable membrane. The holes in this membrane allow water to pass through, but not larger molecules like the sugar sucrose. The student added the same amount of water on each side of the membrane. Then, at time = 0 minutes, they added sucrose to one side, as shown in Figure 1C–15. Explain what results you would expect at time = 30 minutes.

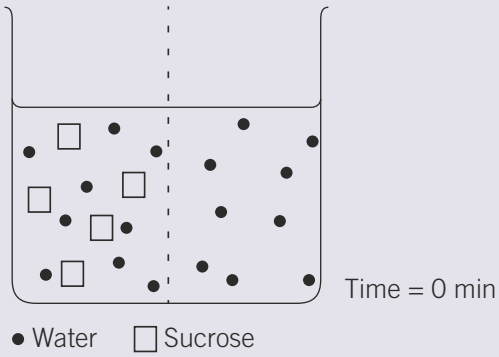


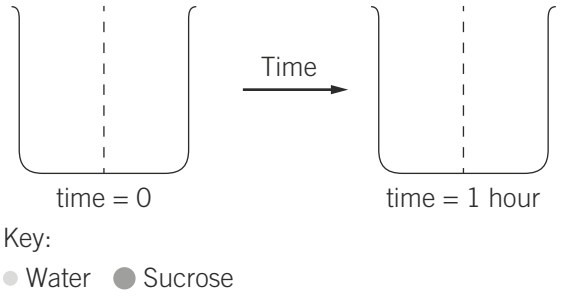
Figure 1C–15 Set-up of student experiment investigating the movement of molecules

Answer: The water level will rise on the left.

- Define – Diffusion is the net passive movement of a substance from a region of high concentration to a region of low concentration until equilibrium is reached. Osmosis is the net passive movement of free water from a region of high free water concentration to a region of low free water concentration across a semi-permeable membrane until equilibrium is reached.
- Explain: Therefore, because sucrose has a high concentration on the left, it will want to move to the right by diffusion, but it is too large to fit through the holes in the membrane, so it cannot move. Water, on the other hand, can fit through the holes and so it moves by osmosis from the right (high free water/low solute concentration) to the left, where the solute concentration is high.
- Relate back to the question: Due to water moving to the left where the sucrose is and cannot move, the water level on the left will have increased by time 30 minutes.

Section 1C questions

1 State why plasma membranes are crucial to the survival of cells.
2 Why are some membranes called 'selectively permeable'?
3 Give examples of substances that move through the
  a phospholipid bilayer **b** protein channels.
4 A tray of cookies was baking in the school Home Economics room. Explain why people closest to the kitchen were first to notice. Include any relevant definitions.
5 Diffusion is one way that substances can move across the plasma membrane.
  a Define simple diffusion.
  b List the differences between osmosis and diffusion.
  c Are osmosis and simple diffusion passive or active forms of transport?
6 As part of a project, a student was asked to demonstrate osmosis to their peer, who was absent from class on the previous day. The student had a beaker, cellulose membrane (semi-permeable), sucrose (sugar) solution and distilled water. The student put the membrane in the centre of the beaker to separate it into two halves. On one side, they half filled the beaker with sucrose solution, and the other side they half filled with distilled water. Complete the following blank diagram by showing the position of the sucrose and water at time = 0 (left) and time = 1 hour (right).



7 The cells in plant roots use active transport to move mineral ions from the soil into the cytoplasm of the cell.
- **a** Define active transport.
- **b** Justify how this would allow the cells to also take up water.

8 Once a week at the school canteen, potatoes are cut into chips several hours before they are cooked. Due to the early preparation time, something needs to be done to prevent the chips drying out. In order to keep the water content of the chips constant, they are stored in a salt solution. The canteen manager has asked you to investigate what concentration of salt solution would be best to use. You weighed five chips, placing them in different concentrations of salt solution, then reweiged them an hour later. The results are shown in the table.

	Concentration of salt solution (M)				
	0	0.5	1	2	3
Mass of chip (g) at time = 0	2.7	2.6	2.7	2.6	2.6
Mass of chip (g) at time = 1 hour	2.8	2.6	2.6	2.4	2.1

Determine which concentration of salt solution the chips should be kept in, and give reasons for your answer by describing all your observed results.

9 Proteins are large molecules that are needed by all cells.
- **a** Proteins can enter a cell by which process?
- **b** What source of energy is needed for this process?
- **c** Is this an example of passive or active transport?

10 Identify three features of facilitated diffusion.

11 Distinguish between channel proteins and carrier proteins.

12 Summarise what is meant by 'concentration gradient', using examples.

13 In murder mystery stories, chloroform is used to daze or knock out people. Chloroform is a hydrophobic molecule that acts extremely quickly. Give reasons why this property allows it to act so rapidly.

14 Explain how the plasma membrane is involved in endocytosis and exocytosis. Include definitions and diagrams in your answer.

Chapter 1 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources, which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

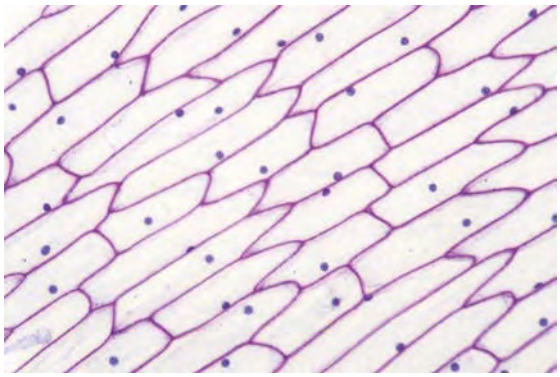
'ITB' in the linked questions columns means there is a question on this success criterion in the Interactive Textbook.

Success criteria – I am now able to:		Linked question
1A.1	Recall the four common factors for all organisms	7☐
1A.2	Outline the function of the four common factors for an organism	ITB
1A.3	Recall and define the following terms: fluid mosaic model, hydrophilic, hydrophobic, plasma membrane, semi-permeable membrane	9☐
1A.4	Recall and define the function of different components of the plasma membrane	9☐
1A.5	Draw and identify the different components of the plasma membrane	4☐, 10☐, 16b☐, 18☐
1A.6	Use evidence from a diagram to explain the structure of the plasma membrane	16a☐
1B.1	Recall and define the following terms: eukaryote, nucleoid, prokaryote	5☐, 6☐, 17☐
1B.2	Recall and define the following terms: cell wall, chloroplast, cilia, flagella, Golgi apparatus, lysosome, mitochondrion, nucleus, ribosome, rough endoplasmic reticulum, smooth endoplasmic reticulum, vacuole, vesicle	2☐, 14☐
1B.3	Identify whether a cell is a eukaryote (including animal or plant) or a prokaryote, based on its cellular features (organelles)	1☐, 17☐
1B.4	Draw animal, plant and bacterial cells, including the identification of organelles	14☐, 17☐
1B.5	Compare the structure of plant and animal cells	1☐, 8☐, 15☐
1B.6	Explain what organelles would be present in different cell types so that the cell can perform its particular function	ITB☐
1C.1	Give reasons why substances need to move through the plasma membrane	19☐
1C.2	Define semi-permeable, hydrophilic and hydrophobic	19☐

Success criteria – I am now able to:		Linked question
1C.3	Summarise the modes of transport used by substances crossing the plasma membrane, including the nature of substances moving, whether energy is required, and the component of the membrane involved in the transport	11☐, 12☐, 19☐, 20☐
1C.4	Define tonicity	ITB☐

Multiple-choice questions

1 The microscope image shown here is most likely from a
- **A** plant.
- **B** bacterium.
- **C** animal.
- **D** protist.



2 Which of the following is the best description of the function of the rough endoplasmic reticulum?
- **A** It modifies and packages proteins.
- **B** It transports proteins to the Golgi apparatus.
- **C** It contains enzymes to perform cellular respiration.
- **D** It is the site of protein synthesis.

3 The cell theory states that
- **A** all organisms are only made up of protein.
- **B** the cell is the largest single component of an organism.
- **C** a cell lasts for the lifetime of an organism.
- **D** all cells arise from pre-existing cells.

4 A carbohydrate attached to a phospholipid is called a
- **A** carbohydrate.
- **B** carbo-protein.
- **C** glycolipid.
- **D** lipid-hydrate.

5 Which of the following most accurately describes eukaryotic cells?
- **A** They have membrane-bound organelles, and some have flagella and circular DNA.
- **B** They have linear DNA, and some have cell walls and small circular pieces of DNA called plasmids.
- **C** They always have linear DNA and a cell wall.
- **D** They have membrane-bound organelles, and sometimes a cell wall, and sometimes a flagellum.

6 Which of the following is an example of a prokaryotic cell?
- **A** a skin cell
- **B** a bacterium
- **C** an enzyme
- **D** a plasma membrane

7 Which of the following lists features common to all organisms?
- **A** nucleus, plasma membrane, cytosol
- **B** ribosome, cytosol, Golgi apparatus
- **C** cytosol, plasma membrane, ribosome
- **D** Golgi apparatus, plasmid, enzymes

8 Which of the following are found only in plant cells?

A chloroplast, nucleus, vacuole
B vacuole, ribosome, cell wall
C Golgi apparatus, plasma membrane, mitochondria
D chloroplast, cell wall, permanent vacuole

9 Which of the following best describes the structure of a plasma membrane?

A phospholipids with a hydrophilic tail and hydrophobic head
B phospholipids with three fatty acid tails
C glyco-carbohydrates attached to phospholipids
D two layers of phospholipids with proteins embedded between the two layers

10 Which statement best describes the structure of the plasma membrane?

A phospholipid bilayer with proteins and carbohydrates
B phospholipid bilayer with carbohydrates
C protein bilayer with phospholipids
D phospholipid bilayer with lipids

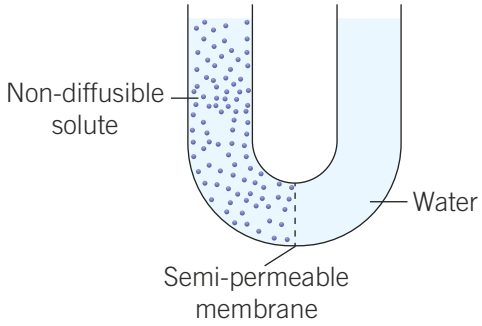
11 ATP is a high-energy molecule. Which of the following pairs of processes both require an input of energy?

A cell movement, diffusion
B active transport, cell growth
C osmosis, cell reproduction
D cell repair, facilitated diffusion

12 Which of the following statements is *incorrect*?

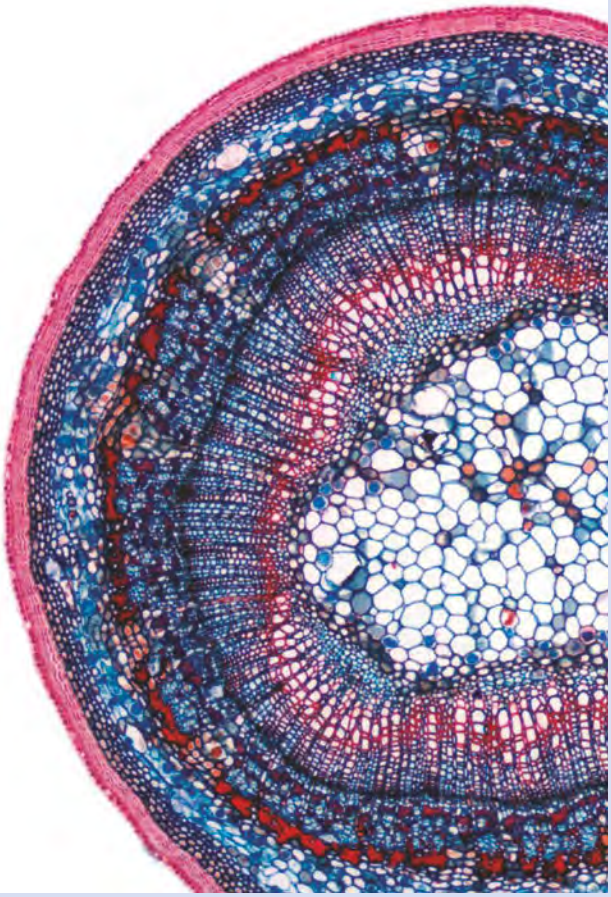
A Cells are unable to directly control the movement of water through the plasma membrane.
B One role of the plasma membrane is to regulate the movement of substances into and out of cells.
C Water molecules move from low to high free water concentration via osmosis.
D Osmosis is the movement of water from low to high solute concentration through the plasma membrane.

13 The U-shaped tube in the figure below is divided by a semi-permeable membrane that selectively allows water to pass through but not large solutes, such as starch.



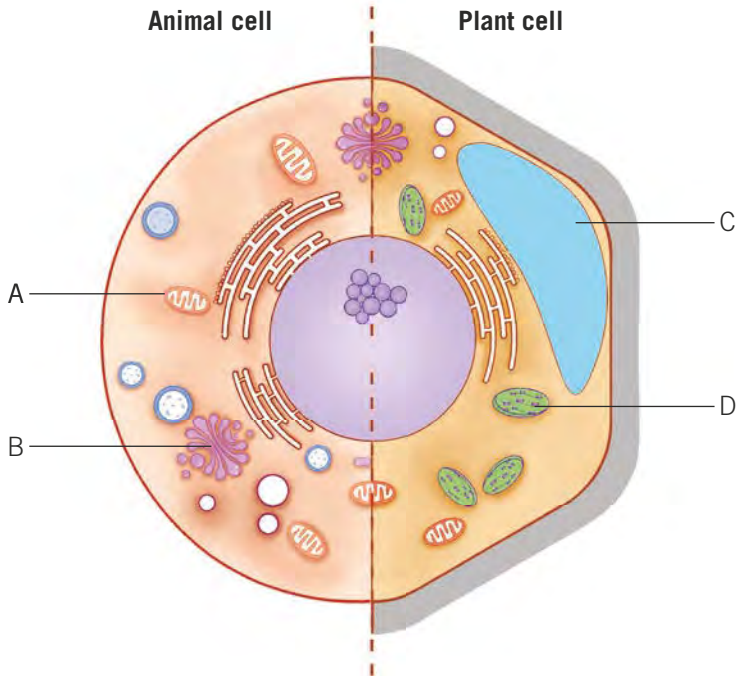
Which of the following will occur?

A Water will move from right to left by osmosis.
B Water will move from left to right by osmosis.
C Starch will move from right to left by osmosis.
D Starch will move from left to right by osmosis.



Short-answer questions

Use the diagram below to answer Questions 14 and 15.



14 Identify the components labelled A, B, C and D. (4 × 1 mark)

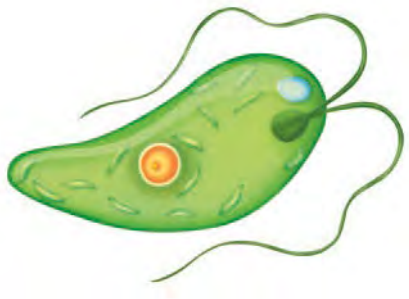
15 Using evidence from the diagram, explain why the right-hand side of the diagram has been labelled 'Plant cell'. (2 marks)

16 A phospholipid from a plasma membrane is shown below.

a One of the fatty acid tails has a bend in it. Outline what is causing the bend. (1 mark)

b Draw and fully label a section of a plasma membrane, including all the key components that make up the plasma membrane. (4 marks)

17 Aisha and Simon disagree about whether the specimen shown here is a eukaryote or a prokaryote. Simon is arguing that it must be a prokaryote because it is unicellular and has a flagellum. Aisha says it is a eukaryote, because no eukaryotes are multicellular, and some have flagella.



a Provide two characteristics of a prokaryote that have already been mentioned in this question. (1 mark)

b Provide two characteristics of a eukaryote has, that have already been mentioned in this question. (1 mark)

c Is the specimen in this example a prokaryote or a eukaryote? Provide a justification for your answer. (1 mark)

18 Draw a plasma membrane, labelling the following parts: phospholipid bilayer, cholesterol, glycoprotein, glycolipid. (2 marks)

19 The plasma membrane controls the entry and exit of substances into and out of the cell.

a Give reasons why cells need to move substances through the plasma membrane. (2 marks)

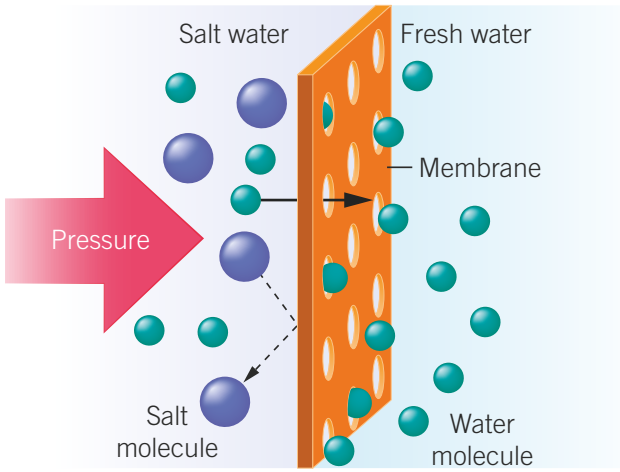
b Not all substances can enter and leave a cell through the plasma membrane. Name and define the term that describes this characteristic of the plasma membrane. (3 marks)

c Complete the table below by filling in the gaps. (3 marks)

Nature of substance	Example	Mode of transport	Passive or active
Small uncharged			
	Protein		

d Explain the process of endocytosis. (2 marks)

20 Reverse osmosis is often used in desalination plants as a means of separating salt from water. Using the following figure and your understanding of movement through membranes, distinguish between osmosis and reverse osmosis. (2 marks)



UNIT 3 HOW DO CELLS MAINTAIN LIFE?

CHAPTER 2 FROM DNA TO PROTEINS

Introduction

Nucleic acids are one of the key building blocks of life. Since 1953, when Francis Crick, James Watson, Maurice Wilkins and Rosalind Franklin discovered the double helix structure of DNA, we have been fascinated by DNA and how our understanding of it can help us in many aspects of life. Indeed, think about all the ways that we now use our knowledge of DNA every day. It is a key part of forensics, in helping identify criminals and solve crimes, or in paternity disputes. We use it to overcome fertility problems and help couples have children. We can harness it to generate large quantities of medical products, such as insulin. Most importantly, by coding for proteins, DNA acts as the genetic blueprint that makes all organisms unique and allows them to function. This chapter investigates nucleic acids and proteins, as well as the processes through which the information contained within the genetic code is converted into proteins. It also looks at how gene expression is regulated in cells, with a focus on a specific prokaryotic example.

Curriculum

Area of Study 1 Outcome 1
The relationship between nucleic acids and proteins

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Nucleic acids as information molecules that encode instructions for the synthesis of proteins: the structure of DNA, the three forms of RNA (mRNA, rRNA and tRNA) and a comparison of their respective nucleotides	**2A Nucleic acids** **2A.1** List the differences between DNA and RNA **2A.2** Draw schematic diagrams of a nucleotide, a single strand and a double strand of nucleic acid **2A.3** State the differences between mRNA, rRNA and tRNA **2A.4** Describe how information is encoded in DNA and instructs protein synthesis
• The genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes	**2B The genetic code and gene expression** **2B.1** Describe the steps involved in transcription **2B.2** Describe the steps involved in processing pre-mRNA to mRNA **2B.3** Describe the steps involved in translation **2B.4** Draw a schematic diagram showing all parts of protein synthesis **2B.5** Define what is meant by degeneracy in the universal triplet code **2B.6** Convert a DNA sequence to an mRNA sequence and then, using a codon table, to an amino acid sequence

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Study Design	Learning intentions – at the end of this chapter I will be able to:
• Amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein	**2C Proteins** **2C.1** Draw the structure of a generalised amino acid **2C.2** Draw the structure of a generalised dipeptide and identify the peptide bond **2C.3** Define primary, secondary, tertiary and quaternary protein structures **2C.4** Draw and/or identify representative structures of primary, secondary, tertiary and quaternary protein structures
• Proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways • The role of rough endoplasmic reticulum, Golgi apparatus and associated vesicles in the export of proteins from a cell via the protein secretory pathway	**2C.5** Define proteome **2C.6** List the functions of proteins and provide examples of each **2C.7** Describe the process of protein secretion from a cell, including the organelles involved **2C.8** Draw a diagram outlining the protein secretory pathway within a cell
• The structure of genes: exons, introns and promoter and operator regions	**2D Gene structure and expression** **2D.1** Define exon, intron, promoter, operator, regulatory gene and structural gene **2D.2** Draw a schematic diagram outlining the relative locations of these elements within a gene
• The basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of a regulatory process	**2D.3** Define operon **2D.4** Describe how the *trp* operon functions in scenarios of low tryptophan and high tryptophan

Glossary

Amino acid
Anticodon
Biomacromolecule
Coding region
Codon
Complementary
Condensation reaction
Degenerate
Enzyme
Exon
Gene expression
Genome
Intron
Monomer
Mutation
Nucleotide
Operator
Operon
Peptide bond
Polymer
Polypeptide
Promoter
Proteome
Regulatory gene
Repressor
Structural gene
Terminator
Transcription
Translation
Universal triplet code

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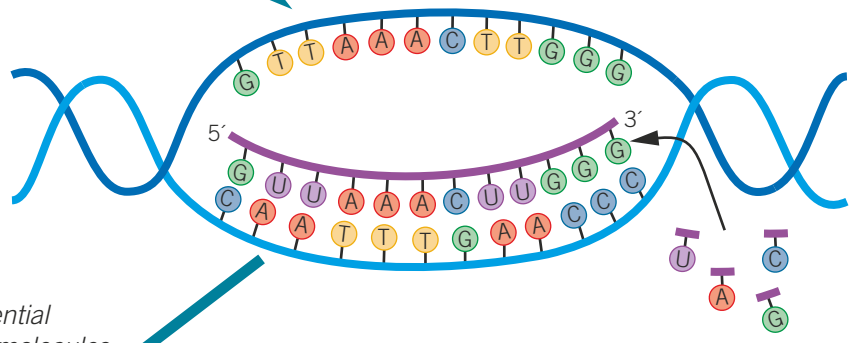
Concept map

2A Nucleic acids

The genetic material behind life

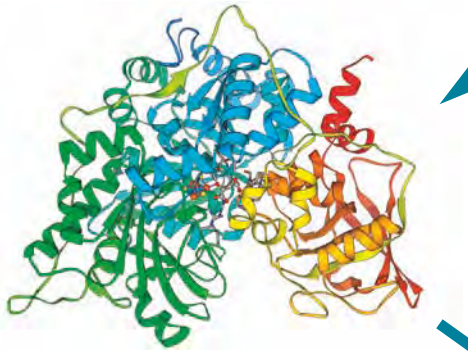
The central process in making life possible

2B The genetic code and gene expression



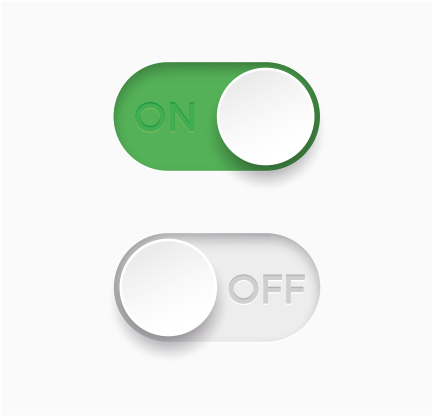
Essential biomacromolecules

2C Proteins



Keeping the processes in check

2D Gene structure and expression



See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

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2A Nucleic acids

Study Design:
Nucleic acids as information molecules that encode interactions for the synthesis of proteins: the structure of DNA, the three forms of RNA (mRNA, rRNA and tRNA) and a comparison of their respective nucleotides

Glossary:
Complementary
Monomer
Nucleotide
Polymer

ENGAGE

What are nucleic acids?

In Unit 1 Biology, you learned about factors that are essential for life. One of those factors is the ability to store and pass on genetic material. The biomolecules that contain all this information are known as nucleic acids. Nucleic acids are found in two forms: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). You may have heard people discuss nucleic acids in various ways, without realising that this was what they were referring to. Phrases such as 'You are a combination of your Mum and your Dad' or 'It's all about genetics' refer to the function that DNA (and nucleic acids in general) has in our body. The concepts regarding how genetic information is passed on through generations were discussed in more detail in Unit 2.

LINK UNIT 1

LINK UNIT 2

EXPLAIN

Understanding nucleic acids

VIDEO 2A–1 INTRODUCING NUCLEIC ACIDS

This chapter will extend your understanding of nucleic acids as you learn about their role in a key process within cells. In this process, shown in Figure 2A–1, organisms convert the information contained within their genetic material (in the form of nucleic acids) into functional molecules (in the form of proteins).

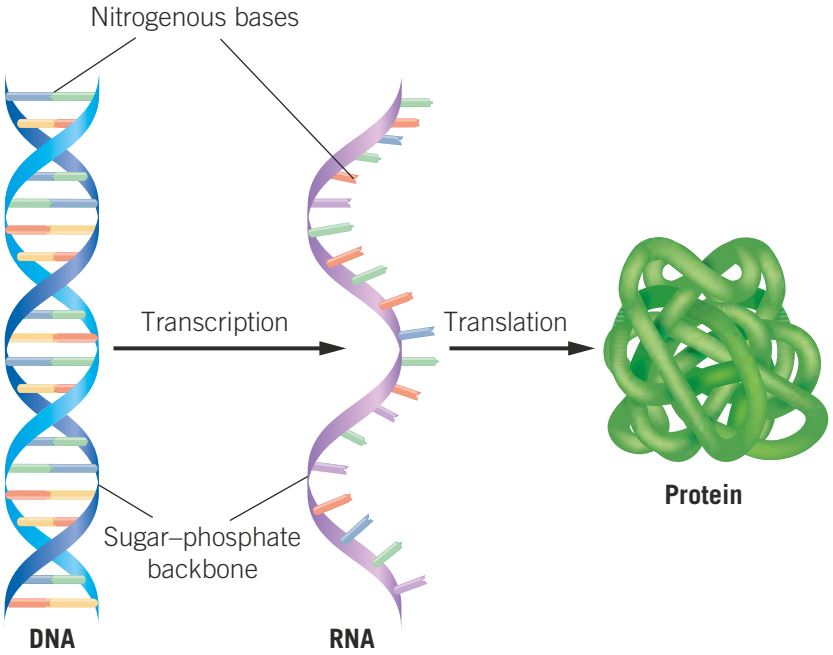

Figure 2A–1 The process through which the genetic information contained within DNA is converted into the functional units in our bodies: proteins

LINK 2B THE GENETIC CODE AND GENE EXPRESSION

However, you first need to study nucleic acids. This section examines the similarities and differences between these molecules, as well as their structure and function.

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Polymer
a molecule made up of a large number of smaller, repeating units

Monomer
a molecule that forms bonds with other identical molecules as the repeating units that make up a polymer

Nucleotide
the monomer (building block) of nucleic acids which are joined together to form DNA or RNA (polymers); consists of a phosphate group, sugar and nitrogenous base

Differences between DNA and RNA

DNA and RNA have many similarities. Both are made up of long strands (**polymer**) of a repeating unit (**monomer**) made from a sugar and a phosphate group, each with a nitrogenous base sticking out to the side. This monomer is called a **nucleotide**.The sugar group of one unit joins the phosphate group of another, to form a long chain, informally called the sugar–phosphate 'backbone' (Figure 2A–1).

It is important to also be able to recognise the differences between DNA and RNA. There are three main points of distinction, which you can remember using the acronym SBS (sugar, bases, strands):

1 *Sugar:* as their names indicate, DNA (deoxyribonucleic acid) contains deoxyribose sugars, while RNA (ribonucleic acid) contains ribose sugars. As shown in Figure 2A–2, deoxyribose has one less oxygen atom than ribose.

2 *Nitrogenous bases:* these are almost the same in both DNA and RNA. Both have cytosine (C), guanine (G) and adenine (A). However, each also possesses a unique base. In DNA, it is thymine (T). In RNA, it is uracil (U). The bases for both DNA and RNA can be seen around the outer edges of Figure 2A–3.

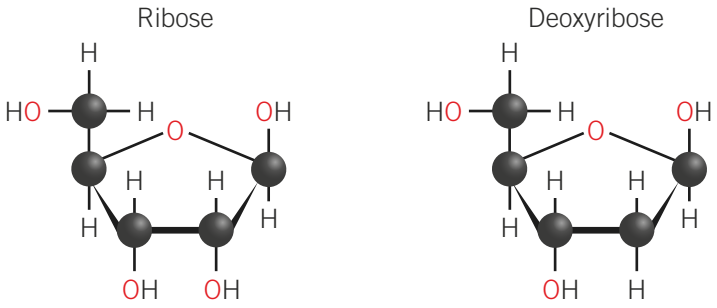

Figure 2A–2 The sugar molecules in RNA strands are ribose and in DNA are deoxyribose. The black spheres represent carbon atoms, and both sugars have five. In subsequent diagrams, these carbon ring structures will be drawn as pentagons.

NOTE

You will not be assessed on the chemical structure of either the sugar or the nitrogenous base in this course.

3 *Sugar–phosphate strands:* DNA is double-stranded, whereas RNA forms only a single strand. This is illustrated in the centre of Figure 2A–3.

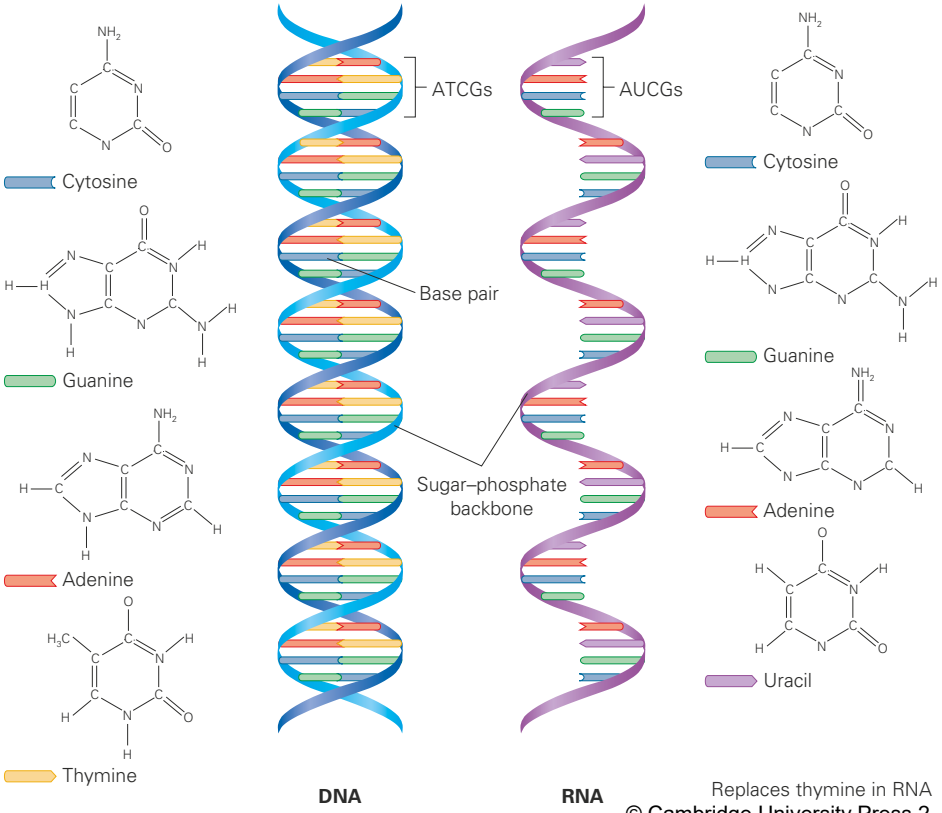

Figure 2A–3 The nitrogenous bases within DNA and RNA. DNA is composed of the pyrimidines cytosine (C) and thymine (T), as well as the purines adenine (A) and guanine (G). RNA contains the same bases except for thymine, which is replaced by another pyrimidine called uracil (U). Pyrimidines always bond to purines, and vice versa.

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Structure of DNA

DNA has probably the best known structure of any biological molecule. The double helix is instantly recognisable as that of DNA. However, before looking at this, you first need to understand the building blocks of DNA. Many molecules in our body, such as DNA, carbohydrates and proteins (which will be discussed later in this chapter), are polymers. A polymer is a molecule that is made up of many smaller, repeating units linked together. Each of these smaller individual units is called a monomer.

Formation of the monomer

In DNA, the monomers (building blocks) are known as nucleotides. Each nucleotide is made up of three components: a sugar, a phosphate group and a nitrogenous base. As mentioned earlier, in DNA this nitrogenous base can be adenine (A), cytosine (C), guanine (G) or thymine (T).

Formation of the polymer

These monomers join together to form a single strand of DNA, via bonds between the sugar of one nucleotide and the phosphate of the next nucleotide (Figure 2A–4). As shown in this diagram, one end of the single strand ends with a phosphate, and is known as the 5′ (pronounced 'five prime') end. The other end finishes with a sugar, and is known as the 3′ (pronounced 'three prime') end.

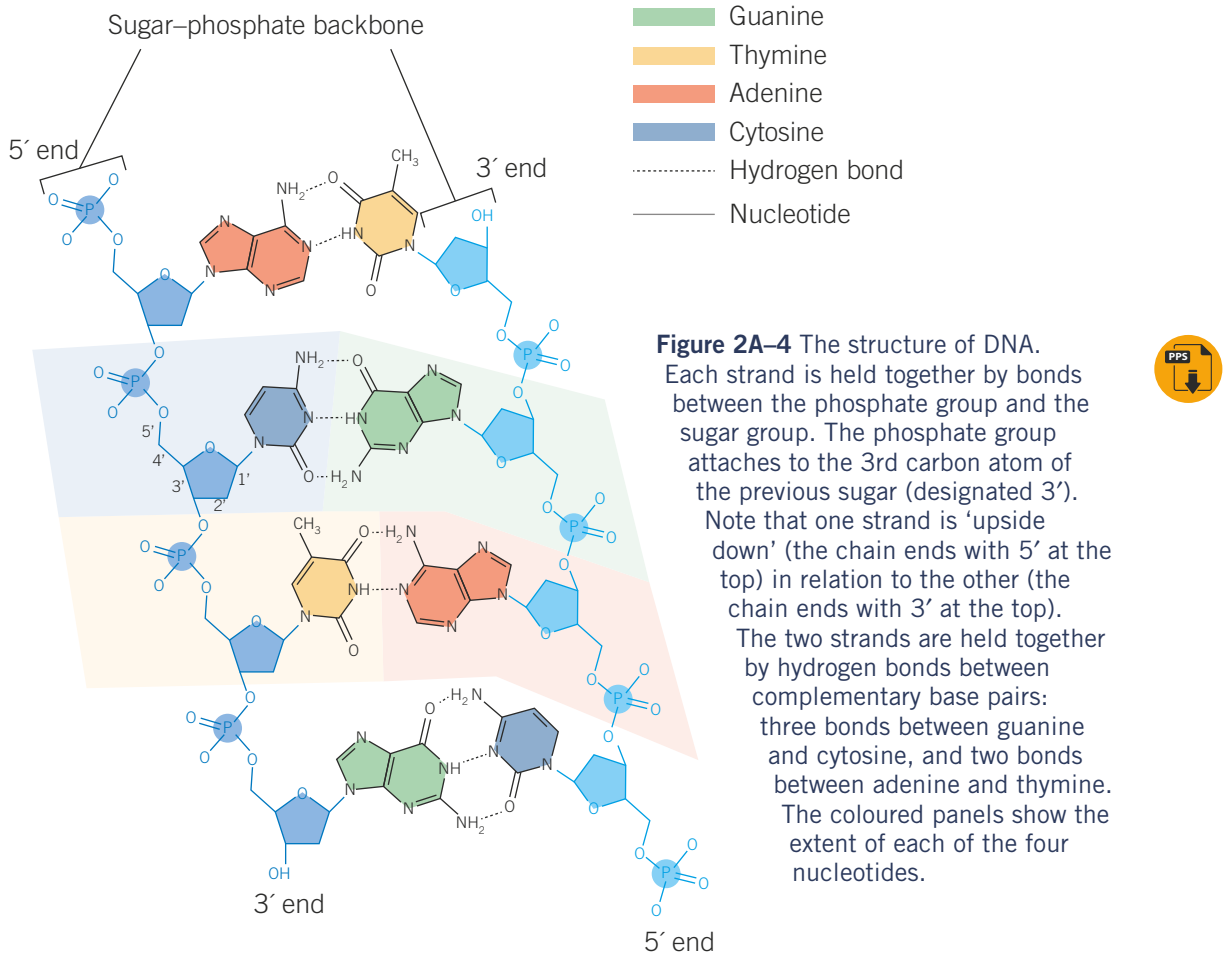

Figure 2A–4 The structure of DNA. Each strand is held together by bonds between the phosphate group and the sugar group. The phosphate group attaches to the 3rd carbon atom of the previous sugar (designated 3′). Note that one strand is 'upside down' (the chain ends with 5′ at the top) in relation to the other (the chain ends with 3′ at the top). The two strands are held together by hydrogen bonds between complementary base pairs: three bonds between guanine and cytosine, and two bonds between adenine and thymine. The coloured panels show the extent of each of the four nucleotides.

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Complementary the term used to describe the fact that a nitrogenous base can only pair with one other nitrogenous base (cytosine is complementary to guanine, adenine is complementary to thymine)

As discussed earlier, one of the defining characteristics of DNA is that it is double-stranded. The two strands are joined by hydrogen bonds between **complementary** nitrogenous bases.

These nitrogenous bases cannot join randomly. Each base is only able to partner with one other base. In DNA, the complementary base pairs are arranged as follows: adenine bonds to thymine (A–T), and guanine bonds to cytosine (G–C). Note from Figure 2A–5 that a different number of hydrogen bonds holds each of the two pairs of bases together: adenine and thymine are joined by two hydrogen bonds, whereas guanine and cytosine are joined by three. For this reason, DNA segments that contain a higher percentage of G–C pairs are stronger and harder to break apart.

Think about the fact that adenine always pairs with thymine, and what consequence this has for the amount of adenine and thymine in a DNA molecule. Consider whether the same would be true for cytosine and guanine. If there are 1000 adenine and 800 cytosine bases in a DNA molecule, how many thymine and guanine bases will there be? This feature was known long before the structure of DNA had been worked out.

Check-in questions – Set 1

1 a What is the unique base that is not in DNA, but is in RNA?
  b State two other differences between DNA and RNA.
2 a What general term is used to describe a molecule that is made by linking many smaller repeating units?
  b What is the term for each individual unit in such a molecule?
3 a What are the complementary nitrogenous bases in DNA?
  b A double-stranded molecule of DNA contains 20% guanine. What percentage of adenine, thymine and cytosine does this same molecule contain?
  c How many hydrogen bonds hold together each of the pairs in part **a**?

WORKSHEET 2A–1 NUCLEIC ACIDS

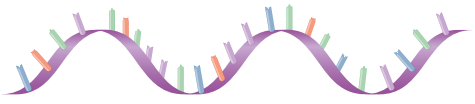
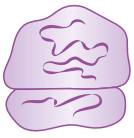
Types and structure of RNA

1B CELL TYPES AND CELL ORGANELLES LINK

2B THE GENETIC CODE AND GENE EXPRESSION LINK

As you saw in Figure 2A–1, ribonucleic acid (RNA) is the intermediate step in converting the information coded within DNA into proteins. This process is complicated and, as such, three types of RNA are involved, as shown in Table 2A–1. Their roles in transcription and translation are described in detail in the next section.

Table 2A–1 The three types of RNA and their structures

Type of RNA	Diagram
Messenger RNA (mRNA) Formed through the process of transcription. This process is discussed in more detail later in this chapter, it involves making a single-stranded piece of RNA that is complementary to a section of DNA. mRNA is created specifically to carry the genetic information from regions of DNA, known as genes, to be used to synthesise proteins.	
Ribosomal RNA (rRNA) The main component of ribosomes. As you learned in Chapter 1, ribosomes are the organelles responsible for protein synthesis within a cell.	
Transfer RNA (tRNA) An important molecule involved in the process of translation, which is covered in detail later in this chapter. It has a clover-leaf shape and is involved in delivering and linking amino acids during protein synthesis.	

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2A SKILLS

VIDEO 2A–2 SKILLS: ANSWERING COMPARATIVE QUESTIONS

Answering comparative questions

In Biology, you will often encounter questions that ask you to compare and contrast different concepts. You might be asked to compare the different types of nucleic acids (DNA vs RNA) or the different types of RNA (mRNA vs rRNA vs tRNA). Although comparison questions may seem straightforward, students often don't receive full marks for their answer. The main reason for this is that students often interpret the question as follows:

Question:

Compare the structures of DNA and RNA.

Interpretation:

List the differences between DNA and RNA.

A comparison question requires you to answer it in two parts. First, you have to discuss the aspects that are similar between the two items, then you have to analyse the aspects that are different. This is the best way to ensure that you get maximum marks for a comparison-style question. Keep your comparisons succinct and do not repeat the question. Let's use the example above to look at ways to approach this type of question.

Question:

Compare the structures of DNA and RNA.

Answer – strategy 1:

Group your responses by similarities and differences.

Similarities:

- Both are made up of nucleotides containing a sugar, a phosphate and a nitrogenous base.

Differences (make it clear that there is a comparison, by using terms such as 'whereas', 'although', 'in contrast' and 'unlike'):

- DNA has thymine, whereas RNA has uracil.
- DNA is double stranded, whereas RNA is single stranded.
- DNA has deoxyribose sugar, whereas RNA has ribose.

Answer – strategy 2:

Draw a table listing key features and the responses for each item.

	DNA	RNA
Strands	Double	Single
Monomer	Nucleotide	Nucleotide
Bases	A, T, C, G	A, U, C, G
Sugar	Deoxyribose	Ribose

As you can see, using either strategy helps ensure that your answer is complete and thorough. Also, setting your answer out in either of these two ways helps the examiner see clearly that you have identified all the key elements required for full marks.

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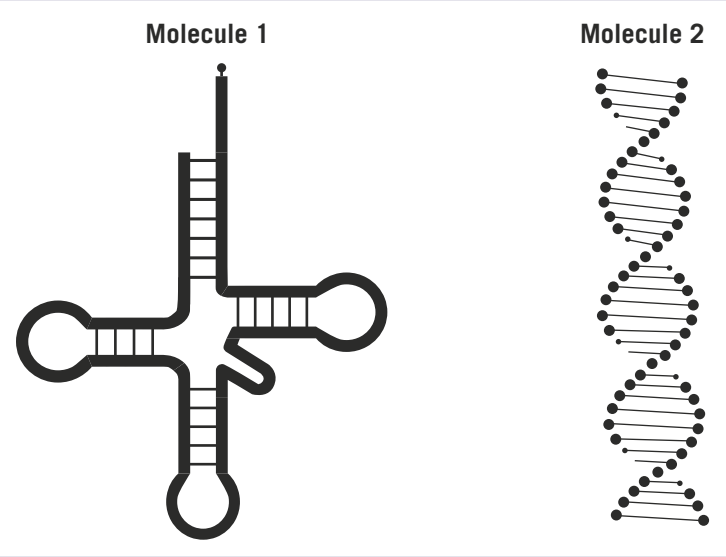
In a similar fashion, it is important to be specific in what you are comparing and to answer the question appropriately. In the 2019 VCAA Biology exam short-answer section, there was a question that asked students to identify the differences between the monomers of two provided images. Unfortunately, many students ignored the request to identify the **differences between the monomers**, and just identified the differences between the molecules themselves. As further practice, it would be a good idea to attempt this question, using the strategies described above.

Question 1

Diagrams of two molecules that are required for the production of proteins within a cell are shown below.



a Complete the table below to describe two differences between the monomers of the two molecules.

	Molecule 1	Molecule 2
Difference 1		
Difference 2		

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Section 2A questions

1 What are the full names of DNA and RNA?

2 You have two pieces of DNA. Piece 1 has 30% adenine in its sequence and Piece 2 has 40% adenine in its sequence.

- **a** Which would be easier to break, and why?
- **b** What would be the percentages of the other nitrogenous bases in each piece of DNA?
- **c** Explain how you determined the percentages in part **b** of this question.

3 A single strand of DNA has the sequence TAT AGC ATG CAC CCA TGA. What is the sequence of the complementary strand of DNA?

4 What type of RNA is directly associated with transporting amino acids?

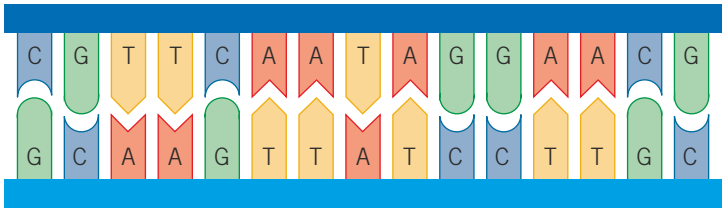
5 Consider the composition of nucleic acids.

- **a** What is their monomer or building block?
- **b** What are the three components of the monomer you named in part **a**?
- **c** Draw the generalised structure of the monomer you named in part **a**.
- **d** Draw a single strand of DNA with six nucleotides, including at least one of each type. Label each specific part of the nucleotide with its identifying letter.
- **e** Add in the complementary strand to your drawing for part **d** with another six nucleotides connected to the first six, to make a double strand. (It is necessary to show the number of bonds between complementary nucleotides.) Label each new nucleotide with its identifying letter.

6 **a** Explain the similarities and differences between DNA and RNA.

- **b** Describe the differences between mRNA, rRNA and tRNA.

7 Consider the following piece of DNA and then answer the questions below.



- **a** How many sugar units are present?
- **b** How many nucleotides are present?
- **c** What is the number of base pairs?

8 Two students, Jasmine and Ahmed, are organising a quiz on nucleic acids. In preparing the questions, they find that they have different answers. Jasmine thinks that nucleic acids are single stranded, whereas Ahmed claims that they are double stranded.

- **a** Is it possible that both students are correct? Explain.
- **b** To help settle their disagreement, Jasmine and Ahmed look up some research studies online. In one journal article, they find an experiment that showed that in the molecule there were identical numbers of the nitrogenous bases guanine and cytosine. Based on this information, which student would you say was more correct?

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2B The genetic code and gene expression

Study Design:
The genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells and translation by ribosomes

Glossary:
Amino acid
Anticodon
Codon
Degenerate
Enzyme
Exon
Gene expression
Genome
Intron
Mutation
Peptide bond
Polypeptide
Promoter
Terminator
Transcription
Translation
Universal triplet code

ENGAGE

A small piece of the puzzle

When researchers completed the Human Genome Project, it was a huge step forward in medical research. In particular, being able to know the genes within our DNA code allowed greater insights into what was happening within our bodies. However, it actually only provided a small piece of the puzzle.

As an example, imagine that you are doing an ecological study of the turtles that live on the Great Barrier Reef.

Figure 2B–1 A green sea turtle swimming at the Great Barrier Reef, Queensland

Genome
the collection of all of the genes contained with the DNA of an organism

Concluding that turtles are found on the Great Barrier Reef would be similar to the information we gained from identifying the human genome – that is, we know that this exists in this location. However, if you really were studying these turtles, what kinds of things would you be interested in? You might want to know the numbers of turtles living on the Great Barrier Reef, and perhaps what other species they are interacting with. In relation to our DNA, we are not so much interested in the genes themselves, but the products they make: mRNA and proteins. In this section, we will investigate the processes through which these molecules are made from our DNA: transcription and translation.

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EXPLAIN

Transcription: DNA to mRNA

Gene expression is the conversion of the genetic code within the DNA of a gene sequence into the functional unit known as a protein (protein synthesis). Recall Figure 2A–1 in the previous section for an overall picture of the information conversion process. **Transcription** is the first step of protein synthesis. In eukaryotes, transcription occurs in the nucleus and involves transforming DNA into pre-messenger RNA (pre-mRNA). In everyday usage, the term 'transcription' means the act of taking some information and writing it down – so, the information is the code in the DNA, and it is 'written down' as an mRNA sequence. An mRNA molecule can therefore be referred to as a transcript. Let's look in detail at the steps involved.

Gene expression conversion of the code in DNA of a gene into a protein through protein synthesis

Transcription the process through which DNA is converted to messenger RNA (mRNA) and the genetic code in the DNA is copied to the mRNA

1 Separating the double-helix strands of DNA

As you learned in Section 2A, DNA is a double-stranded molecule. The two strands are held together by hydrogen bonds connecting the complementary base pairs.

Therefore, separating the DNA double helix requires the action of an **enzyme** called RNA polymerase. For RNA polymerase to function correctly, it must first bind to a specific region in the gene, known as the **promoter**. You will learn more about the specific structure of a gene in Section 2D.

LINK 2A NUCLEIC ACIDS

WORKSHEET 2B–1 TRANSCRIPTION AND TRANSLATION

LINK 2D GENE STRUCTURE AND EXPRESSION

2 Copying the DNA code to create RNA

Once bound to the promoter, the RNA polymerase connects free RNA nucleotides to make a strand of 'pre-mRNA' that is complementary to one of the separated strands of the DNA, called the template strand. Calling it 'pre-mRNA' indicates that more processing is needed before it becomes 'mature' mRNA.

NOTE

The nucleotide sequence (code) of the pre-mRNA is the same as that of the other strand of DNA, the one not being transcribed, except that the nitrogenous base uracil substitutes for thymine. This strand is known as the coding strand.

As the RNA polymerase moves along the template strand, it continues to make the complementary strand of pre-mRNA until it reaches the **terminator**. The terminator causes the enzyme to break away from the DNA and the RNA strand to be completed.

Enzyme a type of protein, also referred to as a biological catalyst, that speeds up reactions within an organism by lowering activation energy

Promoter the region of a gene at which RNA polymerase binds, to initiate transcription

Terminator the region of a gene at which transcription stops and the RNA polymerase dissociates from the strand

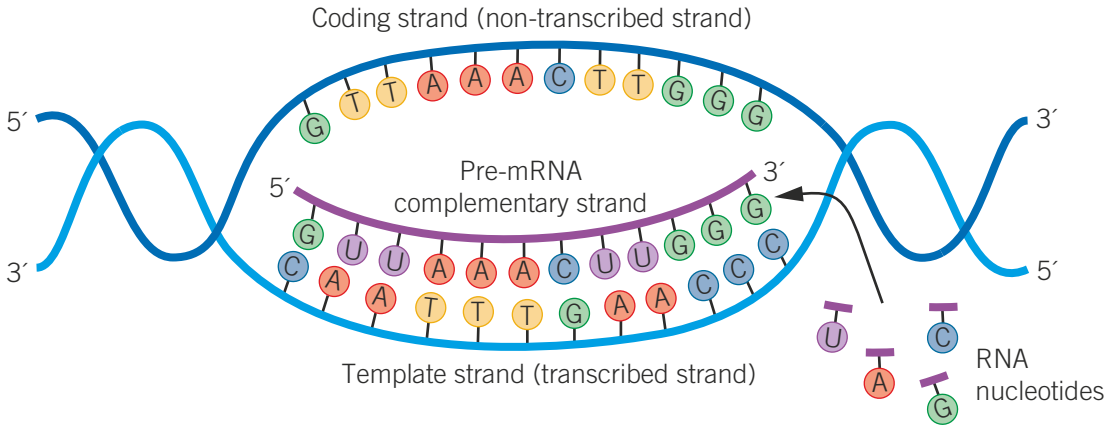

Figure 2B–2 Transcription: the DNA strand that is transcribed into pre-mRNA is the template strand. The other DNA strand (the non-transcribed strand) is the coding strand. Note that the nucleotide sequence of the coding strand is the same as that of the pre-mRNA copy, except that uracil substitutes for thymine. The RNA polymerase enzyme that carries out transcription by connecting free RNA nucleotides in the pre-mRNA strand is not shown here.

VIDEO 2B–1 FROM DNA TO PROTEIN

ISBN 978-1-108-89462-3

Intron
a region of a gene that contains sequences that do not code for the protein to be expressed

Exon
a region of a gene that contains genetic information that codes for the specific protein to be synthesised

Post-transcription modifications (RNA processing)

At this stage of the process, the molecule that has been created is pre-mRNA, not mature messenger RNA (mRNA). For mRNA to be created, three processing steps have to be completed (Figure 2B–3):

1. A 5′ methyl cap is added to help protect it from being degraded by enzymes when it exits the nucleus. It also helps it to be positioned correctly on the ribosome during translation.
2. A poly-A tail (a long string of adenine bases, possibly up to 300) is added to the 3′ end. (This also helps prevent it being broken down by enzymes in the cytoplasm.)
3. Splicing: the **introns** (non-coding regions) are removed from the pre-mRNA, so that all that is left in the final mRNA is a continuous stretch of **exons** (coding regions).

A fourth processing step sometimes occurs, called exon rearrangement. This is where some exons can also be spliced, leaving different exon combinations to be joined together. This results in an increase in the variety of proteins that can be coded for by a single gene.

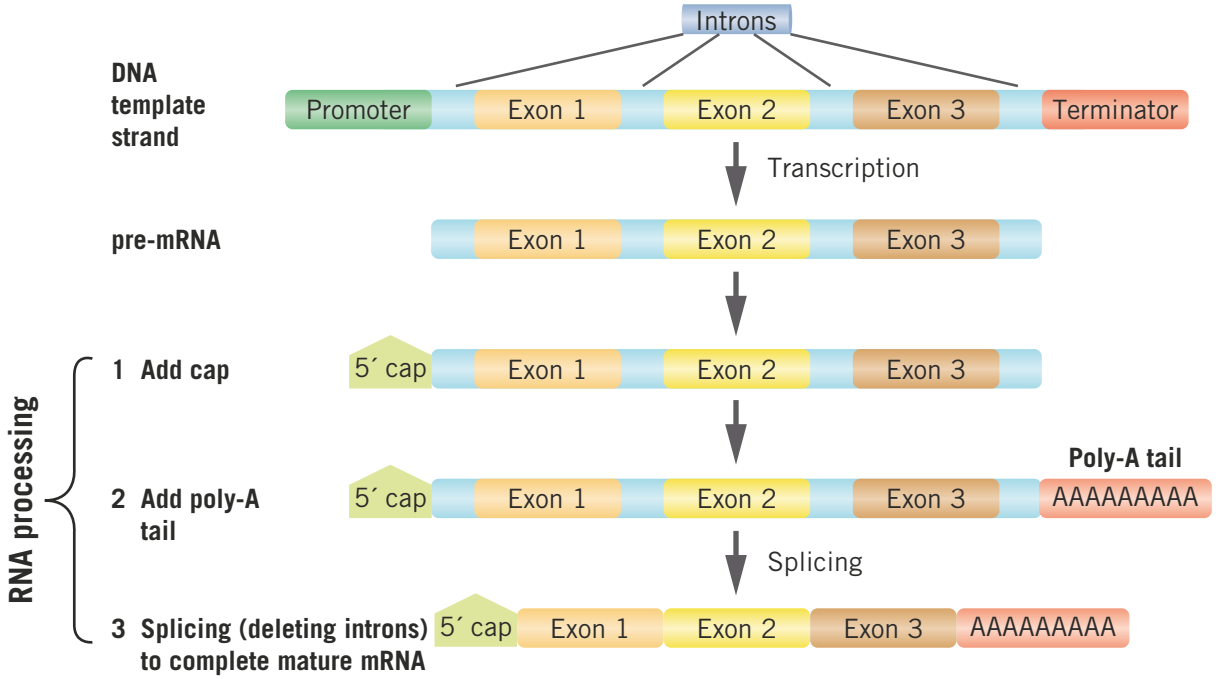

Figure 2B–3 The steps in processing pre-mRNA to mature mRNA, including the addition of a 5′ methyl cap and a 3′ poly-A tail, as well as splicing to delete introns

Check-in questions – Set 1

1. What is meant by the term ‘gene expression’?
2. What are the differences between pre-mRNA (before RNA processing) and completed mRNA (after processing)?
3. Explain the terms ‘coding strand’ and ‘template strand’.
4. What would the mRNA sequence be for the following DNA template strand sequence? CGAGGCTATGCATGCTTACAG?

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Translation: mRNA to protein

Once RNA processing has been completed, the mature mRNA then exits the nucleus through nuclear pores and travels to a ribosome. It is here, at the ribosome, that the protein will be synthesised using the information (code) in the mRNA, through a process called **translation**. In everyday use, a translation is the conversion of information from one language into another. Here it is used to mean the conversion of the information in mRNA into a sequence of amino acids making up a specific protein.

Translation
the process through which the information in mRNA is converted into a sequence of amino acids to synthesise a protein

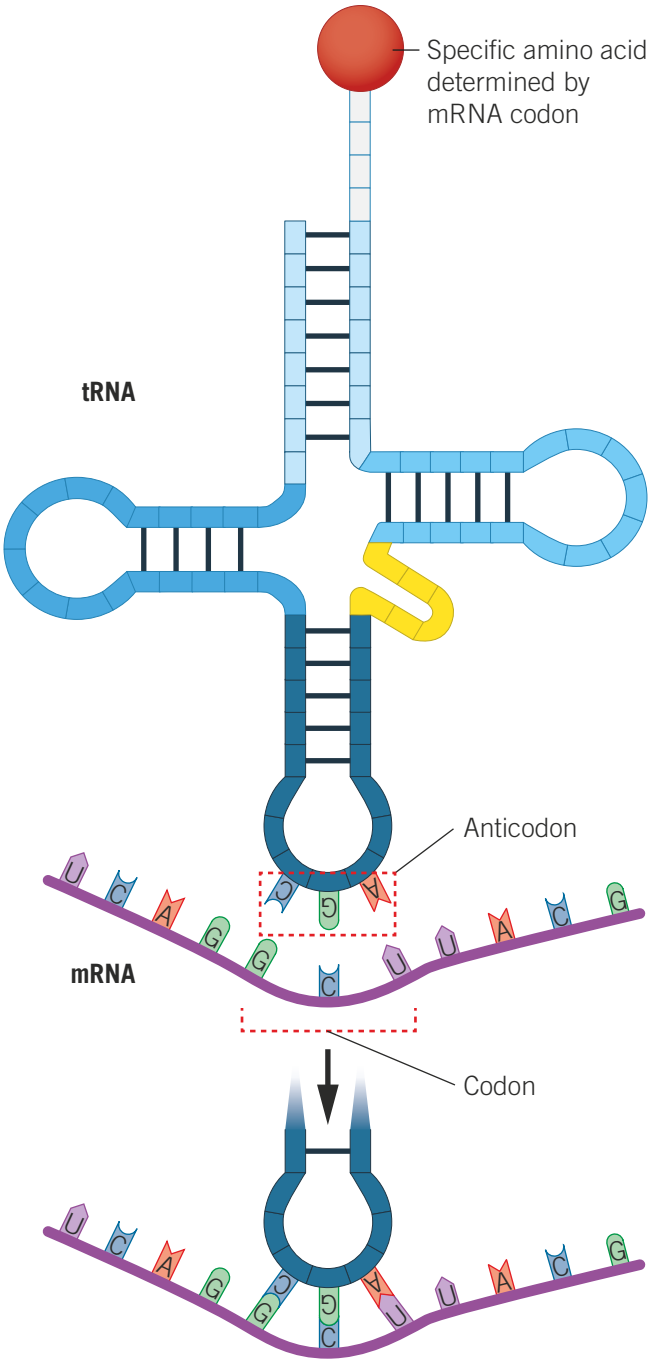

Figure 2B–4 The structure of transfer RNA (tRNA) with a strand of mRNA beneath it, to which it binds as shown at the bottom. Importantly, the tRNA's anticodon sequence is complementary to the codon sequence on the mRNA, to allow for binding. The amino acid attached to this tRNA will be specific to the codon on the mRNA.

Recall from Section 1B that ribosomes exist in two places within the cell: as free ribosomes in the cytosol or attached to the rough endoplasmic reticulum. While protein synthesis can occur in both locations, the proteins formed serve different purposes. Proteins synthesised at free ribosomes are for use within that cell, whereas those produced on the rough endoplasmic reticulum will be secreted to complete their function outside the cell.

Messenger RNA binds to ribosomes and transfer RNA

For the process to begin, the mRNA binds to the ribosome. Once attached to the ribosome, the other crucial molecule involved in translation, the transfer RNA or tRNA, is able to commence its role. The structure of a tRNA molecule can be seen in Figure 2B–4. There are two key areas to focus on. First, it can be seen that the tRNA binds to the mRNA through three complementary bases. The three bases found on the mRNA are referred to as the **codon**, whereas the complementary three bases on the tRNA are called the **anticodon**.

LINK 1B CELL TYPES AND CELL ORGANELLES

Codon
a set of three bases in mRNA that code for a specific amino acid

Anticodon
a set of three bases on tRNA that are complementary to codons in mRNA

At the other end of the tRNA, an **amino acid** is attached. This amino acid is specific to the codon on the mRNA. When two tRNA molecules bind next to each other on the mRNA, their amino acids can be joined, and joining many amino acids together forms the initial structure of a protein, called the primary structure or polypeptide chain.

Amino acid
the monomer that forms polypeptide chains and proteins

Nearly all organisms share the same genetic coding system, based on the three nucleotide codons. This is referred to as the **universal triplet code**.

Universal triplet code
the genetic coding system based on codons with three bases, shared by most organisms

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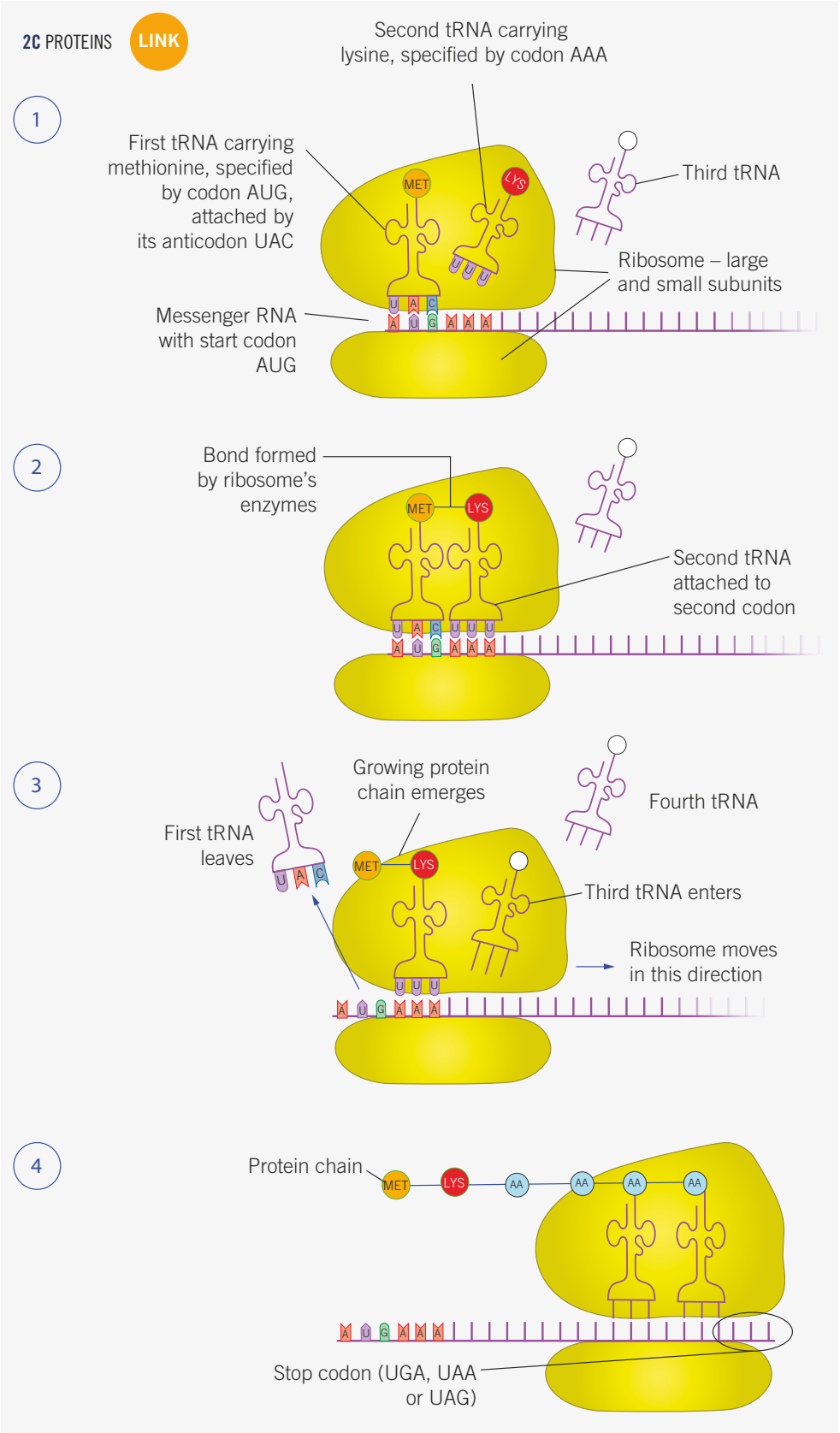

Transfer RNA links amino acids to form peptides

Figure 2B–5 gives an overview of translation. In (1), the first tRNA anticodon binds to the mRNA codon (three bases at a time). A second tRNA attaches to the next codon and there are now two amino acids next to each other. (In this example MET is methionine, LYS is lysine. In (2), the amino acids are joined by a bond (more detail of this is in Section 2C). At this point, there are two tRNA molecules attached to the mRNA. However, it is only possible for two tRNA molecules to be attached to the mRNA at any one time. In (3), the ribosome slides along the mRNA until the next codon is in position to interact with the third tRNA. Before this can happen the first tRNA must detach from the mRNA, but must leave its amino acid attached the next one or the process of protein synthesis would not work. So the amino acid bond must be made before it can detach. (4) The process repeats until a polypeptide chain, part of a protein is formed. The process stops when the ribosome detects a stop codon on the mRNA.

Figure 2B–5 Translation, which occurs at the ribosomes, is the process that leads to protein synthesis. This simplified diagram shows the steps in synthesising a polypeptide chain that starts with the amino acids methionine and lysine (code and amino acids not shown for subsequent steps).

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Codons and the amino acids they represent

As mentioned previously, each set of three bases in the mRNA sequence represents a codon, and the sequence of the codon determines the amino acid that will be added to that position in the polypeptide chain. The sequences of the codons and the amino acids that they represent can be illustrated in a codon table, as shown in Figure 2B–6. It is important that you know how to read this table, as questions will commonly ask you to determine the amino acid sequence based on a given mRNA sequence. The codon table is always provided, so you do not need to memorise this.

Use the table to find the answer to the question: *Which amino acid is coded for by UCG?*

1 Look down the 'First base' column to find the first base, U. The answer is going to be in this large block, outlined in **black**.

2 Look across the 'Second base' columns to find C. The answer is now going to be in this smaller block outlined in **red**.

3 Look down the 'Third base' column to find the third base, G.

4 Look across to see where the row for G intersects the block outlined in red. Check the code is the one you want (UCG).

5 The answer is the amino acid on the right, in the same block: Serine.

First base	Second base: U		Second base: C		Second base: A		Second base: G		Third base
U	UUU	Phenylalanine phe	UCU	Serine ser	UAU	Tyrosine tyr	UGU	Cysteine cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine leu	UCA		UAA	STOP CODON	UGA	STOP CODON	A
	UUG		UCG		UAG		UGG	Tryptophan trp	G
C	CUU	Leucine leu	CCU	Proline pro	CAU	Histidine his	CGU	Arginine arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Glutamine gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Isoleucine ile	ACU	Threonine thr	AAU	Asparagine asn	AGU	Serine ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lysine lys	AGA	Arginine arg	A
	AUG	met (START CODON)	ACG		AAG		AGG		G
G	GUU	Valine val	GCU	Alanine ala	GAU	Aspartic acid asp	GGU	Glycine gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glutamic acid glu	GGA		A
	GUG		GCG		GAG		GGG		G

To find the code for an amino acid, look for its name in the table. Its codes are on the left.

Figure 2B–6 A codon table, showing the sequences of bases for codons that code for each amino acid

An important aspect of the codon table is that more than one codon sequence can result in the same amino acid. This feature is referred to as **degenerate** or redundant genetic code. While this may not seem useful, it is actually important for the survival of organisms. Can you think why this may be the case?

The answer lies in the fact that **mutations** (spontaneous changes in the DNA sequence) can occur quite frequently. Considering this, imagine that every amino acid had only one triplet sequence that coded for it. As soon as any mutation occurred in the DNA sequence, it would result in the amino acid sequence of the protein changing.

Degenerate describes a genetic code in which multiple codons code for the same amino acid; also referred to as redundant

Mutation a permanent change in the nucleotide sequence of a section of DNA

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Altering the amino acid sequence of a protein could change its overall structure, which could have catastrophic effects on the organism. Indeed, this is the case with sickle cell anaemia, which results from just a single base mutation. Thankfully, due to the degenerate genetic code, this outcome is rare. In fact, some studies have suggested that more than 99% of all mutations don't lead to any change in the amino acid sequence. As these types of alterations have no impact, they are referred to as 'silent mutations'. The concept of mutations is covered in more detail in Section 9A.

9A MUTATIONS LINK

Check-in questions – Set 2

1 What is the difference between transcription and translation?
2 What events happen between transcription and translation?
3 What are a codon and an anticodon?
4 One of the codons for the amino acid valine is GUC. What code in the coding strand of the gene would result in valine being added to the polypeptide chain by this codon?
5 What is the role of transfer RNA?
6 According to the codon table (Figure 2B–6), what are the codons for the amino acid serine?
7 What is meant by 'universal triplet code'?

Transcription and translation in prokaryotic cells

Everything we have discussed up to this point is based on what occurs in eukaryotic cells. These processes are very similar in prokaryotic cells, although with some differences. Prokaryotic cells lack a membrane-bound nucleus. Instead, their nucleic acid is contained within the cytosol and is known as the nucleoid. For this reason, both transcription and translation occur within the cytosol. Therefore, one of the key differences of protein synthesis in prokaryotic cells is that there is no clear differentiation between these steps. In fact, in prokaryotic cells, the mRNA begins to be translated even while transcription is still ongoing. An example of this is shown in Figure 2B–7. Similarly, you can see in this diagram that (as discussed earlier in this section), transcription in eukaryotes results in two products: initially pre-mRNA, which then undergoes processing to make the final mature mRNA. In prokaryotic cells, RNA processing does not occur. This makes sense when you remember that this product of transcription is immediately and simultaneously translated into protein.

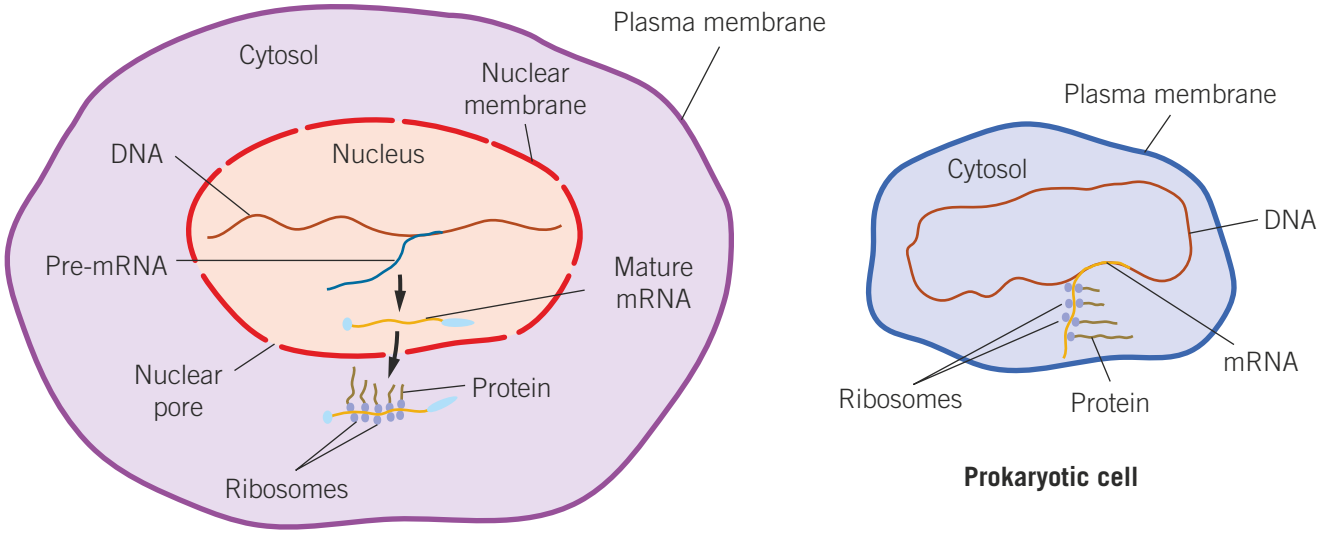

Figure 2B–7 Transcription and translation in prokaryotic and eukaryotic cells

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2B SKILLS

VIDEO 2B–2 SKILLS: ANNOTATING DIAGRAMS

Annotating diagrams

When describing processes like transcription and translation, it can be helpful to use diagrams. However, while these drawings can be very useful, it is important that you annotate them concisely, accurately and appropriately. There are a number of techniques that you can use to do this. One technique is discussed here.

A useful first step is to write a one-sentence description of what happens at each stage. This will help you to order your thoughts and think about what your diagram needs to represent. Also, the sentences can form the basis of the annotations that you use for each diagram. Make your notes brief and succinct.

Let's look at how you might do this for transcription.

Question:

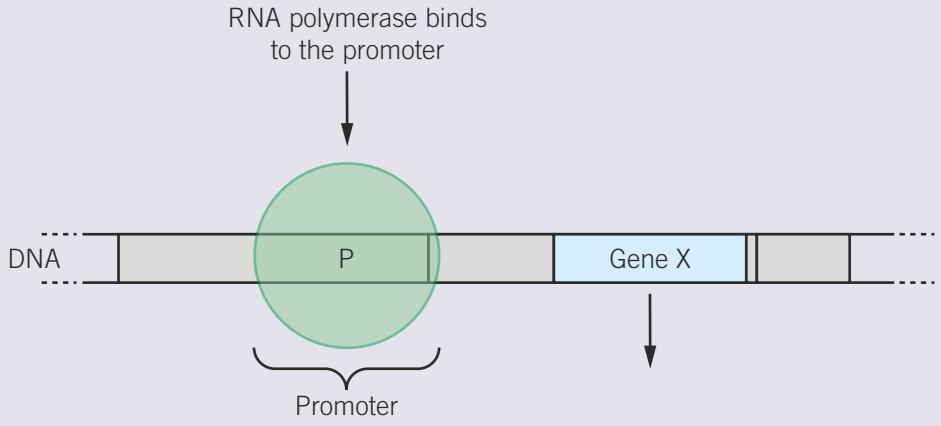
Use an annotated diagram to outline the process of transcription.

First write a brief statement for each step. For example:

Step 1 RNA polymerase binds to the promoter.
Step 2 RNA polymerase unwinds the double helix and joins free RNA nucleotides to make a complementary pre-mRNA copy of the template strand.
Step 3 RNA polymerase reaches the terminator, causing transcription to stop.

Note that technically the processing of pre-mRNA into mRNA and the exiting of the RNA out of the nucleus are not part of transcription, so should not be included.

You can then use these steps as signposts for the series of images that you want to create, and as the text to annotate the images. Below is an example of what you might do for Step 1.



There are two final things to note about using diagrams and annotating them correctly. First, you do not need to draw an impressive work of art for your diagram to communicate your answer effectively. As shown in the diagram above, you can use a very simple illustration that includes basic shapes for the key elements of the process that you are drawing. Second, clearly indicate the part of your drawing that the annotation refers to. As you can see in the example, it is very easy to use an arrow to highlight the important part at each stage. It may be a worthwhile practice exercise for you to now complete this process using Steps 2 and 3 as a guide.

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Section 2B questions

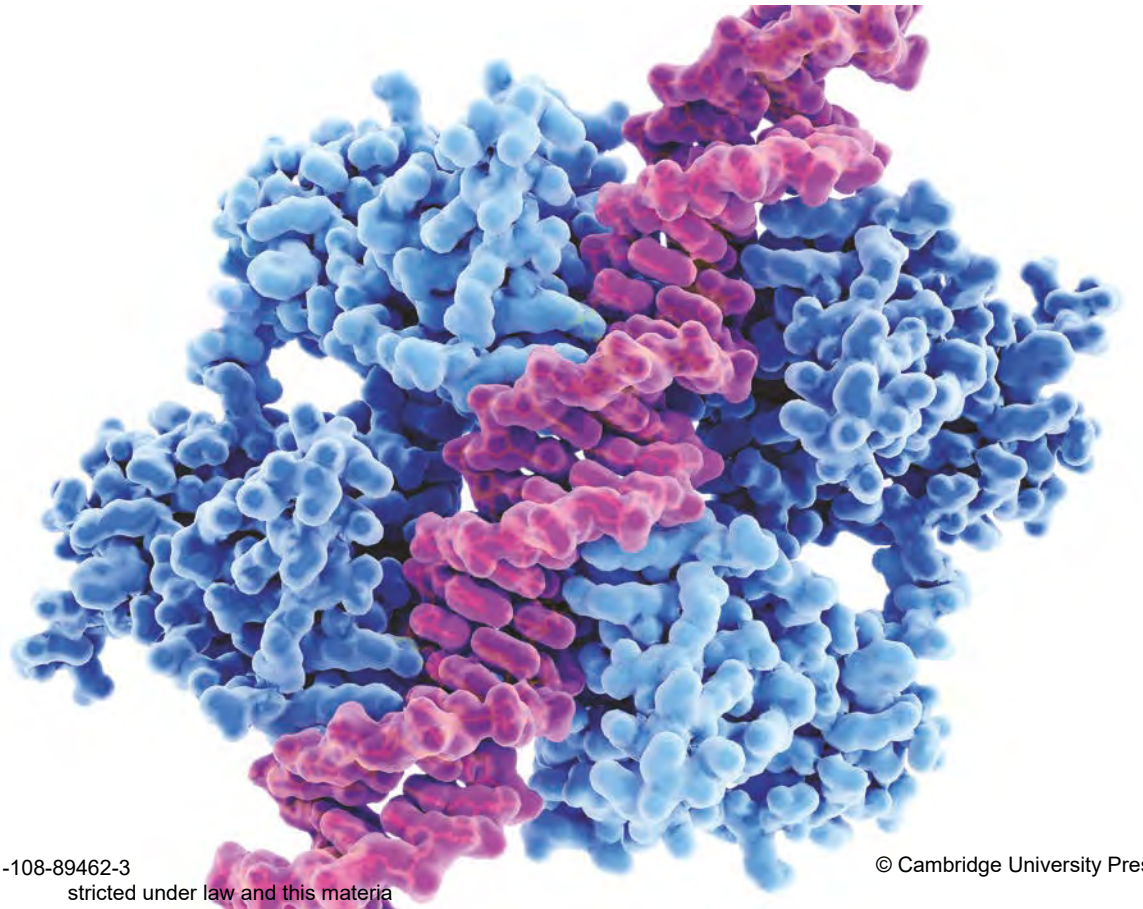
1 The following questions all relate to the process of transcription in eukaryotes.
   - **a** Where in the cell does it occur?
   - **b** What is the first step?
   - **c** What element of the gene stops the RNA transcript from being never-ending?
   - **d** State the two key differences between this process in eukaryotic cells and in prokaryotic cells.

2 Compare a pre-mRNA transcript and mature mRNA.

3 Using the codon table in Figure 2B–6, answer the following questions.
   - **a** What is the amino acid sequence that would be generated from the following mRNA sequence?
     AUG CGA GAG UCA CUG
   - **b** Using the mRNA sequence given in part **a**, write the matching sequence for both the template strand and the coding strand of the DNA.
   - **c** What are the sets of three bases in mRNA that code for amino acids known as?
   - **d** Describe how a single change could cause glutamic acid to become valine.
   - **e** A mutation causes a codon to change from CAA to CAG. What effect does this have on the protein produced, in terms of a change to its amino acid sequence?
   - **f** Explain what is meant by the fact that the genetic code is degenerate.

4 The following questions all relate to the process of translation.
   - **a** Draw an annotated diagram to explain this process.
   - **b** In which two locations in a eukaryotic cell can this process occur?
   - **c** What is the difference between proteins made at each of these locations?

5 Draw a labelled diagram to show RNA processing (post-transcription modifications) for a gene with three exons, turning pre-mRNA into mature messenger RNA (mRNA).



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2C Proteins

Study Design:

- Amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein
- Proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as biological catalysts in biochemical pathways
- The role of rough endoplasmic reticulum, Golgi apparatus and associated vesicles in the export of proteins from a cell via the protein secretory pathway

Glossary:

Biomacromolecule
Condensation reaction
Peptide bond
Polypeptide
Proteome

ENGAGE

Biomacromolecules

Organisms contain many **biomacromolecules** that are critical for their function, such as nucleic acids, carbohydrates and proteins. The structure of a gene can be likened to the structure of a book. A book is a series of repeating pages all bound together. We can see a similar pattern with biomacromolecules. Indeed, a common feature of many biomacromolecules is that they are polymers, consisting of repeating units of monomers (Figure 2C–1). In this section, you will learn more about the monomers that make up proteins, how they lead to the structure of a protein, and how proteins are transported out of the cell to perform their function.

Biomacromolecule a large biological polymer, such as a protein, a nucleic acid or a carbohydrate

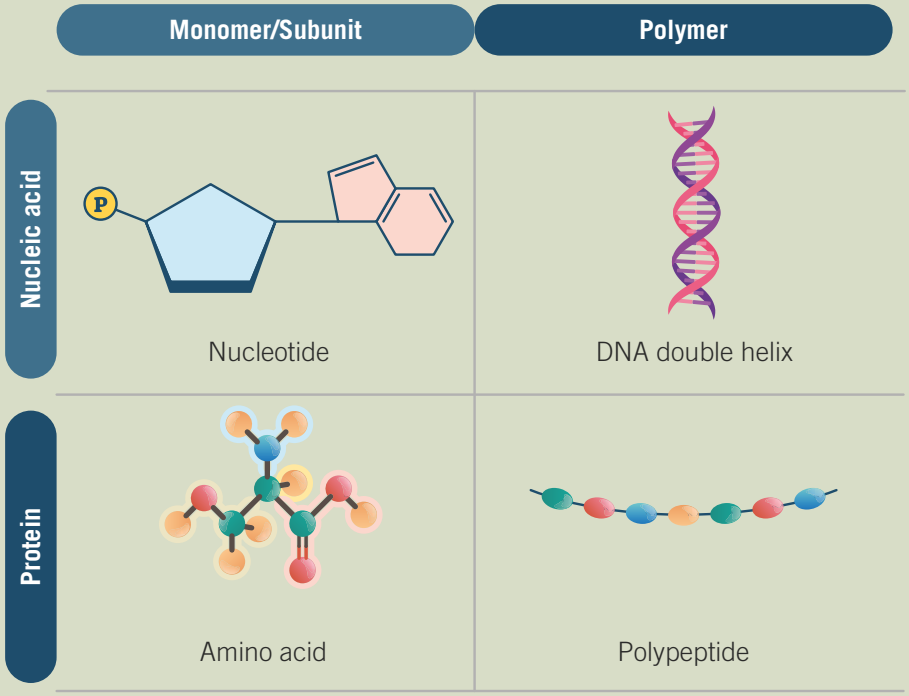

Figure 2C–1 The main biomacromolecules found in organisms are polymers consisting of repeating units of monomers. These polymers form many different cellular structures that are important for the survival of the organism.

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EXPLAIN

Amino acids: the monomers of proteins

The building blocks (monomers) of proteins are known as amino acids. The generalised structure of an amino acid is illustrated in Figure 2C–2. An amino acid has two distinct groups: an amine ($–NH_2$) group at one end, and a carboxyl (–COOH) group at the opposite end. A carboxyl group can also be referred to as a carboxylic acid, and so the term 'amino acid' is derived from these two components: amino ($–NH_2$) and acid (–COOH).

WORKSHEET 2C–1 PROTEINS

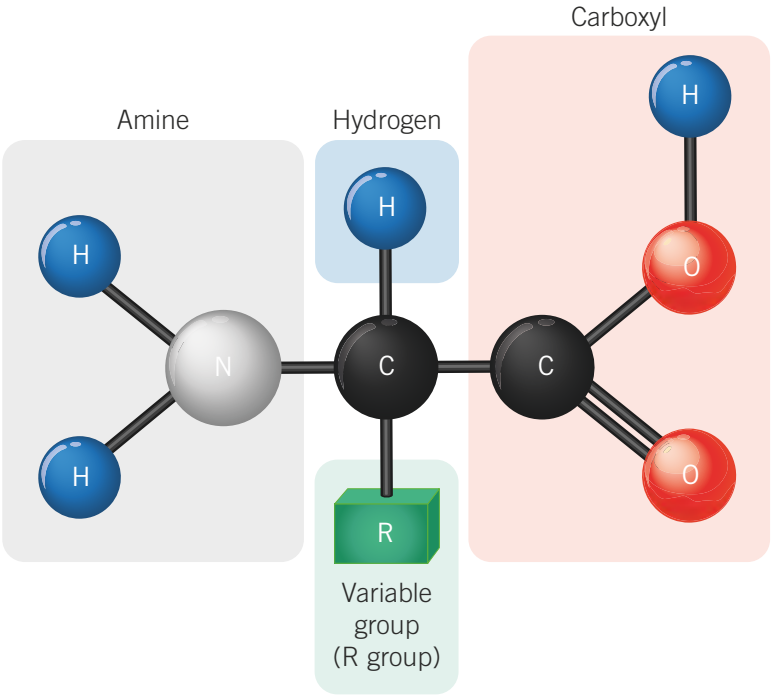

Figure 2C–2 Generalised structure of an amino acid

Peptide bond a chemical bond between two amino acids

Polypeptide a long chain of amino acids forming part of a protein

The other important feature of an amino acid is the variable group, or R group (highlighted in green). All the amino acids are identical in all other parts of their structure – that is, they all have exactly the same amine group (grey), hydrogen (blue) and carboxyl group (red). The only thing that differentiates the 20 amino acids found in the body is the variable group.

Peptide bonds

Amino acids are the monomers that make up proteins. For this to occur, the amino acids need to join to each other when the **polypeptide** chain is synthesised during translation. This occurs through the formation of the **peptide bond**.

2B THE GENETIC CODE AND GENE EXPRESSION LINK

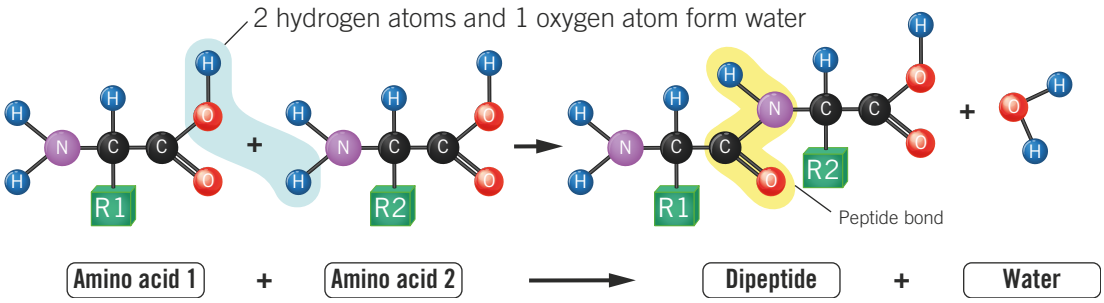

Figure 2C–3 A condensation reaction between two amino acids to form a dipeptide with a peptide bond. This reaction is repeated numerous times to form a polypeptide chain.

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It can be seen in Figure 2C–3 that the reaction between two amino acids leads to the loss of water. A reaction such is this is known as a **condensation reaction**. It is important to note that condensation reactions require energy, in the form of ATP, to occur. This helps explain why mitochondria are also important for supporting protein synthesis, as they are the site of energy release through aerobic cellular respiration. The peptide bond, highlighted in yellow, shows the link between the carbon atom from amino acid 1 and the nitrogen atom from amino acid 2. When looking at a polypeptide chain, it is important to be able to identify the peptide bond, as this will help you determine the number of amino acids present in that protein.

LINK 1A PLASMA MEMBRANE

LINK 5C CELLULAR RESPIRATION

Condensation reaction
a reaction in which two molecules are joined to make a larger molecule, resulting in the loss of a smaller molecule as another product (in organisms, this is usually water)

Check-in questions – Set 1

1 What part of an amino acid makes it unique?
2 Thinking back to translation, what molecule is involved in delivering the amino acid to the site of protein synthesis, and what is that site?
3 What kind of chemical bond joins amino acids together?
4 Complete this equation for the condensation reaction between glycine and alanine, by adding the names of the products (not their chemical formulas):
glycine + alanine → _____ + _____

A gene sequence leads to a unique amino acid sequence

To understand how unique proteins are formed, it is easiest to visualise this process using a flow chart. Figure 2C–4 illustrates how a specific gene sequence leads to a unique amino acid sequence that is specific for the protein encoded for within the genome. However, as seen in the next part, the actual sequence of amino acids that comprise a protein is not the whole story when it comes to understanding the role of amino acids in organisms.

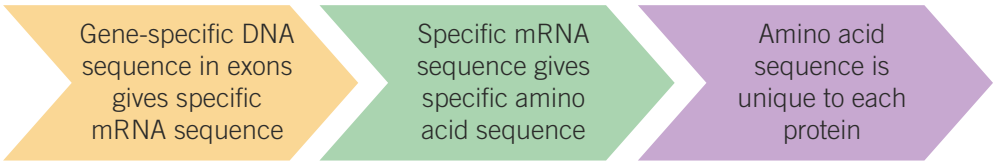

Figure 2C–4 The process through which a specific DNA sequence contained within a gene gives rise to a specific amino acid sequence that is unique to the protein encoded for by this gene.

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Four levels of protein structure

VIDEO 2C–1 PROTEIN STRUCTURE

The *structure* of a protein is critically important to its function. There are four levels of protein structure, each building on the foundation laid by the previous level. These are illustrated in Figure 2C–5.

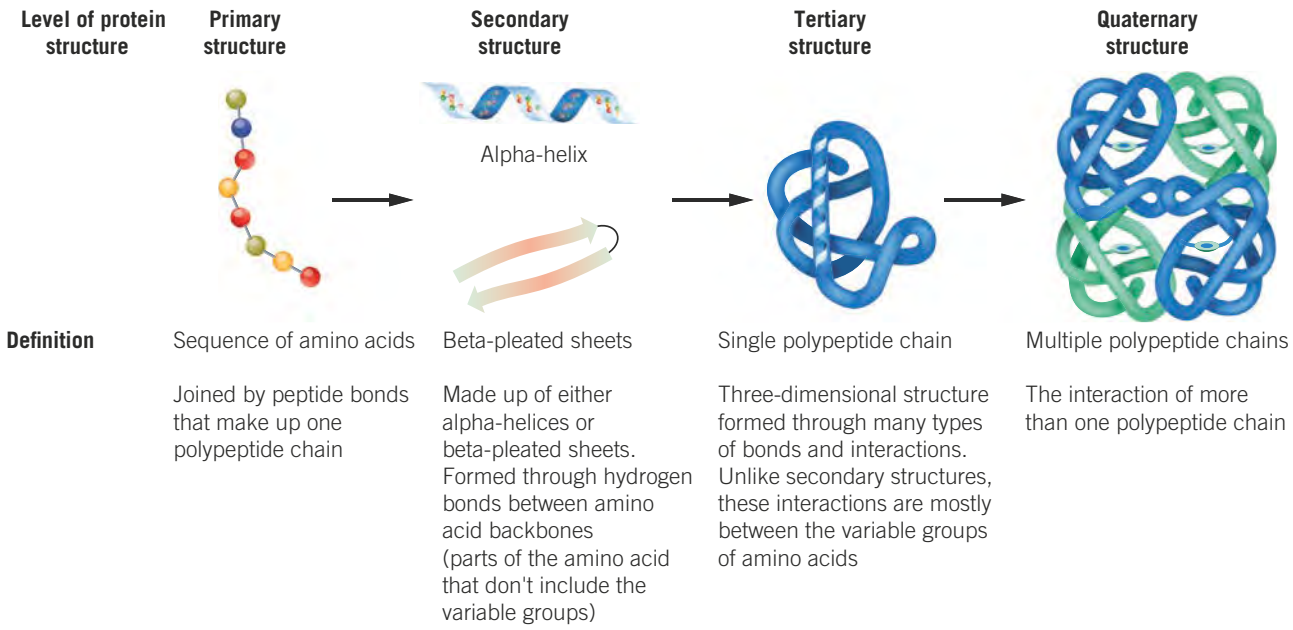

Figure 2C–5 The four levels of protein structure: primary, secondary, tertiary and quaternary. The structure becomes more complex as the levels increase and is essential to the protein's function.

All proteins have a primary, secondary and tertiary structure. However, only certain proteins also have a quaternary structure. An example of a protein that contains a quaternary structure is haemoglobin, present in red blood cells and responsible for transporting oxygen around your body. Haemoglobin is a protein that consists of four subunits (two alpha and two beta, shown in blue and green respectively). Each of these subunits has its own polypeptide chain, but the protein is only functional when they are all combined.

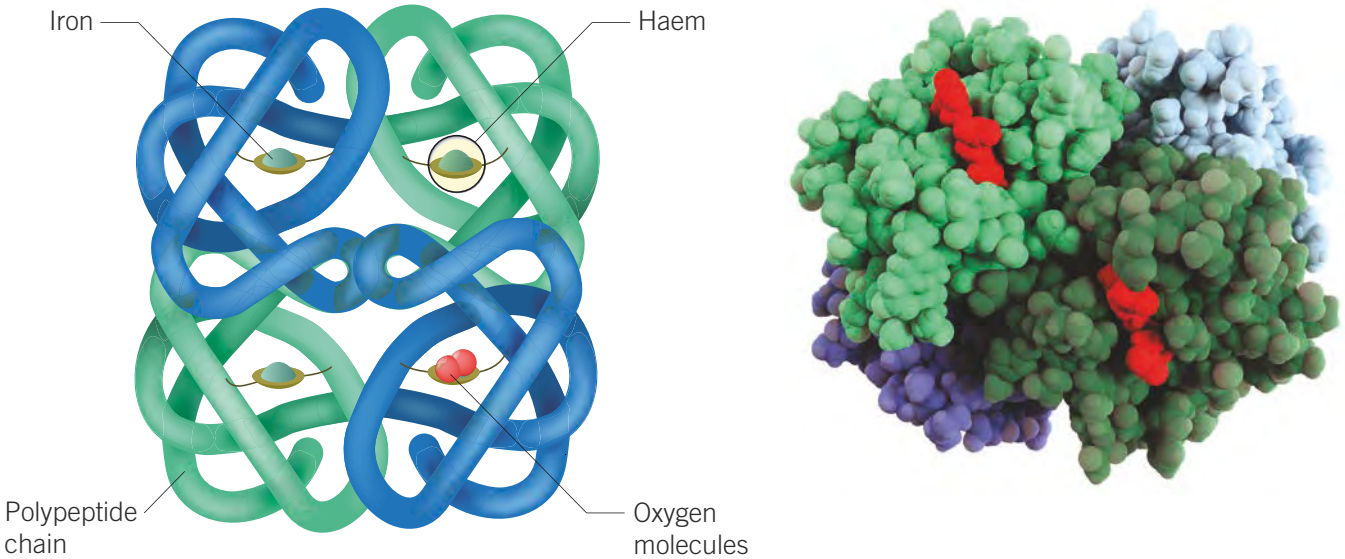

Figure 2C–6 Haemoglobin has a quaternary structure (left) made up of four polypeptide chains as well as a haem group, which is not an amino acid and which contains iron. Each haem group can carry an oxygen molecule (two oxygen atoms). On the right is a molecular model of haemoglobin.

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The proteome of an organism

At the start of Section 2B, we discussed the Human Genome Project and how it has advanced our understanding of human genetics. The genome is all the genes within an organism; similarly, the **proteome** is the complete collection of proteins within an organism at at a given time. A vast number of proteins make up the proteome and they undertake a variety of roles in the body. These functions can be grouped into key categories:

1 hormones (e.g. insulin)
2 immunity (e.g. antibodies)
3 transport (e.g. haemoglobin)
4 structure (e.g. collagen)
5 movement (e.g. actin)
6 enzymes (e.g. pepsin).

Proteome
the complete collection of proteins within an organism at a given time

LINK 2B THE GENETIC CODE AND GENE EXPRESSION

LINK 7D THIRD LINE OF DEFENCE

LINK 4A THE ROLE OF ENZYMES

Check-in questions – Set 2

1 What are the monomers that make up proteins?
2 What are the four levels of protein structure?
3 Define each of the four levels of structure of a protein.

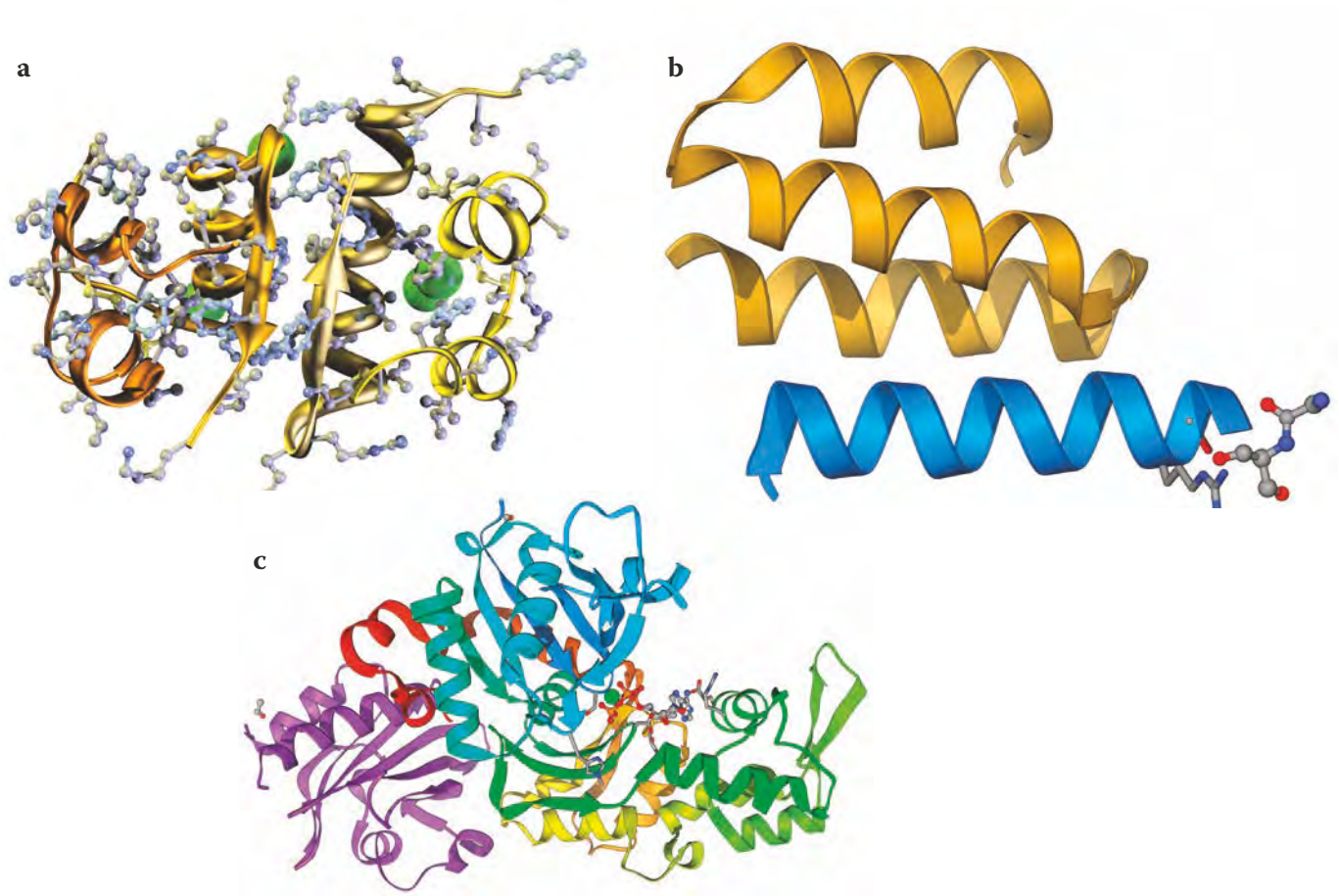


Figure 2C–7 Examples of the quaternary structure of proteins: **a** insulin, a hormone; **b** collagen, a structural protein; **c** actin, a protein in muscle responsible for movement.

The protein secretory pathway

1B CELL TYPES AND CELL ORGANELLES LINK

As you read earlier in this chapter, proteins are synthesised at ribosomes within a cell. However, many proteins need to be able to exit the cell to perform their function. For this to occur, the cell has developed what is known as the protein secretory pathway. This pathway uses many of the organelles that you learned about in Unit 1 and revisited in Section 1B. Essentially, this pathway involves the rough endoplasmic reticulum, the Golgi apparatus and vesicles (Figure 2C–8).

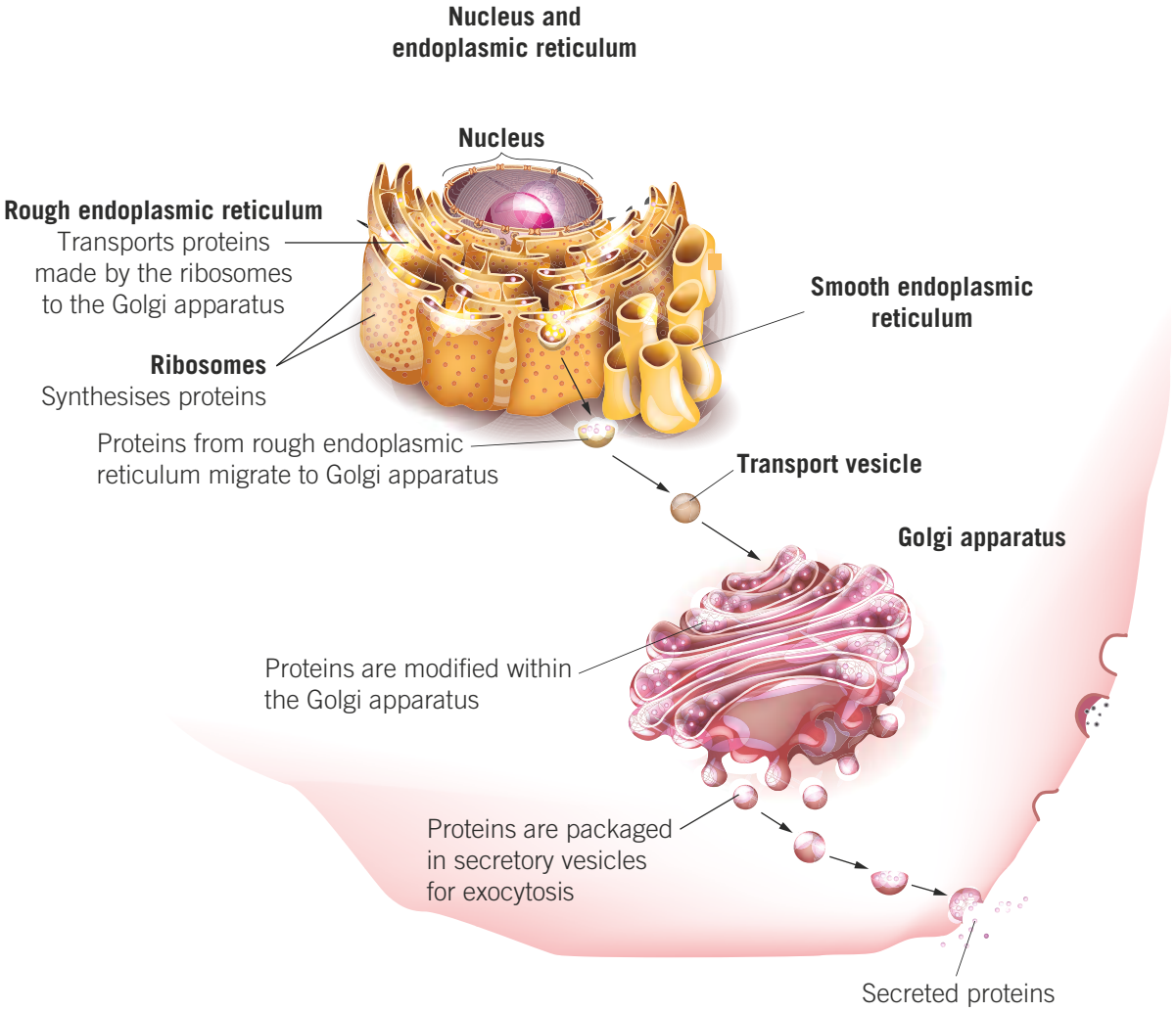


Figure 2C–8 The rough endoplasmic reticulum, Golgi apparatus and vesicles all play critical roles in the protein secretory pathway.

Proteins are synthesised at ribosomes. You may recall that ribosomes can exist freely within the cytoplasm, but are also found on the rough endoplasmic reticulum. The endoplasmic reticulum plays a role in transporting proteins to the Golgi apparatus. At the Golgi apparatus, the proteins undergo modification. The final protein products are then packaged into secretory vesicles and transported to the plasma membrane. At this stage, the proteins exit the cell via exocytosis, as the vesicle fuses with the plasma membrane and secretes the proteins. The proteins then complete their functions elsewhere in the organism.

2C SKILLS

VIDEO 2C–2
SKILLS: MEMORY TOOLS

Memory tools

As you will see throughout this year, Units 3 & 4 Biology requires you to remember a lot of content. It can be very helpful to have strategies for recalling this information – these are referred to as memory tools. One such device that can be handy is the creation of a mnemonic. A mnemonic is a pattern of letters or associations that can help you remember something.

The list of protein functions given earlier can be used as a mnemonic. If you look back to the functions numbered 1–6 and take the first letter of each, you end up with H I T S M E or 'hits me':

Hormones
I mmunity
T ransport
S tructure

Movement
E nzymes

An easy way to commit this to memory is to use the phrase, 'I almost always forget the different functions of proteins and then it HITS ME'.

Another piece of content from this chapter that some students find difficult to remember is which nitrogenous bases are found in DNA and RNA, and how they pair together. A mnemonic can help you.

Step 1: Take all the words that are important to what you are trying to remember.

For example:
DNA: Adenine, Thymine, Guanine, Cytosine
RNA: Adenine, Uracil, Guanine, Cytosine

Step 2: Simplify this to their initials.

For example:
D, A, T, G, C
R, A, U, G, C

Step 3: Come up with a phrase using these initials.

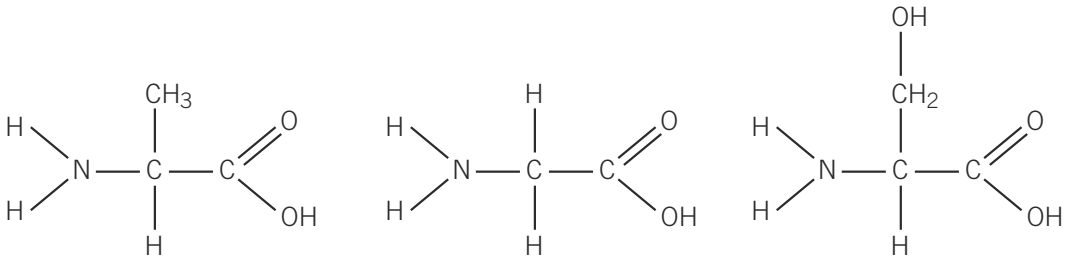
For example:
Doing Anything Tricky Gets Confusing
Reading And Understanding Grows Confidence

In this example, notice that the phrase helps you remember that DNA contains thymine and RNA contains uracil. Also, it ensures that the nitrogenous bases that pair together are joined together in the phrases (that is, A to T/U and G to C).

If you find other information in this year's course difficult to remember, try developing a mnemonic to help you commit it to memory.

Section 2C questions

1 The following structures represent three common amino acids found in proteins.



a Using the structures above, draw a tripeptide that would have the sequence glycine–alanine–serine.

b In the diagram you drew for part **a**, circle the peptide bonds.

c The formation of this tripeptide involves what type of reaction? Also identify what is required for this reaction to occur, as well as what is produced from this reaction.

2 What are the two different forms or shapes that comprise the secondary structure of a protein?

3 What are the six main functions of proteins?

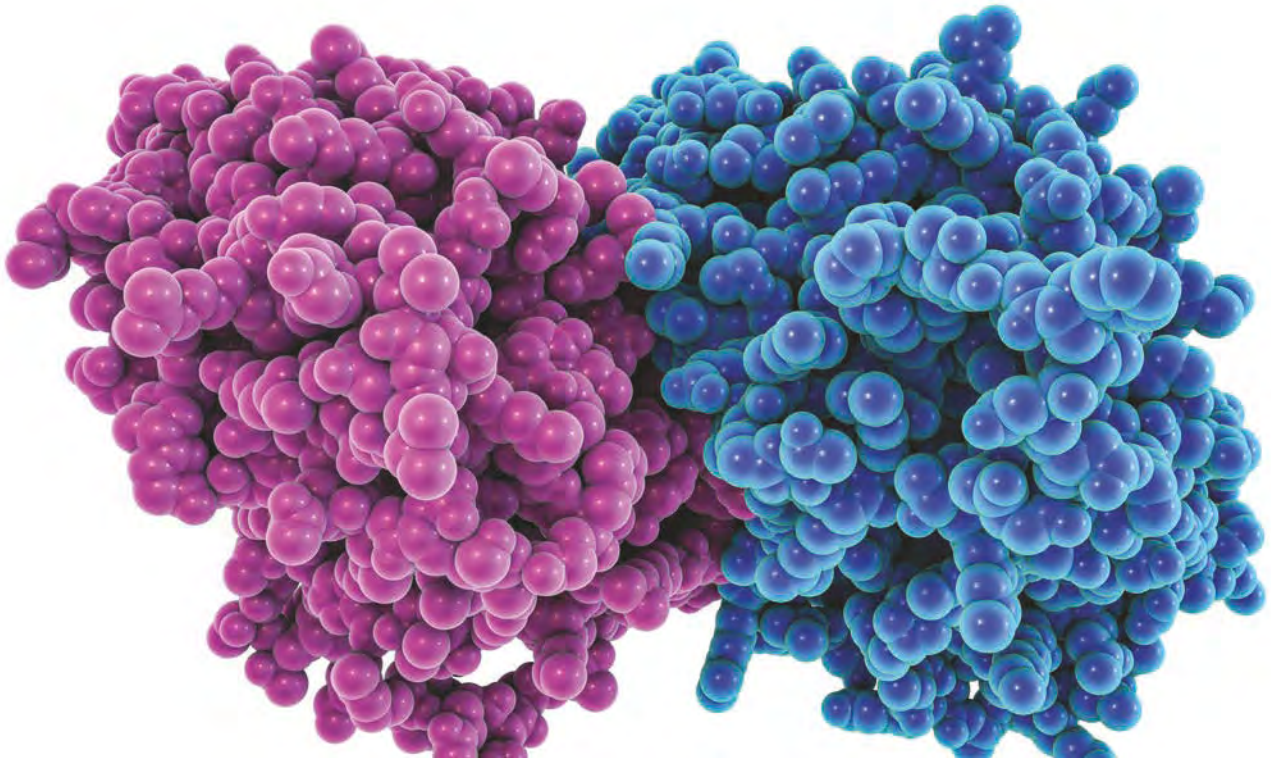
4 The following questions relate to protein structure.

a Define the primary structure of a protein.

b Draw diagrams to represent the four levels of protein structure.

5 Compare the processes of transcription and translation. You may do so in a table format. Key features that you should focus on include the cellular location of the process, the starting molecule, the final product and the other key molecules that are involved.

6 Describe the roles of the rough endoplasmic reticulum, Golgi apparatus and vesicles in the protein secretory pathway.



2D Gene structure and expression

Study Design:
- The structure of genes: exons, introns and promoter and operator regions
- The basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of a regulatory process

Glossary:
Coding region
Operator
Operon
Regulatory gene
Repressor
Structural gene

ENGAGE

How does it all work?

In Section 2A, you learned some general facts about nucleic acids. Have you ever stopped to think about how much genetic material you have in your body? The Human Genome Project found that the total length of the human genome is over 3 billion base pairs. Contained within that huge amount of information are an estimated 20 000–25 000 genes. Remember from Section 2C that genes are the coding information used to synthesise proteins. Recent estimations have suggested that between 80 000 and 400 000 proteins are made within the human body. Considering the complexity of this (3 billion base pairs, 25 000 genes and more than 80 000 proteins), how does it all work without problems arising all the time? The answer lies in the structure of genes and how it relates to their expression. This is covered in depth in this section.

LINK 2A NUCLEIC ACIDS

LINK 2C PROTEINS

EXPLAIN

Understanding the structure of genes

WORKSHEET 2D–1 GENE STRUCTURE AND OPERONS

To understand the structure of genes, think about the structure of your favourite book or movie. Any good story or film has a beginning, a middle and an end. In the middle, there are parts of the plot that are important, and sometimes there are parts that just seem to be 'filling in time'. In essence, the structure of genes in eukaryotic cells is the same. Figure 2D–1 shows the overall structure of a eukaryotic gene. We will look at those components of a gene separately and discuss the role played by each.

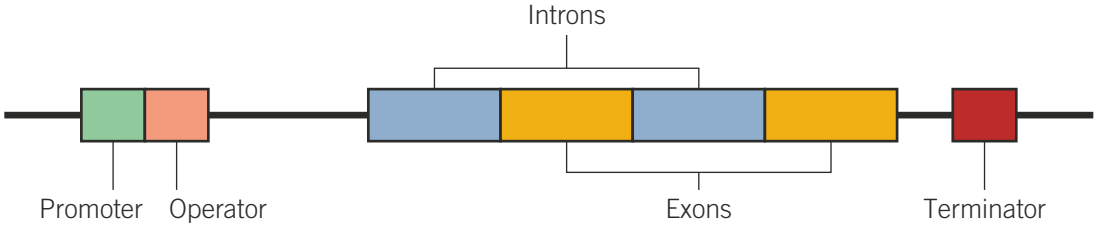


Figure 2D–1 The generalised structure of a eukaryotic gene, which comprises a promoter, an operator, introns, exons and a terminator.

Promoter: the beginning of the story

LINK 2B THE GENETIC CODE AND GENE EXPRESSION

In Section 2B the underlying process of going from DNA to protein was described. Remember that, for this to occur, the gene encoding the protein must be converted from DNA to messenger RNA (mRNA), through a process known as transcription. As you may recall, the process of transcription starts with a promoter. The promoter is the binding site for RNA polymerase, the enzyme that moves along the DNA template strand, producing a complementary pre-mRNA strand.

Introns and exons: the coding region (the middle of the story)

Coding region the introns and exons of a gene that are transcribed into pre-mRNA

2B THE GENETIC CODE AND GENE EXPRESSION LINK

Introns and exons are what is transcribed from the DNA into pre-mRNA during the process of transcription (Figure 2D–2). Together, introns and exons form the protein **coding region** of DNA. As shown in Figure 2D–2, there is an additional step that occurs after DNA is transcribed into pre-mRNA, which is the processing to mature mRNA (covered in Section 2B). However, what is important to note is that the introns do not feature in the final mRNA product. This is because introns do not actually contain any of the sequence related to the genetic code for the protein. **Int**rons are the **int**ervening sequences (or **int**erruptions) found between exons. They have previously been referred to as 'junk DNA', but it is likely that they serve other purposes that we are not yet fully aware of. **Ex**ons, on the other hand, contain the parts of the genetic code that will be **ex**pressed as a protein.

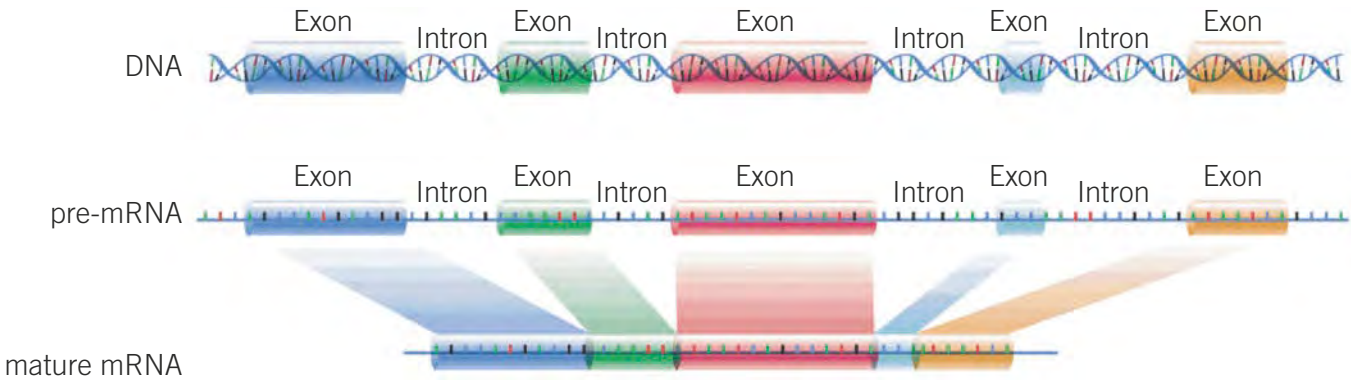


Figure 2D–2 Introns and exons form the coding region of a gene. They are transcribed into pre-mRNA and then further processed into mRNA. As you can see here, mature mRNA contains only the sequences from the exons.

Check-in questions – Set 1

1 Name the part of a gene that is responsible for starting transcription.
2 A sequence from the coding region of a gene is being converted to RNA. The sequence is TAGGATCGGATCCTTGAT. What would be the sequence of the mRNA that is made from this?
3 The coding region is made up of two distinct parts. What are they?

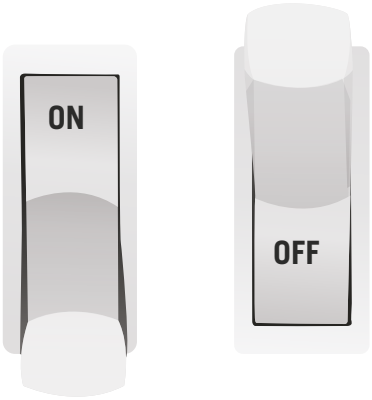
Terminator: the end of the story

Up to this point, our 'story' (the process of turning a gene into a protein) has a beginning (promoter) and a middle (introns and exons). It also needs an ending. The end of the process is marked by a part of the genetic sequence called the 'terminator'.

The terminator is the sequence at the end of a gene that signals for transcription to stop. Without the terminator, the process would continue along the DNA strand, creating a 'never-ending story'. As you may appreciate from the discussion of protein synthesis and protein structure earlier in this chapter, this is critically important. Extending the sequence even just a little, or even changing a single base within an exon, can have catastrophic consequences for the protein that is made and the organism as a whole.

Operators: the genetic 'off-switch'

Our genetic story now has a beginning, a middle and an end. However, one final problem still needs to be addressed. The promoter, or 'on-switch' of the whole process, could theoretically restart transcription repeatedly. This would lead to huge numbers of mRNA copies being produced and lots of protein being synthesised. As you may remember from Units 1 and 2 Biology, organisms keep things tightly regulated, so that everything is kept at appropriate levels. The process of transcription is no different.



LINK UNIT 1

LINK UNIT 2

Once the correct levels of mRNA have been produced, transcription is stopped through the function of another sequence within genes, known as the **operator**. As you can see in Figure 2D–1, the operator is found close to the promoter. The operator itself does not function independently; it works by interacting with another protein, known as the **repressor**. If the repressor is bound to the operator, RNA polymerase is blocked from binding to the promoter and transcription cannot commence. However, if the repressor is not bound to the operator, transcription can occur. The repressor is an example of a regulatory protein, which is coded for by a **regulatory gene**. These genes and the proteins they code for are specifically involved in altering the expression of other genes. This is different from proteins that have a function throughout the body, such as those discussed in Section 2C, which are known as structural proteins (coded for by **structural genes**).

Operator
a section of DNA code where the repressor protein can bind

Repressor
a regulatory protein that binds to DNA, inhibiting transcription

Regulatory gene
a region of DNA that codes for a regulatory protein, which controls the expression of other genes

Structural gene
a region of DNA that codes for a protein that performs a specific function for a cell or organism

In the next section, the regulation of transcription is discussed, using the example of the *trp* operon.

Check-in questions – Set 2

1 What is the role of the operator in a gene?
2 What might happen to the cell if a gene had no operator or terminator?
3 When is the first time that you would expect to see uracil during the process of going from a gene to a protein?

Regulation: the *trp* operon

To help you understand how gene expression is regulated, this section examines a specific system to highlight these mechanisms. There are many well-defined examples, but we will focus on just one, the *trp* **operon**, which is required for this course.

Operon
a series of genes under the control of a single promoter and operator

The *trp* operon is involved in the production of tryptophan (trp), which is an amino acid and therefore important for the synthesis of proteins. The structure of this operon is shown in Figure 2D–3.

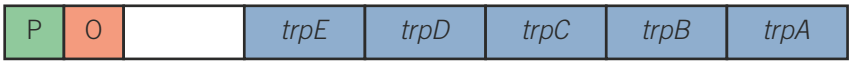


Figure 2D–3 The organisation of the *trp* operon, which shows that the five structural genes (*trpE–trpA*) are all under the control of a single promoter (P) and operator (O).

VIDEO 2D–1 THE TRP OPERON

The five genes that make up the *trp* operon are involved in coding for the enzymes necessary for the synthesis of tryptophan. Thinking back to what you learnt in Unit 1 Biology regarding homeostasis, you might appreciate that this operon functions differently depending on how

LINK UNIT 1

much tryptophan is available. As a general rule, resources are limited within organisms, and as such, organisms don't perform processes that are unnecessary. Considering this, you could imagine that, at times when tryptophan levels are high, the organism would not want to be producing more. On the other hand, as tryptophan is an important amino acid, when levels become low, it is critical that more can be produced. Let's look at both these scenarios and see how this regulation is maintained at the genetic level.

Scenario 1: tryptophan levels are low

For each of these situations, it is useful to start by thinking about what the aim of the organism is in the current circumstances and how it will achieve that in the context of the *trp* operon. For scenario 1, this will be as follows:

Aim: To raise the levels of tryptophan by producing more.

Solution: Increase transcription of the genes necessary to produce tryptophan (that is, those within the *trp* operon).

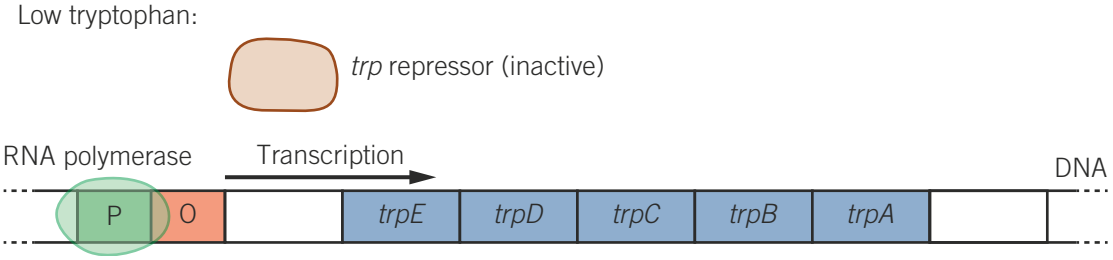


Figure 2D–4 When tryptophan levels are low, RNA polymerase binds to the promoter and initiates transcription of the *trp* operon genes. This leads to the synthesis of tryptophan.

As you can see in Figure 2D–4, this process involves RNA polymerase binding to the promoter and many copies of the *trpE* to *trpA* genes being transcribed, which will then be translated into the relevant proteins. These proteins will then help synthesise tryptophan.

Scenario 2: tryptophan levels are high

Once again, let's start by looking at the aim and solution in this scenario, and then discuss the mechanisms by which that solution is achieved.

Aim: To stop making tryptophan.

Solution: Stop transcription of the genes necessary to produce tryptophan (that is, those within the *trp* operon).

As things stand from Scenario 1, the cell is currently producing mRNA copies of the genes contained within the *trp* operon, which will lead to the synthesis of the proteins required to make tryptophan. This is occurring because RNA polymerase can bind to the promoter of the *trp* operon and transcribe the genes contained within it.

As you learned earlier in this section, the role of the operator within a gene is to be the 'off-switch' for transcription, which is exactly what is required in this scenario. So how does the operator work?

The purpose of the operator is to provide a binding site for the repressor. In this way, it works in a very similar way to the promoter, which provides a binding site for RNA polymerase. However, once the repressor has bound to the operator site, it prevents RNA polymerase from binding to the promoter. In this way, the transcription of the operon is prevented.

While this may seem straightforward, it is important to recognise that the repressor protein is present at all times. Considering this, how does an organism prevent transcription from being stopped in scenario 1 and only stopped in scenario 2? The answer can be seen in Figure 2D–5. When tryptophan levels are high, tryptophan binds to the repressor, causing a change in shape that allows it to bind to the operator. This prevents RNA polymerase from binding to the promoter, which stops transcription and leads to a decrease in the synthesis of tryptophan.

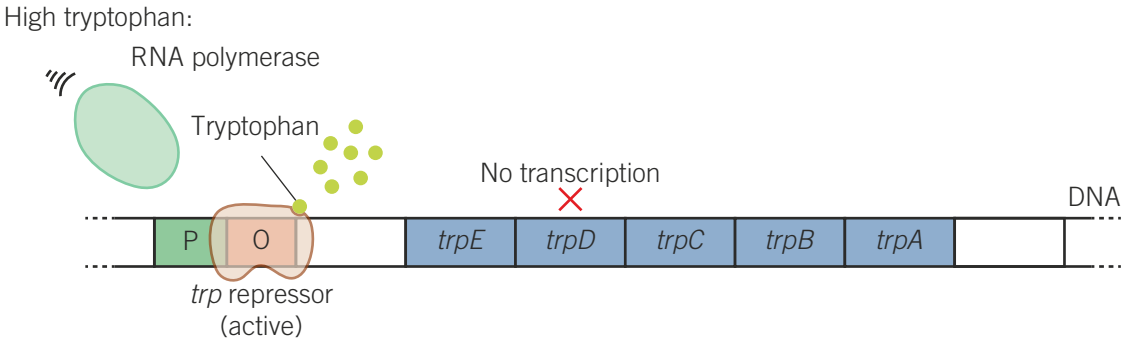


Figure 2D–5 When tryptophan levels are high, the repressor binds to the operator to prevent transcription of the trp operon genes.

2D SKILLS

Knowing your definitions

As you will have noticed, there are many terms in Units 3 & 4 Biology that you need to be able to define. Indeed, almost all questions in this year's assessments will require you to know a definition. You may be asked directly (for example, the question specifically asks you to define a term) or indirectly (for example, you aren't prompted for a definition in the question, but you are required to know it, to provide a complete and accurate answer). Considering the importance of this skill, here are some strategies to help enhance your ability to master this concept:

1. *Separate your definitions.* You will likely end up with a lot of notes by the end of the year. Having your exact definitions mixed in with all your other content will make it harder to learn them and easier to overlook them. Create flashcards or use an online tool that allows you to have a specific place to store terms and their meanings, so you can easily refer to them and learn them when needed.
2. *Always clarify.* There is no point making notes and accomplishing strategy 1, if the definitions that you are writing down are inaccurate. Doing so would reinforce wrong information whenever you study. If you have any definitions that you want to check or are unsure of, refer to this textbook or ask your teacher. It is always better to be sure than sorry! This also leads to strategy 3.
3. *Ask the experts.* Past VCAA examiner's reports can be an effective way to find appropriate and accurate definitions of terms that you need to know. These reports often contain definitions that the assessors deem worthy of full marks. This is a great way of making sure the definitions you are using are the right ones.

An example of this can be found in the 2018 examiner's report, looking at Section B Question 6a. The question asks for the functional difference between a structural gene and a regulatory gene. This requires you to know the definition of a structural gene as one that 'codes for a protein that becomes part of the structure or function of an organism', whereas a 'regulatory gene controls another gene'.

VIDEO 2D–2
SKILLS: KNOWING YOUR DEFINITIONS

Section 2D questions

1 Genes are stretches of DNA sequences that encode for what?

2 The coding region of a gene contains two components. Which component appears in the complete mRNA after transcription?

3 What part of the genetic structure ensures that transcription ends, so that only the genetic sequence corresponding to the protein is present in the RNA?

4 What would the likely result be if there was a genetic issue in the promoter region?

5 Draw a diagram that represents the general structure of an operon. Make sure you include all the key components (promoter, operator, introns, exons and terminator).

6 It is important that the expression of genes is tightly regulated, so proteins are only synthesised when necessary.

- **a** What part of a gene regulates the amount of protein synthesised?
- **b** What molecule binds to the part of the gene that is the answer to part **a**, in order to stop gene expression?
- **c** The molecule that is the answer to part **b** is coded for by what type of gene?
- **d** Scientists identify a strain of *E. coli* that has a mutation in the DNA sequence of the *trp* operon. Further analysis of these bacteria shows that they have abnormally high levels of tryptophan. Use this information to explain where in the operon the mutation has most likely occurred and what impact it has had.

7 Microarrays are technology that can be used to compare the expression of genes between two different samples. They are often used to identify genes that may play a role in disease. The colour of the spot on the microarray indicates the relative level of gene expression between the two samples:

- Black indicates that the gene isn't expressed in either sample.
- Yellow indicates that the gene is expressed at the same level in both samples.
- Green indicates that the gene is expressed at higher levels in sample 1 than in sample 2.
- Red indicates that the gene is expressed at lower levels in sample 1 than in sample 2.

The following data was obtained from a microarray. The diseased sample was sample 1 and the healthy sample was sample 2. The analysis was completed three times.

	Microarray #1	Microarray #2	Microarray #3
Gene V			
Gene W			
Gene X			
Gene Y			
Gene Z			

- **a** Which gene or genes are unlikely to be involved in this disease?
- **b** Explain your answer to part **a**.
- **c** Is Gene Y likely to cause the disease or protect against it? Explain.
- **d** What is the most likely explanation for the result for Gene Z in Microarray #3?
- **e** What is the importance of using the healthy sample in this experiment?

Chapter 2 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
2A.1	List the differences between DNA and RNA	7☐
2A.2	Draw schematic diagrams of a nucleotide, a single strand and a double strand of DNA	12☐
2A.3	State the differences between mRNA, rRNA and tRNA	6☐
2A.4	Describe how information is encoded in DNA and instructs protein synthesis	12☐
2B.1	Describe the steps involved in transcription	13☐
2B.2	Describe the steps involved in processing pre-m RNA to mRNA	2☐
2B.3	Describe the steps involved in translation	3☐
2B.4	Draw a schematic diagram showing all parts of protein synthesis	13☐
2B.5	Define what is meant by degeneracy in the universal triplet code	12☐
2B.6	Convert a DNA sequence to an mRNA sequence, and then, using a codon table, to an amino acid sequence	1☐
2C.1	Draw the structure of a generalised amino acid	12☐
2C.2	Draw the structure of a generalised dipeptide and identify the peptide bond	12☐
2C.3	Define primary, secondary, tertiary and quaternary protein structures	5☐
2C.4	Draw and/or identify representative structures of primary, secondary, tertiary and quaternary protein structures	12☐
2C.5	Define proteome	13☐
2C.6	List the functions of proteins and provide examples of each	9☐
2C.7	Describe the process of protein secretion from a cell, including the organelles involved	8☐
2C.8	Draw a diagram outlining the protein secretory pathway within a cell	13☐
2D.1	Define exon, intron, promoter and operator, regulatory gene and structural gene	4☐
2D.2	Draw a schematic diagram outlining the relative locations of these elements within a gene	11☐
2D.3	Define operon	11☐
2D.4	Describe how the *trp* operon functions in scenarios of low tryptophan and high tryptophan	10☐

Multiple-choice questions

1 What is the correct RNA sequence that would be copied from the following DNA template sequence: CGA AGT TTA ATT CGC AGT?
- **A** CGA AGT TTA ATT CGC AGT
- **B** GCT TCA AAT TAA GCG TCA
- **C** GCU UCA AAT TAA GCG UCA
- **D** GCU UCA AAU UAA GCG TCU

2 Which of the following is a step involved in RNA processing?
- **A** splitting
- **B** splicing
- **C** addition of 3′ cap
- **D** addition of a poly-G tail

3 Where in the cell best describes where translation occurs?
- **A** ribosome
- **B** nucleus
- **C** endoplasmic reticulum
- **D** cytosol

4 The part of the gene sequence that is responsible for turning off transcription is the
- **A** promoter.
- **B** operator.
- **C** intron.
- **D** terminator.

5 The level of protein structure that involves beta-pleated sheets is the
- **A** primary structure.
- **B** secondary structure.
- **C** tertiary structure.
- **D** quaternary structure.

6 The molecule that is the intermediate step between DNA and protein is
- **A** amino acid.
- **B** rRNA.
- **C** mRNA.
- **D** tRNA.

7 One of the features that distinguishes between DNA and RNA is the
- **A** phosphate group.
- **B** nitrogenous base cytosine.
- **C** covalent bonds between nucleotides.
- **D** sugar found in the nucleotide.

8 What is the correct order in which proteins move through the protein secretory pathway?
- **A** endoplasmic reticulum, Golgi apparatus, vesicle, secretion from the cell
- **B** Golgi apparatus, vesicle, endoplasmic reticulum, secretion from the cell
- **C** secretion from the cell, endoplasmic reticulum, vesicle, Golgi apparatus
- **D** nucleus, cytoplasm, ribosome, secretion from the cell

9 Which of the following is a correct match between a protein function and an example of a protein that performs that function?
- **A** hormone, pepsin
- **B** immunity, collagen
- **C** transport, haemoglobin
- **D** structure, antibodies

10 When levels of tryptophan are high, what molecule binds to the operator?
- **A** tRNA
- **B** RNA polymerase
- **C** DNA polymerase
- **D** repressor

Short-answer questions

11 The *lac* operon contains the genetic code for the proteins necessary to break down lactose, the sugar found in milk. Three coding genes make up the lac operon – *lacZ*, *lacY* and *lacA*. For this question, use the *trp* operon as a model but note that it functions and responds to lactose in the **opposite** way that the *trp* operon does for tryptophan.

a What is an operon? (1 mark)

b Draw a representative diagram of the gene structure for the *lac* operon, including all the key elements. (2 marks)

c A bacterium is in an environment with high levels of lactose (and no glucose). Describe the process that would happen in this situation. (4 marks)

d Explain how the process you described in part **c** would change over time. (3 marks)

12 A central principle in biology is that information in DNA is transferred to RNA and then to protein.

a State the monomer that makes up nucleic acids, and draw a representative diagram of one of these structures. (3 marks)

b Amino acids are the monomers that make up proteins. Draw the structure of a generalised amino acid. (1 mark)

c Glycine is the simplest of amino acids and it only has a single hydrogen atom as its variable group. Using this information, draw a dipeptide showing two glycine amino acids joined together by a peptide bond. (2 marks)

d Using the codon table in Figure 2B–6, state how many codons code for glycine. What is the term for this and why is it important? (3 marks)

e The primary structure of a polypeptide is the linear sequence of amino acids. Draw a diagram to show this structure, as well as the three other levels of protein structure. (2 marks)

f State the nature of the information contained in DNA, and outline how it instructs protein synthesis. (2 marks)

13 Proteins play many vital roles within the body. When they are synthesised, some are used within that cell and others are secreted for use elsewhere in the body.

a Define the term proteome. (1 mark)

b Brefeldin A is a drug that interferes with the function of the Golgi apparatus. Draw a diagram showing the protein secretory pathway, clearly indicating the steps in the process. On the diagram, indicate where Brefeldin A would interact and what you may observe. (3 marks)

c A cellular response to Brefeldin A may be to stop transcription. Describe the steps involved in transcription. (4 marks)

UNIT 3

HOW DO CELLS MAINTAIN LIFE?

CHAPTER 3

DNA MANIPULATION TECHNIQUES AND THEIR APPLICATIONS

Introduction

As you learned in Chapter 2, DNA is the fundamental building block of life on Earth. Every organism has DNA inside its cells, and the specific sequence of nucleotides that makes up this DNA determines what proteins are made. Some species have structural similarities, and therefore the DNA of these species can be similar. Within a species, DNA is a macromolecule that is passed from generation to generation.

Since the discovery of DNA and the determination of its structure, biologists have developed techniques to isolate specific sections of DNA, called genes, to investigate some of the following key ideas:

- exploring the molecular basis of disease
- developing products for medicinal purposes
- solving crime and paternity disputes
- understanding how plants and animals develop and function, and how they have evolved over time
- managing and conserving endangered species.

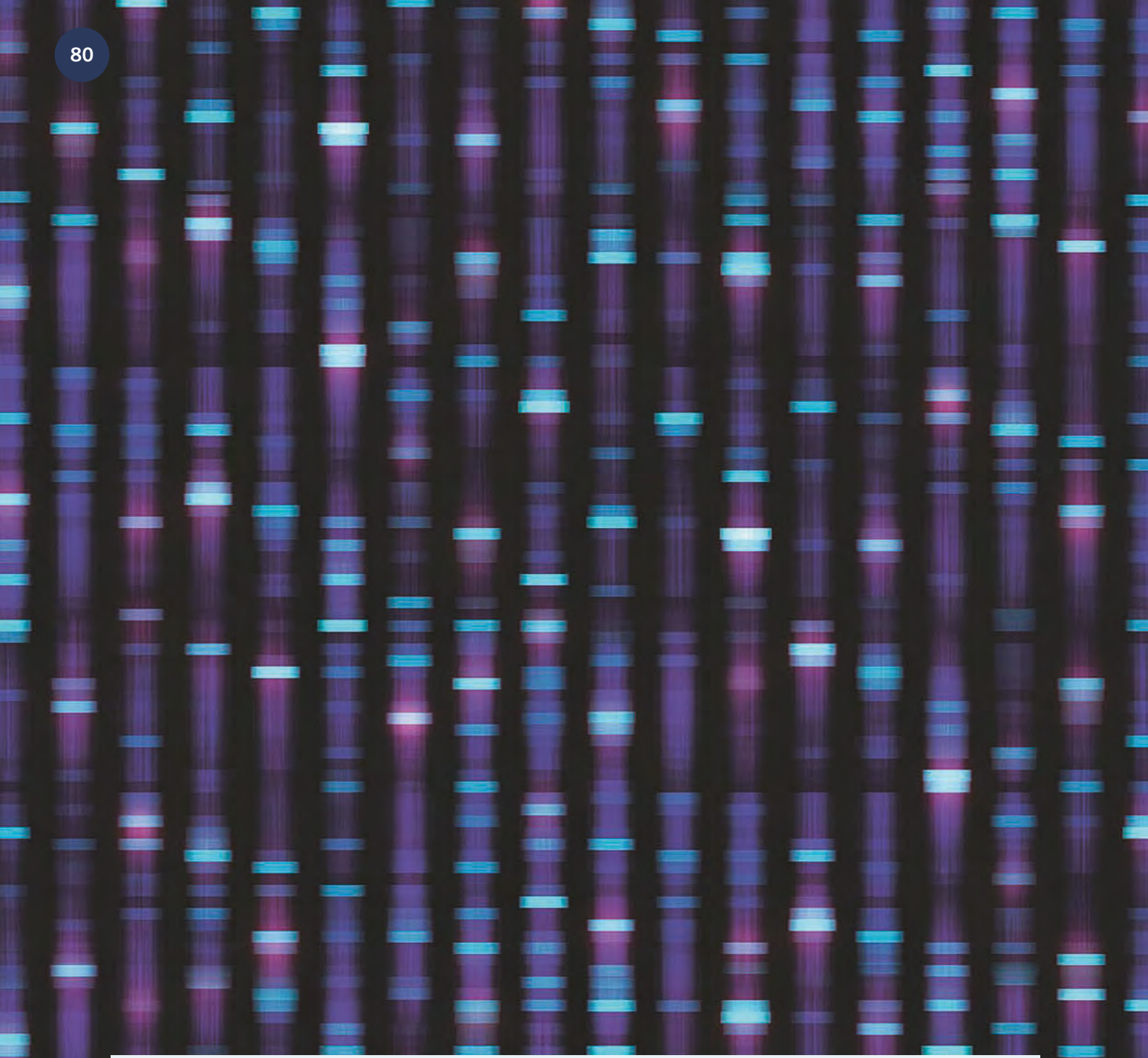
This chapter explores some key techniques used in these modern science investigations. It also focuses on a new technology, CRISPR-Cas9, and its function in editing a bacterial organism's genome.

Curriculum

Area of Study 1 Outcome 1
DNA manipulation techniques and applications

Study Design	Learning intentions – at the end of this chapter I will be able to:
• The use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA and endonucleases to cut DNA	**3A Common DNA tools and techniques** **3A.1** Outline the role of DNA polymerase in synthesising DNA **3A.2** Explain the reason for and difference between using DNA polymerases from different organisms **3A.3** Explain the action and importance of DNA ligase **3A.4** Explain the action of endonucleases in cutting DNA of different organisms **3A.5** Draw and compare the differences between blunt end and sticky end endonucleases **3A.6** Explain the advantages of using certain endonucleases over others in DNA techniques

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling	**3A.7** Describe the steps, materials and conditions needed for the polymerase chain reaction **3A.8** Draw a diagram of the steps in the polymerase chain reaction **3A.9** Draw and label a gel electrophoresis set-up **3A.10** Explain how DNA is separated using gel electrophoresis, including the need for a control/standard
• Amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling	**3B** **Applications and implications of DNA manipulation techniques** **3B.1** Explain what DNA profiling is and give examples of its use in society **3B.2** Interpret diagrams of gel electrophoresis for DNA profiling
• The use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin	**3B.3** Define plasmid, recombinant plasmid, transformation, gene cloning and antibiotic resistance **3B.4** Recall the difference between a plasmid and a recombinant plasmid **3B.5** Explain the use of recombinant plasmids in gene cloning **3B.6** Outline the various DNA techniques and their roles in gene cloning **3B.7** Explain and interpret the requirements for bacterial growth under different conditions **3B.8** Outline in words and diagrammatically the production of human insulin using gene cloning and bacteria
• The use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease	**3C** **Genetically modified and transgenic organisms** **3C.1** Outline the difference between a GMO and a TMO **3C.2** Explain how GMOs and TMOs are used in crop productivity **3C.3** Explain how GMOs and TMOs are used in providing resistance to disease **3C.4** Identify potential ethical, social, biological and economic issues with their use
• The function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome	**3D** **The future of genome editing** **3D.1** Explain what CRISPR-Cas9 is **3D.2** Identify potential ethical, social, biological and economic issues with the use of CRISPR-Cas9

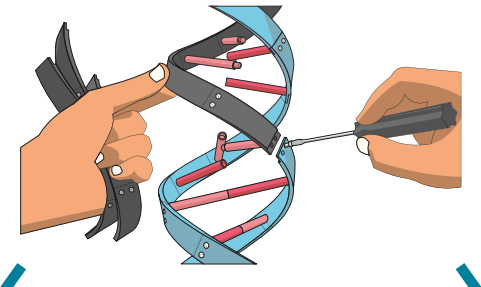


Glossary

Allele
Antibiotic
Autoimmune disease
Blunt ends
Cas9
CRISPR
CRISPR-Cas9
Digestion
DNA ligase
DNA profiling
DNA standard
Ethics
Gel electrophoresis
Gene cloning
Genetically modified organism (GMO)
Genetic screening
Genetic transformation
Genome editing
Guide RNA (gRNA)
Homologous chromosomes
Palindrome
Plasmid
Polymerase chain reaction (PCR)
Primer
Recognition site
Recombinant DNA
Restriction enzyme
Stakeholder
Sticky ends
Transgenically modified organism (TMO)
Transformed bacteria
Variable number tandem repeats (VNTR)
Vector

Concept map

3A Common DNA tools and techniques

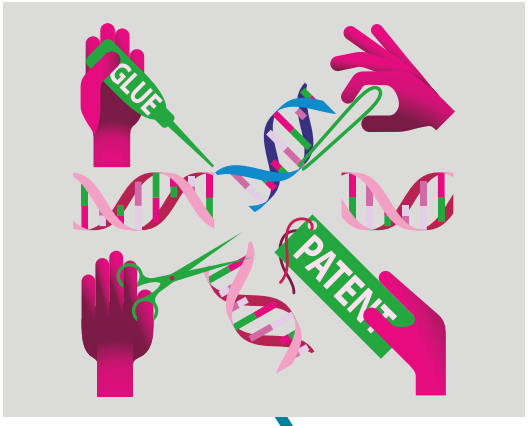


Use in real-world applications

Comparison to evolution of DNA that has occurred in bacteria for centuries

Use in creating GMOs and TMOs

3B Applications and implications of DNA manipulation techniques

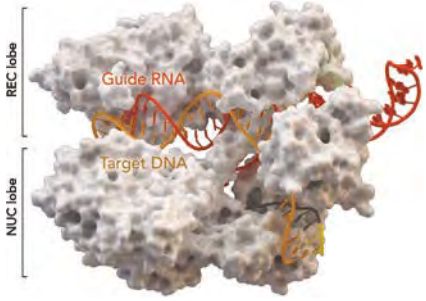


3C Genetically modified and transgenic organisms

A possible new way of undertaking these applications

3D The future of genome editing

A new age of genetic modification



See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

3A Common DNA tools and techniques

Study Design:

- The use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA and endonucleases to cut DNA
- Amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling

Glossary:

Blunt ends
Digestion
DNA ligase
DNA standard
Gel electrophoresis
Palindrome
Polymerase chain reaction (PCR)
Primer
Recognition (restriction) site
Restriction enzyme
Sticky ends

ENGAGE

Forensic science on TV

As you may have seen in crime dramas like *CSI* or *NCIS*, the investigators are able to obtain DNA samples from a crime scene, get them back to the lab and have a result all within a few hours. Often, they carry out a number of DNA techniques to help solve a crime, within a 40-minute episode. In reality, even though the same methods are used, the process is much slower. Some of the DNA analysis tools explored in this section, such as restriction enzymes, gel electrophoresis and the polymerase chain reaction, take many hours to run. In addition to the time that these methods take, prior analysis and preparation of materials are required. The TV versions also do not account for the fact that some techniques need to be run one after the other, in order to obtain meaningful results.

Figure 3A–1 Actor David Caruso as Horatio Caine in *CSI: Miami*, analysing a crime scene

EXPLAIN

The importance of DNA

2B THE GENETIC CODE AND GENE EXPRESSION LINK

DNA is a macromolecule that is passed from pre-existing cells to new cells. In this way, offspring inherit their DNA from their parent(s). As you learned in Section 2B, each cell of an organism contains the entire complement of the organism's DNA, also known as their genome. This chapter focuses on applying your knowledge of the structure and function of DNA and how different molecular tools can be used to manipulate this DNA.

Molecular tools

Restriction enzymes

Restriction enzymes (also known as restriction endonucleases) are proteins that act like molecular scissors, cutting the sugar–phosphate backbone of DNA at a specific region, known as a **recognition (restriction) site** (Figure 3A–2).

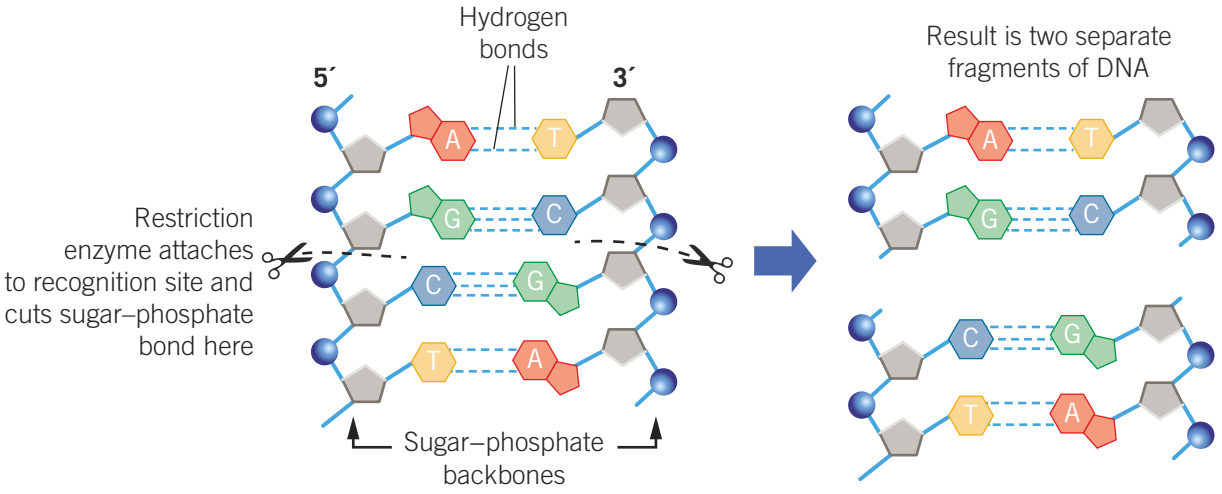


Figure 3A–2 A DNA chain is cut at a recognition site by a restriction enzyme. In this case the cut has been made in the same place on both sugar–phosphate chains in the DNA. Other restriction enzymes may make cuts at different places on the two chains.

Restriction enzymes occur naturally in bacteria (some are also found in viruses, archaea and eukaryotes), where they serve the same 'scissor-like' purpose but for a defence mechanism. They cut the DNA of a bacteriophage (a virus that infects bacteria, shown in Figure 3A–3) into fragments. The bacterium's own DNA is protected from these restriction enzymes by chemical modification and enzymes that modify the bacterial DNA so it is not recognised by the restriction enzyme, or by simply not having the recognition site in the first place.

Restriction enzyme
a bacterially produced protein that cuts DNA at a specific sequence of nucleotides called a recognition site; also known as a restriction endonuclease

Recognition (restriction) site
a specific sequence of nucleotides that is the location for a restriction enzyme to cut

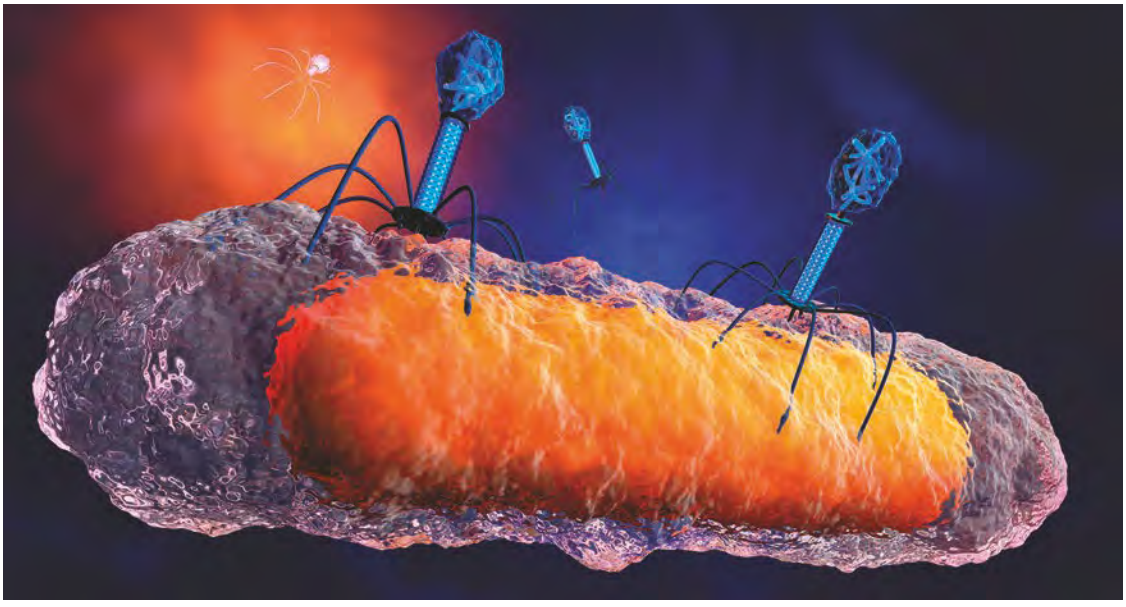


Figure 3A–3 Bacteriophages (with nucleic acid in the capsid head) infecting a bacterial cell

Differences between restriction enzymes

There are more than 3000 different restriction enzymes, with only around 600 of these commercially available to scientists. Each restriction enzyme recognises a specific recognition site in the DNA. Restriction enzymes are therefore named based on the following international guidelines:

- abbreviation, in letters, of the name of the bacterium in which they are naturally found
- Roman numerals after the letters to classify the specific enzyme from the same bacterium (as usually there is more than one).

As an example, HaeIII is the third endonuclease isolated from the bacterium *Haemophilus aegyptius*. Table 3A–1 shows HaeIII and four other restriction enzymes, to highlight the differences between them.

Table 3A–1 Different restriction enzymes with their respective recognition sites and resulting fragments following **digestion**

Restriction enzyme name	Recognition site (including location of cut in this sequence)	Resulting fragments of DNA	Type of ends produced (sticky or blunt)
AluI	5′ … A G ↓ C T … 3′ 3′ … T C ↑ G A … 5′	A G C T T C G A	Blunt
HaeIII	5′ … G G ↓ C C … 3′ 3′ … C C ↑ G G … 5′	G G C C C C G G	Blunt
BamHI	5′ … G ↓ G A T C C … 3′ 3′ … C C T A G ↑ G … 5′	G G A T C C C C T A G G	Sticky
HindIII	5′ … A ↓ A G C T T … 3′ 3′ … T T C G A ↑ A … 5′	A A G C T T T T C G A A	Sticky
EcoRI	5′ … G ↓ A A T T C … 3′ 3′ … C T T A A ↑ G … 5′	G A A T T C C T T A A G	Sticky

Digestion (in the context of restriction enzymes) a reaction using an enzyme to break down large molecules

Sticky ends short lengths of unpaired nucleotides in DNA resulting from a staggered cut by a restriction enzyme

Blunt ends short lengths of fully paired nucleotides in DNA resulting from a straight cut by a restriction enzyme

Palindrome a sequence that reads the same in both directions

2A NUCLEIC ACIDS LINK

VIDEO 3A–1 DIFFERENT TYPES OF RESTRICTION ENDONUCLEASES

Note the red arrows in column two of Table 3A–1. These indicate the location in the sugar–phosphate backbone where the specific restriction enzyme digests (cuts) the DNA. This location within the recognition site determines whether the fragments of DNA produced from this will result in **sticky ends** or **blunt ends** (shown in columns three and four). Note also that each recognition site is usually 4–6 base pairs long and is a **palindrome**. This means they read the same in a 5′ (5 prime) to 3′ (3 prime) direction on the template strand as they do on the coding strand, remembering that the two strands of DNA run in opposite directions, as you learned in Section 2A.

For example, in EcoRI (in Table 3A–1), the coding strand reads GAATTC and the template strand reads GAATTC, if looking at both from the 5′ end first.

DNA ligase

In genetic engineering, sometimes two fragments of DNA need to be joined. This is essentially the reverse effect of restriction enzymes, which digest DNA. To join fragments of DNA, an enzyme is required, as is the case for most processes that occur within the body. This enzyme is called **DNA ligase**. Its role is to catalyse the joining of pieces of double-stranded DNA at their sugar–phosphate backbone. This joining of fragments of DNA can create a longer linear strand of DNA, or even a circular DNA molecule. It can also join fragments of DNA from different species. These techniques are explored in Section 3B.

DNA ligase
an enzyme that joins two pieces of DNA at their sugar–phosphate backbone

LINK **3B** APPLICATION AND IMPLICATIONS OF DNA MANIPULATION TECHNIQUES

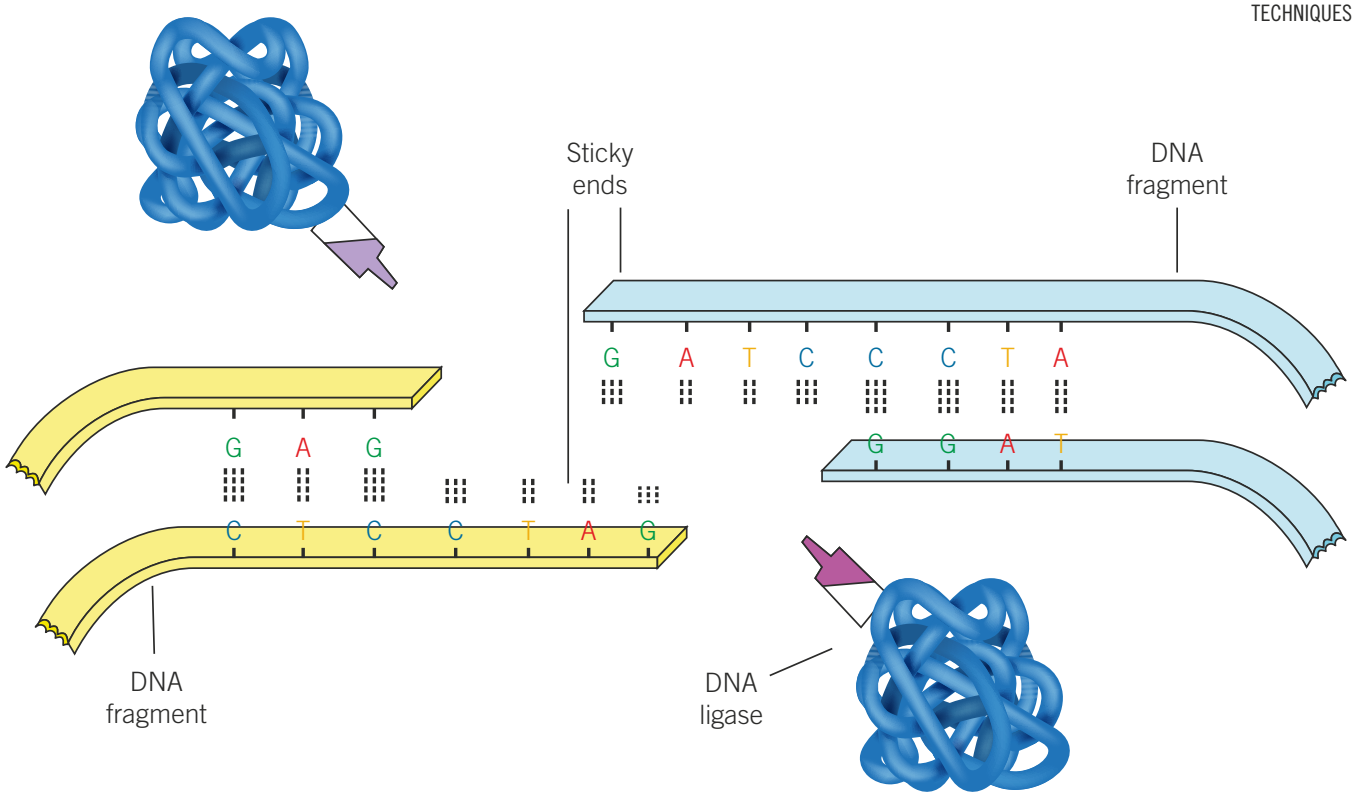


Figure 3A–4 DNA ligase acts as a 'glue' that joins two fragments of DNA together at the sugar–phosphate backbone.

The types of ends matter for DNA ligase

As you will recall, blunt end fragments of DNA are produced when the restriction enzyme cuts straight across the sugar–phosphate backbone of both DNA strands. As they have no overhanging nucleotides, DNA ligase is able to join them with any other fragment of DNA that also has a blunt end, though with more difficulty than when there are overhangs. This is random and not overly specific.

Sticky end fragments are produced when the restriction enzyme cuts between the same pair of nucleotides in the palindromic recognition site on each strand of DNA. This results in overhanging nucleotides that DNA ligase can only join to another fragment of DNA containing complementary overhanging nucleotides. If not, the overhanging nucleotides would not form complementary base pairs due to hydrogen bonding between the nitrogenous bases A and T, and C and G, as you learned in Section 2A. Therefore, this fragment must also have been cut with the same restriction enzyme (shown in Figure 3A–4), making sticky ends of DNA more specific than blunt ends.

WORKSHEET 3A–1 RESTRICTION ENZYMES

LINK **2A** NUCLEIC ACIDS

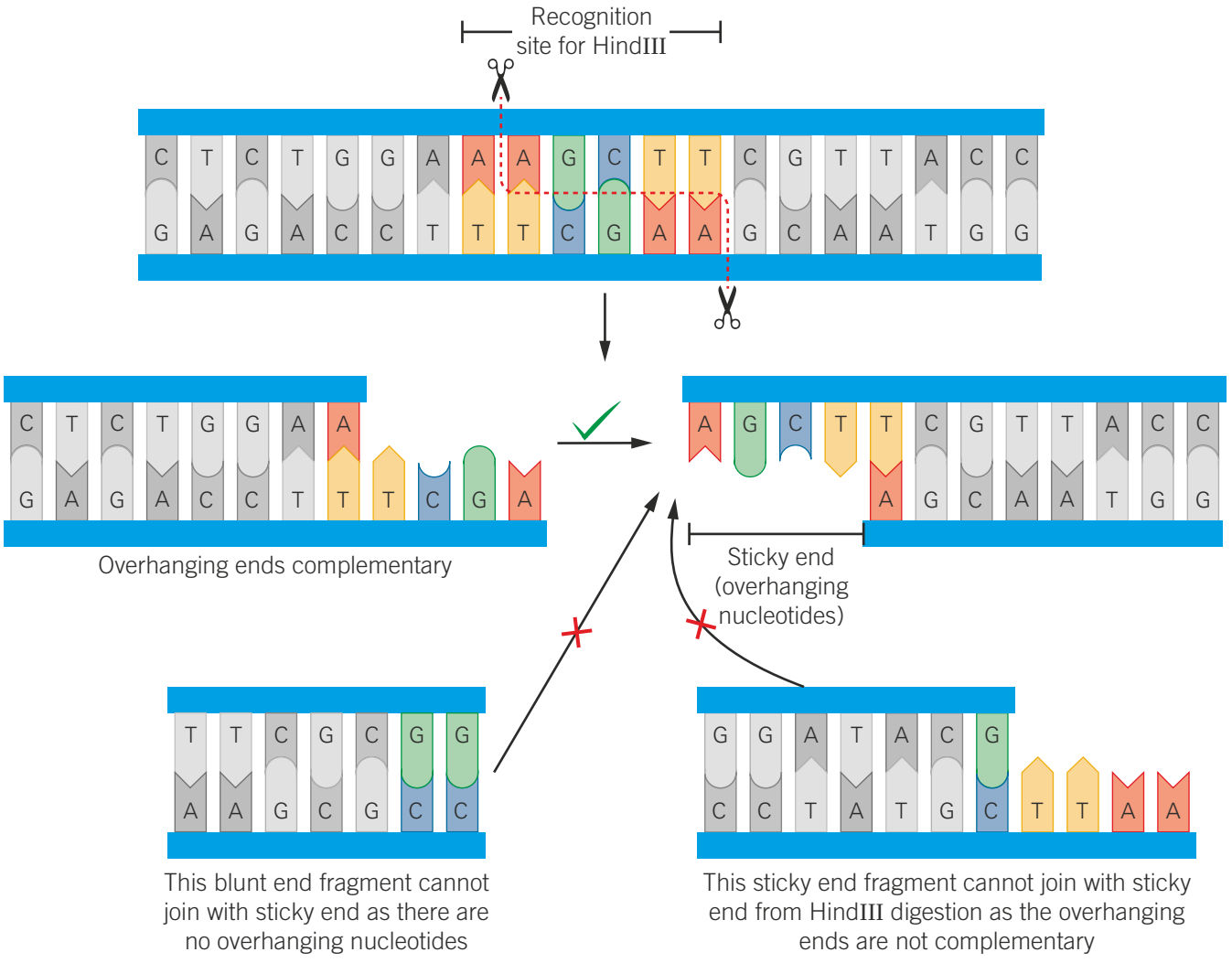


Figure 3A–5 A sequence of DNA cut with restriction enzyme HindIII resulting in two fragments with overhanging nucleotides (sticky ends), and which fragments of DNA can or cannot join to one of these fragments

Check-in questions – Set 1

1 Define the following key terms: restriction enzyme, recognition site.
2 Identify the difference between blunt ends and sticky ends.
3 What is the name of the enzyme that can join two fragments of DNA together? How does this join the two fragments of DNA?

Polymerase chain reaction

Polymerase chain reaction (PCR) a technique used to amplify a sample (template) of DNA

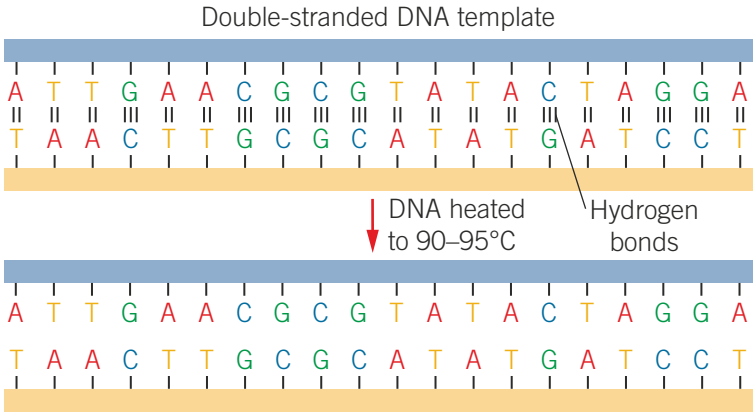
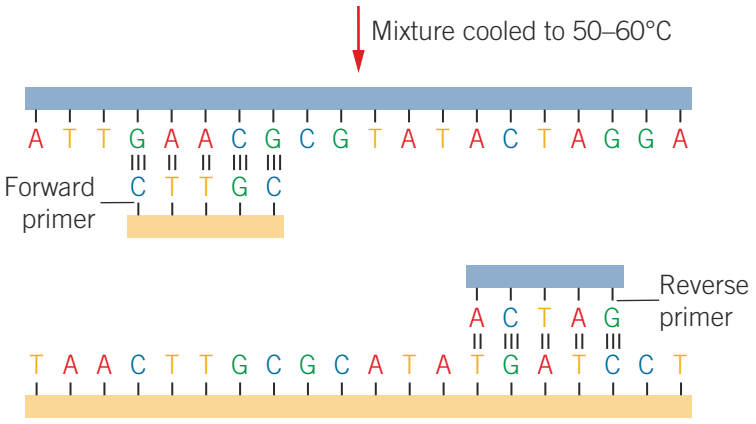
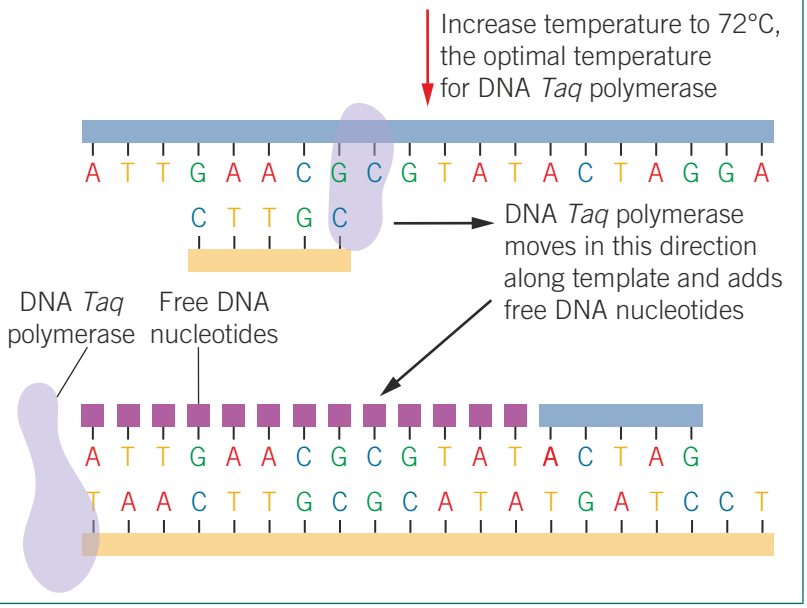
In order to work effectively with DNA, it is necessary to have more than a single copy or a few copies of it. Therefore, a simple method is needed to amplify the DNA to produce large quantities in a short amount of time. This method is called the **polymerase chain reaction (PCR)**. As you will see later in this section, PCR is particularly useful for forensic scientists to amplify certain regions of the DNA from traces of blood found at a crime scene.

VIDEO 3A–2 STAGES OF PCR

WORKSHEET 3A–2 POLYMERASE CHAIN REACTION

The polymerase chain reaction uses a mixture of ingredients that are heated and cooled in cycles. After each cycle, the number of copies of the DNA is doubled. At the beginning of the process, the ingredients are added to a microtube, which is then placed in a thermocycler (a machine that acts as a heating block to rapidly change the temperature for each stage). The stages of the cycle are outlined in Table 3A–2.

Table 3A–2 Stages and ingredients required in one cycle of PCR: this cycle is repeated 35–45 times

Name of stage, key ingredients and description	Diagram
Denaturation Key ingredients: DNA template (to be copied) Heating separates the DNA strands by breaking hydrogen bonds between complementary base pairs.	
Annealing Key ingredients: Forward and reverse **primers** (synthetic single-stranded pieces of DNA that specify the start and finish points of DNA replication) Cooling allows primers to bind to the complementary regions of the template DNA through hydrogen bonding (at the opposite end of each strand, marking the start of the region of each DNA strand to be copied).	
Extension (elongation) Key ingredients: Free nucleotides and a heat-resistant DNA polymerase (DNA *Taq* polymerase) DNA *Taq* polymerase binds to each primer and moves along each strand of the template DNA, adding free nucleotides through complementary base pairing. This creates two copies of the original DNA template.	

PCR is an extremely sensitive process. Any contamination in the original DNA template or mistake made in a cycle means the same mistake will be copied over and over again through each cycle. Therefore, for this process to work effectively, great care is required in the preparation of samples and the regulation of temperature.

Primer
synthetic single-stranded piece of DNA (or RNA) complementary to a specific sequence of nucleotides

Check-in questions – Set 2

1 How many DNA molecules would be produced after one double-stranded DNA template completed six cycles of PCR?
2 What are the names of the three stages in a single cycle of PCR? At what temperature does each stage occur?
3 What is the name of the enzyme used in stage 3 of the PCR process?

Gel electrophoresis

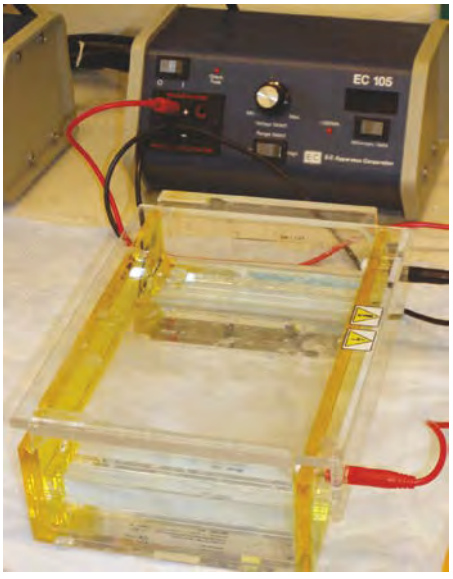
Gel electrophoresis a technique used to separate different-sized fragments of DNA (or protein)

Biologists usually work with large amounts of DNA and long strands of DNA. This means that when a sample of DNA is mixed with restriction enzyme(s), fragments of differing lengths are produced. Often, biologists are only interested in one section of this DNA and so they need to isolate the relevant fragment. This is made even more difficult because DNA is too small to see and is colourless. This is where **gel electrophoresis** proves valuable.

WORKSHEET 3A–3 GEL ELECTROPHORESIS

The basic process of gel electrophoresis is as follows:

1 Digested samples containing the DNA fragments and a dye are loaded into wells at one end of an agarose gel.
2 The gel is covered in a solution containing ions, which allows the movement of DNA fragments when an electric field is applied to the gel through a negative electrode at the well end and a positive electrode at the other end. DNA is negatively charged due to the phosphate groups.
3 The negatively charged DNA migrates from the wells at the negative electrode of the gel towards the positive electrode. DNA from each well stays in the 'lane' running from each well. The DNA fragments separate according to their size and the charge they carry. Smaller DNA fragments travel faster (and therefore further) than larger fragments.
4 The dye in the samples travels faster than the DNA and, when the 'dye front' (its position) reaches the positive electrode, the power is turned off and the gel plate is removed for analysis.
5 At the end, when the agarose gel has been removed, a different dye that attaches to DNA can be added and this reveals where the DNA fragments are.



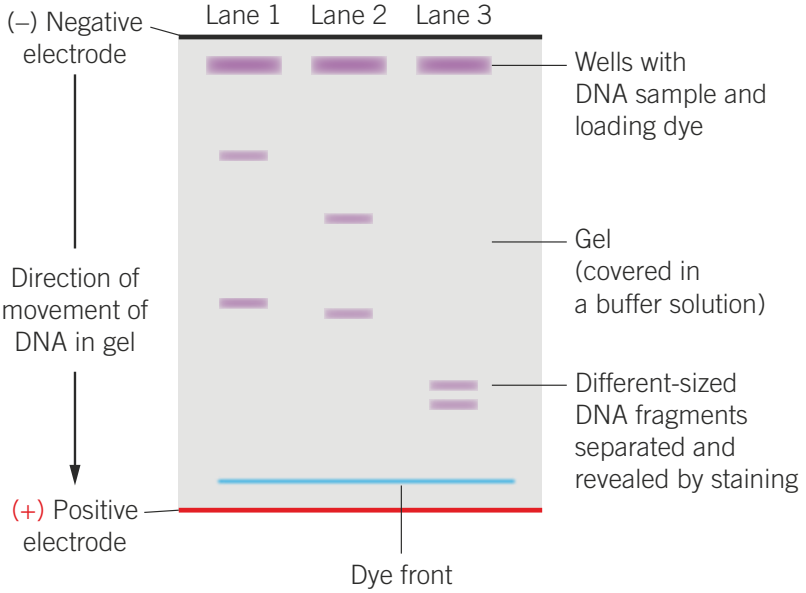


Figure 3A–6 Left: In a laboratory, an agarose gel is placed in a container, covered in solution and connected to a power source. Right: Standard appearance of an agarose gel as a result of gel electrophoresis. Larger fragments do not travel as far from the wells as smaller fragments.

NOTE

All DNA fragments of the same size in a sample will migrate to the same distance from the well and end up at the same point in that lane. The particular fragment that a scientist is seeking can then be cut out from the gel and used in experiments.

The reason for adding 'loading' dye to the sample at the beginning is that DNA is colourless and during the 'run' the operator can't see how far it has moved. This allows a scientist to determine the time at which to stop the gel electrophoresis process, and therefore the time at which the DNA fragments have had the longest to separate. The 'invisibility' of the DNA is also the reason for using a different dye to reveal it on the agarose gel. Dyes that fluoresce under ultraviolet light can also be attached to the DNA. The glowing patches can then be digitally photographed for analysis.

The importance of a control

In gel electrophoresis, conditions can vary each time a gel is run. These conditions include the:

- voltage of the power source
- percentage of agarose in the gel (agarose is a polysaccharide added to the gel to control the rate at which fragments move through the gel)
- concentration of the solution (a 'buffer') covering the agarose gel
- temperature of the environment
- pH of the buffer solution
- length of time the sample of DNA is allowed to run for in the gel
- length of the gel (distance between the negative and positive electrodes).

As such, the distance that particular-sized fragments of DNA will migrate through the gel will not be the same every time. This means scientists cannot use something as simple as a ruler to measure the distance travelled by a fragment of DNA to determine its size.

One lane in the gel must always be used to run a control. In gel electrophoresis, this is called a **DNA standard**, or DNA ladder. A DNA standard is a DNA sample that has been digested with a particular restriction enzyme that produces many different-sized fragments of DNA with known lengths. These DNA standards are usually purchased and come with a pictorial representation of the fragments expected to be seen on a gel and their respective sizes, usually in base pairs (bp) or kilo base pairs (kbp). This allows the scientist to compare the sizes of the fragments in their DNA sample to the fragments with known size from the DNA standard (Figure 3A–7).

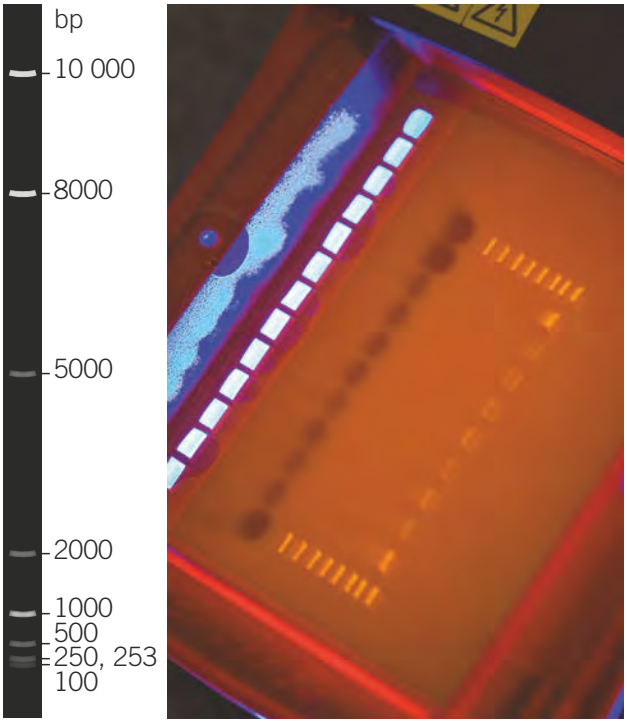


Figure 3A–7 Using a DNA ladder. Left: Eight fragments of known length in base pairs. Right: What this looks like when run through an agarose gel and separated by gel electrophoresis in the far left and far right lanes. The lengths of fragments in the other lanes are compared with these.

DNA standard a DNA sample that contains fragments of DNA of known size that is used to compare the sizes of unknown DNA fragments in base pairs or kilo base pairs; also known as a DNA ladder

The end result

The result of gel electrophoresis is a series of parallel bands of DNA fragments at different distances along the gel. The sizes of these unknown fragments are determined by comparing them to the sizes of known fragments of DNA in the DNA standard.

Check-in questions – Set 3

1 What charge does a DNA molecule have?
2 What is required to be run on an agarose gel to determine the lengths of unknown DNA fragments?
3 What are three examples of factors that could affect the speed at which fragments of DNA migrate through a gel?

3A SKILLS

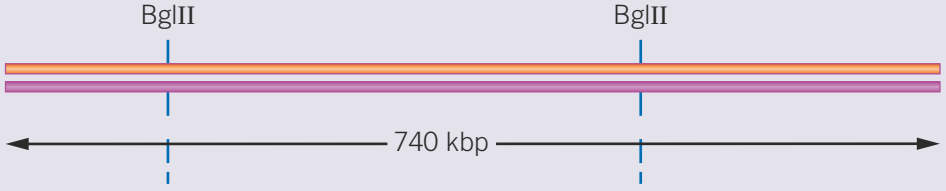
VIDEO 3A–3 SKILLS: APPLYING KNOWLEDGE OF DNA STRUCTURE TO RESTRICTION ENZYMES

2A NUCLEIC ACIDS LINK

Applying knowledge of DNA structure to restriction enzymes

Recall from Section 2A that the structure of DNA in prokaryotes and eukaryotes is different. In prokaryotes the DNA is circular, whereas in eukaryotes the DNA is linear. This has a huge impact on the number of fragments that can be produced from digestion with restriction enzymes. It also means that reading the question correctly is crucial. For example:

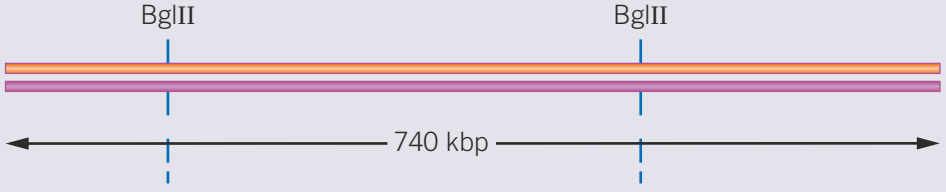
Question:
In this linear piece of DNA, the restriction enzyme BglII has two recognition sites. How many fragments would be produced?



Answer:
Three

However, if the question was worded slightly differently, a different answer would be required. For example:

Question:
The following DNA taken from a prokaryote was digested with the restriction enzyme Bg*l*II, which has two recognition sites. How many fragments would be produced?



Answer:
Two

The key to reading this question is to understand that DNA from prokaryotic cells is circular. Even though the figure shows linear DNA, it is there to test whether you have read the question carefully and understand the differences between different types of cells. The parts of each question marked in yellow above are examples of what would be good for you to highlight or underline as you are reading through the question.

Use of a heat-resistant DNA polymerase in PCR

The DNA polymerase used for PCR comes from the thermophilic bacterium *Thermus aquaticus*, which has evolved to live in extremely hot environments. As such, the optimal temperature for its enzymes is approximately 72°C. As the optimal temperature for this enzyme is so high, it is not denatured (three-dimensional functional shape permanently altered – more on this in Section 4B) even when heating to temperatures above 90°C.

As you can imagine, this is very different to the optimal temperature of the same DNA polymerase enzymes typically found in humans and other animals, which have an optimal temperature of 37°C.

Questions will be along the lines of: 'Why can't human DNA polymerase be used in the PCR process?'. The answer to this is as explained above, and it means you need to be able to apply your understanding of different concepts across topics. You should always provide a comparison in this case between the two, and reference to the specific stage that would affect the enzyme here.

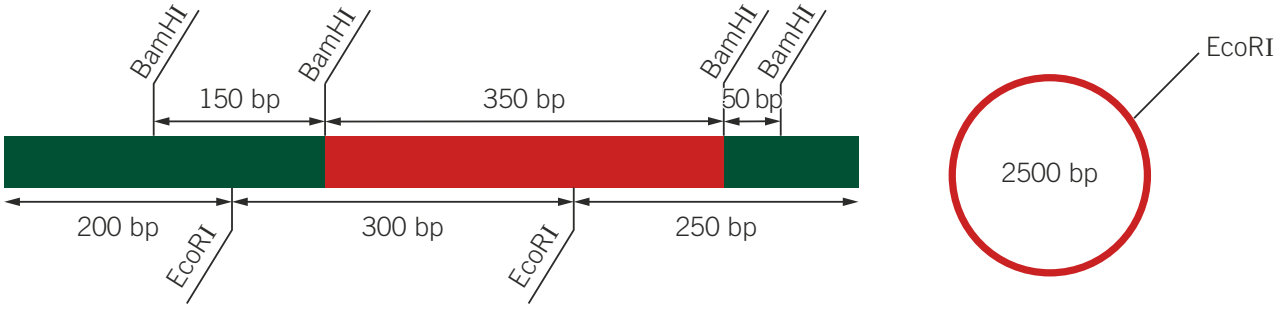
VIDEO 3A–4 SKILLS: USE OF A HEAT-RESISTANT DNA POLYMERASE IN PCR

LINK **4B** FACTORS IMPACTING ON ENZYME FUNCTION

Section 3A questions

1 Outline the differences between the action of the following three enzymes: ligase, polymerase restriction and endonuclease.

2 The following two DNA molecules show the location of cut sites for different restriction enzymes.

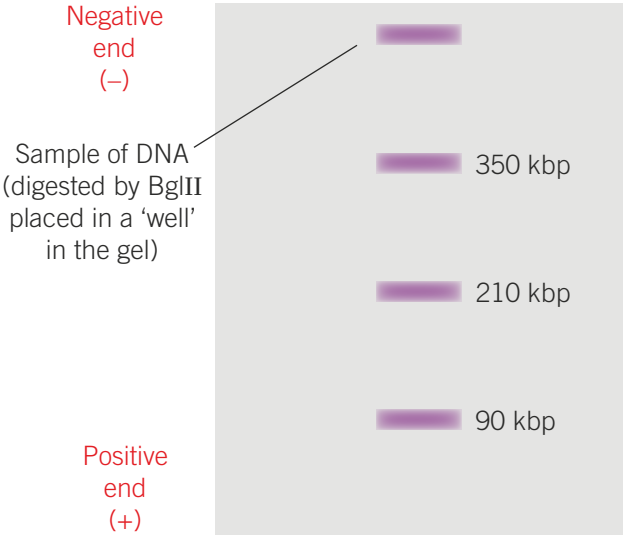


a What is the name of the region where a restriction enzyme cuts the DNA?

b State the number of fragments and the size of those fragments produced if only EcoRI cuts each of the DNA molecules shown.

c What type of cell would each molecule of DNA come from?

d Identify two ways in which the monomer of DNA is different from the monomer of RNA.



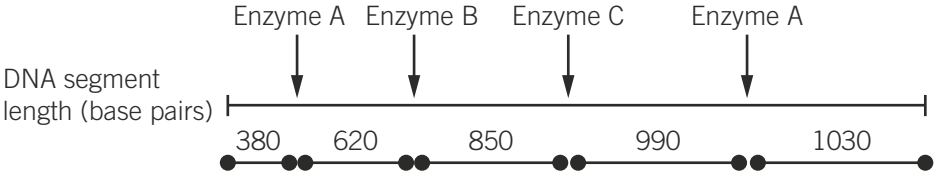
3 The diagram on the left shows a gel containing a sample of DNA digested with BglII loaded into a well at the top of the gel.

- **a** Why does DNA migrate to the positive electrode of a gel?
- **b** What would happen if the genetic engineer accidently confused the electrodes and placed the positively charged electrode at the end of the gel where the DNA was loaded?
- **c** What would happen if the genetic engineer left the gel to run for too long?
- **d** What is missing in this gel that the genetic engineer should have run? What is the purpose of this?
- **e** Explain how gel electrophoresis sorts DNA fragments.

4 Explain the difference between a forward primer and a reverse primer.

5 Why can PCR not amplify the number of RNA molecules in the same way as the process is used to amplify the number of DNA molecules? Use your knowledge of the ingredients in PCR, the structure of RNA and DNA, and your knowledge of protein synthesis from Chapter 2 to answer this question.

6 The diagram below shows a specific gene.



- **a** If the DNA was cut with only enzyme A, what is the length of the fragments that would be produced?
- **b** Explain how the number of fragments would change if this was plasmid DNA, provided it is still only digested with enzyme A.
- **c** A scientist planned to run the digested sample on an agarose gel using gel electrophoresis. Understanding the importance of including a control in their experiments, they ran a DNA standard in lane A, as shown on the left.
  The fragments in this DNA standard have the following sizes: 5000, 2000, 1000, 500 and 250 bp. In lane A, label each fragment with the appropriate size from this list.
- **d** Label the positive and negative ends of the gel.
- **e** Using your answer to part **a** and the known fragments in the DNA standard you identified in part **c**, draw the position of the fragments from the digestion with enzyme A in lane B.
- **f** In lane C, draw bands to represent the fragments that would be present if the DNA was digested with enzymes A, B and C.
- **g** The scientist noticed that the fragments of size 990 bp and 1030 bp were very close together in lane C. What could the scientist do in order to separate these fragments so they are easier to distinguish?

3B Application and implications of DNA manipulation techniques

Study Design:

- Amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling
- The use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin

Glossary:

Allele
Antibiotic
Autoimmune disease
DNA profiling
Ethics
Gene cloning
Genetic screening
Genetic transformation
Homologous chromosomes
Recombinant DNA
Stakeholder
Transformed bacteria
Variable number tandem repeats (VNTR)
Vector

ENGAGE

Bacterial resistance to antibiotics

The traditional method of producing insulin for treating diabetes involved extracting insulin from the pancreas of pigs and cows. A problem with this technique was that some people developed antibodies against animal insulin, which made it ineffective. Insulin is now produced by recombinant DNA techniques, in which the human insulin-producing gene is inserted into bacteria.

LINK 7D THIRD LINE OF DEFENCE

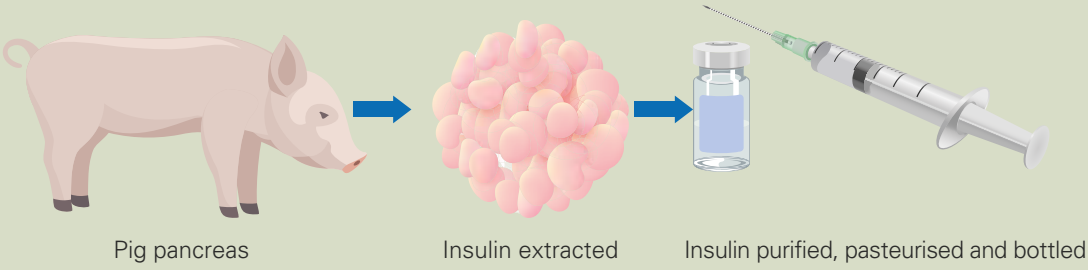


Figure 3B–1 Insulin was originally taken from the pancreas of pigs and cows.

However, bacteria can transfer plasmids (circular rings of DNA) between each other and also to different species of bacteria. For this reason there has been some trepidation on the part of scientists in using bacteria to make copies of human genes. Their concerns centre on the fact that, even though these bacteria are modified in strict isolation and under tightly controlled conditions, the process may inadvertently create a strain of genetically modified bacteria that are resistant to antibiotics.

LINK 8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT

As you will see in this section, in the process of gene cloning, the use of antibiotic-resistance genes in plasmids to identify bacteria with the inserted human genes is gradually being replaced by the use of enzymes that make fluorescent substances. The most common example, which you can do in the school laboratory, is using an enzyme from jellyfish that produces a green fluorescent protein (GFP). As the name suggests, these jellyfish fluoresce green when exposed to UV light. In the same way that the gene of interest would be inserted into the plasmid, the gene for GFP is inserted, thereby avoiding the need to use antibiotic resistance genes.

EXPLAIN

The application of DNA manipulation techniques

DNA profiling

3A COMMON DNA TOOLS AND TECHNIQUES LINK

The main use of the process of gel electrophoresis, which you studied in Section 3A, in forensic science is in **DNA profiling**, or DNA fingerprinting.

In DNA profiling, analysts need to find unique patterns that will identify an individual. Regions of DNA that code for certain proteins or enzymes are not useful for DNA profiling, because they code for products or functions that are the same in everyone. So analysts instead study stretches of non-coding regions of DNA. These are called 'short tandem repeats' (STRs) if they are 2–6 base pairs long, or **variable number tandem repeats (VNTRs)** if they are longer, typically 20–60 base pairs. The number of repeats can vary between individuals. If only one region was focused on, the number of repeats of the sequence could be the same in two individuals. Therefore, in humans, 13 of these VNTR regions are studied in DNA profiling, so the chance of two individuals having exactly the same number of nucleotide repeats at each location would be very low. In fact, only identical twins have the same VNTRs at each region. Each individual has some VNTRs (and STRs) that come from their parents. The VNTRs can be inherited from either the mother or the father and can be a combination of both. An individual's VNTRs will never have sequences that are not present in their parents. Each individual has two copies of each VNTR, because they are **alleles**, one from each pair of **homologous chromosomes** (chromosomes of the same size, centromere and banding pattern from the mother and father).

DNA profiling a method of DNA analysis in which regions of DNA from different individuals are analysed and compared

Variable number tandem repeats (VNTRs) a region of a chromosome that shows variation between individuals in length and number of repeats of nucleotide sequences; also referred to as short tandem repeats (STRs) when 2–6 base pairs long

Allele an alternative form of a gene

Homologous chromosomes chromosomes that have matching structural features (size, banding pattern, centromere location) and gene loci

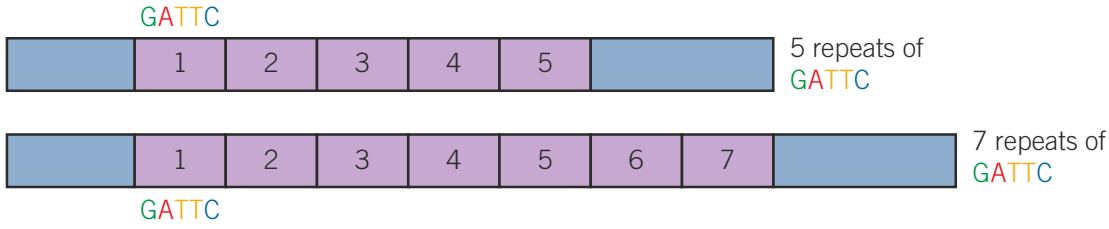


Figure 3B–2 A pair of homologous chromosomes, each with a different number of repeats for the nucleotide sequence GATTC. One chromosome is inherited from the mother and the other from the father.

Therefore, VNTRs can be used to identify a criminal from biological evidence found at a crime scene, or to identify the father of a child in a paternity test. The steps in this process are as follows:

1. DNA is extracted from the cells obtained from an individual (e.g. blood, semen or hair from a crime scene).
2. The DNA of the 13 different VNTR regions (or a set number of these) is amplified by PCR.
3. The DNA is cut with specific restriction enzymes (or multiple restriction enzymes) that have recognition sites at either side of the VNTR regions.
4. The DNA fragments are loaded into wells on agarose gel and separated by gel electrophoresis.

Smaller alleles, or nucleotide sequences of DNA, have fewer repeats of the short sequence and will therefore migrate faster and further through the agarose gel than larger alleles with more repeats. This is shown in Figure 3B–3.

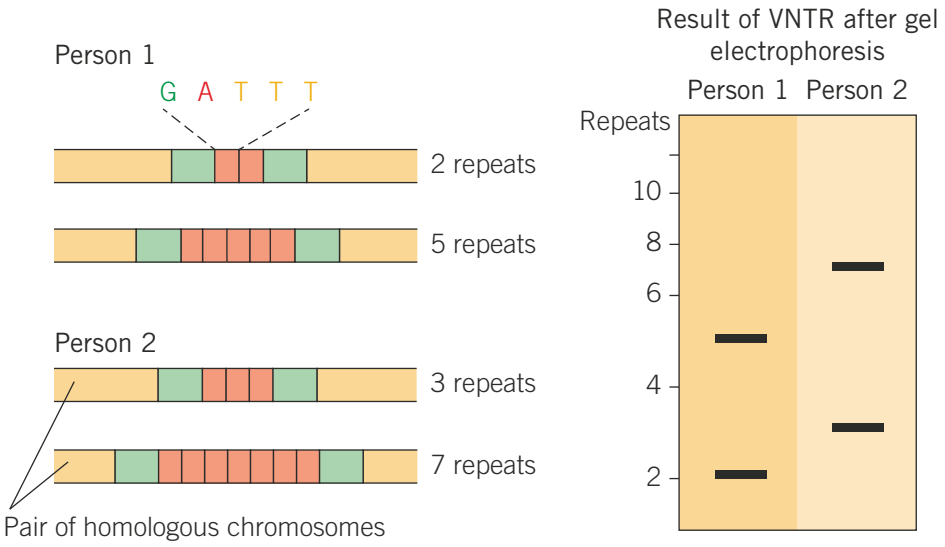


Figure 3B–3 Left: A single VNTR region from two individuals showing varied number of repeats. Right: Results of gel electrophoresis, where the different-sized DNA fragments have separated.

In addition to forensic scientists using DNA profiling to identify the perpetrator of a crime, or geneticists using it to determine a paternal parent, this technique can be used to:

- identify victims following a mass disaster
- identify human or animal fossils that can provide information about possible evolutionary links
- develop a DNA database of wildlife. If there are only a few bands that differ between individuals, indicating little genetic diversity, conservation measures can be implemented to maximise that species' chances of survival. Hence, breeding programs could be initiated by zoos where genetically different individuals are bred to increase the genetic diversity of the species.

LINK 10B FOSSILS

LINK 10C EVIDENCE OF EVOLUTIONARY RELATIONSHIPS

Check-in questions – Set 1

1 Name two uses of DNA profiling.
2 Identify three DNA manipulation techniques that would be used to prepare a sample of DNA for DNA profiling.
3 What does the abbreviation VNTR stand for, and what does it refer to?

NOTE

The DNA used for DNA profiling can be either nuclear DNA or mitochondrial DNA (which will be investigated further in Section 10C).

Genetic screening

Genetic screening is a form of DNA profiling used to analyse an individual's DNA for a particular version of a gene (allele) they might be carrying. Genetic screening can be conducted on adults when they suspect they may be carrying an allele for a disorder they could pass onto their offspring. It can also be conducted on a foetus to test for the presence of a disorder (prenatal testing) or on a newborn baby (postnatal testing). It is used to identify cases where a baby may develop disorders such as those listed in Table 3B–1.

Genetic screening DNA profiling to determine whether an individual is carrying a particular gene for a disorder

Many other common medical conditions can be detected as part of postnatal testing, including phenylketonuria (PKU) and hypothyroidism. The benefit of screening for these conditions is that it allows immediate treatment and/or changes in lifestyle to reduce the negative effects of the condition.

Table 3B–1 Examples of disorders that can be detected by prenatal testing

Disorder	Description
Duchenne muscular dystrophy (DMD)	Progressive weakness and loss of skeletal and heart muscle
Huntington disease	Progressive breakdown of neurons in the brain
Haemophilia	Bleeding disorder where the blood does not clot normally
Thalassaemia	Blood disorder where the body makes an abnormal version of (or not enough) haemoglobin (a protein in red blood cells that carries oxygen)
Down syndrome	Physical growth delays with mild intellectual disability
Cystic fibrosis	Abnormally thick mucus produced in lungs and other parts of the body

Ethics moral principles that guide our beliefs about what is right or wrong conduct

Stakeholder an individual or organisation who will be affected by the factor under consideration

Implications of DNA analysis technologies: overview

In selecting a DNA analysis/application technique to use, scientists need to consider the **ethical**, social, economic and biological implications of that technology. It is also important to identify the **stakeholder** and how the technology is likely to affect them, directly or indirectly. The stakeholder is anyone (or an organisation) who will be affected by the technology. Table 3B–2 outlines these implications.

Table 3B–2 Ethical, social, economic and biological implications of DNA analysis technology

Implication	Definition	Relation to stakeholder
Ethical	A sense of right or wrong in producing or obtaining the technology, based on morals and beliefs	The decision to engage with a technology will depend on the individual's personal ethical considerations. Conducting scientific research involving humans or animals (e.g. drug trials) requires approval by an ethics committee.
Social	The influence of the technology on society, rather than just one or two individuals	An individual's decision making is influenced by the context they are in. For example, different countries, or even different communities within the same country, have different attitudes about what is socially acceptable in regards to different technologies. Government legislation is also different from country to country.
Economic	The availability of funds to obtain or produce the technology	The cost of developing and using some technologies can be prohibitive, and so they may not be equally accessible to all. It is also important to consider who is funding the technology. For example, results might be biased by emphasising positive outcomes and downplaying negative outcomes.
Biological	The effect of a technology on other living organisms in a particular environment	The use of some technologies can affect the survival of other organisms within an ecosystem. It may also change the course of evolution, for better or worse.

The questions raised about these issues, and their answers, will depend on their context and which stakeholder's viewpoint is being considered. This is discussed further in the 3B Skills section.

Implications of DNA profiling and genetic screening

WORKSHEET 3B–1 DNA PROFILING AND GENETIC SCREENING, AND IMPLICATIONS

Ethical

Essentially, ethics deal with questions of right or wrong, or 'Should I or shouldn't I?' In the case of DNA profiling and genetic screening, some examples of questions to be dealt with are:

- Informed consent for DNA sampling – this is an issue for children under the age of 18 years, who at the time of having a DNA sample collected rely on parents to provide consent on their behalf.
- Secure storage of DNA information – who has access to this information and what would happen if it fell into the wrong hands? For example, could insurance companies make it difficult or costly for an individual to obtain health or life insurance if they were identified as having a genetic predisposition to developing a disease?
- Reliability of DNA analysis results as evidence of a foetus having a genetically inherited disorder – if a foetus is identified as having the genetic potential to develop a particular disorder, the parents are then faced with the decision of whether to abort the foetus. Different governments have their own regulations concerning this.

Figure 3B–4 Parents being counselled on results of genetic testing and their likelihood of having a child with a genetic disorder

An example of the third point is a couple finding out that they might both be carriers of the allele for Huntington disease. This is a late-onset condition that might not become apparent until after they have a child. Would they want to know before having a child, if their offspring could develop the condition? Would this change their own quality of life? Remember, they might *not* develop the disease.

Social

Social implications refer to the impact of the technology on more than one or two individuals. For DNA profiling and genetic screening, some examples of questions to be addressed are:

- The possibility of incorrect identification of a suspect from a sample of DNA obtained from a crime scene – this could be caused by contamination of DNA. This would not only affect the individual who has been wrongfully accused, as well as their partner and/or immediate family, but would also have implications for the judicial system as a whole (fairness/justice). So the question is: Is it right to convict a person based solely on DNA evidence?
- Privacy of the information obtained from a DNA profile or genetic screen – for example, what would the implications be for society if a DNA profile was held of every person, even those not convicted of a crime? In Victoria, a DNA sample cannot be obtained from a person unless they give permission. This gives the person the opportunity to know beforehand the risks associated with storage of the information and, if digital results are hacked or compromised, who might access their personal DNA information.
- Information about the possibility of inheriting a genetic disease – intending parents might be faced with difficult reproductive decisions. This could be heightened depending on whether they are planning to have a child or are already pregnant. They could be faced with the ethical implications of choosing to terminate a pregnancy. This could place strain on their relationship, and they might seek professional counselling.
- People who have a genetic predisposition to certain conditions may find they are discriminated against by employers, insurers or other bodies.

Economic

Economic considerations refer to the cost implications of the technology – how it affects individuals directly, as well as others in the community. For DNA profiling and genetic screening, an example of questions to be addressed is:

- Expense of treatment – if a couple was informed of the risk of their potential offspring inheriting a genetic disorder, their decision about whether to undergo IVF must include the cost of treatment, as IVF is expensive. If the couple chose not to have a child, this would avoid the expense not only for themselves, but for society, of providing the medical and educational support that would be required for an affected child.

Biological

Biological implications can affect not only the organism in question, but more than one organism or species. For DNA profiling and genetic screening, an example of questions raised is:

- Altering the affected genome – for some inherited disorders, it may be possible to correct the mutation that causes the condition, using gene editing. (This also raises ethical issues.) The biological implications could be very significant for humans. For example, gene editing can be seen as 'interfering' with evolution, by altering the future inheritance of traits (alleles), some of which may disappear from the population.

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

Check-in questions – Set 2

1 Define each of the following types of implications:
   - **a** ethical
   - **b** social
   - **c** economic
   - **d** biological.

Gene cloning

The universal nature of DNA

DNA is universal, but what does this mean? 'Universal' refers to the fact that the basic structure of DNA is the same in every organism. The building blocks (monomers) you learned about in Section 2A are identical in structure. The nitrogenous bases (adenine, cytosine, guanine and thymine, and their complementarity) that make up part of these monomers are identical. In essence this means that the DNA of one species can be combined with the DNA of a different species, using a range of DNA manipulation techniques, which were explored in Section 3A.

2A NUCLEIC ACIDS LINK

3A COMMON DNA TOOLS AND TECHNIQUES LINK

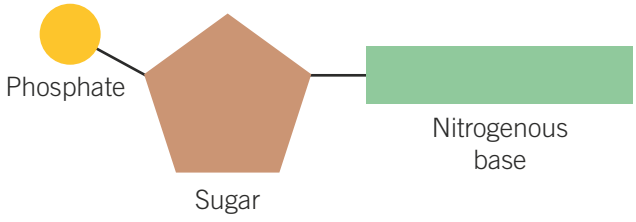


Figure 3B–5 The monomer of DNA

Nowadays, genetic engineers can create genes, and even complete genomes for some species, by using artificial nucleotides in a laboratory setting. They can work backwards from the desired final protein sequence, by looking at the specific chain of amino acids, identifying mRNA codons that code for these and then using stored computer software to synthesise the DNA nucleotides. This built sequence of DNA can then be inserted directly into a plasmid (circular piece of DNA in prokaryotes), all without the need for a physical DNA template.

The basis of genetic engineering is the ability to remove a single gene or, in some circumstances, a group of genes, from one organism and transfer them into another organism. In the new organism, the gene is then able to direct protein synthesis (transcription and translation), producing a fully functioning protein.

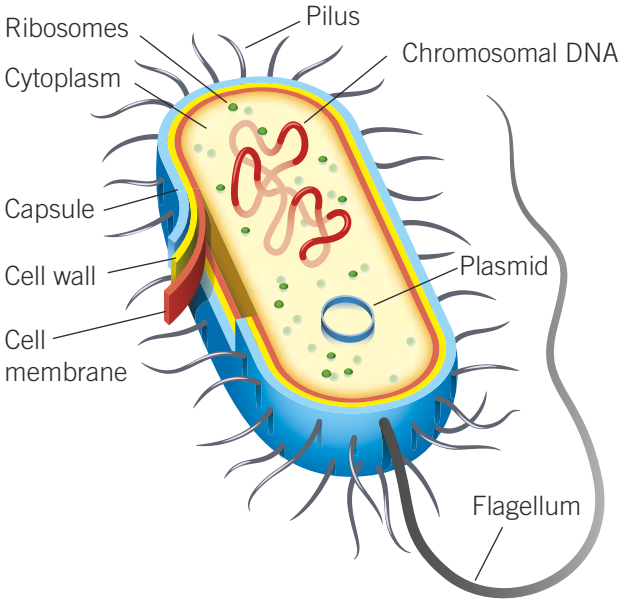


Figure 3B–6 Bacterial cell, showing the difference between the larger circular bacterial chromosome and the smaller circular plasmid.

Vector
a DNA molecule used as a vehicle to carry foreign genetic material from one organism to another

Recombinant DNA
DNA that has been artificially formed by combining DNA from different organisms

Gene cloning
the production of exact copies (clones) of a gene (DNA sequence) using various DNA manipulation techniques

The importance of a vector

Transferring DNA from one cell to another is not simple. A **vector** is required. The vector acts as a 'vehicle' that carries the DNA between the donor and recipient cells. The most common vector is a plasmid. When DNA from the donor cell/organism is placed into the plasmid, this can then be used to transfer the DNA into the recipient bacterial cell. When DNA from the host organism is combined with DNA from the donor organism, it is said to be **recombinant DNA** (in this case, a recombinant plasmid; Figure 3B–7). The organism that has this recombinant DNA is referred to as genetically modified (more on this in Section 3C).

LINK 3C GENETICALLY MODIFIED AND TRANSGENIC ORGANISMS

Plasmids for the purpose of gene cloning can be obtained directly from bacteria by using enzymes to break down the cell walls and then centrifuging (spinning the bacteria at high speeds) to isolate the smaller plasmid from the larger, single bacterial DNA chromosome. Or plasmids that have already been harvested and modified can be purchased. These are ready to be used in processes in the laboratory, such as protein purification or genome engineering (for example, for CRISPR, which is explored in more detail in Section 3D). The advantage of using plasmids is that they carry many different recognition sites for restriction enzymes.

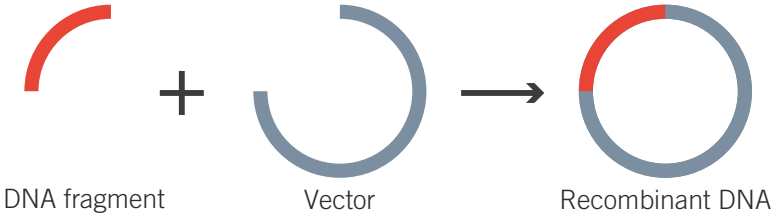


Figure 3B–7 The combination of a foreign DNA fragment with a vector (in this case a plasmid), producing recombinant DNA

LINK 3D THE FUTURE OF GENOME EDITING

The gene cloning process

The **gene cloning** process combines the use of a plasmid with some of the DNA manipulation techniques outlined in Section 3A. The key steps of this process in relation to inserting the human insulin gene into bacteria for insulin protein production, which is vital in the treatment of type I diabetes (T1D), are outlined in Figure 3B–8. If you studied Unit 1 Biology, you will already know that this disorder results from the cells that produce insulin, beta cells in the pancreas, being targeted for destruction by the immune system. This is known as an **autoimmune disease**. You will learn more about these types of diseases in Section 7D.

Autoimmune disease
a disease in which the immune system acts abnormally and begins to attack the body's own cells (self cells)

LINK 3A COMMON DNA TOOLS AND TECHNIQUES

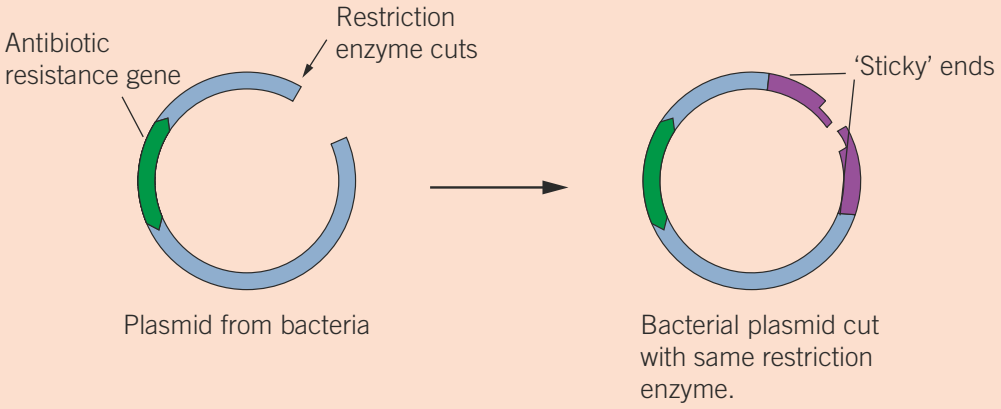
LINK 7D THIRD LINE OF DEFENCE

Insulin is a vital protein-based hormone, responsible for controlling blood glucose levels by reducing the concentration of blood glucose when it becomes too high. The process of gene cloning (outlined in Figure 3B–8) is a way of mass-producing insulin for people with T1D.

Cloning the human insulin gene for type I diabetes

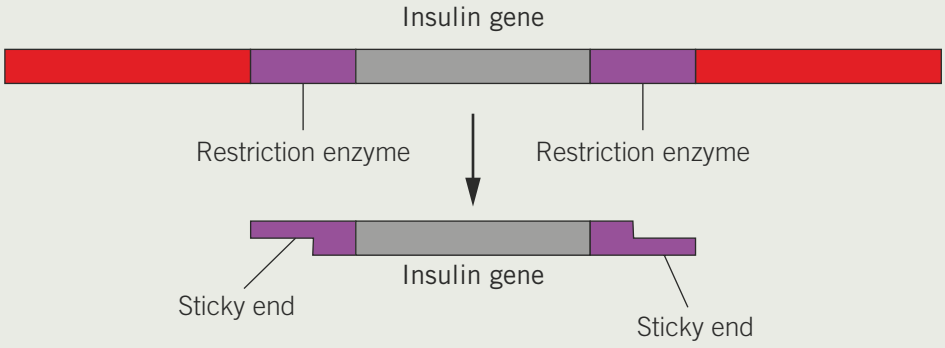
Step 1: Opening up the plasmid

Plasmid is cut with the specific restriction enzyme. This makes the initially circular DNA temporarily linear.



Step 2: Isolating the gene of interest

Gene of interest (the DNA coding for the insulin protein) is cut using a specific restriction enzyme.

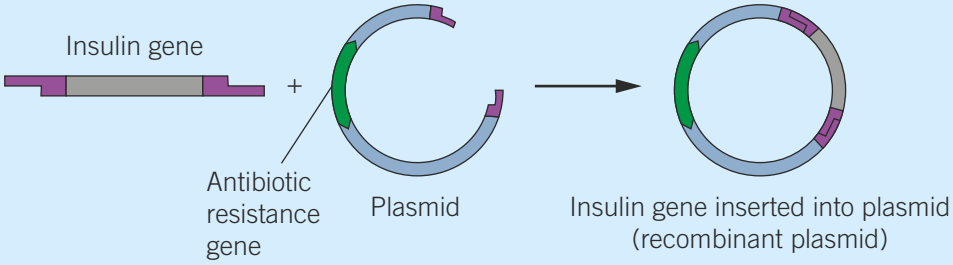


If the restriction enzyme in step 1 was one that resulted in 'sticky end' fragments, then the same restriction enzyme must be used to cut the gene of interest as well. The 'sticky ends' must be complementary.

If the restriction enzyme was one that resulted in 'blunt end' fragments, any other blunt end restriction enzyme may be used.

Step 3: Recombinant plasmids

The plasmid and gene of interest are mixed together with the enzyme DNA ligase so that the sugar–phosphate backbones of each join. This results in a closed circular piece of DNA again. This is called the recombinant plasmid, as it has 'recombined' with a piece of foreign DNA.

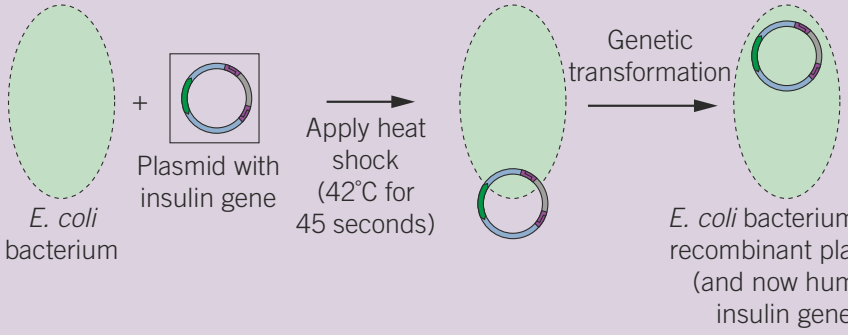


Note: At this step it is common for the circular plasmid to re-join to itself with the help of DNA ligase, therefore without taking up the gene of interest. This is referred to as a non-recombinant plasmid.

Figure 3B–8 Steps in the cloning of the human insulin gene for treating type I diabetes

Step 4: Genetic transformation

Recombinant plasmids (and non-recombinant plasmids) are mixed with bacteria. They are then soaked in a solution containing calcium ions (e.g. calcium chloride) and heat shocked. This opens up the pores in the cell membrane of the bacteria, increasing the chances of them taking up the recombinant plasmid.

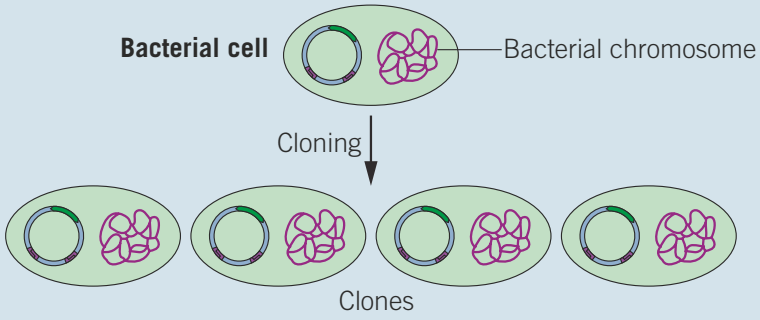


Note: Less than 1% of the bacteria actually take up the recombinant plasmid. Most of the time the bacteria do not take up the plasmid at all, or take up the non-recombinant plasmid (mentioned in the note in step 3). This process is very sensitive and if the heat shock is not done for exactly the right time and at the correct temperature, then no transformation occurs.

Genetic transformation the genetic alteration of a cell, resulting from taking up foreign DNA

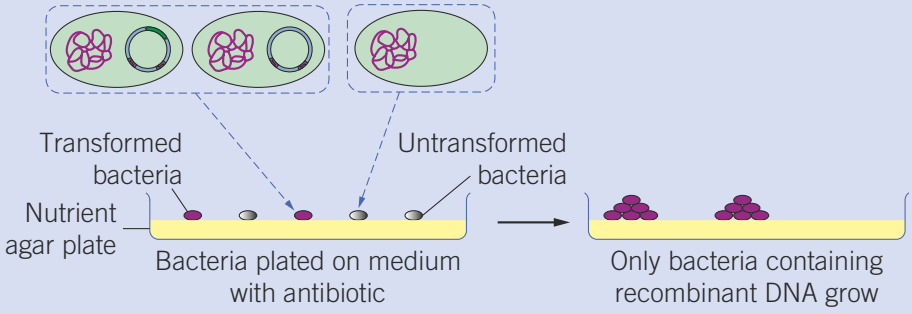
Step 5: Bacteria reproduce

Bacterial reproduction (binary fission) results in clones forming that are genetically identical to the parent cell (this process occurs every 20 minutes, doubling the number of cells each time – and the number of plasmids increases even more, as they replicate independently of the bacterial reproduction). The insulin gene undergoes protein synthesis, resulting in the insulin protein being produced in every bacterial cell.



Step 6: Identifying bacteria with the recombinant plasmid

The traditional method of doing this step is to use the antibiotic resistance genes found naturally in plasmids as a way of locating the **transformed bacteria** (those that have taken up the recombinant plasmid) from untransformed bacteria (those that have not taken up the recombinant plasmid).



The bacteria are spread onto a nutrient agar plate containing an **antibiotic**. Only those transformed bacteria that contain the gene for antibiotic resistance are able to survive. Untransformed bacteria, which have no plasmid, and therefore no antibiotic resistance gene, do not survive in this environment.

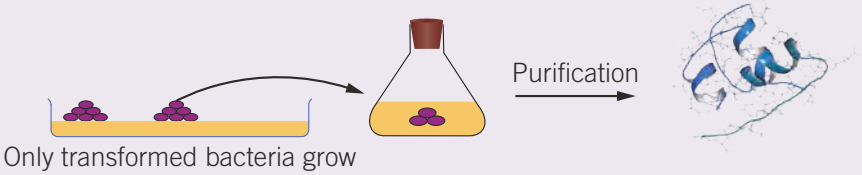
However, there are ethical concerns about using this technique. As the bacteria are able to transfer plasmids between each other, and even to other species of bacteria (bacterial conjugation), it is possible that this may result in antibiotic-resistant bacteria, possibly creating a 'superbug'.

Transformed bacteria bacteria that have taken up foreign DNA; in gene cloning, the foreign DNA is in the recombinant plasmid

Antibiotic a substance that inhibits the growth of bacteria; an example is penicillin

Step 7: Extraction and purification of the insulin protein

The insulin protein from the transformed bacteria that survived on the nutrient agar plate with antibiotic is extracted and purified, ready to provide to diabetic patients.



VIDEO 3B–1 GENE CLONING PROCESS

WORKSHEET 3B–2 GENE CLONING AND IMPLICATIONS

Even though insulin is the only example we need to focus on in this Biology curriculum, some other key human proteins that are produced by gene cloning are:

- human growth hormone
- thyroid-stimulating hormone
- a blood-clotting protein, factor VIII.

Check-in questions – Set 3

1 Identify two enzymes that are required in the process of gene cloning.
2 Describe the difference between a plasmid and a recombinant plasmid.
3 Describe the difference between untransformed and transformed bacteria.

Implications of gene cloning

Ethical

The use of bacteria for gene cloning has overcome the ethical and animal welfare issues associated with extracting proteins, like insulin, from animals such as pigs and cattle, or from the cells of human donors. However, some people view the transfer of genes from one species to another as unethical, regardless of the overall benefit it could provide to society.

Social

There are many advantages of gene cloning for society, including:

- reduced cost of therapeutics made with bacteria, providing greater access to treatments for more individuals in the community
- the creation of employment opportunities in the biotechnology industry, as well as marketing and sales of these products.

A potential disadvantage of gene cloning is its misuse for non-therapeutic purposes, such as creating performance-enhancing drugs, giving athletes an unfair advantage in competitions.

Economic

An economic advantage of using bacteria for gene cloning is that it has made the production of therapeutics, like insulin for diabetics, relatively cheap – large quantities can be produced, with a reliable (constant) supply. However, it is still important to note that this benefit depends on the country and the socioeconomic background of the individual.

Biological

7E ACTIVE AND PASSIVE IMMUNITY LINK

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT LINK

Inserting human genes into plasmids, creating a recombinant plasmid, and performing genetic transformation to make copies of proteins to give to patients, provides a purer or more effective product than using proteins purified from animals. This has made new and safer treatments for disorders and the development of vaccines possible. It also reduces the chances of side effects or of transmitting diseases. The use of vaccines reduces the number of deaths from diseases, and this contributes to an increase in the average life expectancy of the population. This results in the birth rate increasing more than the death rate, and the increase in the world's population increases the strain on the environment and affects other species that share our ecosystems. In the long term, this changes the course of natural selection and our evolution.

9C APPROACHES TO PATHOGENIC EVOLUTION LINK

The risk of antibiotic-resistant bacteria (superbugs) developing has already been outlined. Such a development would be very difficult to overcome. This is covered in more detail in Section 9C.

3B SKILLS

Gene cloning over PCR?

Through all the steps of gene cloning, you might have asked, 'Why doesn't the scientist just isolate the insulin gene of interest and then use PCR to amplify this section of DNA?' The answer is threefold.

1 While PCR makes copies of DNA, genetic engineers need to know that this insulin gene can be converted into a fully functioning protein. For this to occur, there must be a set-up in which protein synthesis can take place, and this requires the different types of RNA and ribosomes that you learned about in Section 2C.
2 Bacteria reproduce by binary fission every 20 minutes – this means that every 20 minutes, the number of bacteria doubles. Even within this time, plasmids replicate independently of the bacteria. Therefore, even more plasmids, and therefore copies of the insulin gene, can be produced in every reproductive cell cycle.
3 PCR requires a number of expensive ingredients: samples of the four nucleotides, a heat-resistant enzyme, and forward and reverse primers. In comparison, the use of restriction enzymes and bacteria, like *E. coli*, is inexpensive.

VIDEO 3B–2 SKILLS: GENE CLONING OVER PCR

LINK 2C PROTEINS

Determining whether transformed bacteria have taken up the recombinant or non-recombinant plasmid

The advantage of plasmids is that they usually contain, or can be constructed to contain, genes that code for antibiotic resistance. This gives bacteria with that plasmid a survival advantage in the presence of the antibiotic that they have resistance to.

Common examination questions in this topic involve how the transformed bacteria are identified. To determine which bacteria are transformed, the bacteria are grown on agar nutrient plates containing an antibiotic (e.g. ampicillin). The recombinant plasmid that has been taken up by transformed bacteria has a gene for resistance. It is important to recall here that it is also possible for transformed bacteria that contain a non-recombinant plasmid (without the gene of interest) to survive, as they still contain the antibiotic-resistance gene. This is shown in Figure 3B–9.

VIDEO 3B–3 SKILLS: DETERMINING WHETHER TRANSFORMED BACTERIA HAVE TAKEN UP THE RECOMBINANT PLASMID OR NON-RECOMBINANT PLASMID

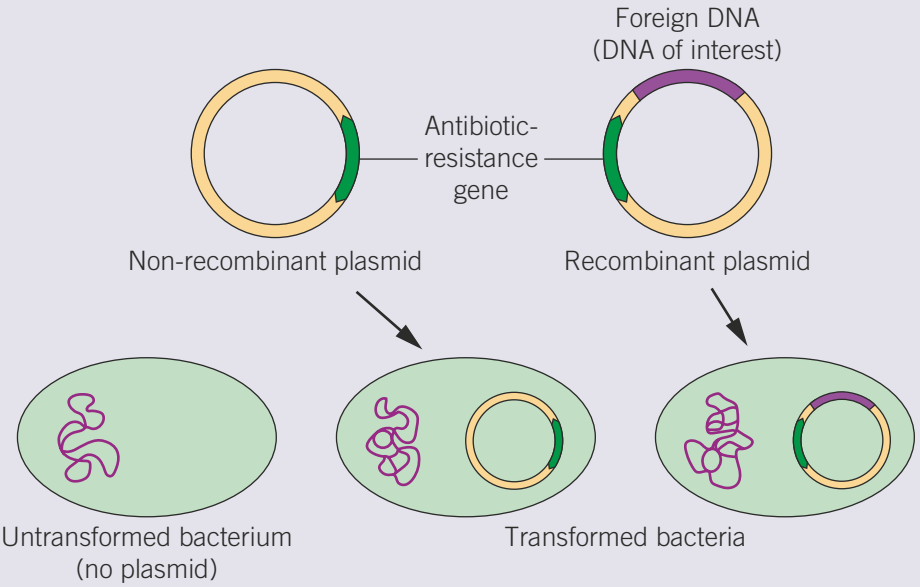


Figure 3B–9 The three possibilities for bacteria after the genetic transformation step in gene cloning

Figure 3B–9 shows that three types of bacteria are possible after genetic transformation:

- containing no plasmid (very common)
- containing the non-recombinant plasmid
- containing the recombinant plasmid.

If all three types of bacteria are grown on an agar plate containing antibiotic, both types of transformed bacteria will survive and reproduce. So how can a scientist work out which bacteria contain the recombinant plasmid and which contain the non-recombinant plasmid?

Hopefully you have worked out that the plasmids could be isolated from the bacteria and run on gel electrophoresis. The recombinant plasmids with the foreign DNA inserted should be larger than the non-recombinant plasmids without the foreign DNA. This is shown in Figure 3B–10.

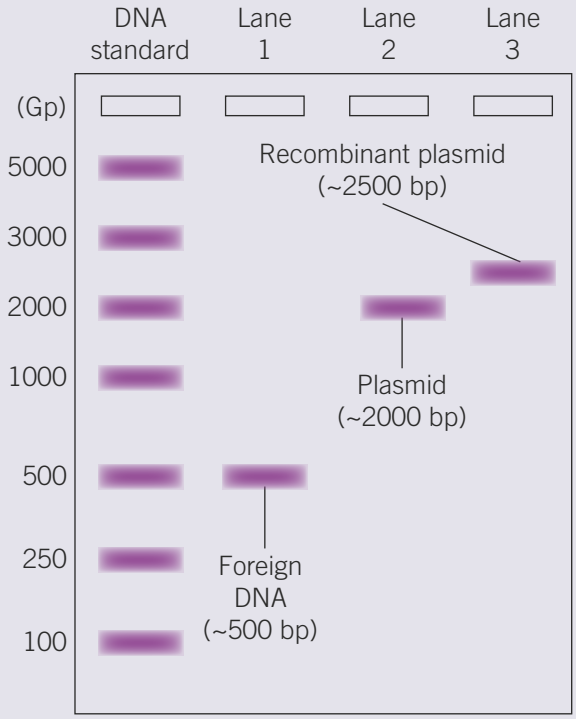
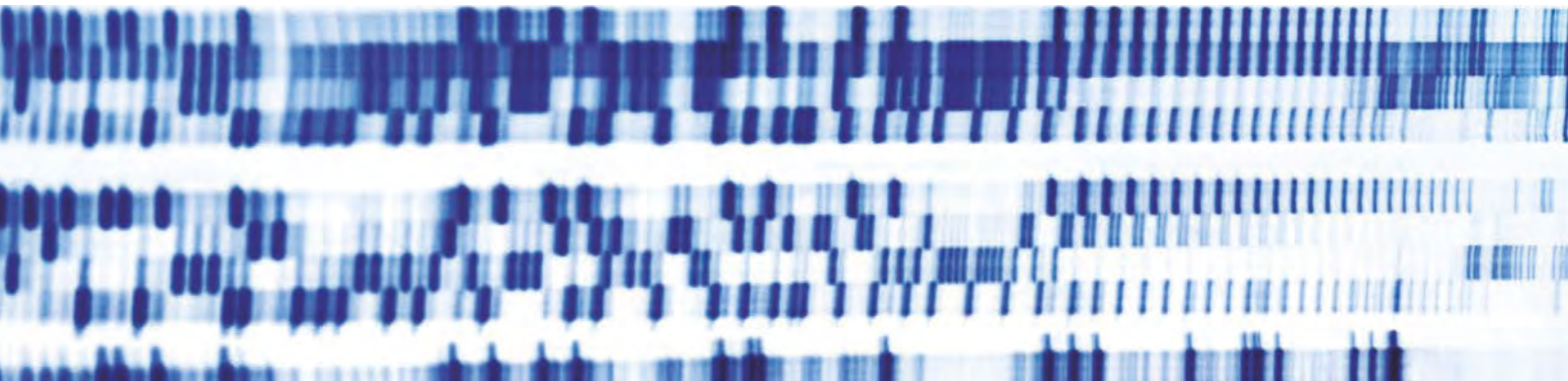


Figure 3B–10 Agarose gel showing results of gel electrophoresis. Lane 3 shows that the recombinant plasmid is equal in size (in base pairs, bp) to the combined sizes of the foreign DNA and plasmid.

VIDEO 3B–4 SKILLS: ANSWERING QUESTIONS ABOUT ETHICAL AND SOCIAL IMPLICATIONS

Answering questions about ethical and social implications

As mentioned in this chapter, when discussing the implications of gene cloning, it is crucial to identify the stakeholder. The following question from the 2019 VCAA Biology exam highlights this.



Question:

The Genomics Health Futures Mission will run a $32 million trial, starting in 2019, to screen over 10 000 couples who are in early pregnancy or who are planning to have a baby. Using a blood test, individuals will be screened for over 500 severe or deadly gene mutations.

Couples will be told they have a genetic mutation if both individuals in the couple carry the same mutation. The trial may lead to a population-wide carrier screening program. The researchers will evaluate cost effectiveness, psychological impact, ethics and barriers to screening. It is anticipated that future tests will be free of charge.

The test may find that a couple who were planning to have a baby or who were already pregnant both carry the same severe or deadly mutation.

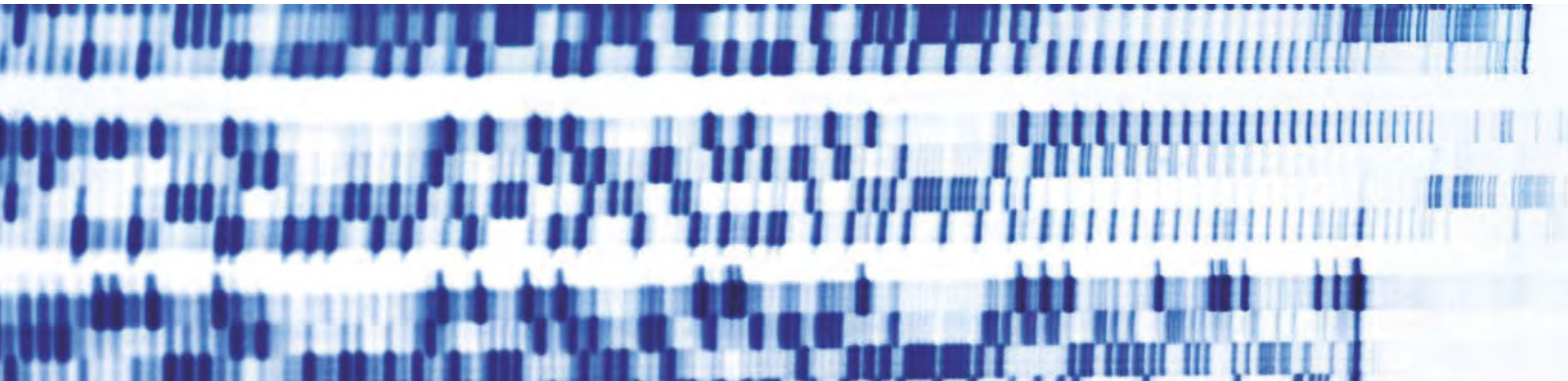
Describe one ethical and one social issue/implication that could arise from this finding.

For this question, the stakeholder is the couple. Many answers would focus on the child as the stakeholder or the future impact on the child, but the question clearly asks for the implication that arises from *this finding*. The table below shows answers that would be acceptable or not acceptable, for each implication.

Answer:

Ethical:

Acceptable answers	Unacceptable answers
Possible termination of the foetus	Child is given up for adoption. *(Wrong, as the couple is only planning or already pregnant but have not had the child)*
Should a severely affected child, who may suffer, be born?	The child may live a poor-quality life. *(Again, the child is not born yet)*
Should alternative reproductive therapies (like IVF) be accessed?	Issues for the child in knowing they have the gene for the disease, as they did not give consent for the test. *(Wrong, as this is not directly related to the parents finding out this information.)*
Who else has access to the information from this finding?	What if insurance companies refuse to provide life insurance to this individual in the future, if they carry this disease-related gene? *(Wrong, as the gene in question would cause health effects in the child, not the parents.)*



Social:

Remember that social implications affect more than the one or two individuals involved.

Acceptable answers	Unacceptable answers
Cost of alternative reproductive approaches – does the government provide a rebate for this?	Genetically modifying babies *(Wrong, as this is not a globally approved method of treatment)*
Psychological impact on individuals and family knowing that the gene is carried	Psychological impact on child growing up with the condition *(Cannot be assumed the child is born with symptoms of the disorder – symptoms might not manifest until later in life)*
Reduction of suffering in potential parents reduces the need for psychological support networks	'Playing God' reduces the suffering of parents *(Cannot use the term 'playing God' without any context)*
Could the government's money be better spent elsewhere, rather than conducting these tests?	Expensive process; parents might not be able to afford the test *(The question stated that this cost was paid for by the government in this trial, so there is no cost to parents. Also, this answer does not refer to how society is affected.)*

It is important to practise answering these types of questions. Examples of questions from the perspective of different stakeholders are provided at the end of this section and in the chapter review and digital questions.

VIDEO 3B–5 SKILLS: LINKING IDEAS IN A SEQUENTIAL NATURE

Linking ideas in a sequential nature

When answering questions, you should do so in a sequential way, although this isn't always easy. It can require you to change the way you think about what is happening. Answering questions in this way helps the examiner see what you are thinking, and to identify that you know your content and are able to link ideas together.

Let's look at how to approach a question of this nature.

Question:

Explain the different types of enzymes used in the process of gene cloning. (2 marks)

Answer:

- To open up the plasmid and isolate the foreign DNA from an organism, the strands need to be cut with restriction enzymes. These are enzymes that identify a specific recognition site and cut the DNA at the sugar–phosphate backbone of the two strands.
- For the plasmid and foreign DNA to be joined together, they may need to be cut with the same restriction enzyme that produces complementary sticky ends.
- To join the two pieces of DNA together, the enzyme DNA ligase is used. This enzyme works in the opposite way to a restriction enzyme, as a 'glue' joining nucleotides at the sugar–phosphate backbone.

Key points to remember when answering these types of questions:

- Incorporate relevant definitions, to show your knowledge of the content.
- Talk a process through, step-by-step: 'This happens, so this happens, and this means, so this occurs ...'.
- Remember, you want to show your teacher or the examiner your knowledge. Even though they will probably know what you mean if you don't explicitly state a particular relevant point in your answer, you will not be awarded marks for it.

Section 3B questions

1 Explain how it is possible for DNA from one organism to be combined with DNA from another organism.

2 In DNA profiling, 13 different VNTR regions are analysed between individuals.

a Why are 13 VNTR regions analysed, instead of just one?

b Give an example of a situation where DNA profiling would be important.

c Outline one ethical and one social implication of these tests. You may choose your stakeholder, but this must be outlined at the beginning of your answer.

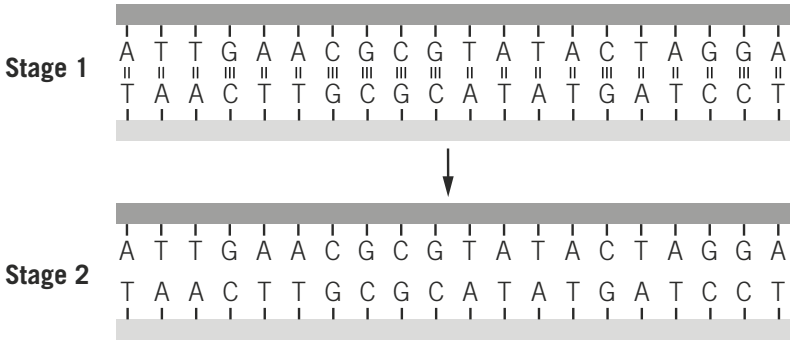
d Explain the DNA manipulation technique used to compare the DNA of these VNTR regions, between individuals. In your answer, clearly highlight how the DNA is separated.

3 Forensic scientists working for Victoria Police collected samples from a crime scene for DNA profiling. However, they found only a small amount of DNA from a blood sample.

a What process must they first use to amplify this DNA?

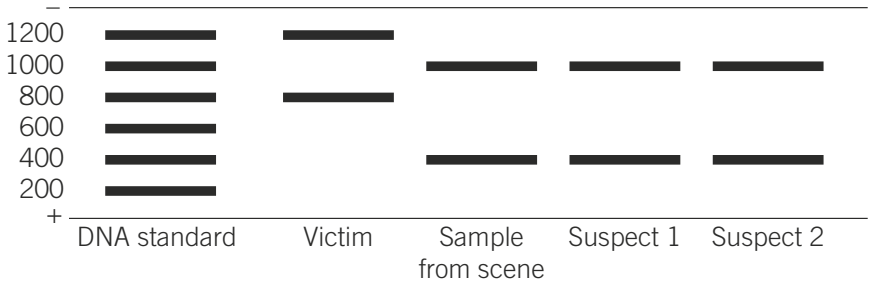
b Below is a diagram showing part of the process you identified in part **a**.

Explain what must be done between stages 1 and 2 on the diagram to separate the strands of DNA and how this occurs.



c Complete the diagram by showing what occurs following stage 2.

Samples of DNA from the victim and those found at the crime scene were compared to samples from two suspects. The DNA was first mixed with restriction endonucleases to isolate the VNTR regions required and run through gel electrophoresis. The following diagram shows just one of the VNTR regions in the agarose gel.



d In which direction does the DNA move through the gel in the diagram below? Explain your answer using your knowledge of the properties of DNA.

e In the lane on the left, a DNA standard was added to this gel. What does the standard consist of and what is its purpose in this case?

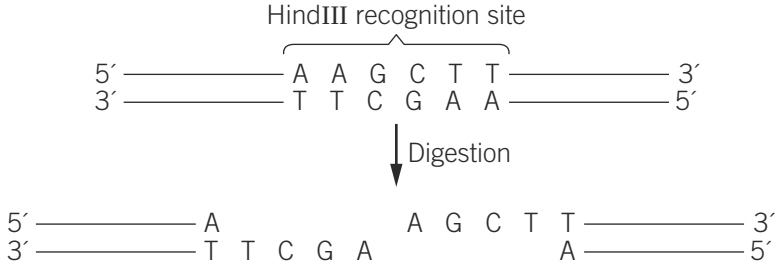
f From the results, what can you conclude?

g What further action would you recommend the forensic scientists do, based on your answer to part **f**?

h Using your understanding of ethical implications and your own opinion, is it justifiable to convict a person based solely on the results of a DNA profile?

(adapted from 2007 VCAA exam)

4 HindIII is a restriction enzyme isolated from the bacterium *Haemophilus influenza*. The figure below shows the effect of this restriction enzyme on DNA.



a What does the 3′ and 5′ arrangement on the two strands of DNA indicate about the strands?

b A plasmid to be used in genetic modification contains this recognition site at four locations. If the plasmid was digested with HindIII, how many fragments of DNA would be formed?

c Are the ends of the fragments produced from the digestion blunt or sticky ends?

d Explain why the types of ends seen in the figure are an advantage in gene cloning.

There are signs that a possible genetically inherited disorder runs in the MacIntosh family. Sufferers produce an abnormal protein structure. The parents in the MacIntosh family, Andrew and Mel, and their son, Jack, undergo genetic screening. DNA is isolated from a sample of their cells and the gene of interest is amplified by PCR.

e Explain the stage of protein synthesis within a cell that would normally convert the DNA into another form of nucleic acid that is still able to code for a protein.

f Where does the process you explained in part **e** occur in eukaryotic cells? Is this location the same for prokaryotic cells?

g Draw a fully labelled diagram of the process you explained in part **e**.

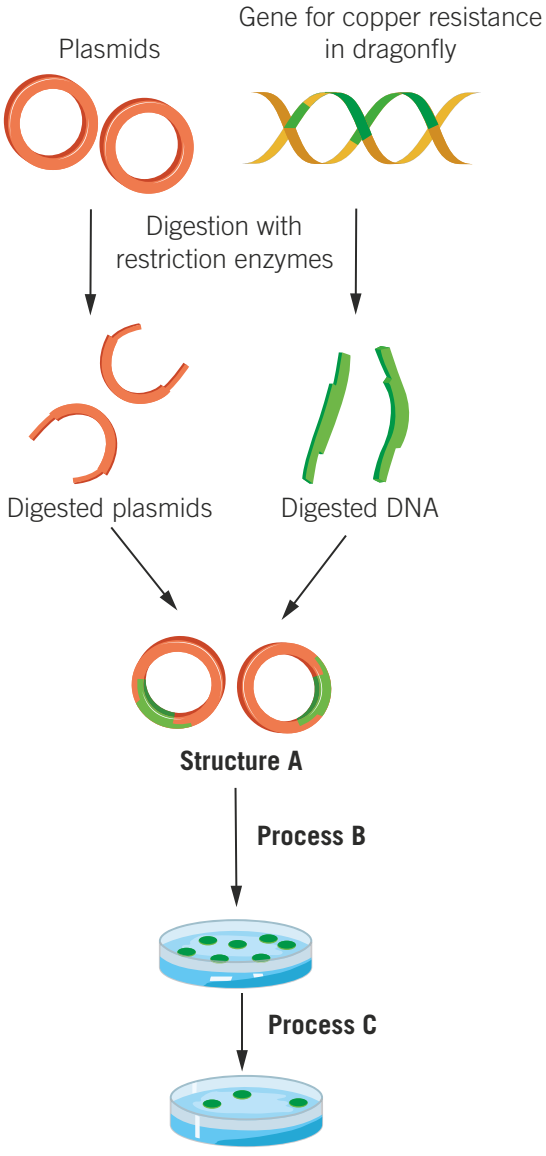
h Primers must be placed into the reaction mixture when amplifying the DNA by PCR. Two different primers are available. Both flank the gene of interest, but one primer is 15 bp in length and the other is 40 bp in length. Which primer should be chosen? Explain your reasoning.

i The normal allele is 60 kbp in length and includes the sequence below at the halfway point of the allele:

5′ … C A T T A G T G T G T A A G C T T T T A C A C T ..... 3′

Use the information provided at the beginning of this question to draw a clear line at the point where HindIII would cut the DNA strand above.

5 A group of dragonflies was found living at the side of a river where there was a high concentration of copper. Very high levels of copper can be toxic to living organisms. However, these dragonflies had a mutation that codes for copper resistance. The diagram on the right is a flow chart of the gene cloning process used to create bacteria that have the copper resistance gene from the dragonflies.



a Identify structure A.

b Bacteria can take up structure A through process B. What is the name of process B?

c Why is heat shock often used to help initiate process B?

d Dragonflies are multicellular eukaryotes. Explain why the DNA from the dragonflies would first need to be modified before it could be placed in bacteria.

e In process C, the bacteria are grown on two agar plates, one without copper and the other with copper. Using a diagram to support your answer, explain the results expected on each agar plate once bacteria are spread onto it.

f What is the purpose of including the agar plate without copper in this experiment?

3C Genetically modified and transgenic organisms

Study Design:
The use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease

Glossary:
Genetically modified organism (GMO)
Transgenically modified organism (TMO)

ENGAGE

The evolution of genetically modified organisms

Genetic modification really took off in 1973 when two scientists, Stanley Cohen and Herbert Boyer, successfully removed an antibiotic resistance gene from one species of bacteria (donor) and placed it into another species of bacteria (recipient). The recipient bacteria then demonstrated antibiotic resistance.

You might think this is where the story begins, but humans have actually been 'modifying' organisms for thousands of years. As far back as 30 000 BC, humans were changing the DNA of a particular species through the process of selective breeding, also referred to as artificial selection, which you will learn more about in Chapter 9. What was this species? It is one you are familiar with, if you look around when you go for a walk, watch TV or visit a pet store: dogs! Our ancestors domesticated wild wolves in Eastern Asia and selectively bred individuals to produce offspring that were initially more passive and compliant.

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

From there, and over thousands of years, everything from hair length, body shape and size, to colour, has been selected for, resulting in the many breeds that exist today. The difference between selective breeding and the current method of genetic modification is that the process is now much faster and does not require many generations to pass before change is noticeable.

With the rise of genetically modified organisms (GMOs) and the accompanying interest in them, various stakeholders have expressed their concerns about the implications, for our own health and, more broadly, that of the world's ecosystems. As a result, regulation of experiments and appropriate safety measures have been established for researchers, who also educate others in the broader scientific community about the significance of their findings.

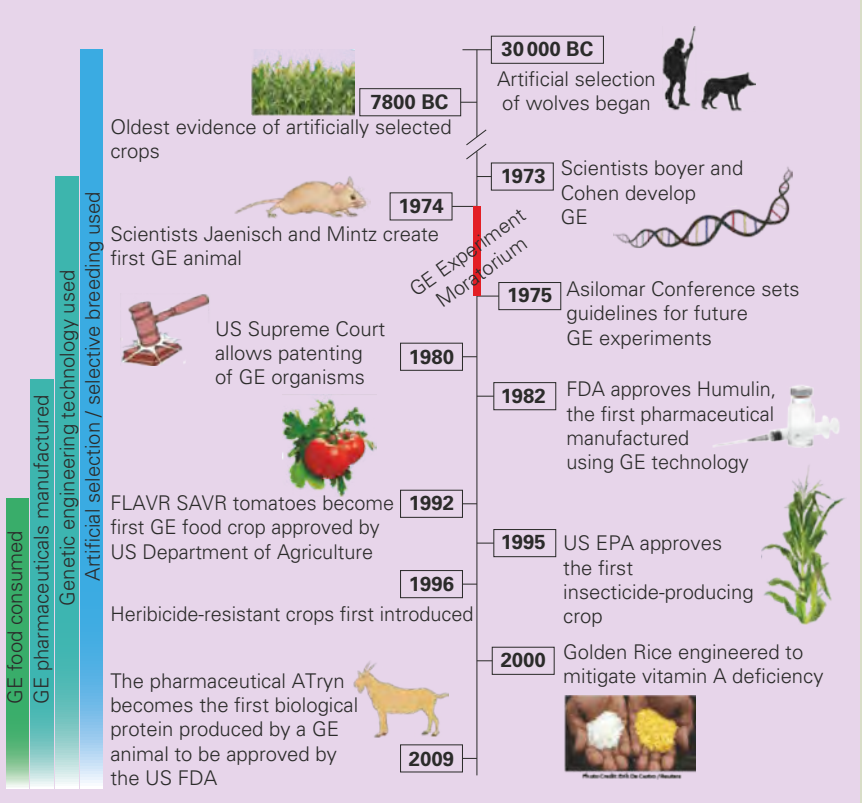


Figure 3C–1 Key developments and advances in the evolution of genetic engineering (GE) and genetically modified organisms

EXPLAIN

Genetically modified vs transgenically modified organisms

As discussed in Section 3B, genetic engineering involves the transfer of DNA from one organism to another, resulting in recombinant DNA. If the recipient organism has had its own genome modified (e.g. genes being switched on or off), or contains DNA from a donor of the same species, then it is referred to as a **genetically modified organism (GMO)**. However, if the recipient organism contains DNA from a different species, then it is referred to as a **transgenically modified organism (TMO)** (*trans* = across, *genic* = gene), or transgenic. The bacteria used in the process of gene cloning for the human insulin gene (in Section 3B) would be referred to as both genetically modified (their DNA has been manipulated) and transgenic (they contain human DNA).

LINK **3B** APPLICATIONS AND IMPLICATIONS OF DNA MANIPULATION TECHNIQUES

Genetically modified organism (GMO) an organism that has had its genome altered

Transgenically modified organism (TMO) a type of GMO that has had genetic material from a different species inserted into its genome

NOTE

Organisms can be both transgenic and genetically modified. Transgenically modified organisms are always genetically modified. However, not all genetically modified organisms are transgenic.

Creating genetically modified plants

The essential steps in producing a GMO are:

1. The desired gene is identified.
2. The desired gene is synthesised artificially from nucleotides using genetic engineering in the laboratory, OR it can be removed from a donor organism (same species) using restriction enzymes.
3. The DNA is amplified using PCR.
4. The gene is inserted into a vector using DNA ligase. The vector can be a plasmid (as was used in the human insulin gene cloning example in Section 3B), a virus or a liposome (a vesicle made of a bilayer of phospholipids).

LINK **3A** COMMON DNA TOOLS AND TECHNIQUES

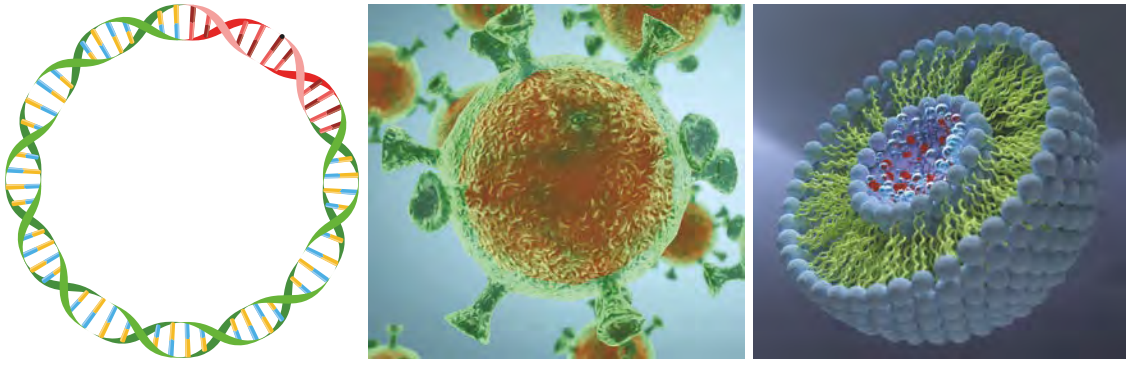


Figure 3C–2 Different types of vectors that can be used in genetic modification. Plasmid (left), virus (middle) and liposome (right)

WORKSHEET 3C–1 CREATING GENETICALLY MODIFIED PLANTS

5. The vector with the desired gene is inserted into a recipient organism. This is more complex than inserting plasmids into bacteria, as the gene has to be inserted into a chromosome.
6. Transformed cells that contain the desired gene are identified. This could be using other genes that are present in the vector, such as an antibiotic-resistance gene or fluorescence gene.
7. The transformed cells are cloned and grown into genetically modified organisms.

Figure 3C–3 Genetically modified cloned plants

Check-in questions – Set 1

1 Outline the difference between GMOs and TMOs.
2 Is the following statement true or false?
'All organisms that are genetically modified are also transgenic.'
3 Name three types of vectors that could be used in the process of creating a genetically modified organism.

Increasing crop productivity

Making photosynthesis more efficient

5E BIOTECHNOLOGICAL APPLICATIONS OF BIOCHEMICAL PATHWAYS LINK

When plants undergo photosynthesis, the process is not 100% efficient, as you will learn in Section 5E. The process generates by-products that are damaging to the cellular components that convert light into chemical energy, and increasing global temperatures are causing this to occur more regularly.

Normally when chlorophyll in the grana of chloroplasts absorbs light, water is split, creating oxygen, hydrogen ions and electrons. The electrons help to power photosynthesis. However, heat can damage a key protein, D1, involved in this process. As such, scientists have genetically modified plants, such as the mustard plant, tobacco and rice, to better repair the damage caused by heat.

2B THE GENETIC CODE AND GENE EXPRESSION LINK

As you learned in Section 2B, DNA codes for proteins. In plant cells, DNA is located in the nucleus, mitochondria and chloroplasts. Chloroplast DNA codes for proteins that are used by the chloroplast, like the D1 protein that is affected by heat. The issue with the chloroplast DNA is that it is much harder to modify than nuclear DNA. Scientists have removed the gene coding for this protein using restriction enzymes, combined it with a section of DNA that is activated during 'heat stress' and moved it into the nucleus.

WORKSHEET 3C–2 GMO'S AND IMPLICATIONS

The result observed in the mustard plant, *Arabidopsis thaliana*, was that it survived extreme heat (41°C) in the laboratory for 8 hours. Other studies with the mustard plant saw an 80% increase in plant biomass.

Figure 3C–4 Field of mustard plants

Similar results were observed in transgenically modified rice crops in Shanghai in 2017, when the city reached temperatures above 36°C for 18 consecutive days. The transgenic crops yielded 8–10% more grain than normal plants. Even at normal temperatures, the yield of the transgenic rice crops was 20% higher. As rice is a staple worldwide, particularly in low-income countries, this increase in yield could help to feed growing populations in a world of continually increasing global temperatures.

Increasing vitamin content: Golden Rice

As previously mentioned, rice plants have been genetically modified over the past two decades. This began in 2000, when scientists genetically engineered rice, producing what they called Golden Rice as a way to combat vitamin A deficiency, which kills over half a million people each year.

Figure 3C–5 Comparison of GMO Golden Rice with 'normal' or standard rice

This strain of rice was transgenically modified when two genes, one from a daffodil and one from the bacterium *Pantoea ananatis,* were inserted. The genes result in the production of beta-carotene, which gives the rice its distinctive yellow colour (hence the name Golden Rice). Beta-carotene is also found in carrots, and when consumed by humans it is converted into vitamin A. As rice is a relatively cheap staple food, especially in developing countries, this transgenically modified (TMO) rice helps to reduce the number of people suffering from vitamin A deficiency. The added advantage of Golden Rice is that research has proved that these crops are unlikely to cross-pollinate unmodified crops, as rice crops usually self-pollinate. And, unlike many other modified crops, the seeds can be re-planted season after season, making it relatively cheap for farmers.

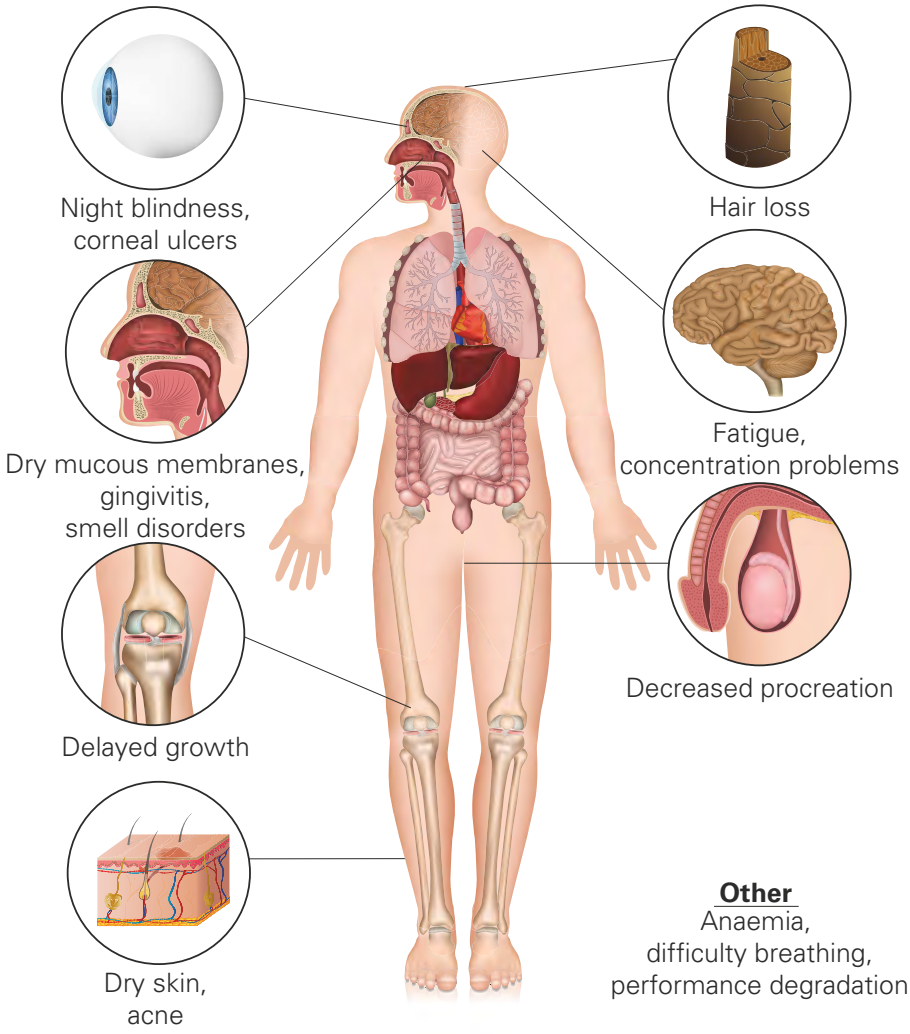


Figure 3C–6 The symptoms of vitamin A deficiency, which Golden Rice can help to reduce

The United Nations predicts that, by 2050, the world will need to produce 70% more food than it currently does, in order to feed the growing global population. This is a vital reason to continue using genetic engineering to increase crop yields.

Improving disease resistance

Effect of fungus on wheat production

A disease known as wheat scab, caused by the fungus *Fusarium graminearum,* has a huge impact on the production of wheat and barley. The disease causes the grain to shrivel and reduces the yield of these plants. The fungus also releases a toxin that remains in grain destined for food consumption. If this toxin is in high concentrations, it can harm humans and animals. Therefore, in many countries, these infected grains must be discarded.

6B CELLULAR PATHOGENS LINK

Figure 3C–7 The effect of the fungus *Fusarium graminearum*, which causes wheat scab. It can affect single (left) or multiple spikelets (centre), or the entire head (right) of the wheat.

As you will learn in Section 6B, fungal infections can be treated by spraying the plants with chemicals, known as fungicides. However, the pathogen infects plants during winter, meaning that rain can wash the fungicide away.

Scientists discovered a gene, called *Fhb7*, in a wild species related to wheat. This gene codes for an enzyme, glutathione S-transferase, which is able to destroy trichothecenes (toxins released by the fungus). This allows the continual reproduction of wheat that has *Fusarium* resistance. The *Fhb7* gene was thought to have originated from a fungus that normally lives inside wild wheatgrass but does not cause disease, as the plant and the fungus exist together in a mutual symbiotic relationship. At some point, the fungal DNA managed to combine with the wheatgrass DNA, and is now incorporated into the wheatgrass genome. As you learned in Section 3B, it is normally bacteria that are associated with the transfer of genes between species of animals and plants – this was one of few examples of a fungus taking this role.

UNIT 2 LINK

3B APPLICATION AND IMPLICATIONS OF DNA MANIPULATION TECHNIQUES LINK

Experiments highlighted the fact that adding the *Fhb7* gene to wheat did not have any negative effects on the grain's yield and provided the wheat with some resistance to fungal toxins.

Implications of genetically modifying plants

Below are outlined the types of implications, and examples of each, that need to be considered when genetically modifying plants for increased crop productivity and/or improved resistance to disease.

Ethical implications

Some of the major ethical implications raised by the use of GMOs and TMOs are listed below. Note that most of these are negative implications.

- Many individuals or groups consider the manipulation of any organism's genetic information to be unnatural.
- Is the consumption of genetically modified (GM) plants safe for humans? Has there been enough research to prove whether there are short- or long-term impacts on an individual's health?
- If cross-pollination occurs between GM crops and non-GM crops growing nearby, then the farmers of the non-GM crops cannot claim that their crops are GM free. Also, the patents for those GM crops could be owned by companies.
- Large biotechnology companies generally control the production and distribution of GM seeds. Some GM seeds become unviable (that is, they will not germinate) after just one generation, which means farmers have to continually re-purchase seeds, which can be expensive. Therefore, although the rewards of increased crop yield and reduced disease are potentially great, much of the farmer's profit is spent on buying new seeds year after year. This can be even more expensive if large conglomerate companies decide to increase their own profit by raising the price of seeds. This specifically has a link to social implications.

Figure 3C–8 A farmer in the United States fills his planter with GM corn seed.

LINK **3B** APPLICATION AND IMPLICATIONS OF DNA MANIPULATION TECHNIQUES

Social/economic implications

Remember from Section 3B that social implications are those that have an impact on not only one or two individuals but a greater proportion of society. Some of the major social implications (both positive and negative) of the use of GMOs and TMOs are listed below. Note that many of these social implications can be linked closely to economic implications.

- Large companies that own the patents for GM seeds control the market and the cost of the seeds, and not all farmers can afford this technology. This increases the inequality between wealthier and poorer struggling farmers. Those who are able to afford the GM seeds initially can grow more crops, sell more and earn more profit to buy seeds again in future seasons.
- The reduced need to use pesticides due to GM crops' improved resistance to disease could put manufacturers of these products out of business because of reduced sales.

Figure 3C–9 Protesters tear up GM canola plants in 2002 at a demonstration against GM crops at a farm in England. The farm is being used as a government test bed for GM crops, which the protesters say will pollute the environment with GM pollen. Eighty protesters trespassed on the farm and tore up the plants, before being arrested.

- Improved crop yield for plants such as rice and wheat could help to reduce malnutrition and hunger, especially in low-income countries. As was seen with Golden Rice, GM food with improved nutritional value may lead to a reduction in deficiencies (like vitamin A deficiency), resulting in healthier populations.
- Plants that have been genetically modified to grow in harsher conditions due to climate change could also help to reduce malnutrition in some countries.
- Giving consumers a choice as to whether they buy GM foods is becoming increasingly important. (For example, the organic food industry has become very big in Western countries.) This makes correct labelling of products as 'GM' or 'GM free' crucial, and has been legislated in Australia.

Biological implications

Any unnatural (or natural) change to an organism's genetic material that changes the way it behaves has the potential to affect other living organisms in the same ecosystem. These effects can be positive or negative. Some of the biological implications of GMO and TMO plants are listed below.

- As GM crops can result in increased growth and yield of plants, less space is required for growth. This means less land is cleared for farming, which reduces the loss of habitat for other organisms.
- A reduction in the use of insecticides or pesticides means less of these chemicals flowing into rivers and streams, and therefore less impact on aquatic life or other organisms that drink this water (including humans, in some countries).
- Increased planting of GM crops could lead to loss of biodiversity, as the genomes of GM crops are identical (or very similar). If selection pressures such as disease or environmental changes occur, the lack of variation in the plant species could mean a drastically reduced survival rate for the crop, resulting in shortages in the food supply for a community or population.
- The genes from GM crops could be inadvertently transferred to another plant of the same (or very similar) species that is not GM, via cross-pollination caused by insects or wind. This links back to the ethical implications mentioned previously.

NOTE

It is important to be aware that much of the work in genetic modification has been conducted on, and is established with, animals. This includes the benefits of the production of increased supply of food (e.g. milk) and resources (e.g. wool). This is not covered in this text, as the curriculum only requires you to focus on genetically modified organisms relating to increased agricultural yield and improved resistance to disease (plants).

VIDEO 3C–1 SKILLS: IDENTIFYING IMPORTANT INFORMATION IN QUESTIONS

3C SKILLS

Identifying important information in questions

Assessment questions about genetically modified and transgenically modified organisms will not require you to know a specific example. Essentially, information about a GM crop and whether it increases crop yield or provides increased resistance to disease will be given in the question.

Questions will tend to focus on any (or all) of the following information, which you have already learned in Chapter 3:

- Whether the organism is a GMO or TMO (or both)

- The advantages (or disadvantages) of the GMO or TMO for humans, animals, insects, other plants, and so on
- The DNA manipulation techniques that may have been used in the development of the GMO or TMO
- The biological, ethical or social implications of using this GMO or TMO, from the perspectives of different stakeholders.

The following example is from the 2018 VCAA Exam.

Should we grow GM crops?
by Mary Nugyen

More than 25 years after genetically modified (GM) food first appeared, growing GM crops remains a hotly debated topic. Some people argue that GM crops are the only way to feed the growing world population and to minimise environmental harm. Other people express different views.

Bt cotton is a type of cotton that contains two genes from a soil bacterium, *Bacillus thuringiensis,* enabling it to produce insect-resistant proteins. Australian farmers of Bt cotton use only 15% of the quantity of the insecticide that was once needed to protect their cotton crops. However, Bt cotton is not as resistant to the main insect pest of cotton crops, *Helicoverpa,* as it has been in the past.

In Australia, Bt cotton is picked by machine, but in India, it is picked by hand. Workers in India have developed skin allergies, which have been attributed to Bt cotton proteins.

Traditionally, farmers have saved money by keeping seed from one year's crop to plant the following year. However, it is illegal for farmers to keep Bt cotton seeds because these seeds have been declared the legal property of the company Monsanto. Every year, cotton farmers must buy more seeds from Monsanto.

Unlike Monsanto, the company that produces the GM food crop Golden Rice allows farmers to replant the rice they harvested the previous year. By inserting a gene from the bacterium *Erwinia uredovora* and another from a daffodil, *Narcissus pseudonarcissus*, into white rice, scientists produced Golden Rice – a rice variety containing higher levels of vitamin A. People who eat Golden Rice avoid vitamin A deficiency. Trials conducted in several countries have shown that Golden Rice is safe to eat.

Here is a question that followed this information.

Question:

Using information from the article, complete the table below by describing one social and one biological implication relevant to the use of Bt cotton and Golden Rice. The same implication should not be used twice.

To develop your answer, use a highlighter (or different colours, as shown in this example) to help identify key information that you can use in your response. It is important to use your reading time to carefully read the questions that follow the stem and look for relevant information in the text. This is shown in the following table.

	Social implications	Biological implications
Bt cotton	*Illegal for farmers to keep Bt cotton seeds, so every year, cotton farmers must buy more seeds from Monsanto,* which is expensive. Could also impact other aspects of life like food and education *Workers in India have developed skin allergies,* which reduces their quality of life	*Australian famers of Bt cotton use only 15% of the quantity of the insecticide,* which would reduce the environmental impact of pesticides
Golden Rice	*The company that produces the GM food crop Golden Rice allows farmers to re-plant the rice,* which can result in an increase in profit for the farmer *Trials conducted in several countries have shown that Golden Rice is safe to eat.* This indicates it would be suitable to grow as food for this population.	*Higher levels of vitamin A* improves nutrition and health of individuals, leading to other benefits for the community *People who eat Golden Rice avoid vitamin A deficiency,* which reduces death and disease

Section 3C questions

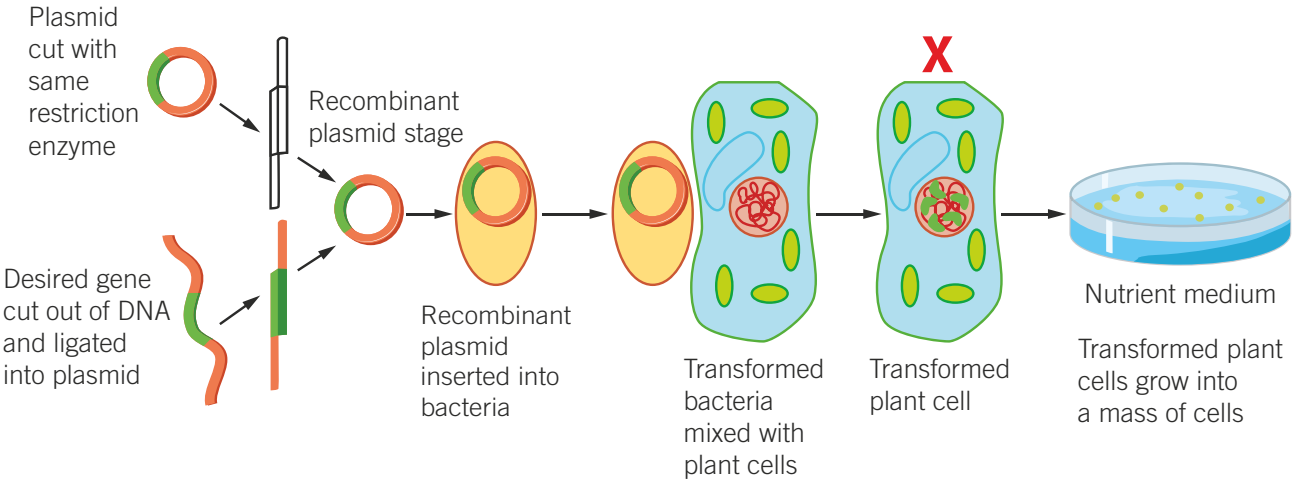
1 Clearly identify the similarities and differences between GMOs and TMOs.

2 The soil bacterium *Agrobacterium tumefaciens* is known to naturally infect plant cells, causing crown gall disease. This is because it carries a plasmid containing genes that cause the growth of a tumour. However, a recombinant plasmid can be inserted into the bacterium that carries a desired gene, but lacks the tumour growth gene. The plant cells are then mixed with the bacteria and the transformed bacteria infect the plant cells. These plant cells are then allowed to grow in a nutrient agar and healthy plants are produced from the plant mass. A diagram of this is shown below.

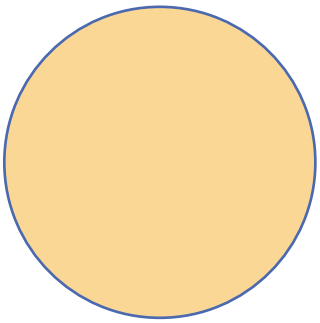
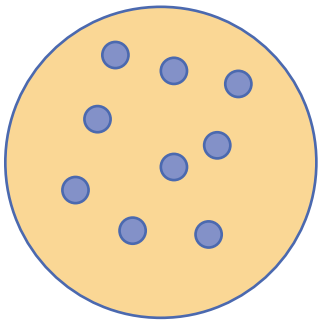
   a In the example shown, is the plant cell genetically modified or transgenically modified? Explain.

   b Looking at the diagram, what type of restriction enzyme has been used to cut the desired gene and the plasmid?

   c What is the name of the process in which the bacterial cells take up the plasmid?



After stage X, the bacteria were grown on agar plates containing different nutrients to confirm that they had accepted the recombinant plasmid. The results of the growth of bacteria are shown in the table below, for two different agar plates.

Agar only, containing no bacterial cells	Agar only, containing bacterial cells	Agar with antibiotic, containing bacterial cells
		

d Copy the table in your notebook and draw the result you would expect to see on the plate consisting of agar with antibiotic and bacterial cells.

e Explain your answer and reasoning behind the drawing you made for part **d**.

f What was the purpose of including the agar plate with no bacterial cells in this experiment?

g Identify one ethical, one social and one biological implication of the technique pictured and discussed. Ensure you provide different examples for each.

3 a Using the information presented in the VCAA exam question from the 3C Skills section pages 116–18, outline an ethical implication for each of Bt cotton and Golden Rice.

b Two students were discussing genetic modification. Tania was arguing that only the Bt cotton crop is transgenically modified, whereas Sakura insisted that both Bt cotton and Golden Rice were transgenically modified. Who is correct? Explain using supportive information from the question.

c Remembering that the DNA code is universal, what would be the similarities and differences between genetic modification and selective breeding?

4 Draw up a table showing what you believe to be the most important social, ethical and biological implications of the use of genetically modified organisms in agriculture. For each type of implication, identify it as either positive or negative.

3D The future of genome editing

Study Design:
The function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome

Glossary:
Cas9
CRISPR
CRISPR-Cas9
Genome editing
Guide RNA (gRNA)

3C GENETICALLY MODIFIED AND TRANSGENIC ORGANISMS LINK

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

ENGAGE

Gene editing to improve adaptability

The ability to change an organism's DNA through genetic modification (as discussed in Section 3C) could be of huge benefit to the international fishery industry. These changes could allow the Australian fishery industry to avoid time-consuming and costly GM regulations as set by the country's jurisdictions, because the slight DNA changes involved result in variations that could occur naturally within a population. As you will see in Section 9B, this variation in a species could make it more adaptable to changes in selection pressures, such as disease or drought. This would allow the industry to plan productively for a world experiencing rapid climate change and growing population.

One of the newest forms of gene editing technology is CRISPR-Cas9, which targets a specific location on a gene and inserts, deletes or replaces DNA at this location. One possible application of this method is to make fish, like salmon, sterile so that they do not spend energy reproducing and instead use their energy for rapid growth. This technology could also be broadened to include environmental productivity, by engineering plants to provide fish with a suitable array of nutrients, enabling further growth. This technology could allow food industries such as fisheries to keep up with growing population demand.

Figure 3D–1 A fish farm in Norway

EXPLAIN

Previous methods of genetic engineering

The first **genome editing** techniques were developed towards the end of the 1900s. For many years, the main technique used viruses as a way of inserting DNA into cells or rectifying 'mistakes' in the DNA of an individual. This was called gene therapy. However, this technique was imprecise, and so there were issues associated with it. The main concern was that there was no control over where the DNA was inserted. For example, it could enter in the middle of another gene in the DNA of the recipient organism and subsequently disrupt the normal code for making that gene's protein. That could have dire consequences for the survival of the organism.

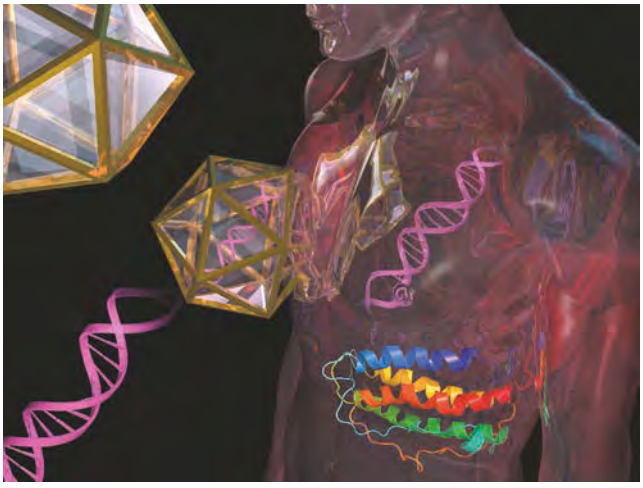


Figure 3D–2 Gene delivery using a viral vector to transfer DNA into a human host is an imprecise method.

The 'evolution' of genetic engineering – CRISPR-Cas9

In 2009, a pattern of DNA now known as **CRISPR** (clustered regularly interspaced short palindromic repeat) was discovered in bacteria, and its use as a gene editing tool began. Breaking down the full name of CRISPR may help you to understand what it is.

- Clustered – positioned close together
- Regularly – present at particular intervals
- Interspaced – a space between two things
- Short – measuring a small distance from end to end
- Palindromic – a word, or in this case a sequence, that reads the same forwards as it does backwards
- Repeat – present more than once.

Therefore, CRISPR is a small sequence of a few nucleotides in DNA that is the same forwards and backwards, repeated one after the other, close together in regular intervals in a genome.

The role of CRISPR-Cas9 in bacteria

These sequences of DNA were discovered in *E. coli* bacteria in 1987. However, it was not until 2007 that their role in defence against bacteriophages (viruses that infect bacteria) was determined. **CRISPR-Cas9** has been used to edit DNA in various organisms, including humans, since around 2012.

CRISPR is a group of nucleotides that codes for short sequences of RNA. A CRISPR-associated enzyme, called **Cas9**, is able to cut at a specific region of the DNA, determined by sequences of RNA, called **guide RNA (gRNA)**. Each gRNA contains 20 nucleotides that are able to bind to a complementary sequence of a strand of DNA nucleotides after it unwinds the DNA. The endonuclease, Cas9, has two active sites (regions that can bind to DNA) and is able to cut both strands of DNA at the sugar–phosphate backbone, like a restriction enzyme would. At this point, the cell's natural DNA repair mechanisms act, doing one (or both) of the following:

- adding nucleotides that alter the sequence of DNA
- inserting a short region of DNA with a specific nucleotide sequence.

Genome editing
(also referred to as gene editing) the insertion, removal or replacement of DNA within the genome of a living cell

CRISPR
a section of DNA containing short repetitions of nucleotides, involved in bacterial defence against viruses

VIDEO 3D–1
CRISPR-CAS9

WORKSHEET 3D–1
STRUCTURE AND FUNCTION OF CRISPR-CAS9

CRISPR-Cas9
an immune system in bacteria that uses CRISPR nucleotide sequences and the Cas9 DNA-cutting enzyme, also modified for use as a genome editing tool

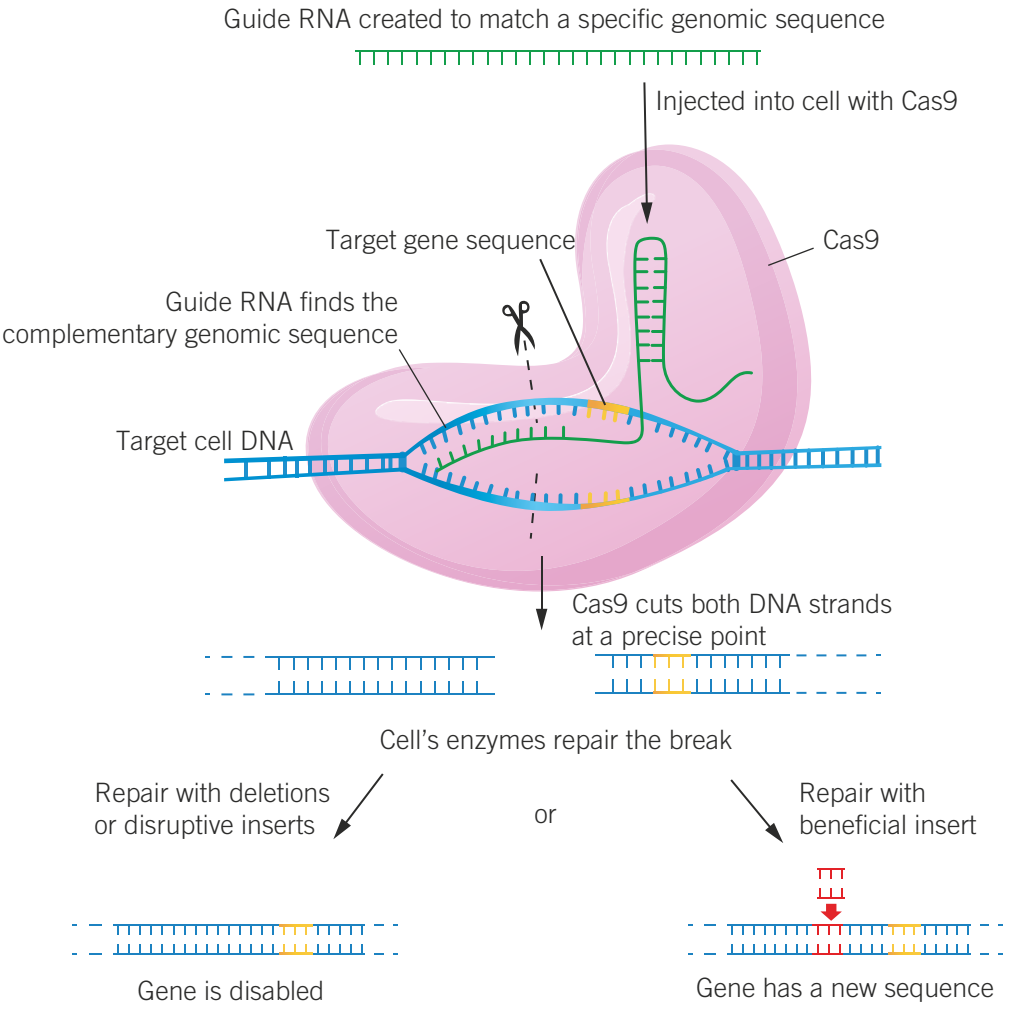
Cas9
an endonuclease (enzyme) that cuts DNA at a specific point determined by guide RNA (gRNA)

Guide RNA (gRNA)
a specific RNA sequence that recognises the desired DNA and directs the Cas enzyme there to cut DNA

However, this process is error prone, and incorrect bases (mutations) are incorporated into the DNA sequence, so the function of the gene is disabled. This is ideal for removing specific genes.

6C NON-CELLULAR PATHOGENS LINK

In bacteria, cutting up viral genetic material ensures that the virus can no longer replicate and damage the bacteria. (You will learn about viruses in Section 6C.) The Cas9 enzyme can include regions of the viral DNA by inserting them into the CRISPR sequence, so the bacteria have a memory of past infections by the same virus. The CRISPR nucleotide sequence is also passed on to daughter bacterial cells when the bacteria reproduce by binary fission, so they also have a memory of the virus.



5E BIOTECHNOLOGICAL APPLICATIONS OF BIOCHEMICAL PATHWAYS LINK

Figure 3D–3 The CRISPR-Cas9 system allows scientists to delete, disrupt or insert DNA within the genome of a living organism. This can be applied to stop an undesirable gene (an allele) being expressed, or to insert a beneficial allele. See Figure 5E–2 for a flow chart applying the process to crop production.

Check in questions – Set 1

1 Is the following statement true or false?
'The role of gRNA is to guide the enzyme CRISPR to the DNA.'
2 What is the role of CRISPR-Cas9 in bacteria?
3 What is one function that CRISPR might be advantageous for?

The future of CRISPR-Cas9

The development of the CRISPR-Cas9 system means that the gRNA nucleotides can be built artificially. Therefore, a scientist can essentially design a sequence to match any specific sequence of nucleotides in DNA and control where in another organism's DNA the Cas9 enzyme will cut. Unlike the use of viruses for gene editing, as described earlier, this technique allows genetic engineers to remove a faulty allele (version of a gene) and insert a normal functioning allele with extreme precision, thereby giving the organism a therapeutic benefit.

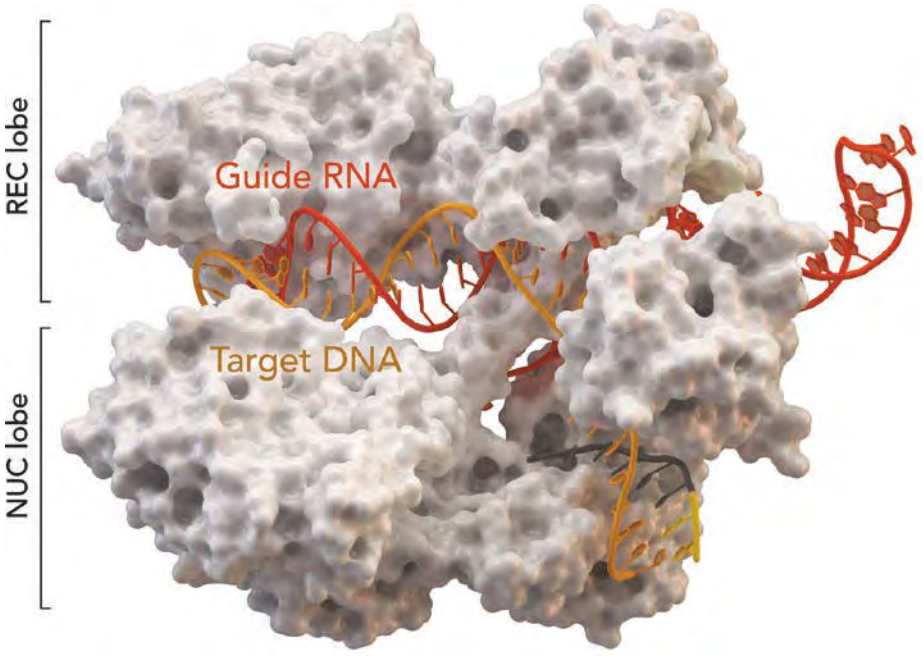


Figure 3D–4 Crystal structure of the CRISPR-associated protein Cas9

Other potential uses of CRISPR-Cas9

When scientists discovered the advantages of CRISPR, including that the process is cheap, easy and accurate, they soon discovered how it could be reprogrammed to edit the genomes of different species. Research in manipulating the role of CRISPR-Cas9 has included the following breakthroughs:

- One of the two active sites of the Cas9 enzyme can be deactivated and new enzymes added. The Cas9 then transports these enzymes to a specific DNA sequence. This means scientists can alter a single nucleotide, so an allele that causes a disorder can be removed, or a nucleotide that results in a stop codon being produced in mRNA transcribed from that DNA can be introduced.
- Adding transcription activators that attach to either the Cas9 enzyme or the gRNA and encourage RNA polymerase to bind more readily to the DNA results in an increased rate of transcription. Alternatively, a gene can be silenced by incorporating more factors that block the action of RNA polymerase in transcription.
- Fluorescent proteins can be added to the Cas9, which allows scientists to locate a specific gene in an organism's genome and/or follow this gene and the chromosome it is on through cell division.
- Several diseases can be treated, including editing cancer genes and potential for removing HIV genes from sufferers.

LINK 2B THE GENETIC CODE AND GENE EXPRESSION

Figure 3D–5 Jennifer Doudna (right) and Emmanuelle Charpentier (left) were awarded a Nobel Prize in 2020 for inventing the revolutionary gene-editing tool CRISPR-Cas9.

5E BIO-TECHNOLOGICAL APPLICATIONS OF BIOCHEMICAL PATHWAYS LINK

As you will explore in Section 5E, CRISPR-Cas9 has demonstrated huge benefits for the agricultural industry in editing plant genomes, for improving photosynthetic efficiency and crop yields, and in the development of biofuels.

3B APPLICATION AND IMPLICATIONS OF DNA MANIPULATION TECHNIQUES LINK

There have been some bioethical issues highlighted about the use of CRISPR-Cas9. In 2015, concerns were raised by reports that this technique could be used to edit the genome of human embryos. The ethical and social implications here are similar to those outlined in sections 3B and 3C.

3C GENETICALLY MODIFIED AND TRANSGENIC ORGANISMS LINK

3D SKILLS

VIDEO 3D–2 SKILLS: QUESTIONS ABOUT CRISPR-CAS9

Questions about CRISPR-Cas9

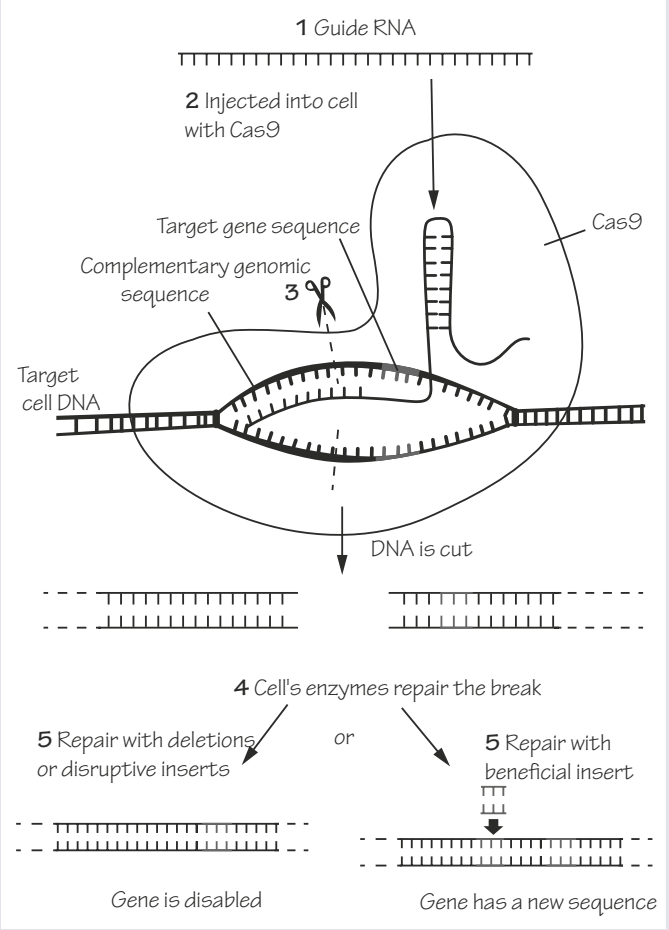
The use of CRISPR-Cas9 as a genome editing tool is new content in the current Study Design. The Study Design states that students need to know:

The function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome

The function of CRISPR-Cas9 in bacteria is immune defence against genes that viruses inject into them to express as proteins and reproduce. A nucleotide sequence from the virus is incorporated into the bacterium's CRISPR sequence as a 'memory', so the next time that virus is encountered, the bacterium produces gRNA, which directs Cas9 to cut the viral gene up into pieces.

This function can be applied to genome editing in live cells in the laboratory, through the following steps and diagram:

1. Guide RNA is created to match the desired gene sequence to be edited.
2. This gRNA, along with Cas9, is injected into the cell.
3. When the guide RNA binds to its target gene sequence, the active sites of the Cas9 enzyme make a precise cut in the organism's DNA.
4. The cell's own DNA repair enzymes repair the break.
5. Scientists can manipulate the repair enzymes to delete or disrupt the gene at the repair site, thereby preventing a harmful gene from being expressed. Or they can insert a new sequence of DNA nucleotides to change a detrimental allele to a beneficial one.



It is highly unlikely that an examination question would ask you to recall the steps of the action of CRISPR-Cas9, although an internal school assessment may.

Here is an example of a question on the application of CRISPR-Cas9 in editing an organism's genome:

Question:

What is the advantage of using CRISPR-Cas9 over other DNA tools that are able to cut the DNA, like restriction enzymes?

Answer:

CRISPR-Cas9 uses guide RNA (gRNA) that can be artificially designed to bind with a specific sequence of nucleotides, enabling Cas9 to cut the DNA at a precise point, whereas restriction enzymes cannot be guided in the same way and will cut DNA at a recognition site that may occur anywhere along the strand. Also, gRNA is 20 nucleotides long, compared to a recognition site, which is only 4–6 nucleotides long. So the gRNA is likely to find only one complementary site in the whole genome, whereas there are likely to be many restriction enzyme recognition sites. The gRNA is more specific in where it allows the Cas9 enzyme to cut the DNA, as opposed to a restriction enzyme.

If you look back at the steps of the action of CRISPR-Cas9 above, step 3 discusses the action of the enzyme Cas9. Other questions relating to enzyme structure and function, the role of the active sites, what they bind to (substrate) and the specificity of these enzymes could also be asked. Enzymes are discussed in Section 4A.

Additionally, questions about transcription (Section 2B) or mutations (Section 9A) could also relate to this concept. Therefore, it is important to use the concept maps provided at the beginning of each chapter and your own developed concept maps across the entire curriculum to find as many links between content as possible. The links to other chapters throughout each section of this book should assist you with this.

LINK **4A** THE ROLE OF ENZYMES
LINK **2B** THE GENETIC CODE AND GENE EXPRESSION
LINK **9A** MUTATIONS

Section 3D questions

1 The CRISPR-Cas9 gene editing technique contains some important aspects. Outline the role of:
   - **a** guide RNA
   - **b** Cas9.

2 Outline the process of gene editing using the CRISPR-Cas9 system.

3 Explain what the issue might be if a scientist does not know the exact part of the base sequence of the target gene in order to use CRISPR-Cas9.

4 Outline one difference between the function of a restriction enzyme and that of CRISPR-Cas9.

5 The gRNA is longer than a recognition site for restriction enzymes. What is the advantage of this for the Cas9 enzyme compared to a restriction enzyme?

6 Identify two possible reasons why scientists would want to use CRISPR-Cas9 technology.

Chapter 3 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
3A.1	Outline the role of DNA polymerase in synthesising DNA	15d☐
3A.2	Explain the reason for and difference between using DNA polymerases from different organisms	11b☐, 15d☐
3A.3	Explain the action and importance of DNA ligase	2☐, 14d☐
3A.4	Explain the action of endonucleases in cutting DNA of different organisms	2☐, 3☐, 10☐
3A.5	Draw and compare the differences between blunt end and sticky end endonucleases	14a☐, b☐, c☐
3A.6	Explain the advantages of using certain endonucleases over others in DNA techniques	14c☐
3A.7	Describe the steps, materials and conditions needed for polymerase chain reaction	1☐, 9☐, 15b☐, c☐
3A.8	Draw a diagram of the steps in the polymerase chain reaction	15c☐
3A.9	Draw and label a gel electrophoresis set-up	16d☐
3A.10	Explain how DNA is separated using gel electrophoresis, including the need for a control/standard	16d☐
3B.1	Explain what DNA profiling is and give examples of its use in society	6☐, 16a☐, b☐
3B.2	Interpret diagrams of gel electrophoresis for DNA profiling	16b☐
3B.3	Define plasmid, recombinant plasmid, transformation, gene cloning and antibiotic resistance	2☐, 12a☐, 14g☐
3B.4	Recall the difference between a plasmid and a recombinant plasmid	2☐, 13a☐, 14h☐
3B.5	Explain the use of recombinant plasmids in gene cloning	12a☐
3B.6	Outline the various DNA techniques and their roles in gene cloning	14e☐
3B.7	Explain and interpret the requirements for bacterial growth under different conditions	4☐
3B.8	Outline in words and diagrammatically the production of human insulin using gene cloning and bacteria	14☐
3C.1	Outline the difference between a GMO and a TMO	7☐, 12b☐, 13b☐
3C.2	Explain how GMOs and TMOs are used in crop productivity	13e☐

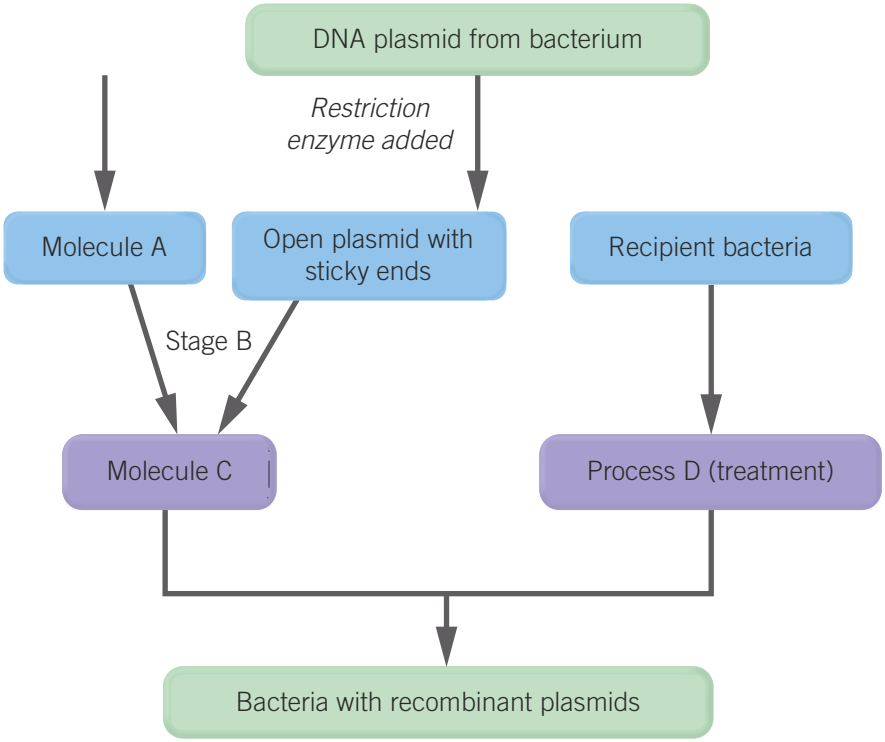
Success criteria – I am now able to:		Linked question
3C.3	Explain how GMOs and TMOs are used in providing resistance to disease	13b☐, c☐, f☐
3C.4	Identify potential ethical, social, biological and economic issues with their use	13d☐, e☐, f☐
3D.1	Explain what CRISPR-Cas9 is	5☐, 12c☐, 13f☐, 15a☐
3D.2	Identify potential ethical, social, biological and economic issues with the use of CRISPR-Cas9	8☐, 13f☐, 15e☐

Multiple-choice questions

1 In PCR, amplification of DNA requires

A amino acids.
B DNA polymerase.
C free nucleotides A, C, G and U.
D a single primer.

The following information relates to Questions 2–4.



2 Which of the following correctly identifies molecule A, stage B, molecule C and process D from the image above?

	Molecule A	Stage B	Molecule C	Process D
A	Plasmid	Ligation	Recombinant plasmid	Transformation
B	Gene of interest	Digestion	Plasmid	Transformation
C	Gene of interest	Ligation	Recombinant plasmid	Heat shock
D	Plasmid	Digestion	DNA ligase	Gene cloning

3 The role of the restriction enzyme in the first step of this process is to cut
- **A** the gene of interest only.
- **B** the plasmid only.
- **C** both the gene of interest and plasmid.
- **D** the recombinant plasmid.

4 Following the last step of the flow chart, the bacteria with recombinant plasmids were grown on agar plates. Which of the following types of agar plates will only demonstrate that these specific bacteria grow after an overnight incubation?
- **A** agar only, incubated at 4°C
- **B** agar containing antibiotic, incubated at 4°C
- **C** agar only, incubated at 37°C
- **D** agar containing antibiotic, incubated at 37°C

5 The CRISPR gene editing process includes the enzyme Cas9, which has a similar function to a restriction enzyme. What is Cas9 required to do in this process?
- **A** cut just the single strand of DNA that the gRNA binds to
- **B** cut both strands of DNA at a specific region
- **C** direct the gRNA to the correct location of a sequence of DNA
- **D** amplify the specific sequence of DNA

6 The main purpose of genetic screening of a foetus as part of the 20-week scan in prenatal testing is to
- **A** determine the likelihood of the individual having a particular disease and to provide early treatment or recommend a change in lifestyle.
- **B** provide the parents with an opportunity to abort the pregnancy at this point.
- **C** use CRISPR-Cas9 to edit the genome of the foetal cells, correcting any potential diseases that would cause mutations.
- **D** have the genome sequence of all individuals on record for future scientific endeavours.

7 All genetically modified organisms (GMOs)
- **A** are also transgenically modified organisms (TMOs).
- **B** have a gene that has been removed by restriction enzymes.
- **C** are designed to provide benefits such as increased crop yield and resistance to disease in plants.
- **D** contain a section of DNA that has been altered by scientists.

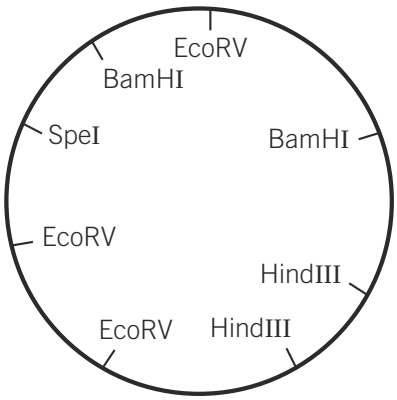
8 In 2015, an international group of scientists gathered to put in place regulations of the use of the CRISPR gene editing technique so that it could not be used to edit the human genome as a way to develop 'designer babies'. This would help to address what type(s) of implication(s)?
- **A** ethical
- **B** biological
- **C** social
- **D** all of the above

9 A short single-stranded piece of DNA or RNA that can be used as a starting point for the synthesis of a new strand is called a
- **A** primer.
- **B** plasmid.
- **C** probe.
- **D** promoter.

10 The figure on the right represents DNA from a bacterium and its recognition sites for different restriction enzymes.

If this DNA was incubated in a tube with

- **A** SpeI, it would result in two fragments of DNA.
- **B** HindIII, it would result in three fragments of DNA.
- **C** SpeI and EcoRV, it would result in four fragments of DNA.
- **D** BamHI and HindIII, it would result in three fragments of DNA.



Short-answer questions

11 Work by genetic engineers in the laboratory involved deliberately altering (mutating) the sequence of nucleotides that codes for the insulin gene.

- **a** Draw a monomer of the insulin gene. In your answer, clearly label all parts. (2 marks)
- **b** How does this monomer differ from the other type of nucleic acid you learned about in Chapter 2? List two key differences. (2 marks)
- **c** Using a diagram to support your answer, describe the key steps involved in the second stage of the synthesis of the insulin protein within normal pancreatic beta cells. (5 marks)
- **d** In terms of the protein structure, what impact would these mutations have on the insulin produced? (2 marks)
- **e** What organelle inside cells is responsible for the correct folding of the insulin protein? (1 mark)
- **f** Using your knowledge of the role of different protein structures, propose a purpose for having these different forms of insulin produced for sufferers of type I diabetes. (1 mark)

12 Identify whether the following statements are true or false in relation to transgenically modified organisms (TMOs). If false, re-write the statement so that it is correct for TMOs.

- **a** A sequence of DNA is inserted from a human into bacteria, using a plasmid as a vector. (1 mark)
- **b** A sequence of DNA from another organism of the same species is inserted into its genome. (1 mark)
- **c** CRISPR-Cas9 is used to correct an allele so that it codes for a normal functioning protein. (1 mark)

13 A transgenically modified species of Bt cotton exists where two genes from the bacterium *Bacillus thuringiensis* have been inserted into the cotton crop's genome. These two genes code for proteins that are lethal to a caterpillar that feeds on the plant, by disrupting its digestive system. For farmers in Australia, this has reduced reliance on pesticides, which greatly reduces the environmental impact of pesticide use, on other organisms. It has also reduced the amount spent on pesticides by farmers. However, it is illegal for farmers to re-plant the Bt cotton seeds in successive seasons, due to legal declarations established by the biotechnology company that sells them to farmers. The seeds are the property of this company. The long-term cost of buying seeds each year could be more than the farmers save on pesticides.

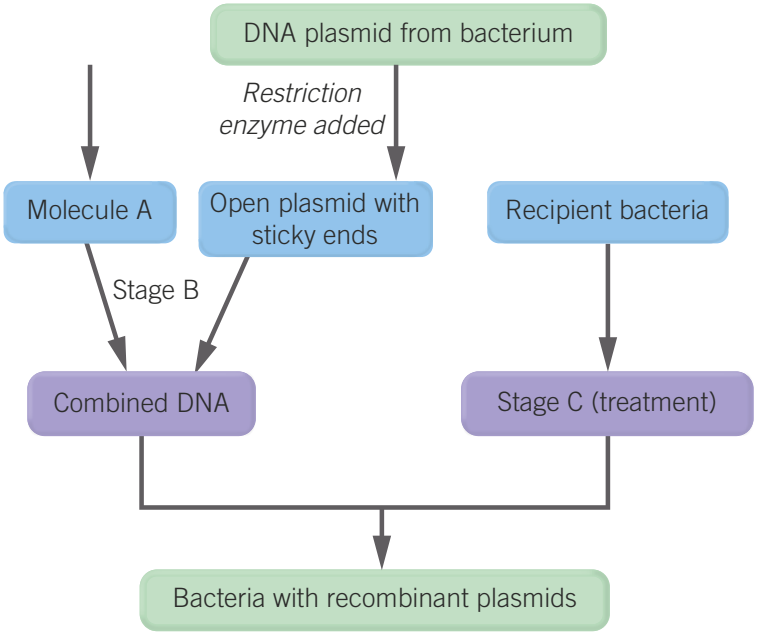
- **a** What would be the likely type of vector used to insert the genes from the bacteria into the cotton crop's genome? (1 mark)
- **b** Is Bt cotton a TMO or a GMO? Explain your answer. (1 mark)
- **c** What is the role of the genes inserted from the bacterium *B. thuringiensis*? (1 mark)

d One disadvantage of the cotton industry is the amount of water required to grow cotton crops. It has been estimated that 20 000 L of water is needed to produce a single t-shirt and a pair of jeans. Give two reasons why the Bt cotton crop might be beneficial in reducing the amount of water needed to grow the cotton. (1 mark)

e Globally, cotton is the most profitable non-food-related crop grown. As a result of this, explain why the use of genetically modified Bt cotton would be an added benefit for farmers. (2 marks)

f The current method of cotton farming and production is environmentally unsustainable, and experts have suggested that it is not viable for long-term future cotton production. Using the knowledge you have gained from this chapter, what is one way that the Bt cotton crop could be genetically modified to solve this problem? (2 marks)

14 A recombinant DNA plasmid is created by inserting a foreign gene into a bacterium, as shown in the figure.



a Explain the importance of sticky ends in the open plasmid. (2 marks)

b What are the other types of ends produced by restriction enzymes that are not used in the process shown? (1 mark)

c Using a diagram, clearly outline the difference in DNA fragments when the restriction enzymes you identified in part **c** are used, and what this would mean for DNA fragments being able to join together. (3 marks)

d Name the enzyme used in stage B to form the permanent combined DNA. (1 mark)

e Describe what type of treatment is given to recipient bacteria at stage C. (2 marks)

In a similar process to that shown in the figure, a gene coding for the green fluorescent protein (GFP) can be removed from the jellyfish genome and combined with a plasmid, which is then inserted into a bacterium.

f Define genome and explain how this is different to an organism's proteome. (2 marks)

g What is the role of a plasmid in gene cloning? (1 mark)

The advantage of incorporating the GFP gene into plasmids is that bacteria that take this plasmid up are able to fluoresce under UV light. Normally in this genetic modification process, an antibiotic, ampicillin, is also used to select for bacteria.

h Draw a diagram of the plasmid, clearly indicating these genes. (2 marks)

15 CRISPR-Cas9 is a relatively new technique that is changing the way gene editing is done in the laboratory and in living organisms. It relies on the use of a specific type of nucleic acid, called guide RNA (gRNA) and an enzyme called Cas9.

a What two properties of gRNA allow it to bind to a specific sequence of DNA nucleotides? (2 marks)

In order for scientists to work effectively with the gRNA that they designed, they need more than one copy of the gRNA.

b What is the name of the technique that the scientist would use to amplify the gRNA? (1 mark)

c Outline the stages of the process you named in part **b**, including all ingredients required. (4 marks)

d From your knowledge of both DNA and RNA, as well as the process you outlined in part **c**, identify what changes might be required to the ingredients used in the process in order to amplify the gRNA, as opposed to the DNA that is normally amplified. (2 marks)

e Outline one ethical, one social and one biological issue with using CRISPR-Cas9 technology to genetically modify genes in human gametes (egg or sperm cells). (3 marks)

16 DNA profiling is a technique used to analyse the DNA similarities and differences between both related and unrelated individuals for the purpose of investigations.

a What is the name of the regions of DNA that DNA profiling typically focuses on? (1 mark)

At a football game, a mother and father accidently became separated from their son. Coincidently, on the same day two other families also lost sight of their sons. At the end of the match, the first couple in question located the security station at the ground to collect their son. However, the security staff wanted to be 100% sure about which of the three children actually belonged to the parents. For this reason, they decided to bring in the police and their forensic science team to perform a DNA profile of each parent and the three lost children. They investigated one region of DNA and compared the size of the DNA fragments in this region on each of their two chromosomes containing this gene (In this hypothetical scenario the children are biologically related to their parents). The results are shown below, for the sizes of those DNA fragments.

DNA region	Mother	Father	Lost child 1	Lost child 2	Lost child 3
'X'	3, 8	6, 10	3, 6	10, 13	8, 10

b From these results, identify which lost child belongs to these parents. Explain your answer. (2 marks)

c Based on your answer to part **b** and your knowledge of DNA profiling, what should the forensic scientists have done differently? (1 mark)

d The method used to generate results in this test was digital. Another technique that could have been used to identify these different-sized fragments of DNA was gel electrophoresis. Draw a fully labelled gel that includes:

- all results for individuals shown in the table above
- wells on the gel
- positive and negative electrodes, including the direction of DNA movement
- a DNA standard that results from individuals can be compared to. (4 marks)

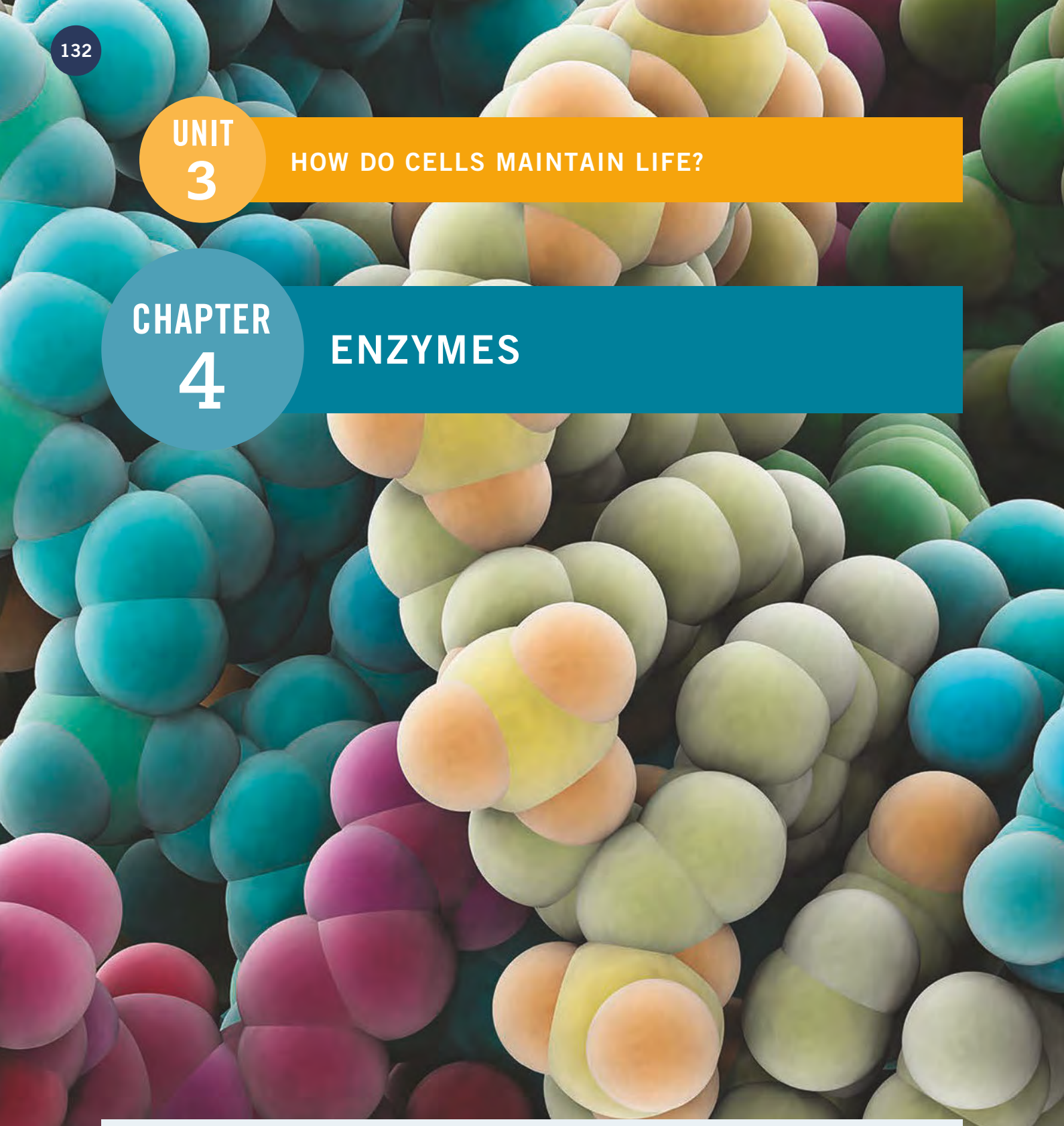
e Explain how the technique in your answer to part **d** would distinguish between different-sized fragments of DNA. In your answer, explain how the material the DNA separates on facilitates this separation. (3 marks)

UNIT 3

HOW DO CELLS MAINTAIN LIFE?

CHAPTER 4

ENZYMES



Introduction

Organisms rely on chemical reactions to stay alive. There are countless reactions that make up the metabolism of an organism. As an example, chemical reactions are needed to digest food to provide nutrients, and to convert these nutrients into a form of energy that can be used. Almost all the reactions within an organism require a special type of protein, known as an enzyme, to occur. This chapter explores biochemical pathways, the role that enzymes play in these pathways, how enzymes function and the factors that affect their activity.

Curriculum

Area of Study 2 Outcome 2
Regulation of biochemical pathways in photosynthesis and cellular respiration

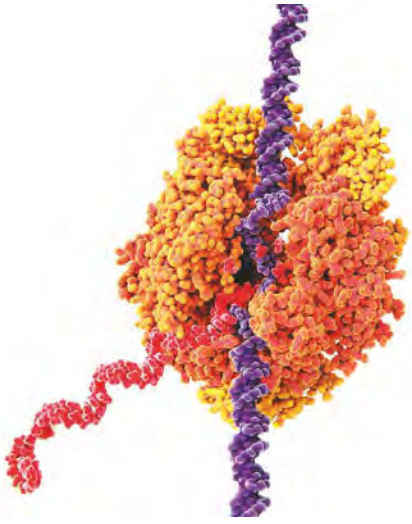
Study Design	Learning intentions – at the end of this chapter I will be able to:
• The general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product	**4A Enzymes** **4A.1** Define biochemical pathway **4A.2** Outline the difference between a reactant (substrate) and a product **4A.3** Explain how a pathway results from the product of one reaction becoming the reactant (substrate) in a subsequent reaction
• The general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration	**4A.4** Describe the lock-and-key and induced-fit models of enzyme function **4A.5** Recall the importance of the active site in explaining the specificity of enzymes **4A.6** Outline some of the key features of enzymes **4A.7** Describe the role of coenzymes in enzyme function
• The general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors	**4B Factors impacting on enzyme function** **4B.1** Explain the effect of temperature on the rate of enzyme activity, referring to the kinetic energy of molecules **4B.2** Understand the difference between denatured and inactive in relation to enzymes. **4B.3** Explain the effect of pH on the rate of enzyme activity **4B.4** Explain the effect of enzyme and substrate concentration on the rate of enzyme activity **4B.5** Define competitive and non-competitive inhibition **4B.6** Explain the effect of competitive and non-competitive inhibition on the rate of enzyme activity **4B.7** Describe how competitive and non-competitive inhibition can be used in rational drug design

Glossary

Activation energy
Active site
Allosteric site
Anabolic
Catabolic
Catalyst
Coenzyme
Competitive inhibition
Denaturation
Enzyme
Enzyme saturation
Inhibitor
Non-competitive inhibition
Substrate

Concept map

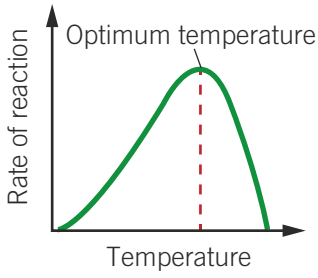
4A The role of enzymes

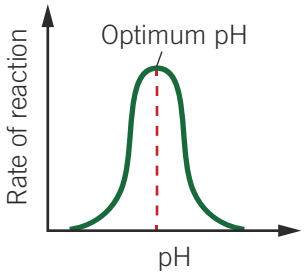


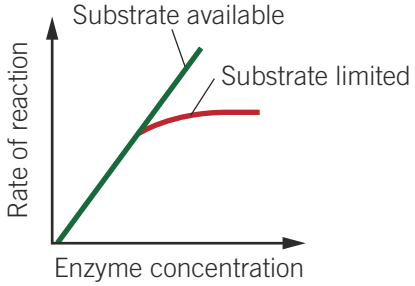
Control of cellular chemical reactions

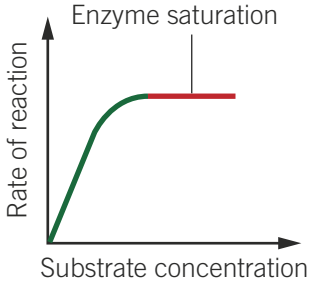
Determining the rate of reaction or rate of product formation for enzyme-catalysed reactions

4B Factors impacting on enzyme function









See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

The role of enzymes

Study Design:

- The general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product
- The general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration

Glossary:

Activation energy
Active site
Allosteric site
Anabolic
Catabolic
Catalyst
Coenzyme
Enzyme
Substrate

ENGAGE

History of enzymes

The term 'enzyme' was first used in 1877 by the German physiologist Wilhelm Friedrich Kühne. However, this was 44 years after the first enzyme, diastase, was discovered by the French chemist Anselme Payen. Although this was the first recorded instance of an enzyme being discovered, enzyme use was documented in history much earlier than this. One such example comes from Homer's *Iliad*, which outlines a process for cheese making that must have involved the use of enzymes. Since these early beginnings, our knowledge of enzymes has changed significantly, due largely to the applications of new technology. In this section, we will examine the structure and function of enzymes and how they are involved in biochemical pathways in organisms.

Figure 4A–1 Wilhelm Kühne (left) and Anselme Payen (right)

Enzyme
a type of protein, also referred to as a biological catalyst, that speeds up reactions within an organism by lowering activation energy

Substrate
a molecule that binds to the active site of an enzyme and then takes part in a reaction; also referred to as a reactant

EXPLAIN

Biochemical pathways

In Chapter 5, you will learn more about the metabolic processes of photosynthesis and cellular respiration. Both these processes are examples of biochemical pathways. A biochemical pathway involves a series of reactions, each catalysed by **enzymes**, where the product of one reaction is the **substrate** (or starting material) for the next reaction.

LINK CHAPTER 5

The easiest way to understand this is by using an illustrated example. Imagine a biochemical pathway that involves four molecules: A, B, C and D. In this pathway, molecule A is converted to B, B to C and, finally, C to D. Each of these transformations occurs via a separate reaction,

and each reaction is catalysed by a separate enzyme: 1, 2 and 3 respectively. This pathway is shown in Figure 4A–2a.

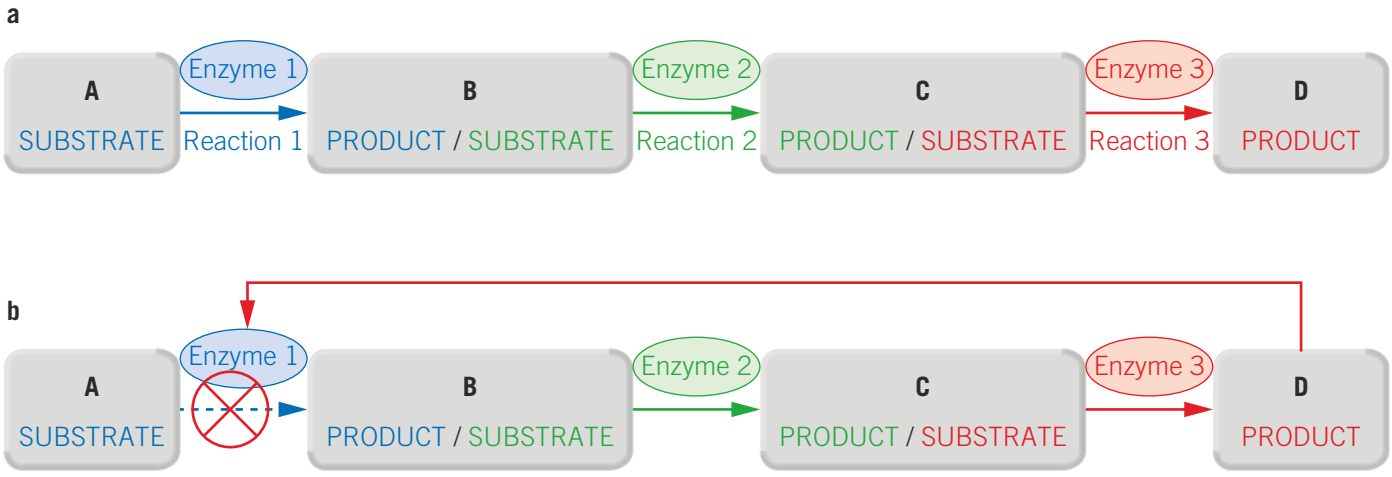


Figure 4A–2 a A general example of a biochemical pathway. **b** A similar pathway, but where the final product inhibits the enzyme that catalyses the first reaction. The colours indicate that molecule B is both the product of enzyme reaction 1 (blue) and the substrate of enzyme reaction 2 (green), and so on.

UNIT 1 LINK

One of the advantages and key features of biochemical pathways is that they are often able to regulate themselves. Generally, this happens through one of the final products (such as molecule D in Figure 4A–2b) inhibiting the function of one of the earlier enzymes in the pathway (such as Enzyme 1 in Figure 4A–2b). This is an example of a negative feedback loop, which you would have learned about in Unit 1. These biochemical pathways and the regulation that keeps them in check are important for a number of reasons. As an example, this mechanism ensures that a cell is only producing molecules that it needs and not one that it already has in sufficient amounts. This makes the processes more efficient and helps save time, energy and resources. This inhibition effect is shown in Figure 4A–2b.

Catalyst
a substance that increases the rate of a reaction by lowering the activation energy and providing an alternative reaction pathway

Activation energy
the minimum amount of energy required for a reaction to proceed

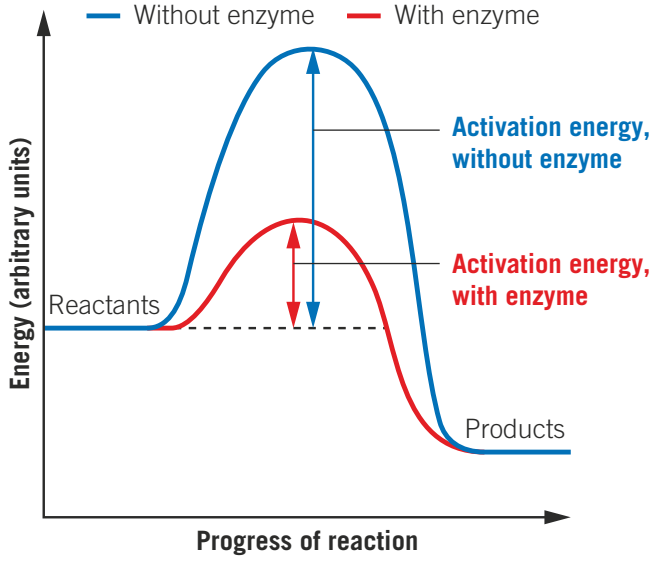


Figure 4A–3 Chemical reactions involve the conversion of reactants into products. For the reaction to occur, it has to overcome an energy threshold, known as the activation energy. This activation energy is decreased in the presence of an enzyme (shown in red).

VIDEO 4A–1 WHAT ARE ENZYMES?

What are enzymes?

Enzymes are a class of proteins and are known as biological **catalysts**. In chemistry and biology, a catalyst is a molecule that increases the rate of a chemical reaction. All chemical reactions have an energy threshold that needs to be overcome in order for the reaction to occur. This threshold is known as the **activation energy** and is shown in Figure 4A–3.

Enzymes speed up or catalyse chemical reactions within organisms by lowering this activation energy (Figure 4A–3). This means less energy is required for the reaction to take place and therefore the reaction can occur more frequently.

In enzyme-mediated reactions, the reactants are referred to as the substrate. For the reaction to occur, the substrate must successfully bind to the enzyme at its **active site**, which will be talked about in depth shortly. The active site allows more successful collisions between substrates in a reaction than would normally occur without the presence of an enzyme.

Active site
the region of an enzyme where the substrate binds for a chemical reaction to take place

Once the substrate has bound to the active site, there are two main categories of reaction that can take place, as shown in Figure 4A–4.

If a larger substrate is broken down into smaller products, the reaction is referred to as **catabolic**. Catabolic reactions release energy, so the products have less energy than the reactants. An example of a biological reaction that is catabolic is cellular respiration, which you will learn more about in Section 5C. On the other hand, if the reaction involves smaller substrates being built up into larger products, the reaction is referred to as **anabolic**. Anabolic reactions require energy, so the energy of the products is greater than the reactants. An example of an anabolic reaction is the formation of a dipeptide from two amino acids, which was discussed in Section 2C.

Catabolic
describes a type of chemical reaction that releases energy and involves breaking down molecules into simpler components

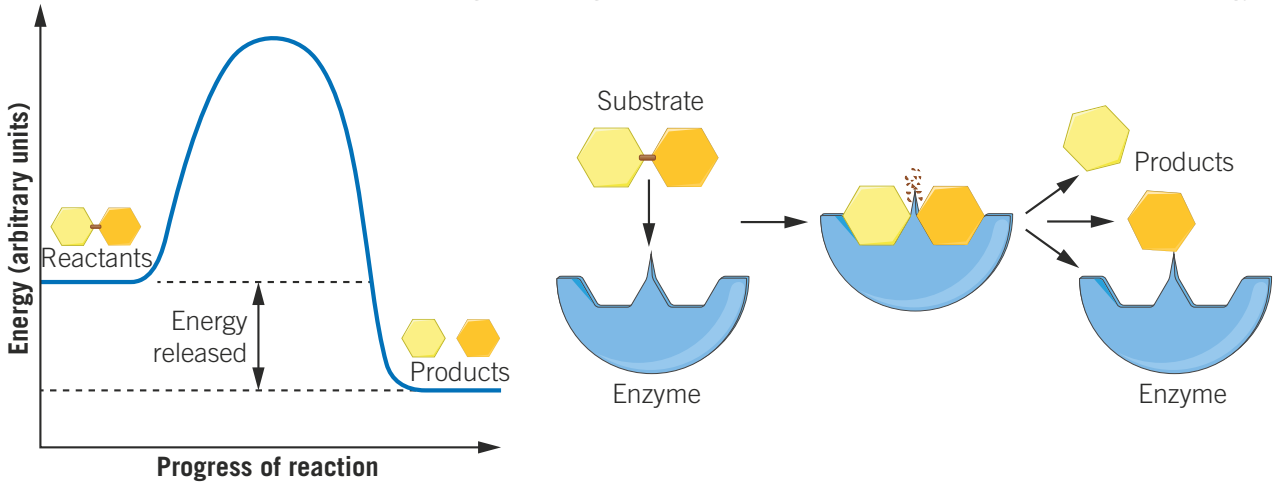
LINK 5C CELLULAR RESPIRATION

LINK 2C PROTEINS

Anabolic
describes a type of chemical reaction that requires energy and involves constructing molecules from simpler components

As you can see in Figure 4A–4, the substrate binds to a specific area on the enzyme. As mentioned previously, this is known as the active site, and its shape and charge are critical for the enzyme to function.

Catabolic reactions: breaking down large molecules into smaller ones with the release of energy



Anabolic reactions: building larger molecules from smaller ones, requiring energy input

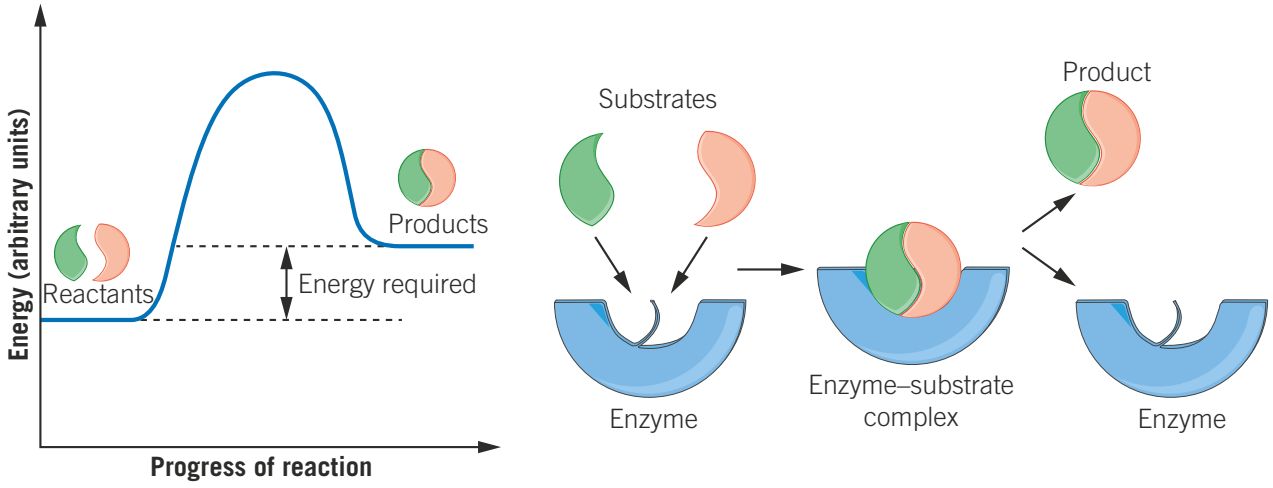


Figure 4A–4 Catabolic reactions involve breaking down molecules and releasing energy. Anabolic reactions involve building up molecules and require energy for this to occur.

Check-in questions – Set 1

1 What are the two main categories of reactions that can be catalysed by enzymes?
2 What is the minimum amount of energy required for a reaction to take place called?
3 Enzymes are a class of what type of biological molecule?
4 Explain how an enzyme can increase the rate of a reaction.

Allosteric site a binding site on an enzyme, where molecules other than the substrate may bind

Structure of enzymes

2C PROTEINS LINK

As you learned in Section 2C, the structure of a protein is critical for it to be able to carry out its function. For enzymes, two main parts of the structure are particularly relevant: the active site and the **allosteric site** (Figure 4A–5).

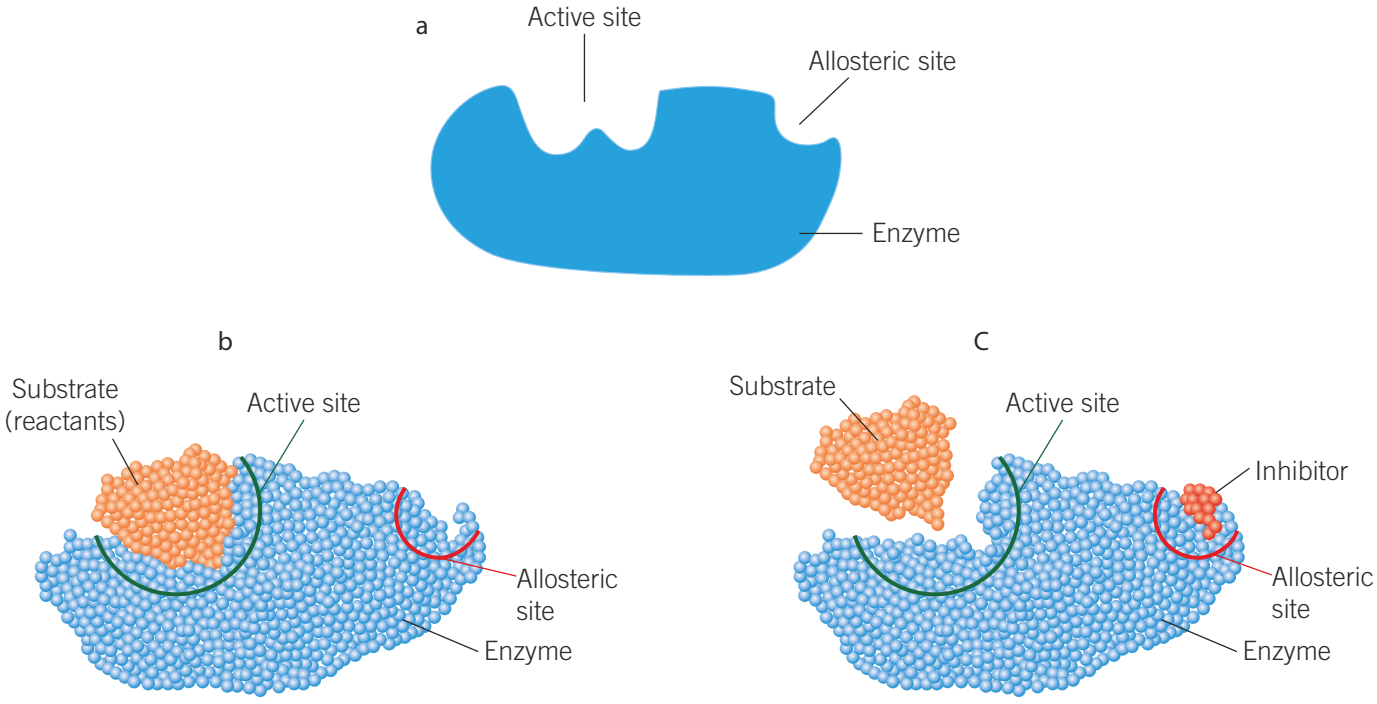


Figure 4A–5 a The generalised structure of an enzyme, showing both the active site and the allosteric site. **b** A 3D structural representation of an enzyme, showing the substrate binding to the active site, which it can do as long as the allosteric site is unoccupied. **c** When an inhibitor (to be discussed in Section 4B) binds to the allosteric site, it changes the shape of the active site so the substrate can no longer bind to it.

4B FACTORS IMPACTING ON ENZYME FUNCTION LINK

The allosteric site is another binding site on an enzyme, but it is not for the substrate to bind to. The allosteric site will be discussed in more detail in Section 4B in relation to enzyme inhibitors.

2C PROTEINS LINK

The active site is the part of an enzyme where the substrate binds. It is important to realise that the shape and charge of the active site are unique to each enzyme and are complementary for the substrate that binds to it. This feature is what provides enzymes with a high level of specificity for their substrate. In Section 2C, we discussed the general structures of amino acids. The R group is the part of the amino acid structure that makes each amino acid unique. These R groups are very important during the interaction of an enzyme with its substrate. The specific amino acid residues found in the active site help determine the specificity of the enzyme, through having R groups that will only interact and bind with the substrate. As the active site is critical for the enzyme to function, we will now discuss the interaction between the enzyme and the substrate in more detail.

WORKSHEET 4A–1 ENZYME FUNCTIONS

Enzyme–substrate interactions

For a reaction to begin, the substrate must first bind to the enzyme. In general, there are two main models used to explain this interaction: the lock-and-key mechanism and the induced-fit model.

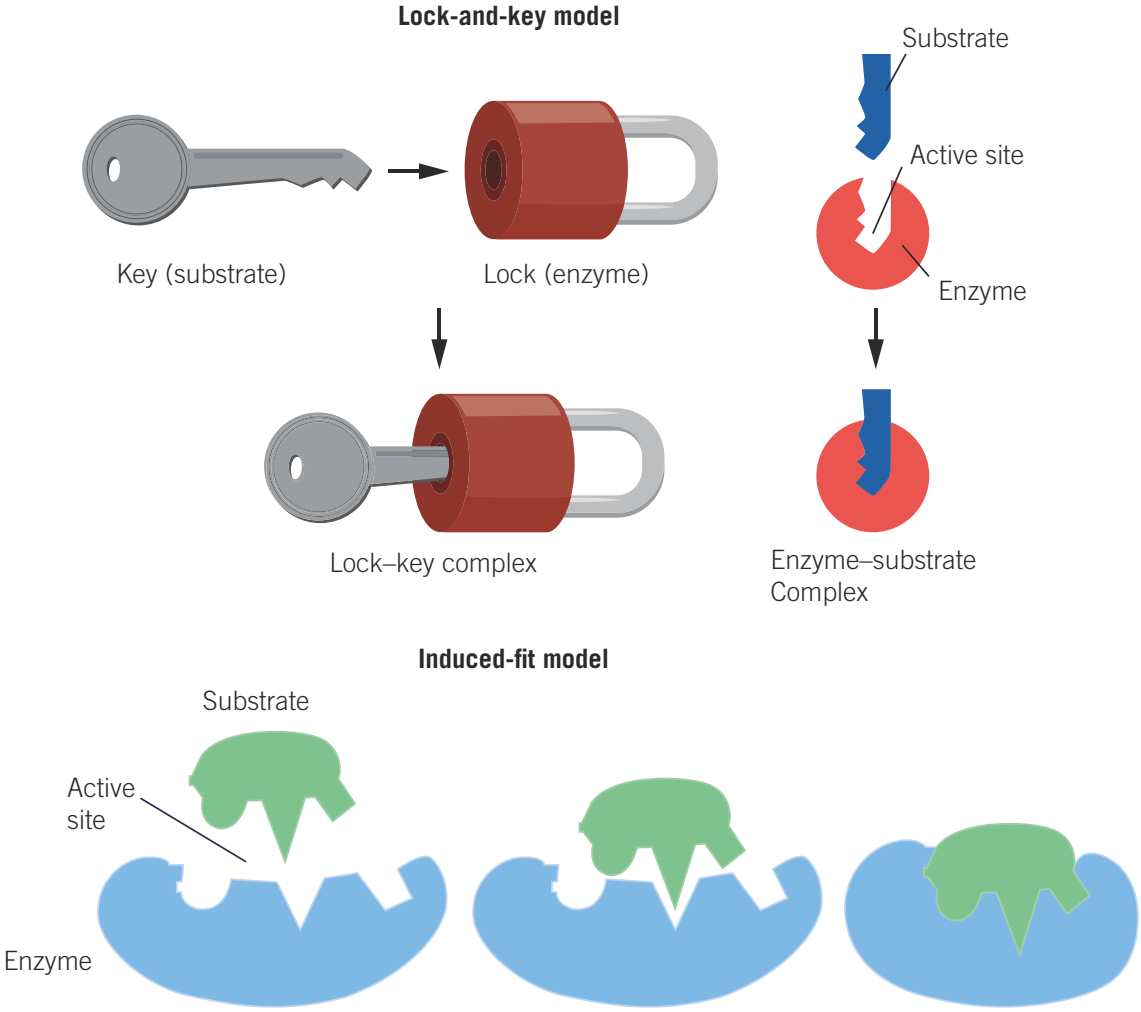


Figure 4A–6 The lock-and-key model (top) and the induced-fit model (bottom) for a substrate binding to an enzyme

The lock-and-key mechanism describes the situation where the shape of the substrate is a perfect fit for the unique shape of the active site of the enzyme. This is illustrated in Figure 4A–6. It can also be seen in this figure that the enzyme will not function unless the correct complementary substrate binds perfectly to the active site. This again highlights the high level of specificity of the enzyme–substrate interaction.

The second mechanism, called the induced-fit model, is very similar to the lock-and-key mechanism, but with one small difference. Initially, the substrate is able to bind to the active site of the enzyme, as the two have complementary shapes and charges. In contrast to the lock-and-key model, the initial conformation of the active site is not a perfect fit for the substrate. However, upon binding of the substrate, the active site of the enzyme is able to alter its shape to mould around the substrate (Figure 4A–6).

Note that there isn't one right or wrong model for the binding of the substrate to an enzyme. In reality, both situations probably occur to some degree within organisms, and different enzymes may use different mechanisms. What is critical is that you understand the overall importance of the complementary nature of an enzyme's active site and its substrate.

Coenzyme
an organic molecule that contains carbon and bind to enzymes to help them to function; examples are NADP, NAD and FAD

The role of coenzymes

Some enzymes require additional help from other molecules to enable them to function optimally. These other molecules are referred to as **coenzymes**, which are organic molecules that contain carbon and hydrogen, which do not get used up as part of the reaction. Examples are NADP, NAD and FAD, all of which will be explored further in Sections 5A and 5C.

Coenzymes can function via two different mechanisms: they can activate the enzyme by interacting with it and altering the shape of the active site; or they can act as electron carriers, taking electrons to the active site and transferring them to the substrate. You will learn more about electron-carrying coenzymes in later chapters, in particular NADP (for photosynthesis), NAD and FAD (for cellular respiration).

5A PHOTOSYNTHESIS LINK

5C CELLULAR RESPIRATION LINK

4A SKILLS

VIDEO 4A–2 SKILLS: CONNECTING CONCEPTS

Connecting concepts

As you will have noticed in this chapter, there is a large overlap with the concepts discussed in Chapter 2, when we talked about genes and proteins. You may have noticed that the nature of protein synthesis is important for the formation of enzymes, which are all proteins. Similarly, you may have realised that the structures of proteins are critical for dictating the shape of the active site, which is very important for how the enzyme functions. In Chapter 9, you will learn about how even minor variations (mutations) in the protein sequence can reduce the activity of an enzyme or, in some cases, cause it to cease functioning altogether.

9A MUTATIONS LINK

Even though you often learn these topics independently, it is important that you are able to connect related concepts. There are a number of approaches you can use to help build a complete understanding, including the ability to identify and explain relationships. These include:

1. *Concept maps.* These are used throughout this textbook, as a way of graphically illustrating the connected nature of the content that you are learning (both within a topic and between topics). Throughout the book there are also markers showing when links between chapters occur. These are designed to help you understand these connections. Developing a large concept map of your own, which can be stuck on your wall, can act as both a reminder of how to join ideas together from different sections of the course and as a checklist for your studies.
2. *Answering connected questions.* It is important to practise answering questions that require you to use your knowledge of multiple areas. You will find questions like this throughout this textbook, particularly at the end of sections, chapters and units. Question 3 at the end of this section is an example.

Section 4A questions

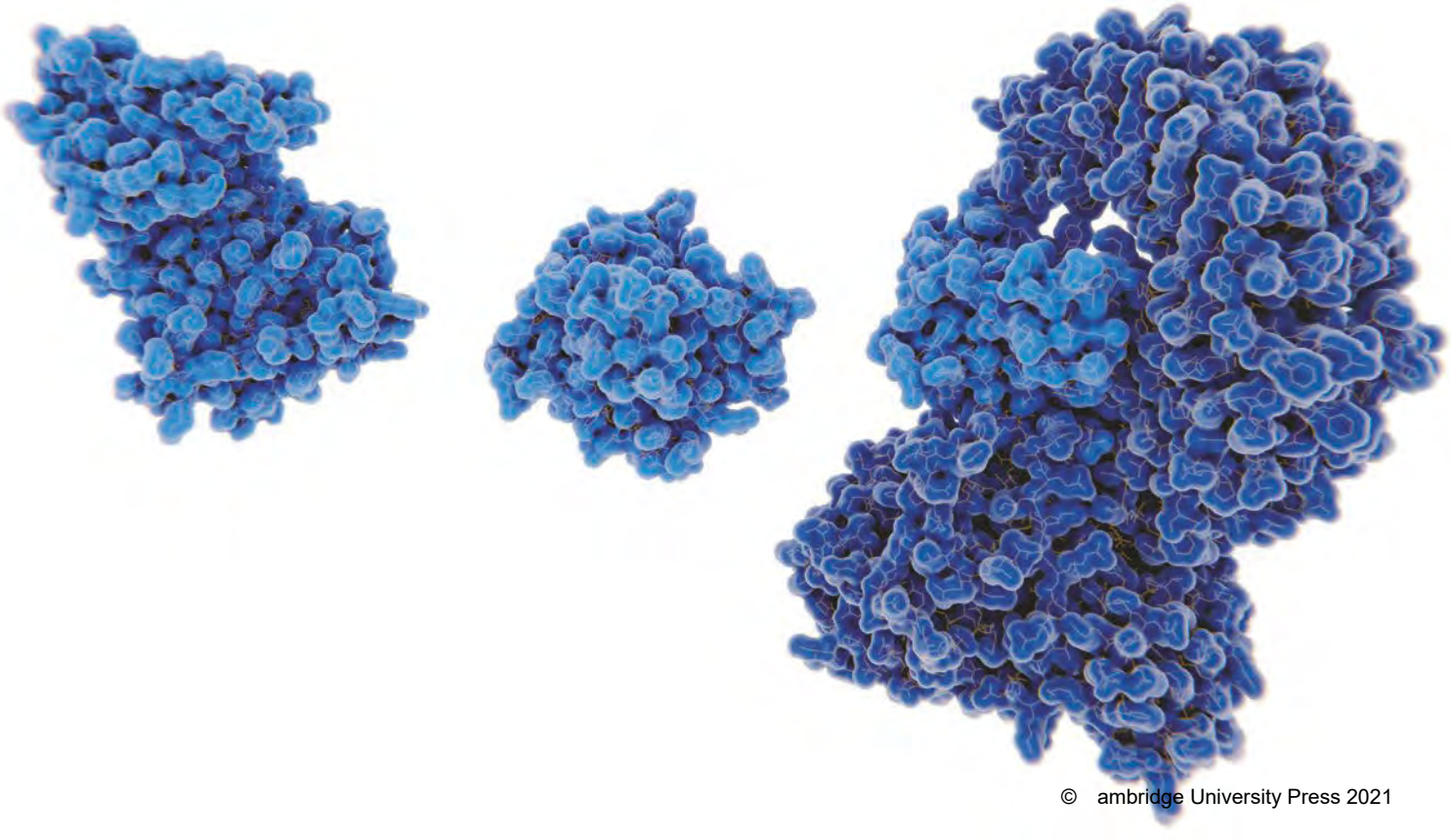
1 A particular biochemical pathway contains five chemical reactions.
   - **a** How many enzymes would you expect to be involved in this pathway?
   - **b** How many substrates and/or products would you expect to be involved in this pathway?
   - **c** Draw a representative image for what this biochemical pathway may look like.

2 In terms of an enzyme:
   - **a** what is the name of the location where a substrate binds?
   - **b** what are the names of the two models for how an enzyme and substrate interact?
   - **c** compare the two models you identified in part **b**.

3 Enzymes are described as having a high level of specificity.
   - **a** Explain what property of enzymes provides them with a high level of specificity.
   - **b** Describe how an enzyme is made by cells.
   - **c** What specific part of the monomers of enzymes gives the active site the specificity for allowing substrates to bind?

4 Some enzymes require help to function.
   - **a** What name is given to a molecule that plays this role?
   - **b** The molecule you named in part **a** helps an enzyme to function in a reaction. Is it able to participate in a subsequent reaction?
   - **c** Name three examples of these molecules that assist enzymes in their role.

5 **a** Describing the change in energy from reactants to products, explain how enzyme reactions can be either catabolic or anabolic.
   - **b** For each type of reaction, draw a simple graph showing the change in energy level.
   - **c** On the graphs you drew for part **b**, label the activation energy for each reaction.
   - **d** Provide one biological example of a catabolic reaction and one example of an anabolic reaction.



Factors impacting on enzyme function

Study Design:
The general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors

Glossary:
Competitive inhibition
Denaturation
Enzyme saturation
Inhibitor
Non-competitive inhibition

3A COMMON DNA TOOLS AND TECHNIQUES LINK

ENGAGE

Some (enzymes) like it hot

In the previous chapter, you learned about the polymerase chain reaction (PCR). This technique is used to amplify DNA, producing many copies of a target sequence, and is regularly employed in areas such as forensic science and paternity testing. The enzyme most commonly used in PCR is called DNA *Taq* polymerase. The word '*Taq*' is derived from the organism that this enzyme was isolated from, *Thermus aquaticus*. *T. aquaticus* is a type of bacterium that can be classed as a thermophile, which means it is capable of surviving at extremely high temperatures. Indeed, *T. aquaticus* was first discovered in hot springs in Yellowstone National Park, USA, and can survive at temperatures of 55–100°C. It is the ability of the *Taq* polymerase enzyme to function at such high temperatures that makes it useful for PCR, which requires significant heat to first separate the two strands of DNA.

However, as you will see in this section, many other enzymes have optimal conditions, outside of which they function slowly, or not at all. Specifically, we will be investigating how factors such as temperature, pH, concentration and inhibition impact on the activity of enzymes.

EXPLAIN

Factors impacting on enzyme function

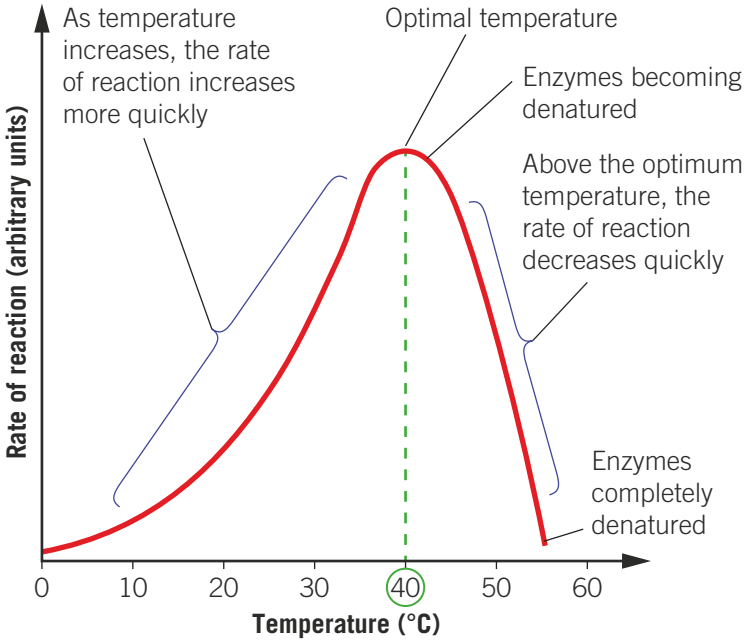


Figure 4B–1 The rate of reaction for a typical human enzyme across a range of temperatures

Temperature

Temperature is one of the most important factors that control the activity of an enzyme. All enzymes have an optimal temperature – that is, the temperature at which they function best. For most enzymes within the human body, it makes sense that this temperature is around 37°C. However, organisms that thrive in hotter environments (such as *Thermus aquaticus*) have enzymes that work optimally at much higher temperatures.

The impact of temperature differs depending on whether it is above or below the optimal temperature. This is highlighted in Figure 4B–1, which shows the rate of reaction for a typical human enzyme across a temperature range.

How can the shape of this curve be explained in terms of what is happening with the enzymes at a molecular level?

Enzyme activity below optimal temperature

Figure 4B–1 shows that at low temperatures the enzyme activity is reduced. As the temperature increases from 0°C towards the optimal temperature, the enzyme activity (and so the rate of reaction) also increases. The explanation for this involves understanding the relationship between temperature and the kinetic energy (related to speed of vibration or other movement) that molecules have. The faster something is moving or vibrating, the more kinetic energy it has. Temperature is a measure of the average kinetic energy of atoms and molecules, and adding heat energy raises their speed, kinetic energy and therefore temperature (Figure 4B–2).

VIDEO 4B–1 FACTORS AFFECTING ENZYME ACTIVITY

WORKSHEET 4B–1 FACTORS AFFECTING ENZYME ACTIVITY

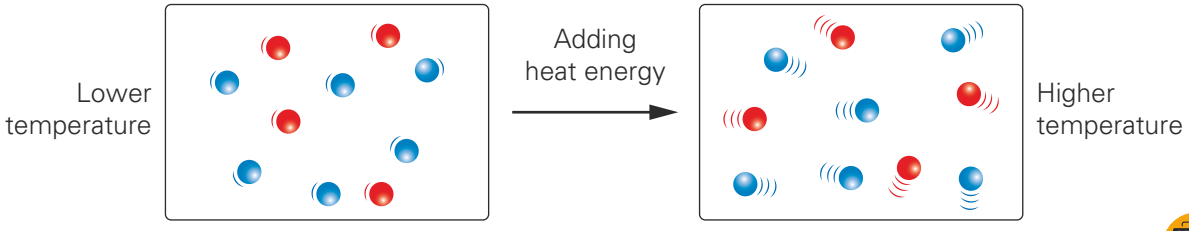


Figure 4B–2 Adding heat energy to a solution leads to increased speed of movement and kinetic energy of particles and higher temperature (or a change of physical state). This helps explain why heating can lead to an increase in enzyme activity, until the optimal temperature is reached.

However, how is the speed at which these molecules are moving around related to the rate at which they function? Imagine that you and a friend are in your Biology classroom, blindfolded and told to walk around for a 10-minute experiment. You are told to keep count of the number of times you collide with each other, as this will represent every time that a substrate and enzyme interact with each other. Now imagine doing this experiment a second time, but instead of walking, you are told to run around as fast as you can.

In which experiment do you think you would bump into each other more often? The most likely answer would be experiment number 2. By thinking about it this way, you can appreciate why an increase in temperature may lead to an increase in enzyme activity.

As discussed in Section 4A, an enzyme requires the binding of a substrate at its active site for it to function. For this to happen, the molecules first need to collide. Importantly, not only do they need to collide, but they need to interact in such a way that the substrate binds successfully with the active site of the enzyme. From this you may realise that, while many things need to go right for an enzyme reaction to occur, the greater the kinetic energy of both the enzyme and the substrate, the more likely it is to occur.

LINK 4A THE ROLE OF ENZYMES

Enzyme activity above the optimal temperature

As can be seen in Figure 4B–1, when the temperature exceeds the optimal temperature, there is a sharp decrease in the rate of reaction. To understand what is happening under these conditions, it is important to remember this important fact: enzymes are proteins. In Section 2C, the role of proteins and the importance of their structure was discussed.

LINK 2C PROTEINS

Denaturation the process by which a protein loses its 3D conformational structure through breaking of hydrogen bonds, caused by an external stress such as temperature or pH

At higher temperatures, the hydrogen bonds and interactions that form the tertiary structure of a protein begin to break down due to increasing vibrations in the molecules. When this occurs, we say that the enzyme is being denatured and the process is called **denaturation**. This process is permanent, so once an enzyme is denatured it will not function properly again. So why does this process have such a profound impact on the activity of an enzyme? It is easiest to illustrate this by looking at what happens in a series of steps:

1 At the optimal temperature, binding of the substrate occurs normally.
2 As the temperature increases beyond the optimum, the enzyme's 3D conformational structure changes.
3 The specific structure of the active site begins to disappear, which means that the substrate is no longer complementary and cannot bind.
4 Enzyme activity becomes zero, as substrate interaction is no longer possible.

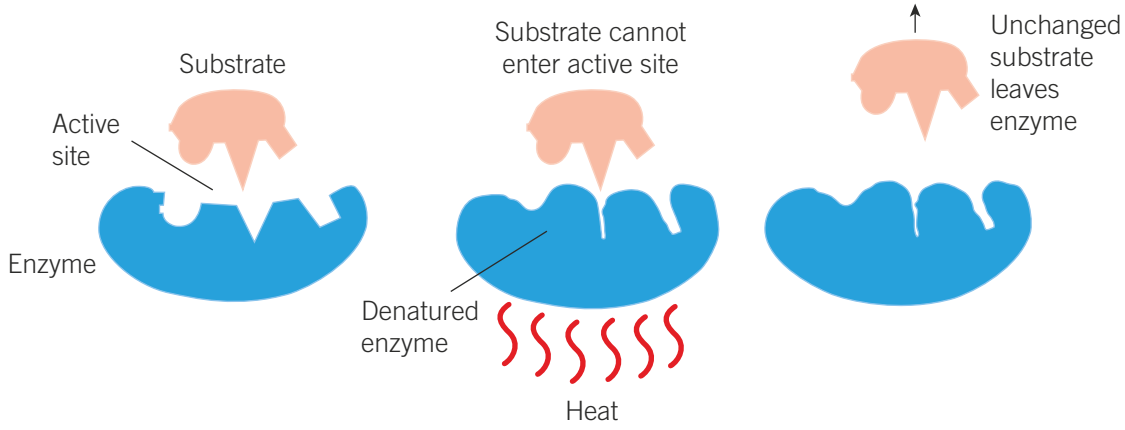


Figure 4B–3 An increase in temperature denatures the enzyme, which leads to a change in the conformation of the active site, so the substrate is unable to bind and the enzyme is unable to function.

Check-in questions – Set 1

1 What level of protein structure determines the shape of the active site of an enzyme?
2 What is the general name for a molecule that binds to the active site of an enzyme? What properties of the active site allow this molecule to bind to it?
3 What is the name of the process whereby a protein permanently loses its 3D conformational structure?
4 What is the main reason that denaturation leads to a loss of enzyme function?
5 Why does enzyme function increase when temperature goes from low to optimal?

pH

The pH gives an indication of how acidic or basic a solution is. A pH of less than 7 is acidic, 7 is neutral and greater than 7 is basic. pH is measured on a scale between 0 and 14 – values closer to 0 are highly acidic, and closer to 14 are highly basic (Figure 4B–4).

The pH of various environments within an organism are tightly regulated through homeostatic mechanisms. As an example, in humans:

- blood pH ~ 7.4
- stomach pH ~ 2.5
- saliva pH ~ 7.0
- bile pH ~ 7.5.

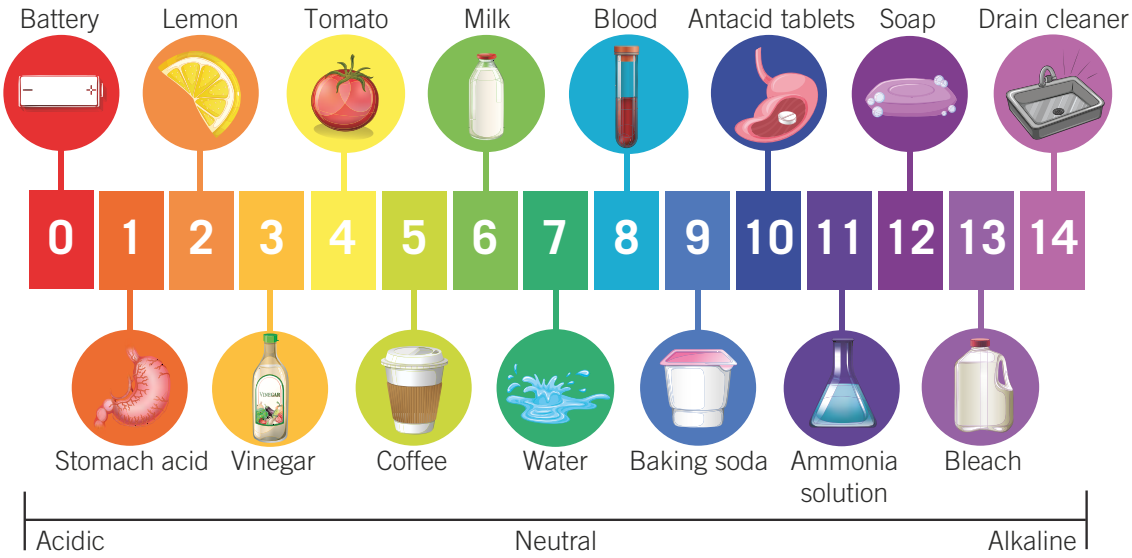


Figure 4B–4 The pH scale runs from 0 (highly acidic, such as a car battery) to 14 (highly basic, such as drain cleaner).

pH is another factor that can affect the function of an enzyme. Figure 4B–5 shows a summary of how a number of enzymes in the body function at different pH levels.

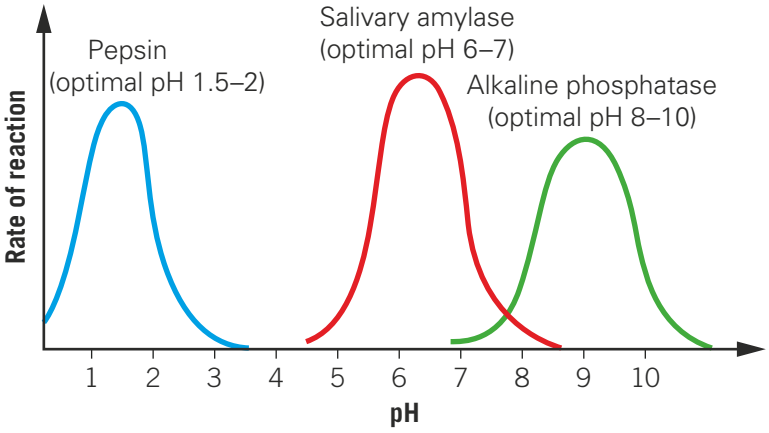


Figure 4B–5 The effect of pH on the rate of reactions catalysed by different enzymes within the human body

Two important points are demonstrated by Figure 4B–5. First, different enzymes within the body function at different optimal pH values. As mentioned previously, the pH values of different environments vary greatly within organisms. Therefore, it is not surprising that enzymes have an optimal pH that suits the environment that they function in. For example, pepsin (an enzyme found in the stomach) has an optimal pH of 1.5–2, which suits the acidic nature of gastric juices. In contrast, salivary amylase works best at a pH of around 6.5, which is appropriate for the neutral pH environment of the mouth.

Second, the rate of reaction on either side of the optimal pH changes quickly. At pH values that are either too acidic or too basic (i.e. below or above the optimal pH), the rate of reaction or enzyme activity level drops quickly. This is due to the fact that in these unsuitable pH conditions, the enzyme begins to denature. This process is the same as for the loss of enzyme function at temperatures above optimal, but in this case the cause of denaturation is a pH that is either too high or too low. Therefore, the steps outlined earlier become as follows:

1 At the optimal pH, binding of the substrate occurs normally.
2 As pH changes to below or above the optimum (becomes too acidic or too basic), the enzyme's 3D conformational shape changes.
3 The specific structure of the active site begins to disappear, which means that the substrate is no longer complementary and no longer binds
4 Enzyme activity becomes zero, as substrate interaction is no longer possible.

This illustrates that having a standard, structured answer can be really helpful in describing common processes. You will find a similar strategy useful in other topics, such as when describing the process of natural selection.

LINK 9B EVOLVING AND NON-EVOLVING POPULATIONS

As well as altering the shape of the active site, changes in pH can also impact on the charge of the active site. The substrate and the active site can bind through the attraction of opposite charges (positive and negative). However, if the pH causes these charges to change, that attraction can decrease or actually lead to the substrate being repelled from the active site. This is another mechanism through which changes in pH away from the optimum can cause a decrease in enzyme activity.

Concentration

As you have seen previously, the activity of an enzyme requires both the enzyme and a substrate. Therefore, the concentration of each of these components affects the overall activity. Let's look at each of these situations separately.

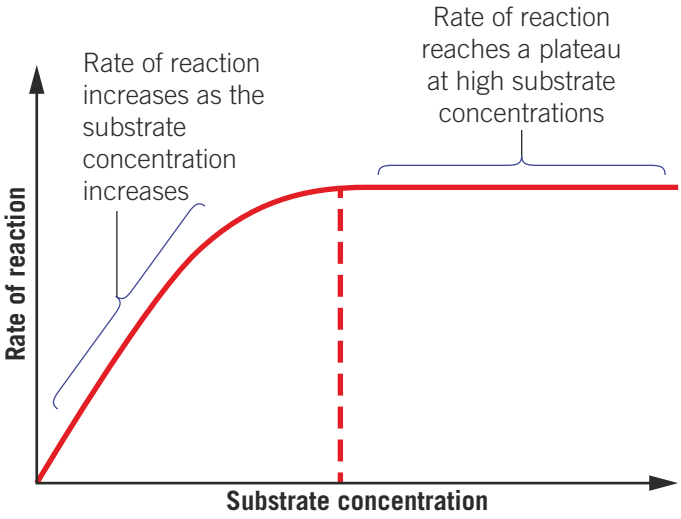


Figure 4B–6 The effect of substrate concentration on the rate of a reaction catalysed by an enzyme

Substrate concentration

The effect of substrate concentration can be seen in Figure 4B–6. In this scenario, assume there is a fixed (limited) number of enzymes available to use in the reaction.

The initial part of this graph indicates that adding more substrate results in an increased rate of reaction. However, at a certain substrate concentration (shown by the dotted line), the curve begins to flatten out. Why do you think this might be?

This can be understood by considering the situation shown in Figure 4B–8. Increasing the substrate concentration increases the rate of reaction until a certain point, but then flattens out or plateaus. At this point, every available enzyme has substrate bound to the active site. This is known as **enzyme saturation**. Adding more substrate at this point (just like adding more than ten students to the room) does nothing to increase the rate of the reaction, as there is nowhere for this excess substrate to bind to.

Enzyme saturation the point at which the rate of reaction reaches a maximum, with no further increase at a specific enzyme concentration

Enzyme concentration

The rates of reactions during photosynthesis and cellular respiration, processes that will be explored in Sections 5B and 5D, can also be affected by altering the enzyme concentration. In organisms, the amount or expression levels of a protein within a cell can be raised or lowered through gene regulation, as you learned in Section 2D.

Figure 4B–7 shows two possible outcomes of changing enzyme concentrations. Initially, it may seem confusing as to how we could obtain two very different graphs.

5B FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS LINK

5D FACTORS AFFECTING THE RATE OF CELLULAR RESPIRATION LINK

2D GENE STRUCTURE AND EXPRESSION LINK

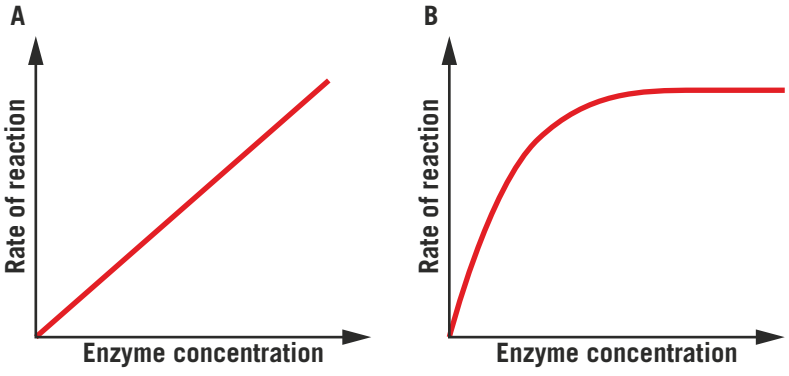
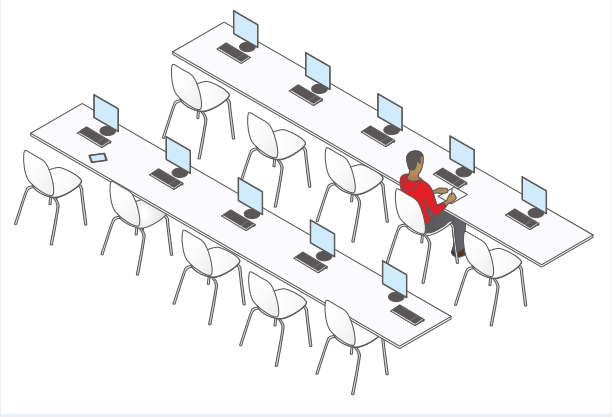
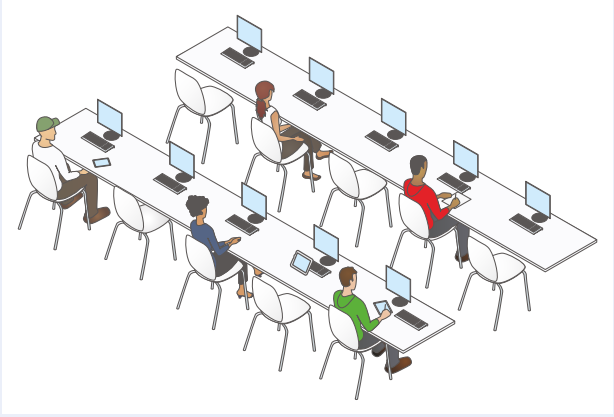


Figure 4B–7 The effect of enzyme concentration on rate of reaction (enzyme function), in the case of unlimited amounts of substrate (graph A) and limiting amounts of substrate (graph B)

1 Imagine a room contains 10 computers. In this room, a single student is working on one of the computers, attempting to type out a complete copy of this textbook. You can imagine that this would be a slow task!



2 If another four students are added to the room, working on four other computers, and each typing a different section, the process would begin to speed up.



3 Taking this even further, each of these students is then encouraged to call a friend and ask them to join in the project. Once they arrived, there would be ten students typing different sections on the ten available computers.

4 But what would happen if they all then invited another friend along to help? There are now twenty people in the room. Would the process speed up even further?

5 The answer to the question in step 4 is no. Before the additional students arrived at step 4, the textbook copying was already happening as fast as it possibly could, because there were students typing at every available computer. At this point, adding more students to the room is ineffective, as there are no more computers for them to work on.

Figure 4B–8 An analogy to explain the principle of enzyme saturation. The computers represent enzyme molecules, and the students represent substrate molecules.

To explain how the two graphs in Figure 4B–7 can be so different, consider Figure 4B–8 where the computers are enzymes and the students are substrates. In the final panel of Figure 4B–8, there were 20 students, but only 10 computers. In this situation, if the number of computers were increased to 20, this would also increase the rate at which the task of typing out the textbook was completed (since each person is working on a different section). The process could continue to be accelerated by adding more and more computers to the room. However, this would only work if there was also an endless supply of students to type on them.

This scenario is represented by graph A in Figure 4B–7, which shows that as enzyme concentration increases, so does the rate of reaction, in a linear fashion. In this instance, we have to assume that there is an unlimited supply of substrate. As this is not often the case in biological processes, such as photosynthesis and cellular respiration, the second scenario (graph B in Figure 4B–7) is more likely. In this situation, increasing the enzyme concentration leads to a faster rate of reaction up to a certain point, and then the curve plateaus. At this point, the substrate concentration has become limiting. This is the same as for substrate concentration; however, in that instance it was the enzyme concentration that was the limiting factor.

Throughout this chapter, graphs have been used to illustrate enzyme function. In all these graphs, the data has been presented using 'Rate of reaction' on the *y*-axis. However, it is important to note that you may be shown data that has the *y*-axis labelled differently. A common alternative is to show the amount of a product generated (e.g. volume of gas produced (mL)) over time for multiple reaction conditions (e.g. different temperatures). In this instance, you need to make the connection that the faster a product is generated, the higher the enzyme activity and the higher the rate of the reaction.

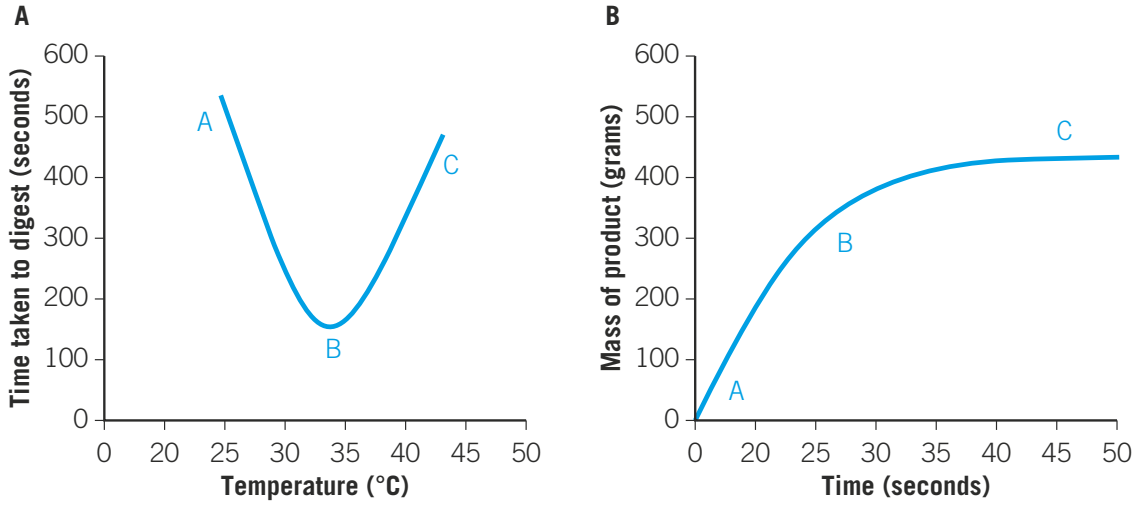


Figure 4B–9 Interpreting rate of reaction in graphs with different *y*-axes.

A The time taken for an enzyme-catalysed digestion reaction at different temperatures. Because time taken to digest is on the *y*-axis, the rate of reaction increases from point A to B where it reaches a maximum (shortest time) and then the rate of reaction decreases from B to C.

B A graph of mass of product over time. Here the rate of reaction is represented by the slope of the graph – the steeper the slope, the higher the rate. The rate starts uniformly, then decreases before it gets to B, shown by decreasing slope. The reaction continues to slow and by point C it has virtually stopped (no more product being formed).

Check-in questions – Set 2

1 What is the term for the situation in which all enzymes have substrate bound to their active site?
2 How does pH affect enzyme function differently to temperature?
3 Why is it important that different enzymes have different optimal pH values?

Enzyme inhibition

Almost all biological processes in the body need to be regulated, and enzyme function is no exception. An important mechanism to regulate the function of an enzyme is to inhibit its activity. There are many ways in which inhibition can happen, but there are two in particular that you need to know for this course: **competitive inhibition** and **non-competitive inhibition**.

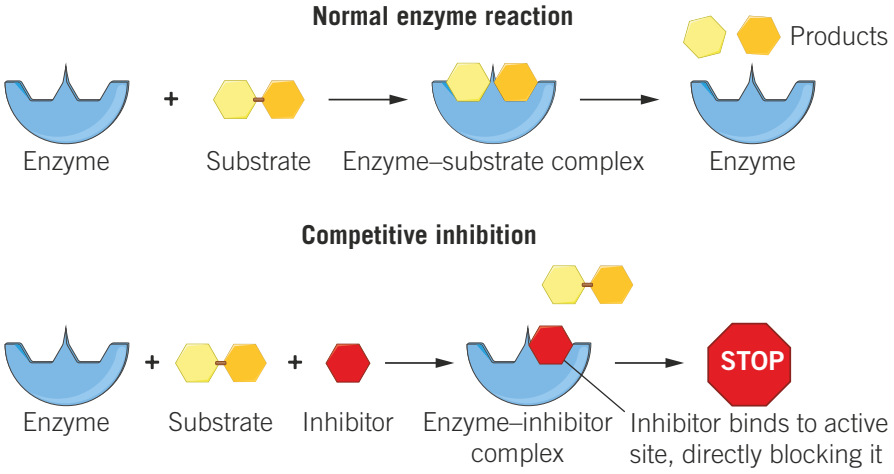


Figure 4B–10 The normal function of an enzyme can be blocked through competitive inhibition, where an inhibitor interacts with the active site, blocking the substrate from binding.

Competitive inhibition

As you will remember from Section 4A, an enzyme's active site is critical to its ability to function. The active site is the location where the substrate binds to the enzyme, allowing the reaction to take place (Figure 4B–10). In the previous section, you learnt how this mechanism is often described as being like a lock and a key. If this analogy is taken further, imagine that a person returned home one day and found that someone had jammed something into the lock of their front door. In this case, the key would no longer be able to fit and the person wouldn't be able to unlock and open their door.

The same principle governs competitive inhibition. In competitive inhibition, an **inhibitor** binds to the active site of the enzyme instead of the substrate. It is able to do this because it has a shape and charge that mimic the substrate (or a complementary shape and charge to the active site), thus fitting the active site (Figure 4B–10). In this situation, the inhibitor and the substrate compete to bind to the active site, which is why this process is referred to as competitive inhibition. With the inhibitor bound, the substrate is unable to access the active site and the enzyme can't function.

Non-competitive inhibition

Non-competitive inhibition has some similarities to competitive inhibition. Both result in the substrate being unable to bind to the active site and, therefore, both also prevent enzymes from functioning to their full potential. However, non-competitive inhibition differs from competitive inhibition in one key way: the part of the enzyme that the inhibitor binds to. As the name suggests, non-competitive inhibition does not involve the inhibitor competing with the substrate for binding to the active site. Instead, a non-competitive inhibitor binds to the allosteric site. When the inhibitor attaches to the allosteric site, it causes the active site to change shape (Figure 4B–11). As you will remember from Section 4A, the interaction between the active site and a substrate is highly specific. Once the active site changes shape through the action of the non-competitive inhibitor, the substrate is no longer able to fit within the active site and, in this way, the enzyme activity is decreased.

It is possible to distinguish between these two types of inhibition by analysing what happens to the activity of an enzyme when the substrate concentration is increased (Figure 4B–12). In the presence of a competitive inhibitor, the rate of reaction can be restored by simply increasing the substrate concentration.

LINK 4A THE ROLE OF ENZYMES

Competitive inhibition the process of disrupting the function of an enzyme by blocking its active site with a molecule other than the substrate

Non-competitive inhibition the process of disrupting the function of an enzyme through a molecule binding to another site on the enzyme, which alters the shape of the active site in such a way that the substrate cannot bind

Inhibitor a molecule that is involved in disrupting the function of an enzyme, either directly (competitive) or indirectly (non-competitive)

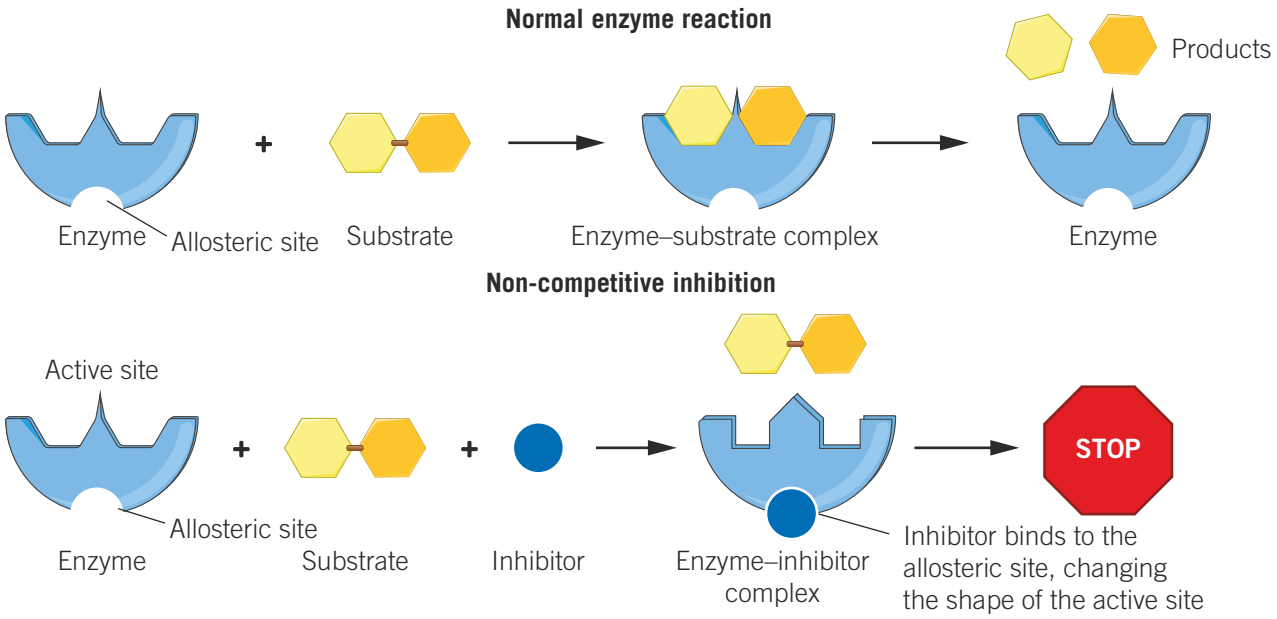


Figure 4B–11 The normal function of an enzyme can be blocked through non-competitive inhibition, where an inhibitor binds to the allosteric site, resulting in a change in the conformation of the active site. Not all enzymes have an allosteric site, but if they have, and if an inhibitor attaches to it, then it causes the active site to change shape.

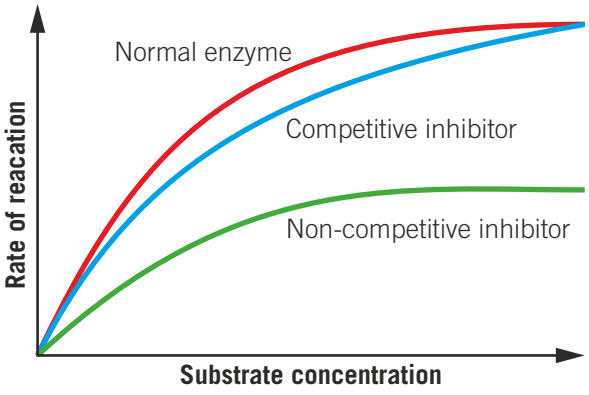


Figure 4B–12 Increasing substrate concentration can distinguish between competitive and non-competitive inhibition.

This is possible because the substrate and the competitive inhibitor are both attempting to bind to the active site of the enzyme. Therefore, adding more substrate to the reaction allows it to 'outcompete' the inhibitor and preferentially bind to the active site of the enzyme. The same thing is not true for a non-competitive inhibitor. Remember in this instance the non-competitive inhibitor is binding to the allosteric site, not the active site, but the 3D conformational shape of the active site is altered. Therefore, increasing the substrate concentration has little effect on the rate of reaction, as the substrate will be unable to bind to the altered active site.

4B SKILLS

VIDEO 4B–2 SKILLS: DATA ANALYSIS

Data analysis

You have seen throughout this chapter that graphs are commonly used to assess the impact of different factors on enzyme activity and function. Using graphs to help illustrate your answers and analysing data to form conclusions are vital skills in your understanding of this material. Let's look at examples of both.

Example 1: Using a graph to help illustrate an answer

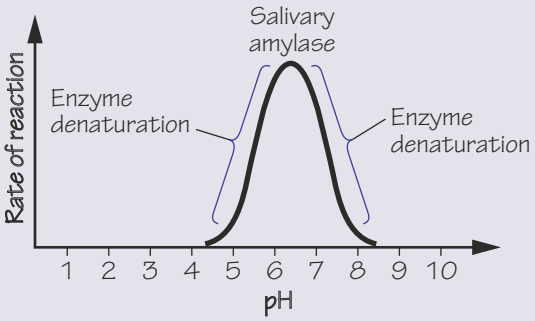
Question:

Explain the effect that pH has on the function of an enzyme.

Strategy: In these situations, it is important to annotate your graph to highlight the key points that you are referring to in the text. For example, as well as having the optimal pH indicated, you could also have text on either side of this saying 'enzyme denaturation'.

Sample response:

The effect of pH on the function of an enzyme is illustrated in the diagram on the right.



It can be seen from this graph that this enzyme has an optimal pH of around 6.5. However, on either side of the optimal pH (too acidic or too basic), the enzyme activity decreases quickly. This is because the enzyme denatures, which means that it loses its 3D conformational structure. As the 3D structure changes due to hydrogen bonds breaking, so does the active site, which means the substrate can no longer bind to the enzyme. As the substrate can't bind, the enzyme can no longer function.

Example 2: Analysing data to form conclusions

Question:

The data in the table is from an experiment measuring the rate of a reaction at different temperatures. The reaction is catalysed by an enzyme and produces carbon dioxide. The reaction was allowed to proceed for five minutes and the data was recorded. Using your knowledge of enzymes, explain the observations of this experiment.

Temperature (°C)	Volume of CO_2 produced (mL)
30	22
35	25
40	22
45	10
50	1

Strategy: The first step in answering this question is to isolate what you are being asked. The question indicates that this is an enzyme-catalysed reaction and that you are observing the effect of temperature. As carbon dioxide is a product of the reaction, you can assume that the greater the volume of carbon dioxide being produced, the higher the rate of reaction, or that the enzyme activity levels are higher. It is important that you answer this question in two parts. First, clearly state the results that are observed, and then explain these results using your knowledge of enzyme theory.

Sample answer:

The data from this experiment indicates that the enzyme has an optimal temperature of approximately 35°C, as this is the temperature at which the highest volume (25 mL) of carbon dioxide is produced. At temperatures higher than 35°C, the amount of carbon dioxide produced decreases greatly. At 50°C, almost no carbon dioxide is produced. This indicates that, at temperatures greater than 35°C, the enzyme begins to denature. During this process, the enzyme loses its 3D conformational structure and so also loses the structure of its active site. Once this happens, the substrate will no longer be able to bind to the enzyme. As the substrate cannot bind to the enzyme, the reaction barely occurs, which explains why almost no carbon dioxide is produced at 50°C.

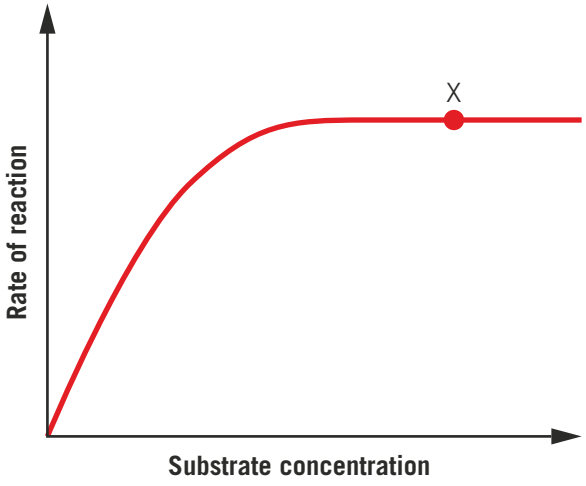
It is critically important that you pay attention to the labelling of graph axes. This will have been discussed before in terms of drawing your own graph, but it is just as important when you are analysing data presented to you in a graphical format. As an example that you will have come across in this chapter, you can see that the *y*-axis is sometimes labelled 'Rate of reaction', and sometimes as 'Amount of product formed'. While both labels are indicators of enzyme activity, you need to ensure that you answer the question by referring to the specific information given.

Section 4B questions

1 Explain the similarities and differences between competitive and non-competitive inhibition.

2 a What type of biomacromolecule are enzymes classified as?
b What is the term for when a protein loses its specific 3D conformational shape?
c Explain what happens to the activity of an enzyme at different pH values.
d Pepsin, a digestive enzyme found in the stomach, is placed in a solution at a pH of 3. What would you expect to happen to the enzyme activity?

3 a Define enzyme saturation.
b Looking at the graph on the right, indicate what would happen at point X if the following changes were made:
i More substrate is added.
ii More enzyme is added.



4 The table below shows data from an experiment that investigated the activity of an enzyme at different temperatures. The reaction led to the solution going from colourless to blue. The intensity of the blue colour was recorded on a scale from + (least intense) to +++++ (most intense).

Temperature (°C)	Colour intensity
35	++++
40	++
45	+
50	Colourless

a Using this data, draw a graph showing relative enzyme activity versus temperature.
b Based on the available data, at which temperature is the most product being produced? Is this the optimal temperature? Explain why or why not.
c Using this data, describe what is happening in the last two experiments, at 45°C and 50°C.
d What could the experimenter change in the set-up of their investigation to get more reliable results?

5 Scientists have discovered a novel bacteria that causes a gastric (stomach) disease in humans. By studying this bacteria further, they identified an enzyme that is critical for the bacteria to be able to survive within the highly acidic environment that they live in.
a Describe two avenues that these researchers could develop that may lead to an effective treatment for this disease.
b After months of trying, the scientists succeed at developing a therapy. They test it in experiments using cells (pH = 7) and get stunning results. However, when they trial it in humans with the disease, the therapy fails completely. What might be a potential reason for this?

Chapter 4 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
4A.1	Define biochemical pathway	12☐
4A.2	Outline the difference between a reactant (substrate) and a product	10☐, 11☐
4A.3	Explain how a pathway results from the product of one reaction being the reactant (substrate) in a subsequent reaction	2☐
4A.4	Describe the lock-and-key and induced-fit models of enzyme function	8☐
4A.5	Recall the importance of the active site in explaining the specificity of enzymes	5☐
4A.6	Outline some of the key features of enzymes	1☐
4A.7	Describe the role of coenzymes in enzyme function	6☐
4B.1	Explain the effect of temperature on the rate of enzyme activity, referring to the kinetic energy of molecules	7☐
4B.2	Understand the difference between denatured and inactive in relation to enzymes	12☐
4B.3	Explain the effect of pH on the rate of enzyme activity	11☐
4B.4	Explain the effect of enzyme and substrate concentration on the rate of enzyme activity	3☐, 11☐
4B.5	Define competitive and non-competitive inhibition	4☐
4B.6	Explain the effect of competitive and non-competitive inhibition on the rate of enzyme activity	9☐
4B.7	Describe how competitive and non-competitive inhibition can be used in rational drug design	12☐

Multiple-choice questions

1 What is the name of the part of an enzyme where the substrate binds?

A active site
B allosteric site
C tertiary structure
D activation site

2 A biochemical pathway comprising eight substances will likely involve how many enzymes?

A six
B seven
C eight
D nine

3 You are performing an experiment where you measure the activity of an enzyme as you increase the concentration of the substrate. At a certain concentration, you notice that the activity is staying constant and no longer increasing. What is the most likely explanation for this?
A The enzyme has been denatured.
B The enzyme is being inhibited competitively.
C The enzyme is being inhibited non-competitively.
D The enzyme has become saturated.

4 Having a molecule that is the same shape as the substrate blocking the active site is an example of
A activation.
B denaturation.
C competitive inhibition.
D non-competitive inhibition.

5 When discussing enzymes, the term 'specificity' refers to the fact that enzymes
A always have 100% activity.
B are able to catalyse many different reactions.
C have one substrate that can bind to them.
D only work under certain conditions.

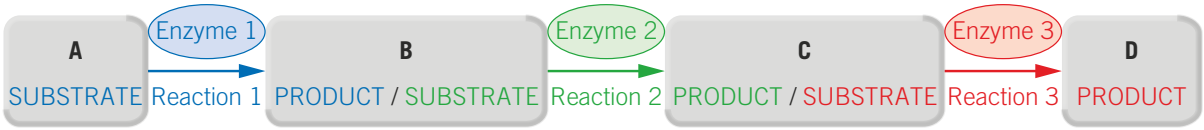
6 An organic molecule that is required by some enzymes to function is called
A a coenzyme.
B an inhibitor.
C a product.
D a denaturant.

7 The reason why high temperatures can reduce enzyme activity is that
A the molecules have increased kinetic energy.
B there are more interactions between the enzyme and the substrate.
C it increases the activation energy of the reaction.
D it causes denaturation of the enzyme.

8 When the active site of an enzyme changes shape after binding to the substrate, this is known as
A the lock-and-key model.
B the induced-fit model.
C a biochemical pathway.
D denaturation.

9 A competitive inhibitor will lead to
A no change in enzyme activity.
B increased enzyme activity.
C decreased enzyme activity.
D denaturation of the enzyme.

10 Looking at the biochemical pathway in the image below, which molecule represents the substrate of reaction 3?



A B
B C
C D
D enzyme 3

Short-answer questions

11 Catalase is an enzyme that breaks down the chemical compound hydrogen peroxide (H_2O_2) into oxygen (O_2) and water (H_2O). The rate of the reaction can be measured by analysing the production of oxygen bubbles in solution.

a Is this reaction catabolic or anabolic? (1 mark)

b What is/are the substrate(s) in this reaction? What is/are the product(s)? (2 marks)

c You want to determine the concentration of hydrogen peroxide necessary for maximum enzyme activity for the amount of catalase you have available (that is, the amount of catalase stays constant). You perform an experiment to test this using 10 concentrations of hydrogen peroxide ranging from 0% to 20% (volume/volume). At the end of the experiment, you have determined the minimum concentration of hydrogen peroxide necessary for maximum activity of the catalase available.
Draw a graph predicting the results for this experiment. You do not need to mark scales on the axes or to show data points, just show the expected shape of the curve or line, remembering to label axes. (3 marks)

d What needed to happen in your experiment to enable you to work out the maximum enzyme activity? Mark this point as Point X on your graph from part **c**. Using your knowledge of enzymes, and reactions between enzymes and substrates, explain what is occurring before Point X and after Point X on your graph. (4 marks)

e Catalase works at an optimum pH of 7. Explain what would happen to the activity of catalase if the pH of the solution in the experiment was lowered to 3. Your response should specifically refer to the different levels of protein structure. (3 marks)

12 Acetylcholinesterase is an enzyme involved in biochemical pathways in the nervous system.

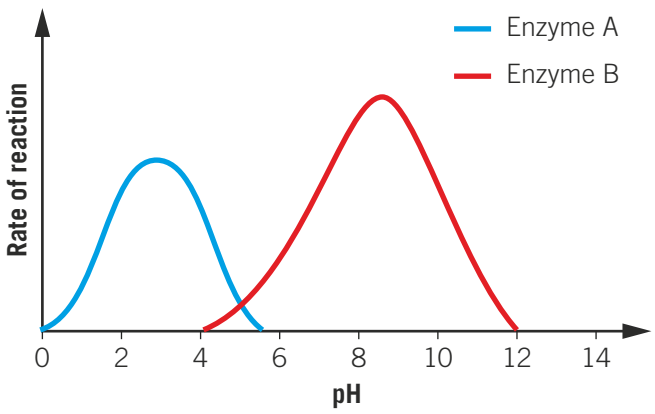
a Define biochemical pathway. (1 mark)

b Acetylcholinesterase is the target of many pesticides, as inhibiting it causes a build-up of neurotransmitters that leads to the death of the organism. Explain two approaches that could be used to develop a pesticide that is effective against acetylcholinesterase. (3 marks)

c Is the example described in part **b** an example of enzyme inactivation or enzyme denaturation? Describe the similarities and differences between these two outcomes. (3 marks)

d Two new insecticides are developed: one uses a competitive inhibitor and one uses a non-competitive inhibitor of an enzyme that is critical for the survival of spiders. In the development of the product, experiments are conducted in which the function of the inhibitors is tested against increasing concentrations of the natural substrate of the enzyme. Based on this test alone, which would consistently produce a more significant decrease in enzyme activity and why? (3 marks)

13 The following questions all relate to the graph on the right, which shows the activity of two enzymes.



a Will both enzymes display activity at a pH of 5? Explain. (2 marks)

b Describe the difference between enzymes A and B at a pH of 6. (2 marks)

c What are the optimal pH values for enzyme A and enzyme B? (1 mark)

d Give a biological reason why you would get different answers to part **c** for enzymes A and B. (3 marks)

UNIT 3

HOW DO CELLS MAINTAIN LIFE?

CHAPTER 5

BIOCHEMICAL PATHWAYS: PHOTOSYNTHESIS AND CELLULAR RESPIRATION

Introduction

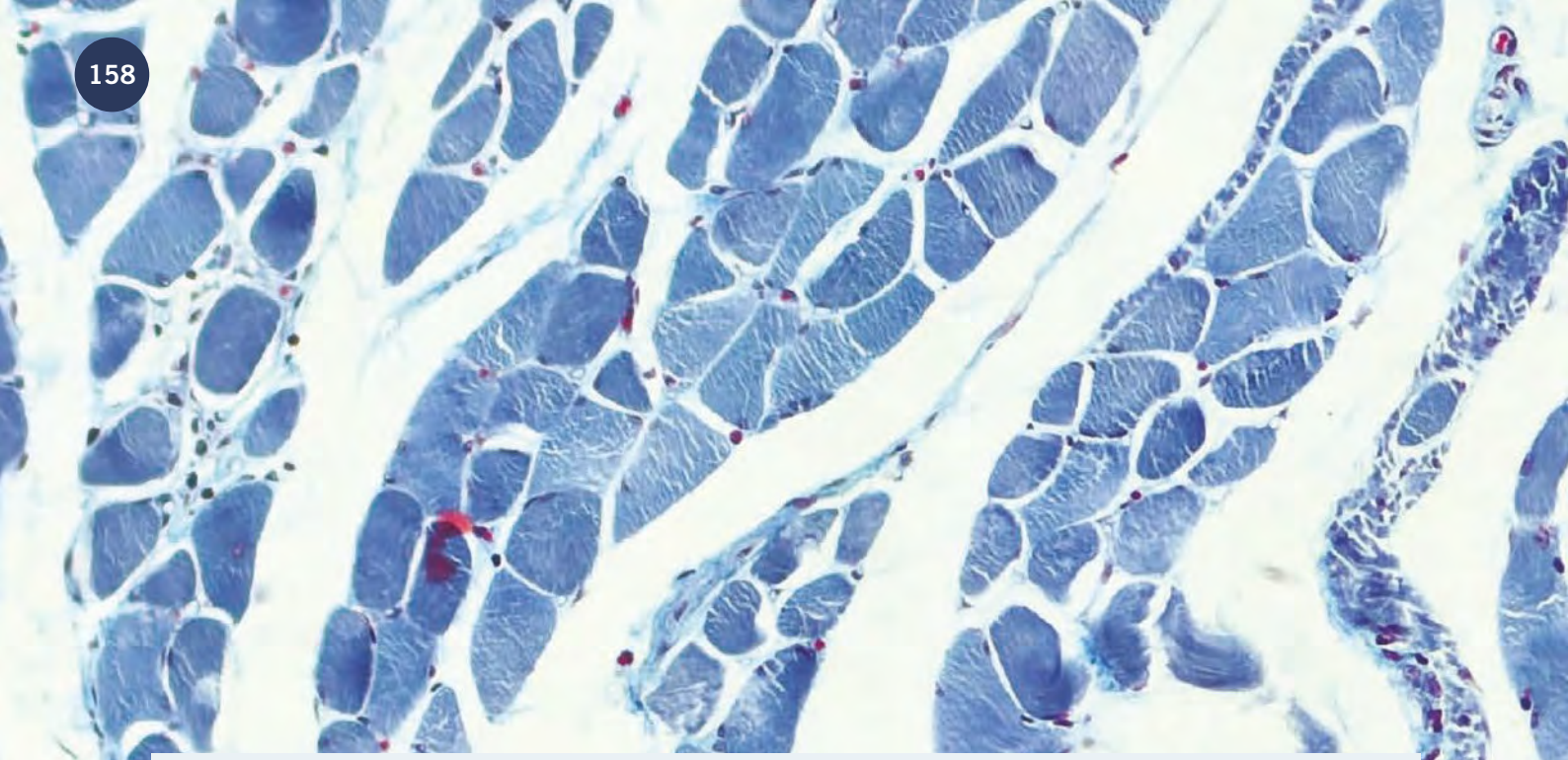
Biochemical pathways, or metabolic pathways, are characterised by a series of reactions that are very tightly regulated by enzymes. Cell survival depends on cells being able to respond to changes in their environment, and without enzymes regulating these biochemical pathways this would not be possible. This chapter examines two biochemical pathways that are integral to life on Earth: photosynthesis and cellular respiration. The general structure of each pathway from reactants to products, as well as the specific location, inputs and outputs of the different stages are explored. With an understanding of the role of enzymes and assisting coenzymes, the efficiency of each process and the factors that affect their rate of reaction will be covered. The uses and applications of biotechnology in the regulation of biochemical pathways are also evaluated in terms of their potential in agriculture.

Curriculum

Area of Study 2 Outcome 2
How are biochemical pathways regulated?

Study Design	Learning intentions – at the end of this chapter I will be able to:
Regulation of biochemical pathways in photosynthesis and cellular respiration • The general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product **Photosynthesis as an example of biochemical pathways** • Inputs, outputs and locations of the light dependent and light independent stages of photosynthesis in C_3 plants (details of biochemical pathway mechanisms are not required) • The role of Rubisco in photosynthesis, including adaptations of C_3, C_4 and CAM plants to maximise the efficiency of photosynthesis	**5A Photosynthesis** **5A.1** State the purpose of photosynthesis **5A.2** Explain the general function of the chloroplasts **5A.3** Draw the chloroplast, label and recall the function of key structures **5A.4** State the word equation and balanced chemical equation for photosynthesis **5A.5** Summarise the inputs, outputs and location of the two stages of photosynthesis in C_3 plants **5A.6** Outline what occurs during the light dependent and light independent stages of photosynthesis. **5A.7** Describe the role of Rubisco and ATP synthase in photosynthesis (C_3 plants)

Study Design	Learning intentions – at the end of this chapter I will be able to:
Photosynthesis as an example of biochemical pathways • The role of Rubisco in photosynthesis, including adaptations of C_3, C_4 and CAM plants to maximise the efficiency of photosynthesis • The factors that affect the rate of photosynthesis: light availability, water availability, temperature and carbon dioxide concentration	**5B Factors affecting the rate of photosynthesis** **5B.1** List the factors that affect the rate of photosynthesis **5B.2** Explain why and how each of these factors may affect the rate of photosynthesis **5B.3** Describe the role of Rubisco in maximising the efficiency of photosynthesis, including a definition of photorespiration **5B.4** Summarise the adaptations of C_3, C_4 and CAM plants that help to maximise the efficiency of photosynthesis **5B.5** Draw, recognise and interpret graphical representations of rates of photosynthesis
Regulation of biochemical pathways in photosynthesis and cellular respiration • The general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product **Cellular respiration as an example of biochemical pathways** • The main inputs, outputs and locations of glycolysis, Krebs Cycle and electron transport chain including ATP yield (details of biochemical pathway mechanisms are not required) • The location, inputs and the difference in outputs of anaerobic fermentation in animals and yeasts	**5C Cellular respiration** **5C.1** State the purpose of cellular respiration **5C.2** Explain the function of the mitochondria **5C.3** Draw a mitochondrion, label and recall the function of key structures **5C.4** Demonstrate an understanding of the energy shuttle and uses of ATP **5C.5** Tabulate the location, inputs and outputs of glycolysis, Krebs Cycle and electron transport chain in aerobic cellular respiration, including ATP yields **5C.6** Outline what occurs during glycolysis, the Krebs Cycle and the electron transport chain in aerobic cellular respiration **5C.7** State the word equation and balanced chemical equation for aerobic cellular respiration **5C.8** Tabulate the location, inputs and difference in outputs of anaerobic fermentation in animals, plants and yeasts **5C.9** State the word equation for anaerobic cellular respiration
Cellular respiration as an example of biochemical pathways • The factors that affect the rate of cellular respiration: temperature, glucose availability and oxygen concentration	**5D Factors affecting the rate of cellular respiration** **5D.1** List the factors that affect the rate of cellular respiration **5D.2** Explain why and how each of these factors may affect the rate of cellular respiration

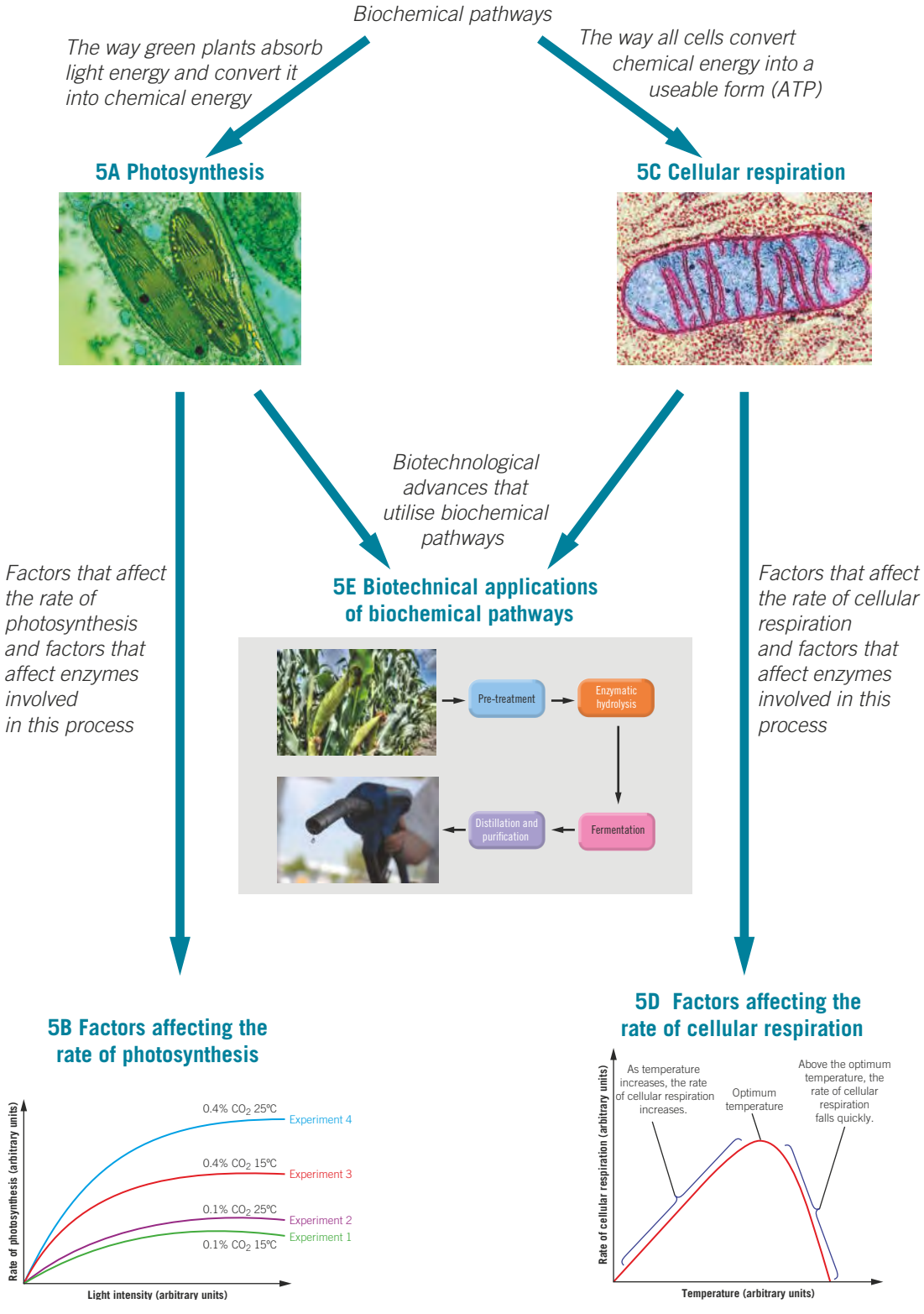


Study Design	Learning intentions – at the end of this chapter I will be able to:
Biotechnological applications of biochemical pathways • Potential uses and applications of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields • Uses and applications of anaerobic fermentation of biomass for biofuel production	**5E Biotechnical applications of biochemical pathways** **5E.1** Define and explain the process involved in CRISPR-Cas9 technologies **5E.2** Summarise the potential applications and uses of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields **5E.3** Define the terms biofuel and biomass **5E.4** Summarise the uses and applications of anaerobic fermentation of biomass for biofuel production

Glossary

ADP
Aerobic cellular respiration
Anaerobic cellular respiration
ATP
ATP synthase
Autotroph
Biofuel
Biomass
C_3 plants
C_4 plants
CAM plants
Cas9
Cellular respiration
Chlorophyll
Coenzyme
CRISPR-Cas9
Crista
Energy shuttle
Fermentation
First-generation biofuels
Gene editing
Glycolysis
Granum
Heterotroph
Light dependent stage
Light independent stage
Limiting factor
Matrix
NAD^+
$NADP^+$
PGA
PGAL
Photolysis
Photorespiration
Photosynthesis
Rate
Rubisco
RuBP
Second-generation biofuels
Stroma
Thylakoid membrane

Concept map



See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

Photosynthesis

Study Design:

- The general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product
- Inputs, outputs and locations of the light dependent and light independent stages of photosynthesis in C_3 plants (details of biochemical pathway mechanisms are not required)
- The role of Rubisco in photosynthesis, including adaptations of C_3, C_4 and CAM plants to maximise the efficiency of photosynthesis

Glossary:

ADP (adenosine diphosphate)
ATP (adenosine triphosphate)
Autotroph
Cellular respiration
Chlorophyll
Coenzyme
Granum
Heterotroph
Light dependent stage
Light independent stage
$NADP^+$
PGA
PGAL
Photolysis
Photosynthesis
Rubisco
RuBP
Stroma
Thylakoid membrane

ENGAGE

The solar-powered sea slug

The green sea slug (*Elysia chlorotica*) has the distinctive appearance of a gelatinous green leaf. The slug eats algae, but it also 'feeds' on sunlight to make its own food! Scientists have found that this slug steals chloroplasts (photosynthetic organelles) and some genes from the algae it consumes, and this allows it to survive for months without eating. Is the photosynthetic process of the sea slug the same as in green plants and algae?

Figure 5A–1 The emerald green sea slug *(Elysia chlorotica)* can get all the energy it needs by 'feeding' on light energy and using photosynthesis to make its own food.

EXPLAIN

Revisiting the link between life and energy

Energy can be defined as the capacity to do work, and transformation means to change form. Recall from Unit 1 that living things are constantly active: gaining oxygen, water and nutrients; removing waste products; and maintaining their internal environments (pH and temperature). It is energy that does the work that sustains these activities.

LINK UNIT 1

Energy exists in different forms, but the forms of energy that are most important to life on Earth are sunlight (light energy), the chemical energy stored in food (like glucose), and heat. Cells and organisms carry out reactions to transform these forms of energy into forms they can use. For example:

- **Autotrophs** capture and use light energy to convert water and carbon dioxide (inorganic) into glucose (organic). This process is called **photosynthesis**. Autotrophs then use the organic compounds they make for their structural and energy needs, like growing and reproducing.
- **Heterotrophs** convert the energy stored in organic materials into energy that is stored in **ATP (adenosine triphosphate)**. This process is called **cellular respiration**.

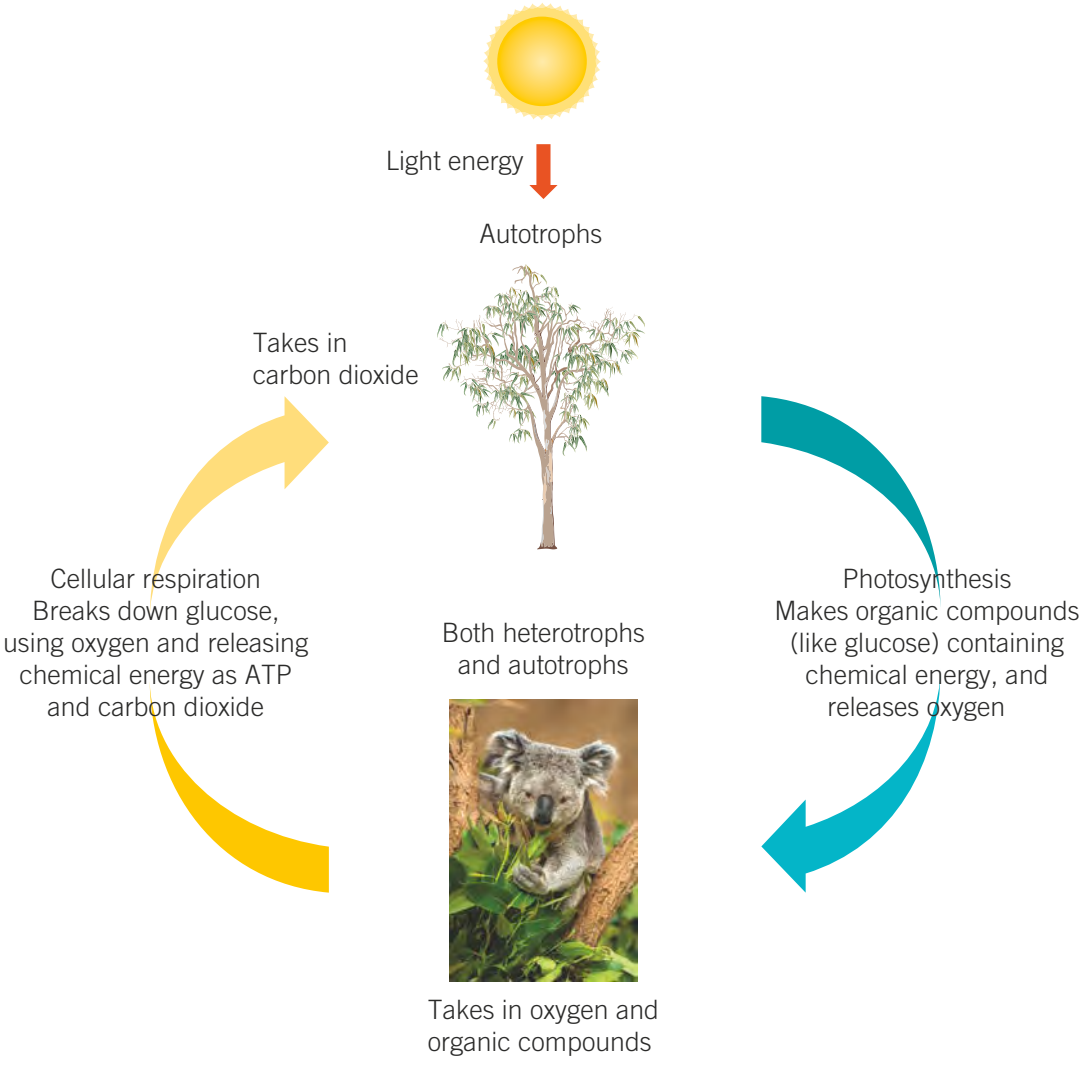


Figure 5A–2 The relationship between autotrophs and heterotrophs and the reactions they carry out to transform different types of energy into forms they can use

Autotroph
an organism that synthesises its own organic materials (food), by capturing light energy and taking in inorganic compounds from its physical environment, to meet its energy needs (*auto* = self, *troph* = food)

Photosynthesis
a chemical reaction in which light energy is used to convert the inorganic compounds carbon dioxide (CO_2) and water (H_2O) into the organic compound glucose; occurs in the chloroplast (*photo* = light, *synthesis* = build or put together)

Heterotroph
an organism that ingests organic materials by feeding on autotrophs or on other organisms and their products, in order to convert energy into the form of energy stored in ATP (*heteros* = other, *trophe* = food)

ATP (adenosine triphosphate)
the main immediate source of chemical energy in a cell, powering most cellular processes; when a phosphate group is removed, energy is released and ADP is formed

Cellular respiration
a series of chemical reactions in which the organic compound glucose is broken down, producing various products (depending on presence or absence of oxygen) and energy stored in ATP

Chlorophyll the green pigment on the thylakoid membranes of the chloroplasts of green plants; absorbs light energy for photosynthesis

1B CELL TYPES AND CELL ORGANELLES LINK

4A THE ROLE OF ENZYMES LINK

VIDEO 5A–1 PHOTOSYNTHESIS

Overview of photosynthesis

The purpose of photosynthesis

Photosynthesis is an anabolic chemical reaction in which the Sun's light energy is used to convert the inorganic compounds carbon dioxide (CO_2) and water (H_2O) into the organic compound glucose ($C_6H_{12}O_6$). Glucose can then be used as a source of energy by cells, enabling growth and reproduction. It is also involved in the synthesis of complex compounds necessary for survival. Any excess glucose can be stored by plant cells in the form of starch, for later use.

For photosynthesis to occur, a photosynthetic pigment called **chlorophyll** (*chloro* = green, *phyll* = leaf) is required. This pigment is found in a specialised organelle called a chloroplast. Photosynthesis consists of a series of steps (a biochemical pathway), with each step controlled by a different enzyme.

The overall process of photosynthesis can be written as both a word equation and a balanced chemical equation:

$$\text{carbon dioxide} + \text{water} \xrightarrow[\text{chlorophyll}]{\text{light energy}} \text{oxygen} + \text{water} + \text{glucose}$$

$$6CO_2 + 12H_2O \xrightarrow[\text{chlorophyll}]{\text{light energy}} 6O_2 + 6H_2O + C_6H_{12}O_6$$

or the simplified version:

$$6CO_2 + 6H_2O \xrightarrow[\text{chlorophyll}]{\text{light energy}} 6O_2 + C_6H_{12}O_6$$

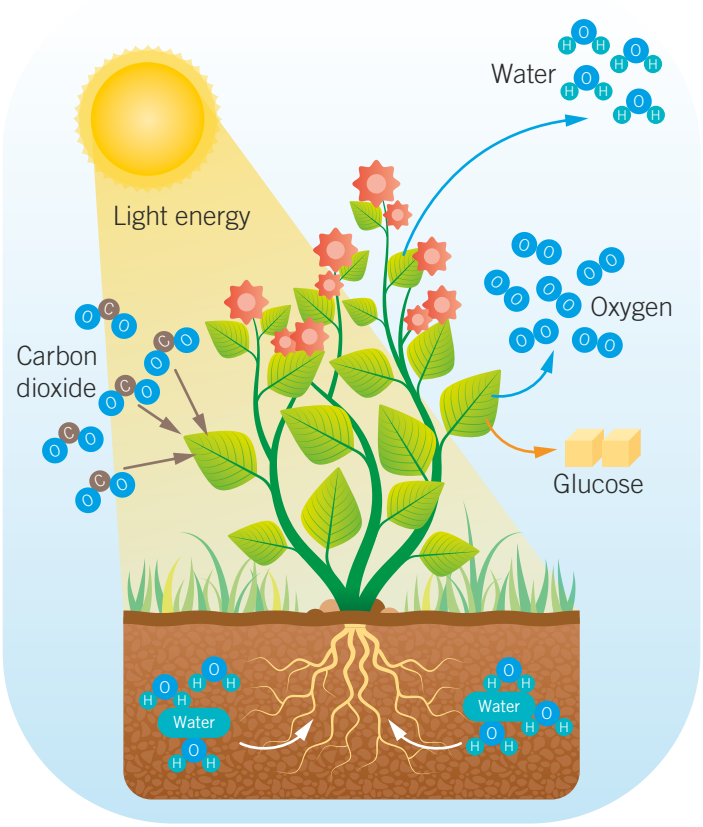


Figure 5A–3 The initial reactants and final products of photosynthesis

ISBN 9 8-1-108-89462-3

The structure of chloroplasts

Plant cells are eukaryotic cells, as they contain membrane-bound organelles. The primary function of the chloroplast is as the site for photosynthesis. Inside the inner membrane of a chloroplast are stacks of what look like pancakes. Each stack is a **granum** (plural *grana*), and each 'pancake' is a thylakoid, a compartment made of a **thylakoid membrane** around a 'lumen', which is like the cytosol. Chlorophyll (the green pigment in plants) is embedded in the thylakoid membranes and absorbs light energy. The more thylakoid membranes there are, the more surface area is available for capturing light energy and for the exchange of the other requirements and waste products in the **light dependent stage** of photosynthesis, which occurs here. The remaining space in the chloroplast is a gel-like fluid called the **stroma**. This contains a large number of ribosomes, due to the large number of enzymes needed for the reactions that occur during the **light independent stage** of photosynthesis, which occurs here.

LINK 1B CELL TYPES AND CELL ORGANELLES
LINK 2C PROTEINS

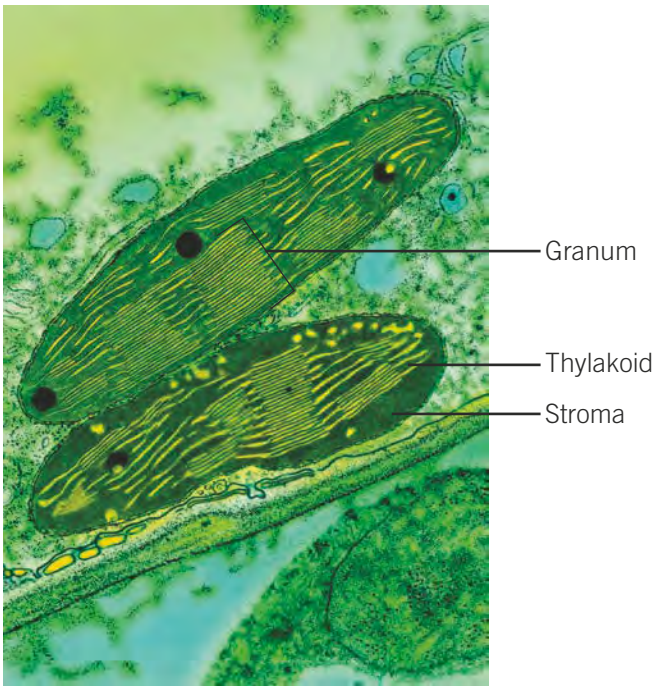


Figure 5A–4 Chloroplasts in a pea plant. Note the stacks of thylakoid membranes that form the grana and the space between them, which is the stroma. The black spots are starch granules.

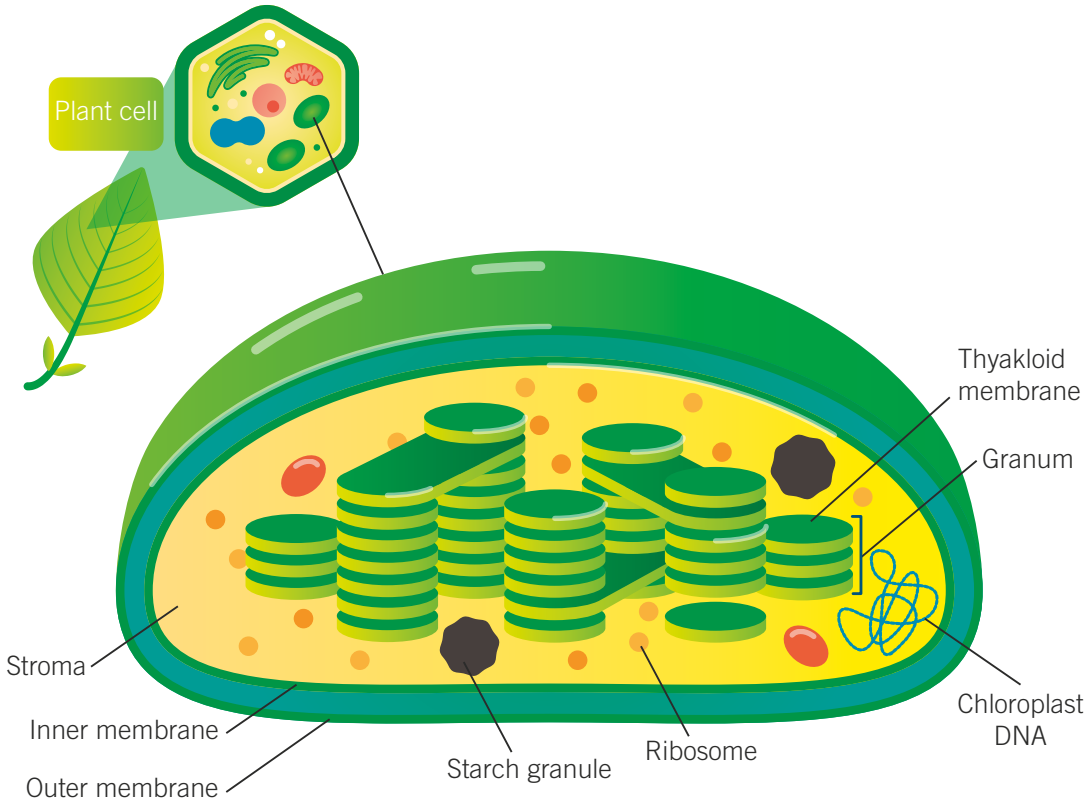


Figure 5A–5 Chloroplasts are the organelles in plant cells that carry out photosynthesis. The grana and stroma of the chloroplast are the sites of the different stages of photosynthesis.

Granum
(plural *grana*) a stack of thylakoid membranes inside the chloroplast of plant and algae cells

Thylakoid membrane
disc-shaped interconnected membrane-bound compartments inside a chloroplast that make up the grana and are the location of the pigment chlorophyll, and therefore the site of the light dependent stage of photosynthesis

Stroma
the gel-like fluid inside a chloroplast which surrounds the grana; site of the light independent stage of photosynthesis

Light dependent stage
the first stage of photosynthesis; occurs in the thylakoid membranes and involves the splitting of water using light energy

Light independent stage
the second stage of photosynthesis; occurs in the stroma of the chloroplast and involves the use of carbon dioxide to create glucose; also called the Calvin Cycle or carbon fixation

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Check-in questions – Set 1

1 What is the purpose of photosynthesis?
2 a Write the word equation for photosynthesis.
  b Write a balanced chemical equation for photosynthesis.
3 Draw a chloroplast.
  a Label the granum, thylakoid membrane, stroma, inner membrane, outer membrane, ribosomes and DNA.
  b State the function of each of the key structures in the chloroplast that have a role in photosynthesis.

The stages of photosynthesis

The process of photosynthesis is a very complex two-stage process. The two stages are:
- light dependent stage (requires the input of light energy to occur)
- light independent stage (requires certain outputs of light dependent stage to occur).

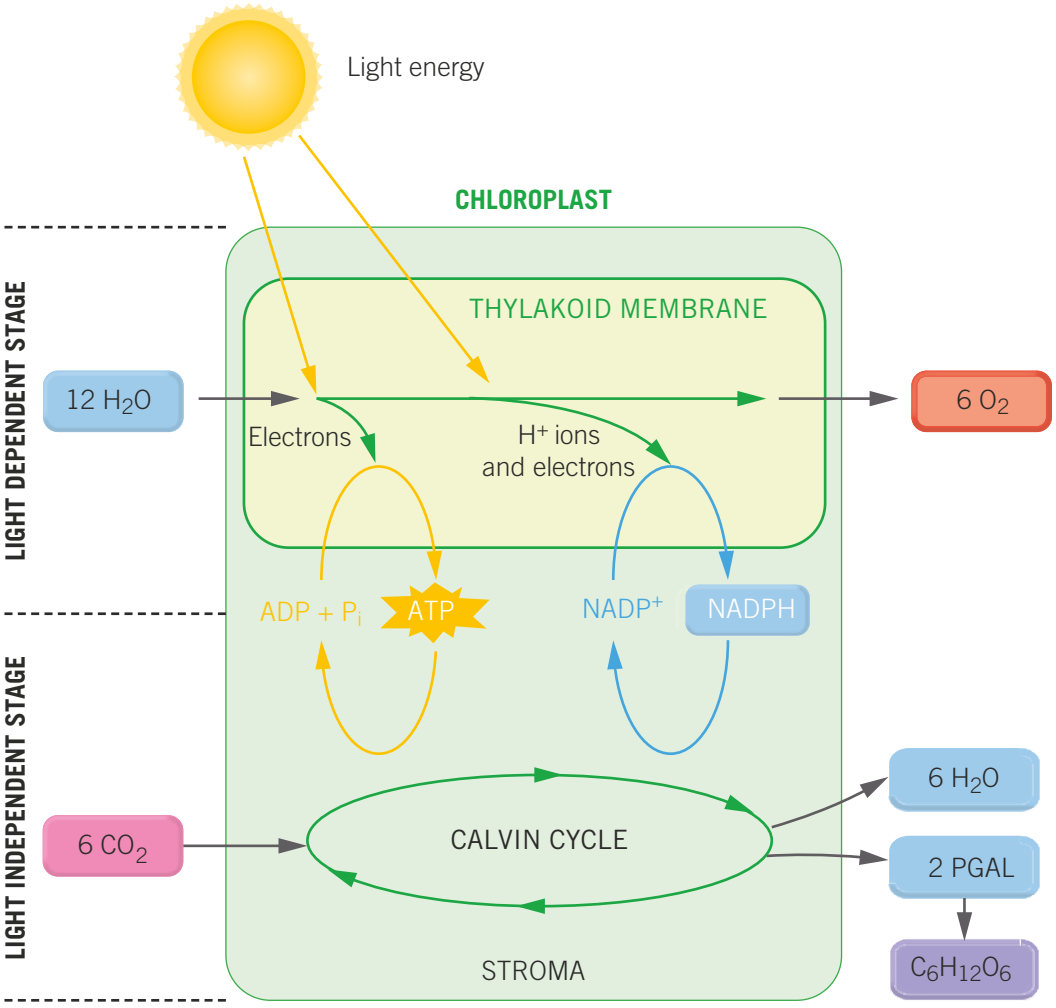

Figure 5A–6 The overall process of photosynthesis consists of many biochemical pathways that can be summarised as two key stages: light dependent and light independent. The steps within these stages are explained in the rest of this section, with more detailed versions of this diagram.

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Light dependent stage

The first stage of photosynthesis occurs in the grana (on the thylakoid membranes) of the chloroplasts. It involves the:

- absorption of light energy by chlorophyll, and
- splitting of water.

Absorption of light energy

Chlorophyll can absorb most wavelengths of the Sun's light energy. However, the red and blue wavelengths of light are usually absorbed the most and therefore used for photosynthesis. Chlorophyll is assisted by accessory pigments, which help to absorb different wavelengths of light energy and then pass it to the chlorophyll molecules. Accessory pigments include the carotenoids: for example, carotene (orange) and xanthophyll (yellow).

Figure 5A–7 demonstrates the relationship between the absorbance of the different wavelengths of light energy by the pigments in leaves, and the rate of photosynthesis when exposed to different wavelengths of light. The rate of photosynthesis is highest when the absorption of light energy by the various pigments in leaves is highest.

WORKSHEET 5A–1 COLOUR AND PHOTOSYNTHESIS

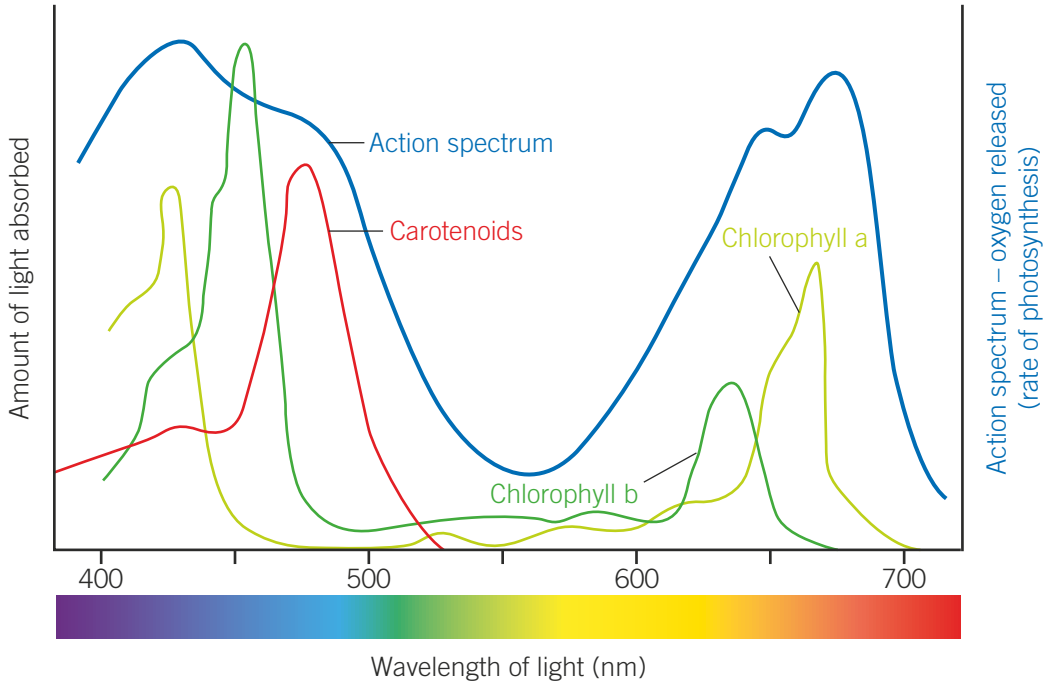

Figure 5A–7 The absorption spectrum (amount of light absorbed at different wavelengths) of the pigments in leaves: chlorophyll (a and b) and the accessory pigment, carotenoid. Also shown is the action spectrum, which demonstrates the overall rate of photosynthesis, measured as mass of oxygen released at each wavelength of light.

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UNIT 1 LINK

4A THE ROLE OF ENZYMES LINK

Splitting of water

The following steps are shown in Figure 5A–8.

1 When light energy is captured, it splits water (**photolysis**), producing oxygen (O_2), hydrogen ions (H^+) and electrons.
2 The oxygen is released, by diffusion, out of the thylakoid membrane to the stroma, then out of the chloroplast and into the atmosphere. It is considered a waste product of photosynthesis.
3 Electrons released when light energy stimulates chlorophyll are used by ATP synthase, an enzyme embedded in the thylakoid membrane, to catalyse the synthesis of ATP from **ADP** and Pi (inorganic phosphate).
4 Hydrogen ions (also called protons) and some of the electrons released in the previous step are taken up by an acceptor molecule called nicotinamide adenine dinucleotide phosphate, or **$NADP^+$**, which then forms NADPH. This is important, as $NADP^+$ acts like a taxi service, carrying the hydrogen ions where they need to go. $NADP^+$ is a **coenzyme**, which means it is an organic non-protein molecule that helps enzymes to catalyse a reaction.
5 The hydrogen ions (via NADPH) and ATP produced during the light dependent stage of photosynthesis are then transported to the stroma for the light independent stage of photosynthesis.

Photolysis
the splitting of water using the light energy from the Sun

ADP (adenosine diphosphate)
a compound composed of adenosine and two phosphate groups that can store energy when another inorganic phosphate group is added, forming ATP

$NADP^+$
a coenzyme that accepts and transfers hydrogen ions from one place to another during photosynthesis

Coenzyme
an organic molecule that contains carbon and bind to enzymes to help them to function; examples are NADP, NAD and FAD

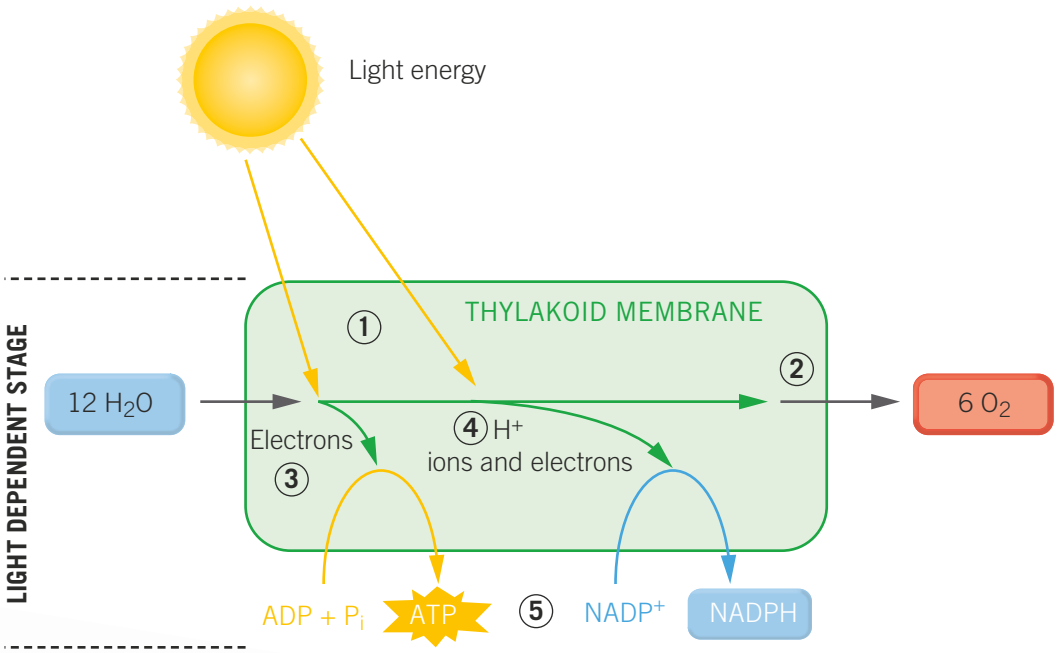

Figure 5A–8 The light dependent stage of photosynthesis involves light energy splitting water and stimulating chlorophyll.

NOTE

This resource shows the biochemical details that are assessable in the course. You do not have to know any finer points of individual reactions in photosynthesis and cellular respiration other than those given in this chapter.

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Light independent stage

The second stage of photosynthesis occurs in the stroma of the chloroplasts and involves the synthesis of the organic compound glucose from carbon dioxide (CO_2). This stage is also referred to as the Calvin Cycle, carbon reduction or carbon fixation (because in this stage, the carbon-based molecules are 'fixed' together into glucose).

1 The cycle begins with a five-carbon molecule called **RuBP** (ribulose bisphosphate), which is added to the CO_2 from the atmosphere with the help of an enzyme called RuBP carboxylase or **Rubisco**.
2 The resulting compound breaks down into two compounds, called 3-phosphoglycerate (**PGA**).
3 PGA is then converted into the carbohydrate glyceraldehyde-3-phosphate (**PGAL**) using NADPH and ATP. Remember:
   - The energy for this process comes from the breakdown of ATP supplied by the light dependent stage.
   - The hydrogen ions carried by the 'loaded' acceptor molecule NADPH are coming from the light dependent stage.
4 PGAL molecules are then used to form glucose ($C_6H_{12}O_6$) and regenerate RuBP (using more ATP), so the cycle can begin again.
5 $NADP^+$, ADP and inorganic phosphate (P_i) are produced and returned to the light dependent stage. H_2O is also formed (not shown in the diagram), as the oxygen created when carbon dioxide breaks down, binds with some of the free H^+ released by the NADPH.

RuBP
ribulose bisphosphate, a 5C (five-carbon) compound that combines with carbon dioxide at the start of the Calvin Cycle of photosynthesis to form PGA

Rubisco
RuBP carboxylase, an enzyme that catalyses the formation of PGA by fixing carbon dioxide to RuBP during the Calvin Cycle of photosynthesis

PGA
3-phosphoglycerate, a 3C (three-carbon) compound formed when the enzyme Rubisco catalyses the attachment of a carbon from carbon dioxide to RuBP during the Calvin Cycle of photosynthesis

PGAL
glyceraldehyde-3-phosphate, a 3C (three-carbon) sugar that leads to the formation of glucose and regenerates RuBP in the process to continue the Calvin Cycle

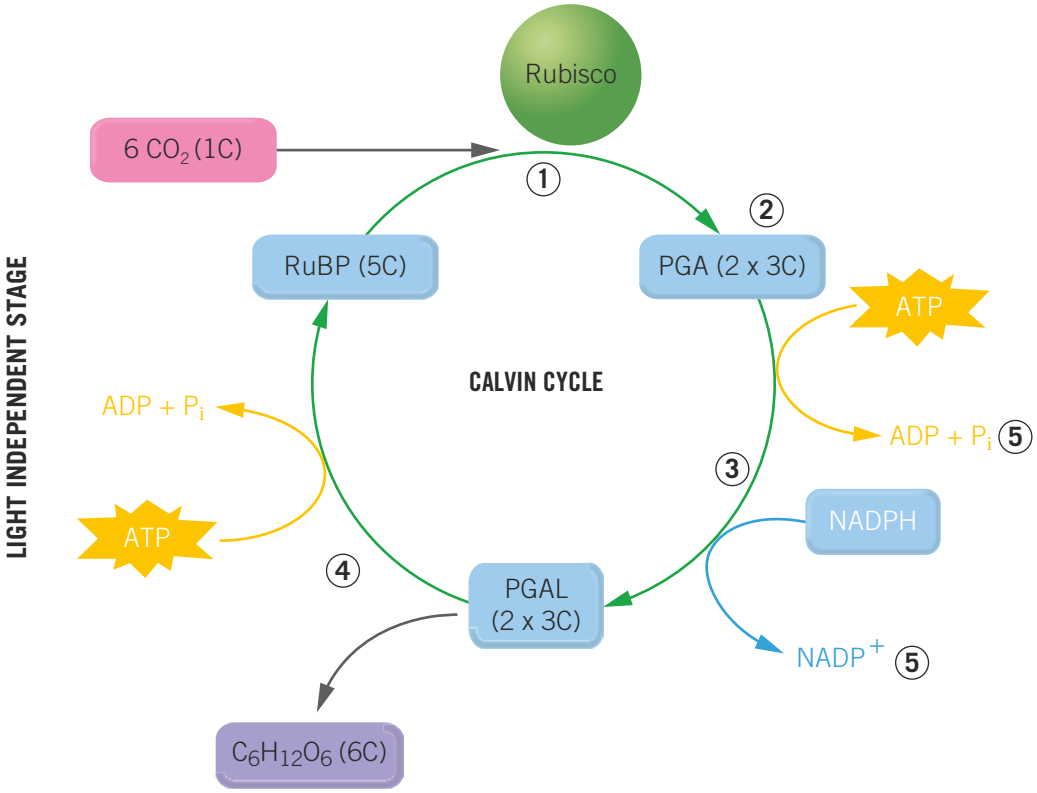

Figure 5A–9 A simplified version of the Calvin Cycle or light independent stage of photosynthesis. Note the inputs and outputs of this stage.

WORKSHEET 5A–2
INVESTIGATING PHOTOSYNTHESIS

You may like to revisit Figure 5A–6 now to remind yourself of the overall process of photosynthesis and how the light dependent and light independent stages fit together.

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Check-in questions – Set 2

1 Light energy is converted into what form of energy during photosynthesis?

2 a Name the collective term for the red/orange/yellow plant pigments that help chlorophyll harness light energy.

b Which two colours of the visible light spectrum are primarily absorbed by chlorophylls a and b?

3 If one cycle of the Calvin Cycle makes one CH_2O molecule, how many cycles will it take to build glucose ($C_6H_{12}O_6$)?

4 a The light dependent reactions of photosynthesis supply the Calvin Cycle with which two molecules?

b Name the waste product of photosynthesis.

c Where does the Calvin Cycle take place?

d Name the stage of photosynthesis where carbon dioxide is used.

e Name the enzyme that catalyses the formation of PGA.

5A SKILLS

VIDEO 5A–2 SKILLS: TERMINOLOGY AND ABBREVIATIONS

Using the correct terminology and abbreviations

Defining key terms correctly in an assessment is essential in Biology. So what do you need to keep in mind for biochemical reactions?

- The equation for photosynthesis (word and balanced chemical equations) *must* include 'light energy' and 'chlorophyll' on the reaction arrow.
- 'Light' is not an acceptable term to use, so practise using 'light energy' in your notes.
- The inputs, outputs and location of the different stages of photosynthesis (and aerobic cellular respiration, covered in Section 5C) need to be understood.
- In an examination, you are allowed to use suitable abbreviations such as ATP, and chemical symbols such as H_2O, CO_2 and O_2. There is some leniency around spelling; however, you must correctly spell any terms that are an explicit answer to a question.

VIDEO 5A–3 SKILLS: APPLYING YOUR KNOWLEDGE

Applying your knowledge

In an examination you will be exposed to new information and research, and will be expected to apply what you have learned over the year to these new situations. Examiners are not trying to trick you; they only want to assess your depth of understanding of particular concepts.

Another way in which you may be asked to apply your knowledge is through interpreting diagrams that may seem unfamiliar. Again, everything you have covered in class is sufficient for you to answer the questions asked; you only need to figure out what the concept is that is being assessed, and what you know about this topic that is relevant.

For example, consider the diagrams in Figure 5A–10. Each represents a different way of presenting the inputs and outputs of photosynthesis, part of a dot point in your Study Design. See if you can determine what the question marks are in each diagram.

To improve your skills in this area, the more practice questions and examinations you can access (practice questions and examinations can be found in the digital resources), the more types of questions you will see assessing the same concepts but in a different way, and the less likely it is that you will be stumped in an examination or SAC.

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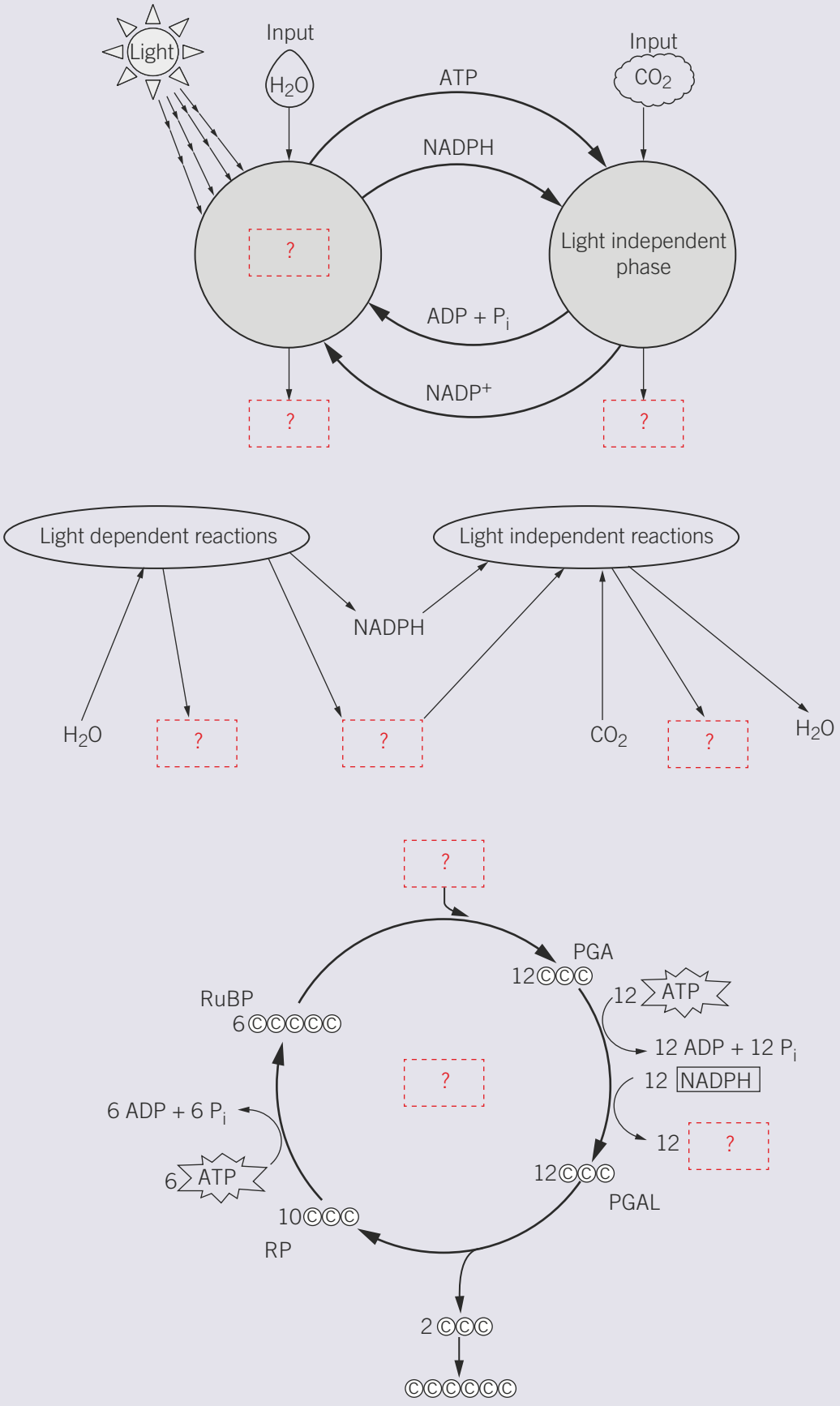

Figure 5A–10 Different representations of the process of photosynthesis. Diagrams may include one stage or both stages, simple boxes or actual structures, detailed information or limited information.

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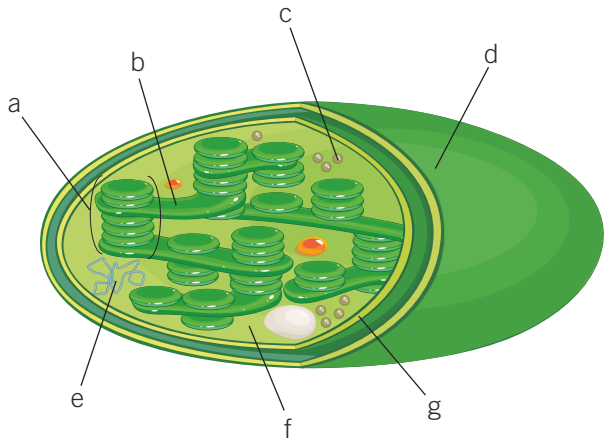
Section 5A questions

1 a Label structures a–g in the diagram of the chloroplast below.



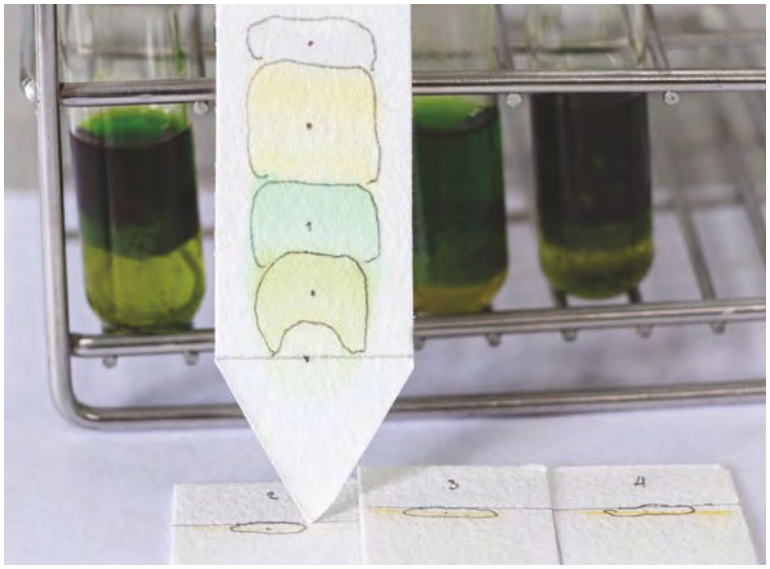
b Explain why the grana have such a large surface area.

2 Describe the relationship between the absorption spectrum and the action spectrum.

3 Outline three changes that occur as a consequence of chlorophyll capturing light energy from the Sun.

4 Draw up a table that summarises the inputs, outputs and location of the light dependent and light independent stages of photosynthesis.

5 Paper chromatography is a scientific technique used to separate components of a mixture. It can be used to investigate the pigments found in plant leaves. The image below shows the results of such an investigation.



a Does it appear that there is more than one pigment in plant leaves? Explain your answer.

b Give reasons why it is advantageous for leaves to contain a number of different pigments.

c When a plant becomes unhealthy, some of its leaves may turn yellow. This is due to the plant breaking down and reabsorbing chlorophyll a and b, away from the unhealthy region. Give reasons why plants may have evolved in this way.

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6 Rubisco is an enzyme involved in photosynthesis. Complete the following table.

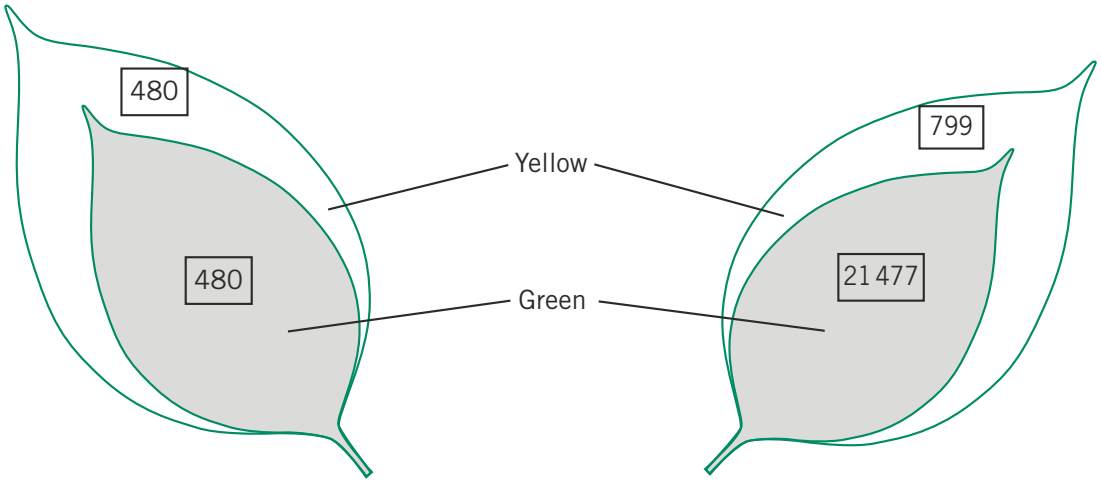
Enzyme	Stage involved in	Converts ...	Into ...
Rubisco			

7 In recent decades, biologists have been able to use radioactive forms of elements to investigate the process of photosynthesis. For example, it has been possible to radioactively label the oxygen in water and in carbon dioxide in order to determine where the oxygen gas released from leaves comes from.

a Scientists supplied a plant with both radioactive water and carbon dioxide, and recorded the results shown below. Looking at these results, what starting material does the oxygen appear to come from? Explain your answer.

Percentage of water supplied that was radioactive	Percentage of carbon dioxide supplied that was radioactive	Percentage of oxygen produced that was radioactive
0.80	0.44	0.79
0.80	0.52	0.79
0.80	0.61	0.80
0.20	0.51	0.21
0.20	0.48	0.19
0.20	0.58	0.20

Two plants with variegated leaves were supplied with radioactive carbon dioxide. Twenty-four hours later, a leaf from plant A and a leaf from plant B were compared by measuring the radioactivity in their leaves. The recorded results are shown below.



Plant A: placed in the dark

Plant B: placed in the light

Radioactivity is shown in arbitrary units in boxes ☐

b What explains the radioactivity measurement in the yellow area of a leaf from plant B?

c Name a coenzyme involved in photosynthesis and its role in this biochemical pathway.

d The experiment was repeated on an extremely hot day and the plants used were exposed to high temperatures. Recall that photosynthesis is regulated by enzymes, which are proteins. Give reasons why the experiment may not be successful in these conditions.

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5B Factors affecting the rate of photosynthesis

Study Design:
- The role of Rubisco in photosynthesis, including adaptations of C_3, C_4 and CAM plants to maximise the efficiency of photosynthesis
- The factors that affect the rate of photosynthesis: light availability, water availability, temperature and carbon dioxide concentration

Glossary:
C_3 plants
C_4 plants
CAM plants
Limiting factor
Photorespiration
Rate

ENGAGE

Greenhouses maximise photosynthesis

Greenhouses are used all over the world for the commercial and household growing of plants. The advantage of using a greenhouse is that the environment inside can be controlled, so the plants grow under the same optimal conditions all year round. But what are optimal conditions? Horticulturalists use their knowledge of the factors that affect the rate of photosynthesis to create an environment that allows plants to grow faster and make more food. They do this by using artificial lights, so that photosynthesis can continue day and night, and also at a higher light intensity. Paraffin heaters can also be used inside a greenhouse, because the burning paraffin produces carbon dioxide as well as heat, and both these factors can encourage a higher rate of photosynthesis.

Figure 5B–1 Greenhouses limit the impact of external factors on the rate of photosynthesis.

EXPLAIN

The rate of photosynthesis

Defining 'rate'

Rate
the speed at which a process occurs, or how quickly the reactants are used up and the products are created

The **rate** of photosynthesis can be defined as the speed at which the process of photosynthesis occurs – that is, the speed at which water and carbon dioxide are converted into oxygen and (mainly) glucose.

Measuring the rate

To measure the rate of a reaction, either the concentration of a product that is formed (per unit of time) or the concentration of a reactant that is consumed (per unit of time) is measured.

5A PHOTO-SYNTHESIS LINK

In the case of photosynthesis, the rate of reaction can be measured by recording the oxygen production (oxygen is a product) or the carbon dioxide consumption (carbon dioxide is a reactant).

- Production of oxygen can be measured by, for example, collecting bubbles of oxygen, in the case of aquatic plants.
- Consumption of carbon dioxide can be measured by, for example, using pH indicators in the water of aquatic plants (because carbon dioxide is acidic, and as it is used up the pH will rise).

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Factors that affect the rate of photosynthesis

A **limiting factor** is any factor that reduces the rate of photosynthesis when there is not enough of it. In the case of photosynthesis, the reactants are limiting factors, because in their absence, the reaction of photosynthesis cannot proceed. For example, a plant requires carbon dioxide, water, chlorophyll and light energy, so these are limiting factors. Additionally, photosynthesis is dependent on enzymes as they catalyse the different stages of the process. This means the factors that affect enzyme activity also affect the rate of photosynthesis – for example, temperature, pH and the concentration of the substrates, as you learned in Section 4B. Here we consider the effect of light, temperature, water and carbon dioxide on the rate of photosynthesis as well as the interplay of these factors – the effects they have on one another.

Limiting factor any factor that slows down the rate of photosynthesis when there is not enough of it, for example, carbon dioxide, water, chlorophyll and light energy

Light availability

Increased light intensity provides more light energy for the light dependent reactions of photosynthesis. Consequently, as light intensity increases, so does the rate of photosynthesis. However, at a certain point, the rate of photosynthesis plateaus. Assuming the plant has access to unlimited amounts of the reactants it needs, something else must be limiting the rate of photosynthesis at these high levels of light. In this case, it is the chloroplasts, as they reach their maximum rate of efficiency; that is, they are working as fast as they can. This means that any further increase in light intensity will have no effect on the speed of photosynthesis, and this is observed as a flattening of the curve or a plateau (as can be seen in Figure 5B–2).

LINK **4B** FACTORS IMPACTING ON ENZYME FUNCTION

VIDEO 5B–1 FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS

At very high light intensities, the plant cannot continue to maintain its rate of photosynthesis, because of the increasing temperature created by large amounts of light energy. Some of the visible light is converted to heat when absorbed, and sunlight includes infrared light (that is, radiant heat). The effect of temperature is covered next.

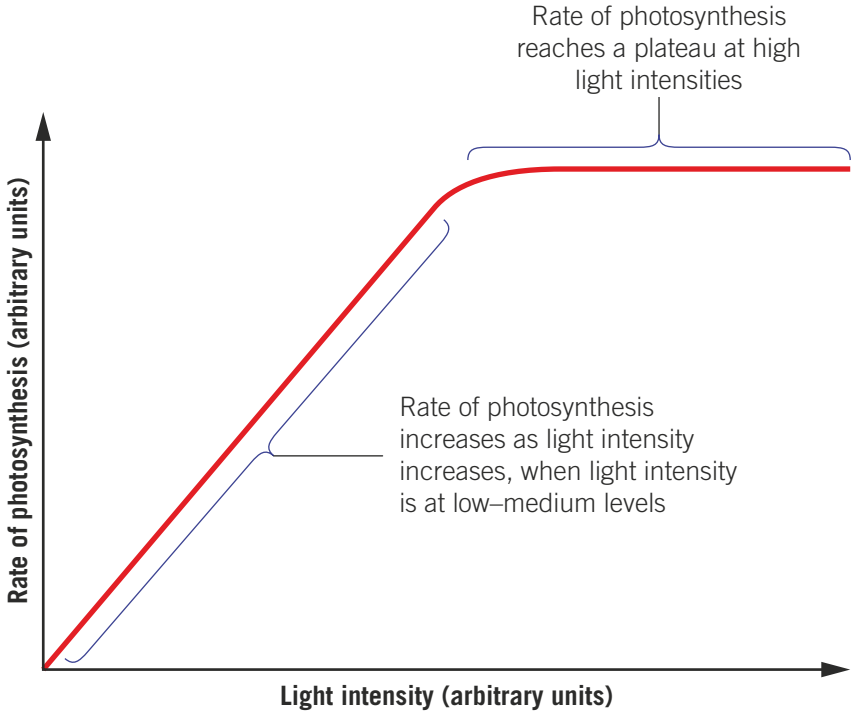

Figure 5B–2 The effect of light intensity on the rate of photosynthesis

WORKSHEET 5B–1 LIGHT INTENSITY AND PHOTOSYNTHESIS

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Temperature

As photosynthesis is regulated by enzymes, and these are sensitive to changes in temperature, the rate of photosynthesis is also affected when temperatures fluctuate. Increased temperatures lead to more kinetic energy and successful collisions between substrates and the active sites of enzymes, so the rate of photosynthesis increases. However, above an enzyme's optimal temperature, the enzyme begins to denature and the active site changes shape. Reactants (substrates) can no longer bind and form the enzyme–substrate complex and the rate of photosynthesis quickly decreases.

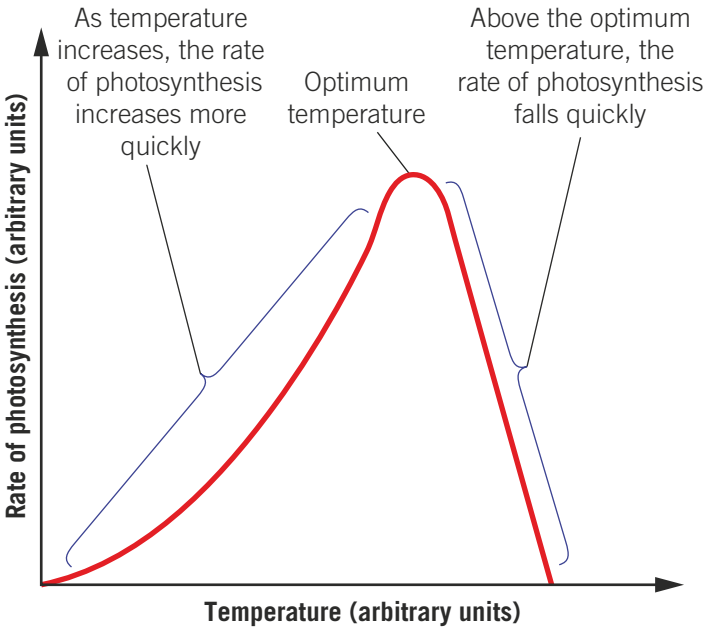

Figure 5B–3 The effect of temperature on the rate of photosynthesis

Water

Water is not generally considered a limiting factor in photosynthesis because the amount needed is generally very small compared to the amount of water plants contain. However, in drought or hot weather, the plant may come under water stress. To prevent water loss, plants will close their stomata (tiny pores on the underside of their leaves). These pores are also the space through which gas exchange occurs, so the closing of the stomata also prevents carbon dioxide entering and oxygen leaving. Therefore, despite unlimited access to light intensity, there may not be enough carbon dioxide available to the plant to carry out photosynthesis. This is summarised in Figure 5B–4 and carbon dioxide concentration is considered after that. You will investigate what this means for the survival of plants when you learn about the adaptations of C_3, C_4 and CAM plants, later in this section.

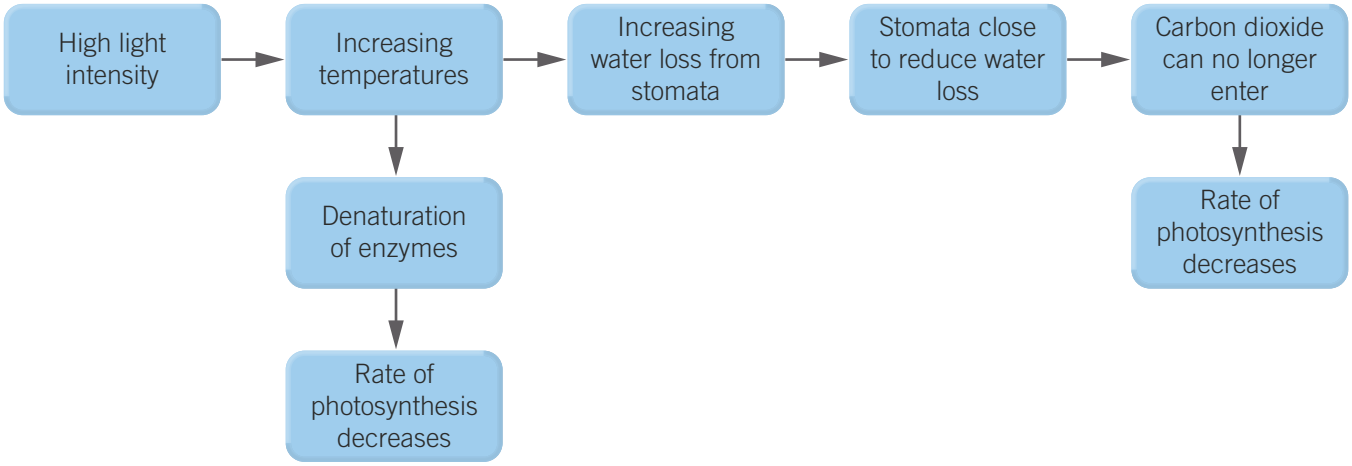

Figure 5B–4 Summarising the impact of high light intensity and its flow-on effects on other factors and on the rate of photosynthesis

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Carbon dioxide concentration

Carbon dioxide is involved in the fixation of carbon atoms to form the organic molecule glucose. More carbon dioxide means more reactant or substrate available for the light independent reactions. However, at a certain level, all the enzyme active sites responsible for carbon fixation are saturated and the reaction rate reaches a maximum. This means that any further increase in carbon dioxide concentration will have no effect on the rate of photosynthesis, which plateaus.

LINK 4B FACTORS IMPACTING ON ENZYME FUNCTION

Figure 5B–5 demonstrates how increasing the concentration of carbon dioxide does not lead to higher photosynthetic rates indefinitely. This is a good outcome, because there is a point at which high levels of carbon dioxide can be damaging to the plant as it creates an acidic environment. Besides, in the plant's internal environment, enzymes can only operate at their optimum pH, so an acidic environment will denature the enzymes and render them inactive.

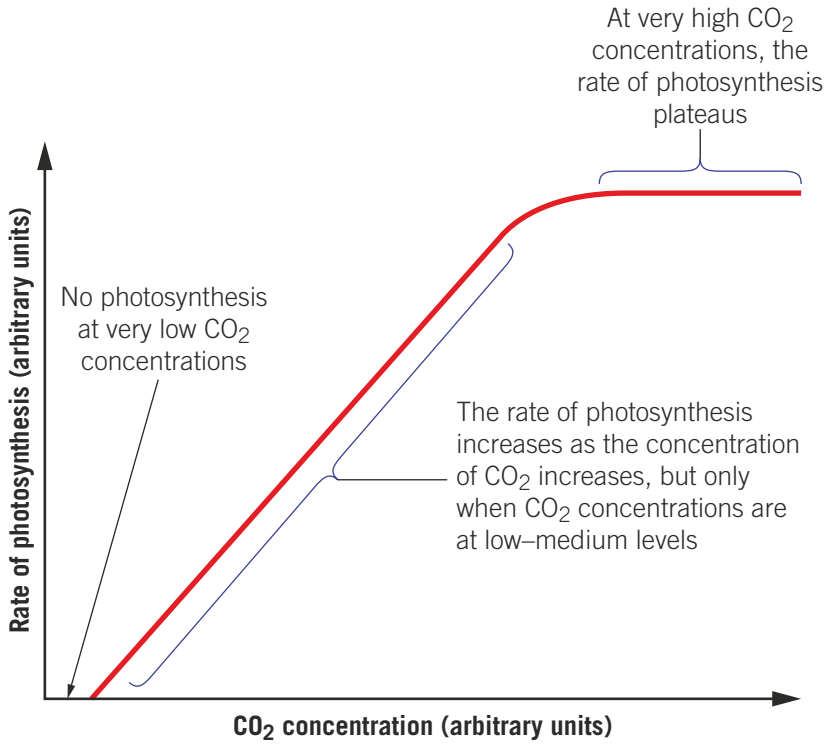

Figure 5B–5 The effect of carbon dioxide concentration on the rate of photosynthesis

Check-in questions – Set 1

1 State what factors influence the rate of photosynthesis and explain why they are able to do so.

2 Summarise the relationship between each of the following factors and the rate of photosynthesis
   a Light intensity
   b Temperature
   c Carbon dioxide concentration

3 Outline the relationship between enzymes and the rate of photosynthesis.

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Adaptations of C_3, C_4 and CAM plants

Maximising the efficiency of photosynthesis

5A PHOTO-SYNTHESIS LINK

C_3 plants plants that fix CO_2 from the atmosphere to form 3-phosphoglycerate or PGA, which contains three carbon atoms; are better suited to cooler and temperate climates; examples are rice, wheat, soybeans and cotton

So far you have learned about the process of photosynthesis in **C_3 plants**. But what are C_3 plants? They are called 'C_3 plants' because they form the 3C compound PGA during carbon fixation. As you learned in Section 5A, they do this by fixing carbon dioxide from the atmosphere to RuBP using the enzyme Rubisco. Approximately 85% of Earth's plants use C_3 photosynthesis. C_3 plants include crops like rice, cotton, rye, wheat, soybean, oats and all trees. They are usually found in temperate or cool and wet climates of approximately 15–25°C.

All plants, not just C_3 plants, need to fix carbon dioxide using the Calvin Cycle during the light independent stage of photosynthesis, in order to make glucose. This means that all plants use the enzyme Rubisco to catalyse carbon fixation. Rubisco, however, is not very efficient, particularly in C_3 plants. Oxygen acts as a competitive inhibitor for Rubisco, so the rate of photosynthesis is reduced in the presence of oxygen. The series of reactions that occur when oxygen (not carbon dioxide) is the substrate for Rubisco is called **photorespiration**. Photorespiration:

- forms a product that cannot be used to make glucose
- is an inefficient pathway, as it wastes energy
- reduces the efficiency of the Calvin Cycle, reducing the levels of photosynthesis by close to 25% in C_3 plants.

4B FACTORS IMPACTING ON ENZYME FUNCTION LINK

Photorespiration the series of reactions that occur as a consequence of Rubisco using O_2 as a substrate instead of CO_2; an inefficient process that cannot produce glucose

Plants get rid of oxygen to the atmosphere during photosynthesis, so normally oxygen won't bind to Rubisco and photorespiration shouldn't be a problem. However, on hot and dry days, or in desert and grassland environments, there is very little water, so C_3 plants close their stomata to conserve water. But closing their stomata also limits their access to carbon dioxide in the atmosphere and prevents them getting rid of the waste product oxygen, produced during the light dependent stage of photosynthesis. This is when Rubisco starts to bind to the oxygen that is building up in the leaves, and photorespiration begins. The oxygen acts like a competitor to the carbon dioxide, and photorespiration competes with photosynthesis.

C_3 plants have no special adaptations to reduce such competition.

Figure 5B–6 Most plants use C_3 photosynthesis

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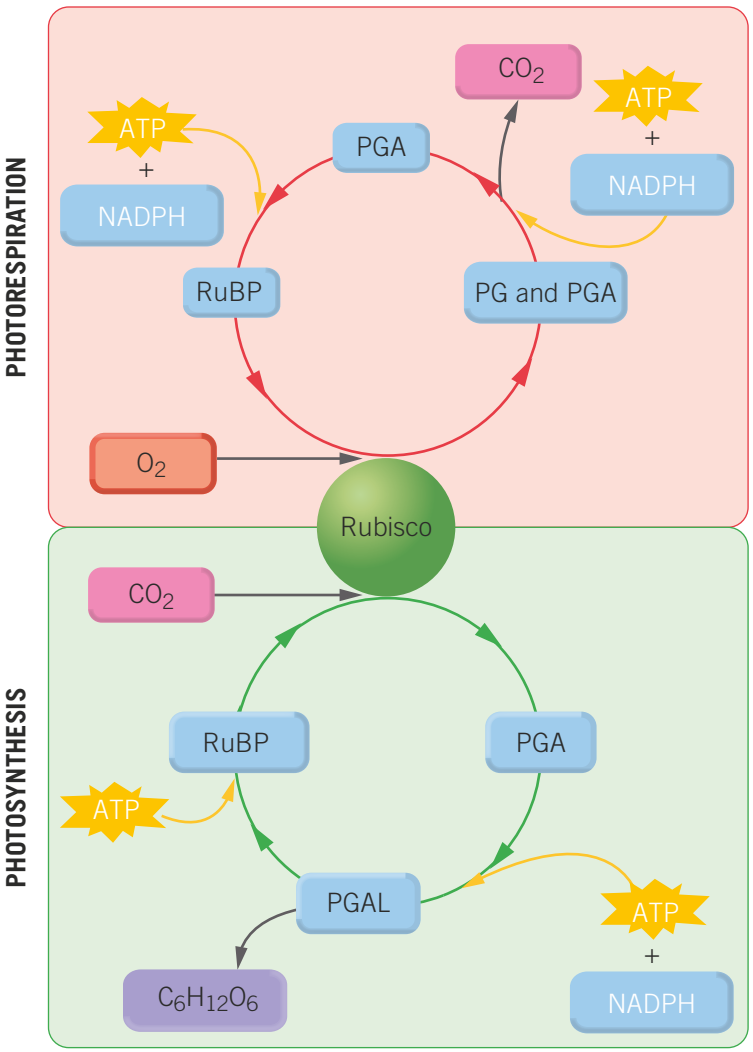

Figure 5B–7 A comparison of the Calvin Cycle in photosynthesis (when Rubisco binds to CO_2) and photorespiration (when Rubisco binds to O_2)

C_4 and CAM plants

C_4 plants and **CAM plants** have special adaptations that allow them to overcome the problem of competition between carbon dioxide and oxygen. Carbon dioxide is stored as a 4C compound and is only released to the Calvin Cycle *where* oxygen levels are low, in the case of C_4 plants, or *when* oxygen levels are low, in the case of CAM plants.

C_4 plants
plants that fix CO_2 to form malate, which contains four carbon atoms; better suited to grasslands; examples are maize and sugar cane

LINK 9B EVOLVING AND NON-EVOLVING POPULATIONS

CAM plants
plants that fix CO_2 to form malate, which contains four carbon atoms; better suited to deserts; examples are cacti and pineapples

In addition, C_4 and CAM plants use the enzyme PEP carboxylase to combine carbon dioxide with a 3C compound to make the 4C compound, and this enzyme has a high affinity for carbon dioxide. This means it can extract a greater proportion of it from the air. This also makes it more efficient than the C_3 process.

These adaptations have arisen by natural selection (which is covered in Chapter 9) and enable C_4 and CAM plants to outcompete C_3 plants in hot and dry environments. Table 5B–1 gives the details.

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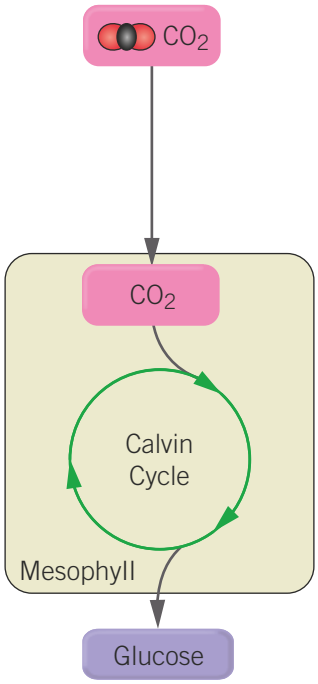
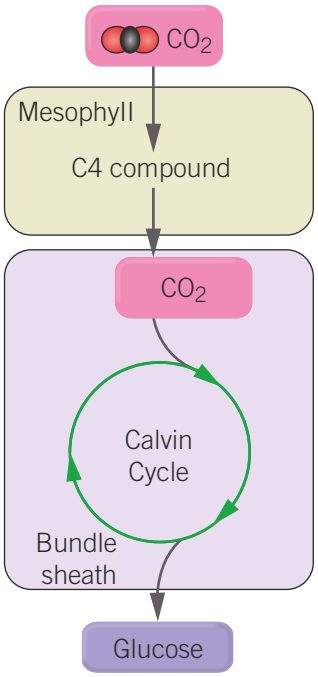
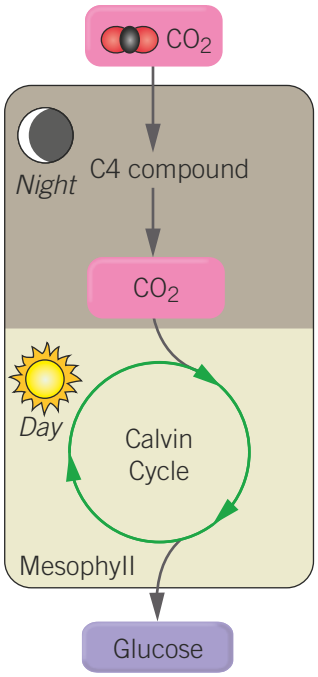
Table 5B–1 Comparison of C_3, C_4 and CAM plants

	C_3	C_4	CAM
Examples	Rice, cotton, wheat, soybean and trees	Corn, sorghum and sugar cane	Cacti, agave, pineapple
Environment	Temperate or cool and wet climates 15–25°C	Grasslands or hot and sunny environments 30–40°C	Deserts or very hot and dry environments > 40°C
Calvin Cycle	*Carbon fixation and Calvin Cycle occur together in the* mesophyll (photosynthetic tissue in leaves). Carbon fixation is the first part of the Calvin Cycle and forms the 3C compound PGA. This involves fixing carbon dioxide from the atmosphere to RuBP using the enzyme Rubisco. However, oxygen competition and photorespiration can reduce the efficiency of the process.	*Carbon fixation and Calvin Cycle occur in different tissues.* Carbon dioxide from the air is fixed to a 4C compound in the mesophyll and is then moved into deeper tissue ('bundle sheath'), where there are low oxygen concentrations. Here, carbon dioxide from the C_4 compound can enter the Calvin Cycle without competition from oxygen.	*Carbon fixation and Calvin Cycle occur at different times of the day within the same cell.* Carbon dioxide from the air is fixed to a 4C compound during the night, when stomata can be open. Therefore, there is a supply of carbon dioxide form the C4 compound that can be used in the Calvin Cycle during the day when the stomata are closed.
Stomata	Stomata open during the day but close when temperatures are high and water availability is low.	Stomata open during the day but close when temperatures are high and water availability is low.	Stomata closed all day, open only at night
Diagram			

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Check-in questions – Set 2

1 Outline the events that happen in C_3 plants during carbon fixation.
2 Summarise why Rubisco is considered inefficient.
3 Explain why C_4 plants are better suited than C_3 plants are to environments that have high light intensities and high temperatures.
4 In what ways is the C_4 carbon fixation pathway different from that of CAM plants?

WORKSHEET 5B–2 FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS

WORKSHEET 5B–3 COMPARING C_3, C_4 AND CAM PLANTS

5B SKILLS

Questions about experiments and experimental design

The Study Design demands that you not only develop key knowledge, but also develop an understanding of key scientific skills. Your teacher and assessors will use your practical work, your SACs and the examination to determine your strength in this area. So, what do you need to know? Working through Chapter 12 is a good starting point, but let's summarise some key terms and their meanings here, in the context of the information provided in a VCAA 2018 examination question.

VIDEO 5B–2 SKILLS: QUESTIONS ABOUT EXPERIMENTS AND EXPERIMENTAL DESIGN

LINK CHAPTER 12

Information provided:

Elsa read that red algae survive at greater water depths than green algae do because of a pigment in the red algae called phycoerythrin. This pigment enables the algae to absorb more of the geen light available at greater water depths. Elsa decided to investigate this by carrying out an experiment.

Using a standard technique, the single-celled algae were trapped in jelly balls. One set of balls contained green algae and another set contained red algae.

To measure the rate of photosynthesis, Elsa used a stopwatch and the pH indicator phenol red. Phenol red changes colour in solutions with different concentrations of carbon dioxide. In low carbon dioxide concentrations, phenol red is pink and in higher carbon dioxide concentrations it is yellow.

Elsa placed the jelly balls into test tubes and covered them with a solution containing dissolved carbon dioxide. Phenol red indicator was added to each solution.

Figure 5B–8 shows the set-up of Elsa's experiment.

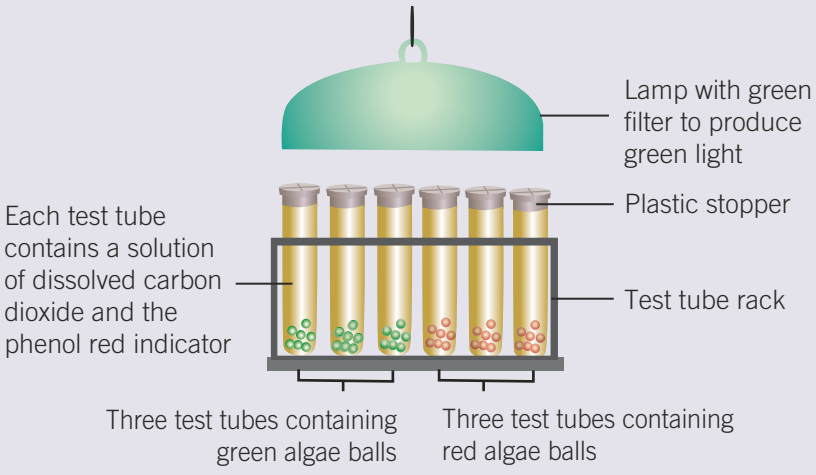

Figure 5B–8 Information from a question in the VCAA 2018 examination © VCAA

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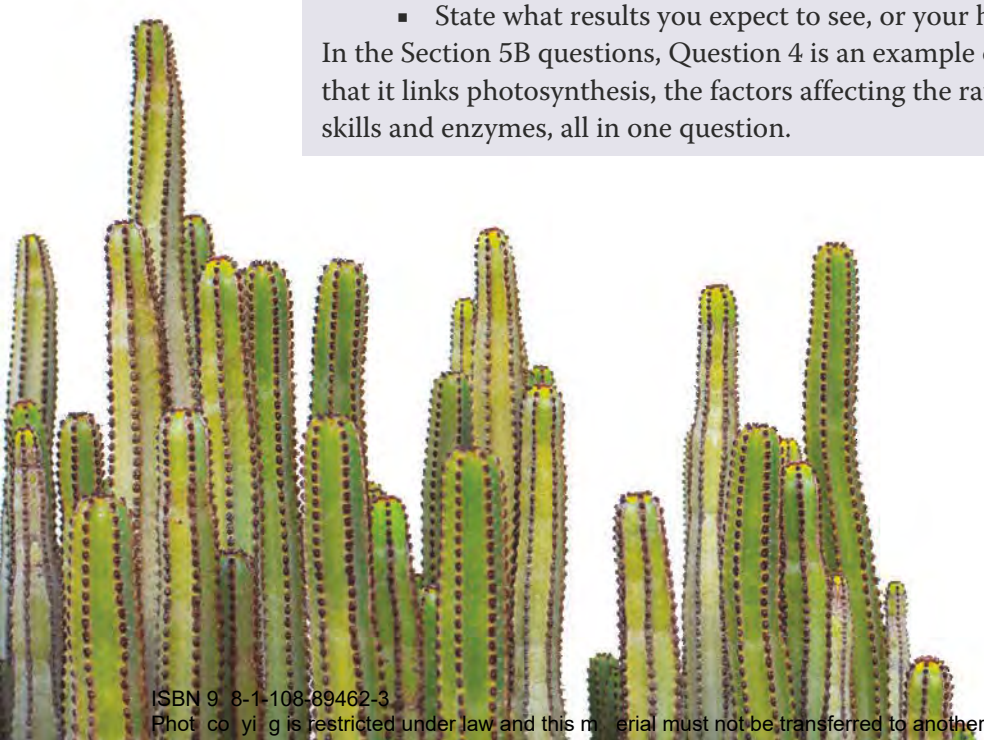
Some key terms and their meanings, in the context of the exam question are discussed below.

- *Hypothesis:* a prediction of the outcome of the experiment; testable
  - ► Example: It is hypothesised that the red algae will photosynthesise (or use CO_2) faster than the green algae when exposed to green light.
- *Independent variables:* the variables for which the quantities are changed by the experimenter; what is being investigated
  - ► Example: type of algae or whether the algae are red or green
- *Dependent variable:* the variable that changes in response to the independent variable; what is being measured
  - ► Example: time for the colour change to occur (seconds), pH/colour of solution. Both indicate the level of CO_2.
- *Controlled variable:* anything that needs to be kept constant (the same) so it won't affect the results
  - ► Examples: number of balls in each test tube, temperature, number of algae present in each test tube, number of drops of phenol red added, the initial pH (level of CO_2)
- *Results that support or do not support the hypothesis:* results that support the hypothesis are evidence that the prediction was correct. If the results do not support the hypothesis, then they are not evidence that the prediction is correct.
  - ► Example: The hypothesis would be supported if the red algae balls caused a faster change in colour of the indicator than the green algae balls.
  - ► Example: The hypothesis would not be supported if the colour of the indicator changed faster with the green algae balls than with the red algae balls.
- *Scientific experiment design:* If Elsa's teacher asked her to set up an experiment under different conditions, there are key steps that are expected in the methodology.
  - ► Examples:
    - Use a large number of test tubes, at least five in each group.
    - Repeat the experiment.
    - Mention any specialised equipment needed.
    - Clearly state the independent variable (deliberately changed), dependent variable (measured) and controlled variables (kept constant).
    - State what results you expect to see, or your hypothesis.

In the Section 5B questions, Question 4 is an example of this type of question. Notice that it links photosynthesis, the factors affecting the rate of photosynthesis, experimental skills and enzymes, all in one question.



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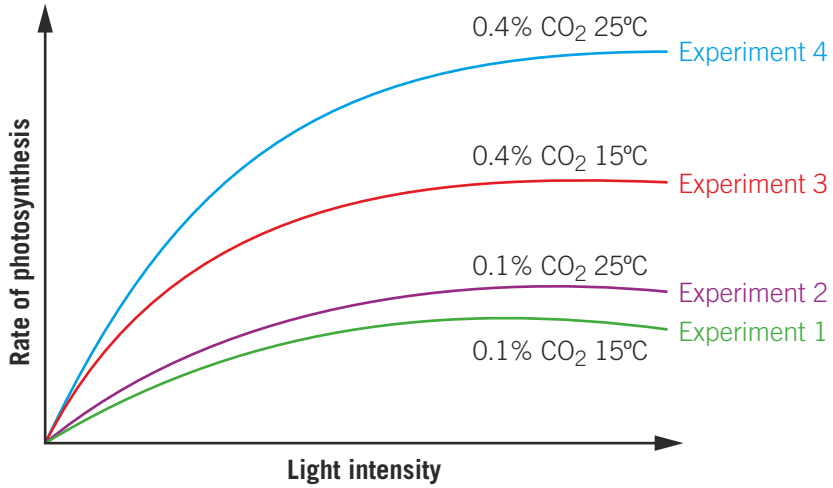
Section 5B questions

1 State three factors that may limit the rate of photosynthesis.

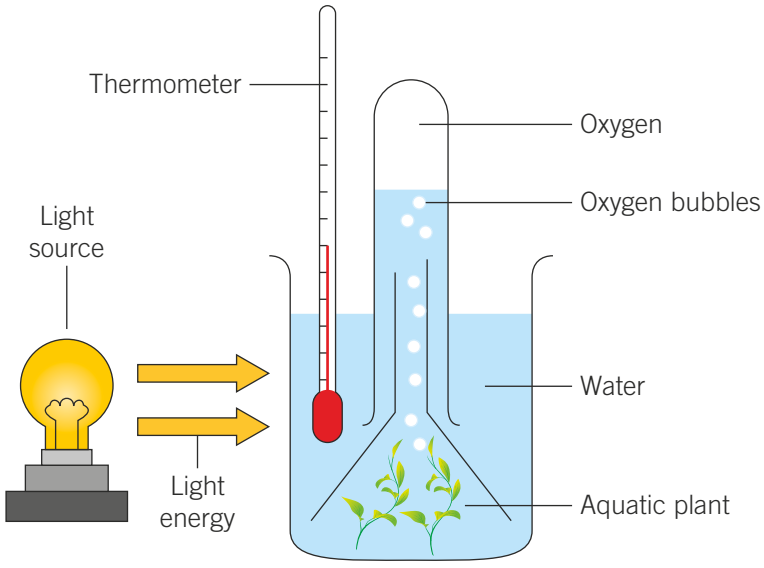
2 Explain why the rate of photosynthesis decreases as the concentration of carbon dioxide decreases.

3 Consider the results of the experiments shown below. Identify which factor – carbon dioxide concentration or temperature – has the greatest impact on the rate of photosynthesis. Explain your answer.



The results of four experiments measuring the rate of photosynthesis against light intensity at two different levels of carbon dioxide and two different temperatures

4 Students were investigating the relationship between the amount of oxygen bubbles produced by an aquatic green plant and the light intensity it was exposed to. They set up two beakers with identical-sized sections of aquatic plant and placed one in the shade and one next to a light source. The students recorded the number of bubbles given off by the plant in 2 minutes. They then moved the plant 10 cm away from the light source and repeated their recordings. They continued moving the plant back in 10 cm intervals until the plant was 50 cm away from the light source.



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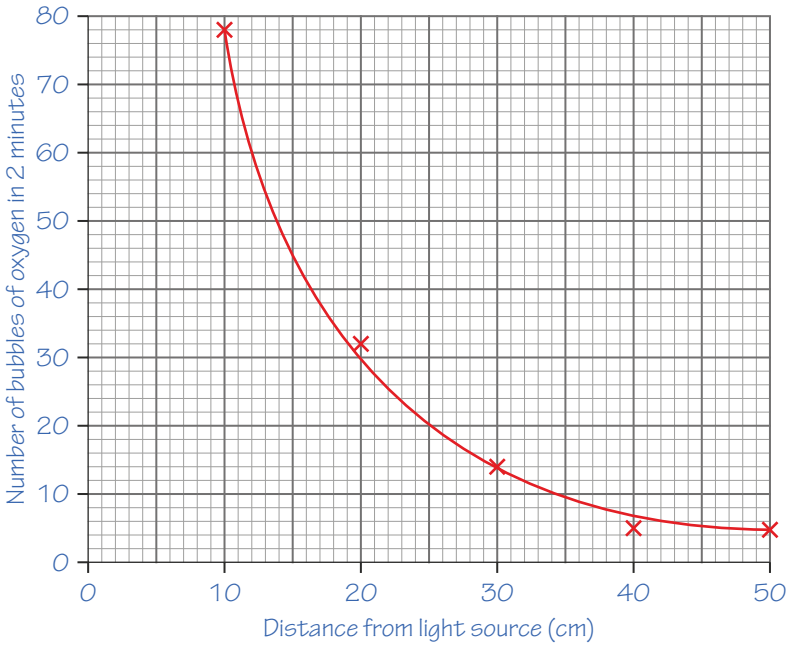
Their results for the plant that was placed in the light were graphed.



a Name the organelle that produces oxygen in aquatic plants.
b Outline how and where oxygen is produced by this organelle.
c What reaction does the production of oxygen inform us about?
d Write a balanced chemical equation for this reaction.
e Which beaker is the students' control?
f State the independent and dependent variables of the students' investigation.
g Explain the trend in the graph.
h What would you expect to happen to the levels of oxygen production if a student accidentally left the beaker containing the aquatic plant next to the lamp for a length of time that caused the water to become very hot? Give reasons for your answer.
i Sketch a graph showing the relationship between temperature and the rate of photosynthesis.
j Suggest two possible improvements to the students' investigation.

5 Rubisco is one of the most important and abundant enzymes on Earth.
a The series of reactions that occur when oxygen (not carbon dioxide) is the substrate for Rubisco is called photorespiration. Give reasons why this is the case.
b CAM plants can minimise photorespiration by carrying out gas exchange and carbon fixation at night. Outline how this helps these plants avoid water loss.

Figure 5B–9 Agave is an example of a CAM plant

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Cellular respiration

Study Design:

- The general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product
- The main inputs, outputs and locations of glycolysis, Krebs Cycle and electron transport chain including ATP yield (details of biochemical pathway mechanisms are not required)
- The location, inputs and the difference in outputs of anaerobic fermentation in animals and yeasts

Glossary:

Aerobic cellular respiration
Anaerobic cellular respiration
ATP synthase
Crista
Energy shuttle
Fermentation
Glycolysis
Matrix
NAD^+

ENGAGE

The discovery of an animal that doesn't need oxygen to live

In 2020, researchers discovered a unique organism, less than ten cells in size, that doesn't need oxygen to produce energy to survive. The microscopic parasite, called *Henneguya salminicola*, lives in salmon muscle tissue, an environment largely devoid of oxygen. It has evolved so that it doesn't need oxygen to produce ATP: it has literally stopped this critical biochemical pathway. Consequently, it has dropped its mitochondrial genome. Simply, the parasite is saving energy, as it doesn't need to copy genes and produce proteins for a process it doesn't use. Remember that it is the mitochondria that are responsible for converting glucose into ATP in most organisms during aerobic cellular respiration.

LINK UNIT 2

What does this relative of the jellyfish do for energy? This is not known, but one thought is that it steals it from the muscle tissue of the salmon. Other than that, researchers do not know for certain what processes or molecules *H. salminicola* uses. Until this new discovery, scientists were unsure whether organisms in the animal kingdom could survive in anaerobic (low-oxygen) environments. It was always assumed that the multicellular, highly developed organisms we know as animals first appeared on Earth because oxygen levels rose. How will an animal be categorised now?

Figure 5C–1 A non-oxygen-using parasitic animal called *Henneguya salminicola* has been discovered in salmon tissue. It forms white patches in the pink salmon flesh.

LINK UNIT 1

LINK 10A CHANGES IN BIODIVERSITY OVER TIME

This section explores the biochemical pathways of cellular respiration, both aerobic (in the presence of oxygen) and anaerobic (in the absence of oxygen). You will learn about the main inputs and outputs of the processes, where they occur and the ATP yield. You may gain some insight into how *H. salminicola* survives, as a result of your study.

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EXPLAIN

Understanding energy

The energy shuttle

Energy shuttle the cycling between the formation of ATP when energy is stored and the formation of ADP and P_i when energy is released; also known as the ATP–ADP cycle

The glucose produced in the light independent stage of photosynthesis is used as a raw fuel by organisms to make energy in the form of ATP (adenosine triphosphate). Before investigating how glucose is used in cellular respiration, let's revise the role of ATP and the **energy shuttle**.

As chemical reactions do not always occur in the same place within the cell, energy needs to be transferred between reactions. ATP enables this by acting as an energy shuttle. ATP consists of adenine (a nitrogen-containing compound), a ribose (five-carbon sugar) and three phosphate groups, as shown in Figure 5C–2.

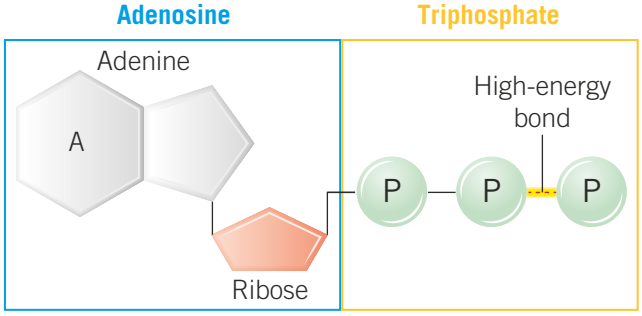

Figure 5C–2 Adenosine triphosphate is the primary source of energy for cells. It contains a high-energy bond between the second and third phosphates, where energy is stored.

When a cell needs energy, the high-energy bond in ATP (the bond between the second and third phosphate) is broken and a phosphate is removed, releasing the energy stored in the bond. This energy can then be used for cellular processes. The remaining molecule now only has two phosphates and so is named adenosine diphosphate, or ADP. Recall from Section 4A that:

4A THE ROLE OF THE ROLE OF ENZYMES LINK

- when complex compounds are broken down into simpler ones in this way, the reaction is described as catabolic
- when energy is released, the reaction is called *ex*ergonic (energy *ex*its).

Cells can store excess energy by adding an inorganic phosphate (P_i) back onto ADP, storing the energy in the bond, forming ATP once again. Again, recall that:

- when complex compounds are synthesised from simpler ones in this way, the reaction is described as anabolic
- when energy is required or is stored in a bond, the reaction is called *en*dergonic (energy *en*ters).

This cycling between ATP and ADP, as energy is released and stored, is referred to as the energy shuttle, or the ATP–ADP cycle.

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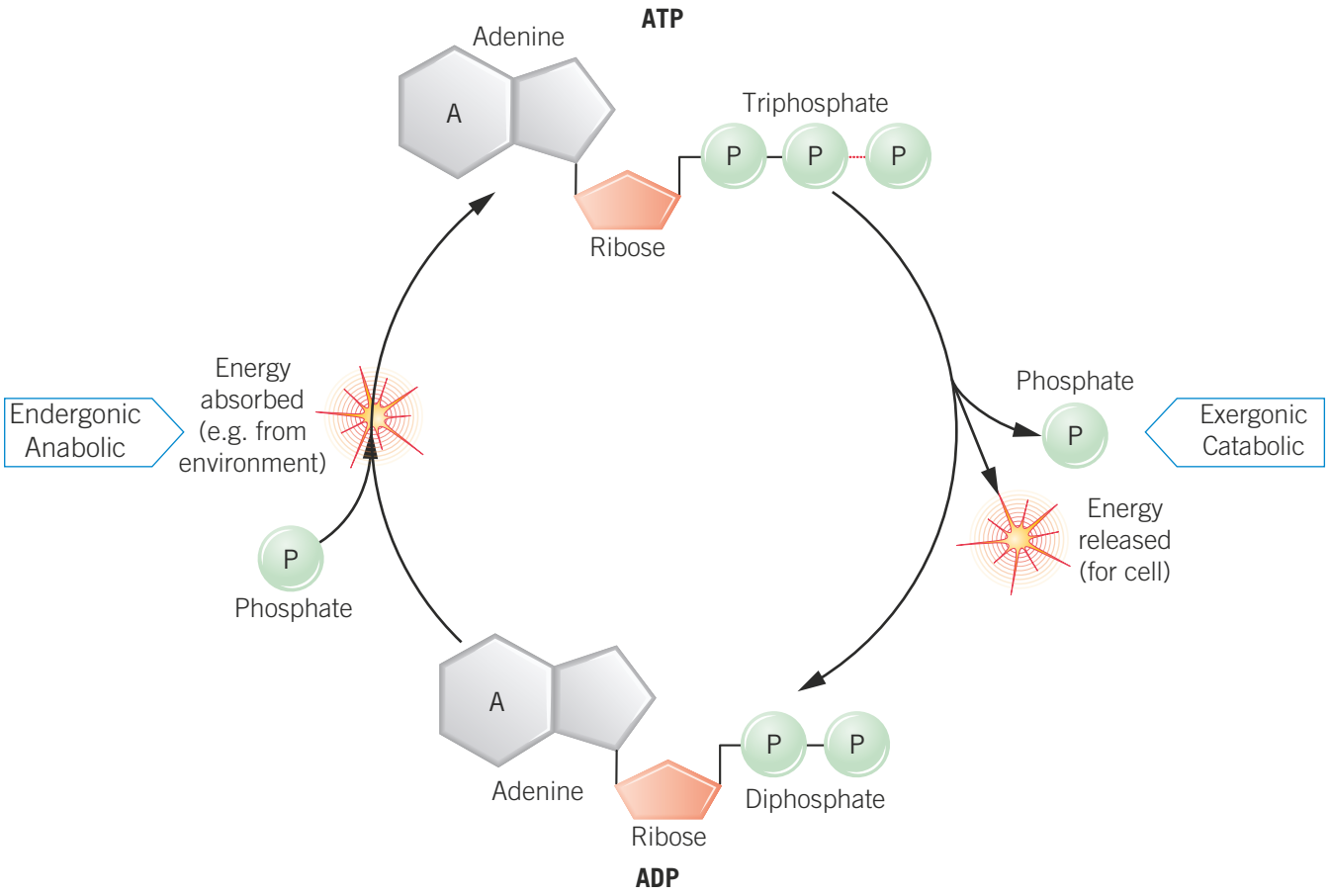

Figure 5C–3 The energy shuttle or ATP–ADP cycle. ATP shuttles energy to the location of cellular processes that require energy, and releases the energy by breaking the bond between the second and third phosphates (exergonic reaction). Excess energy is then stored by adding an inorganic phosphate (P_i) to ADP, to form ATP (endergonic reaction).

PPS

Uses for ATP

LINK UNIT 1

The energy stored in ATP is needed for numerous biochemical processes in cells, including cell growth and repair, muscle movement, the transmission of nerve impulses, moving molecules by active transport, synthesising molecules (like proteins), and so on. Keep in mind that both heterotrophs (animals and fungi) and autotrophs (green plants and algae) need to access the energy stored in glucose and make ATP in order to carry out these processes.

- Autotrophs make the organic compound glucose, which allows them to then make ATP.
- Heterotrophs cannot make their own glucose. They need to first consume the glucose produced by autotrophs, and then use it to make ATP.

Check-in questions – Set 1

1 Summarise why ATP is so important to cells.
2 Draw the ATP–ADP cycle.
3 Outline the relationship between ATP and ADP in terms of energy release and energy storage.

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Glycolysis
the first stage of cellular respiration, where glucose is broken down into two pyruvate molecules in the cytosol, producing 2 ATP and 2 NADH; does not require oxygen

Aerobic cellular respiration
cellular respiration that occurs in the presence of oxygen and involves the transformation of the chemical energy stored in glucose into ATP; includes the Krebs Cycle and the electron transport chain, which occur in the mitochondria

Anaerobic cellular respiration
cellular respiration that occurs in the absence of oxygen and involves the transformation of the chemical energy stored in glucose into 2 ATP; the products depend on the type of organism carrying out the process

The stages of cellular respiration

Overview

Like photosynthesis, cellular respiration occurs as a series of steps (a biochemical pathway), each controlled by a different enzyme. There are two types of cellular respiration, both of which begin with an initial step, called **glycolysis**, in the cytosol of cells.

- **Aerobic cellular respiration**: glucose is broken down in the presence of oxygen to produce carbon dioxide, water and ATP. It includes the Krebs Cycle and the electron transport chain, which both occur in the mitochondria.
- **Anaerobic cellular respiration**: occurs in the absence of oxygen. The products formed depend on the type of organism this process occurs within. Plants and yeasts carry out alcohol fermentation, whereas animals carry out lactic acid fermentation.

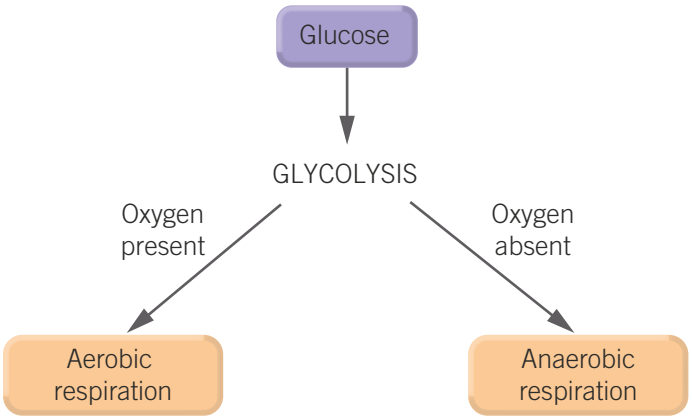

Figure 5C–4 Cellular respiration begins with the process of glycolysis. What happens after that depends on whether oxygen is present.

Glycolysis

The first stage of cellular respiration occurs in the cytosol of the cell. Its purpose is to break down the large glucose molecules (containing six carbons) into two smaller molecules called pyruvate, or pyruvic acid (containing three carbons), so they can be transported to the mitochondria. Glycolysis sounds simple but actually consists of ten different reactions, with each step catalysed by a specific enzyme.

4A THE ROLE OF ENZYMES LINK

5A PHOTO-SYNTHESIS LINK

NOTE

Glyco- refers to the carbohydrate glucose and *-lysis* means something is broken down.

Overall, 2 ATP, 2 NADH (nicotinamide adenine dinucleotide plus hydrogen) and two pyruvate molecules are produced during glycolysis.

NAD⁺
a coenzyme that accepts hydrogen ions and transfers them from one place to another during cellular respiration

Recall from Section 5A that a coenzyme called NADP⁺ can carry hydrogen ions safely between reactions in photosynthesis. In cellular respiration, **NAD⁺** has the same role. The hydrogen ions carried by NADH are required later during the electron transport chain stage in aerobic cellular respiration.

NOTE

You are not required to know numbers of NADH.

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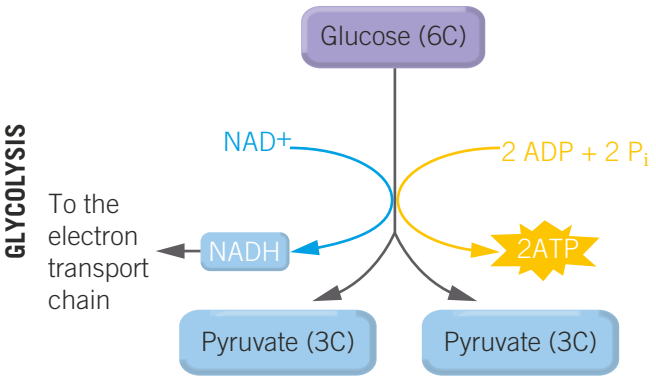

Figure 5C–5 Glycolysis is the process in which glucose, containing six carbons (6C), is split into two pyruvate molecules, each containing three carbons (3C).

Check-in questions – Set 2

1 Glycolysis is the first stage of cellular respiration.
- **a** Briefly state what happens during this stage.
- **b** Complete the following table for glycolysis.

Inputs	Outputs	Location

- **c** Write a word equation for glycolysis.
- **d** Does glycolysis require oxygen?

VIDEO 5C–1 CELLULAR RESPIRATION: AEROBIC RESPIRATION

Aerobic cellular respiration

Overview

If oxygen is present, following glycolysis in the cytosol, the second and third stages of aerobic cellular respiration – the Krebs Cycle and the electron transport chain – occur in the mitochondria.

- The *Krebs Cycle* (or citric acid cycle) occurs in the fluid matrix of the mitochondria and produces two molecules of ATP per molecule of glucose that enters glycolysis.
- The *electron transport chain* occurs on the inner membrane of the mitochondria, the cristae, and produces 32 (or in some cases 34) molecules of ATP per molecule of glucose that entered glycolysis.

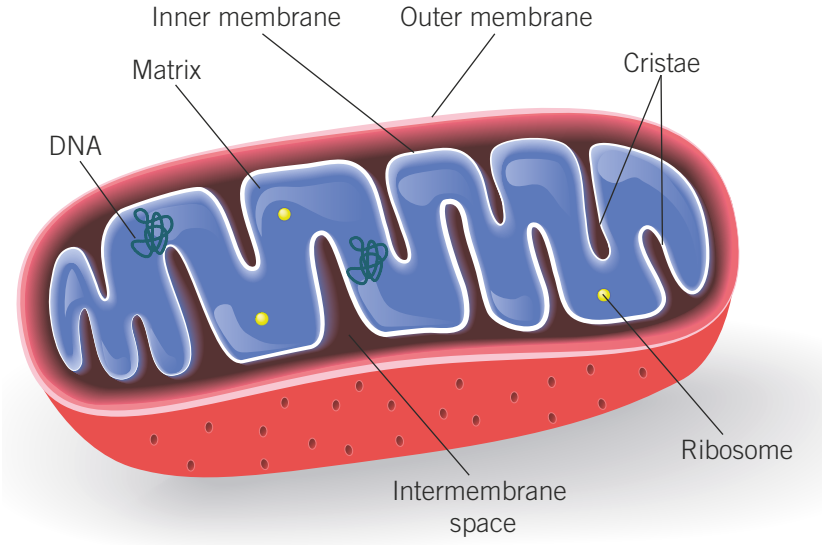

Figure 5C–6 Mitochondria are the organelles in eukaryotic cells that carry out two stages of aerobic cellular respiration: the Krebs Cycle in the matrix and the electron transport chain on the cristae.

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NOTE

Remember that ATP is the product of ADP and Pi but these reactants are not required in the chemical equation.

The overall process of this catabolic chemical reaction can be written as a word equation:

glucose + oxygen ⟶ carbon dioxide + water + energy

or as a chemical equation:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 36 \text{ or } 38 \text{ ATP}$$

The entire story of aerobic cellular respiration can be represented in a diagram, as shown in Figure 5C–7. Refer back to this figure as you learn about the stages that occur in the mitochondria in more detail.

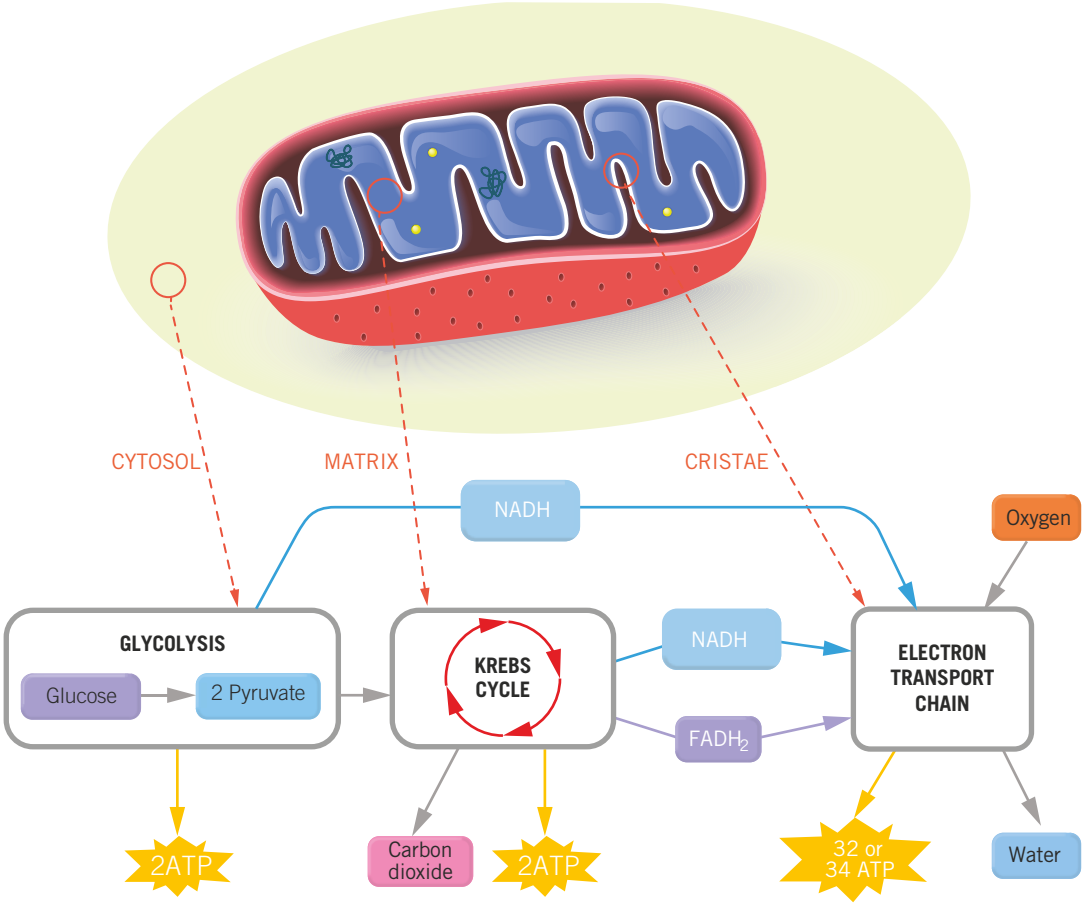


Figure 5C–7 An overview of aerobic cellular respiration: glycolysis, Krebs Cycle and the electron transport chain. See if you can identify where the reactants and the products of the balanced chemical equation enter and exit aerobic cellular respiration. Take special notice of how much ATP is produced in each stage.

NOTE

Heat production

During cellular respiration, energy in the form of ATP is released, but heat energy is also produced. The higher the cell's rate of respiration, the more heat is produced. This is most obvious when you exercise – the mitochondria in your muscles are working hard to release energy to sustain your movement. As a consequence, your body temperature increases. Similarly, on a cold day you may shiver. This is your muscles rapidly contracting and when they do this, the mitochondria are stimulated to increase their rate of cellular respiration, thus producing heat.

The structure of the mitochondrion

When you observe the structure of a mitochondrion, as shown in Figure 5C–8, notice that the inner membrane of the mitochondrion has folds that project into the matrix. Each fold is called a **crista** (plural *cristae*). The more cristae there are, the more surface area there is available for carrying out the important reactions of the electron transport chain. The remaining space in the mitochondrion is a fluid called the **matrix**, which contains many ribosomes due to the large number of enzymes that are required for the Krebs Cycle.

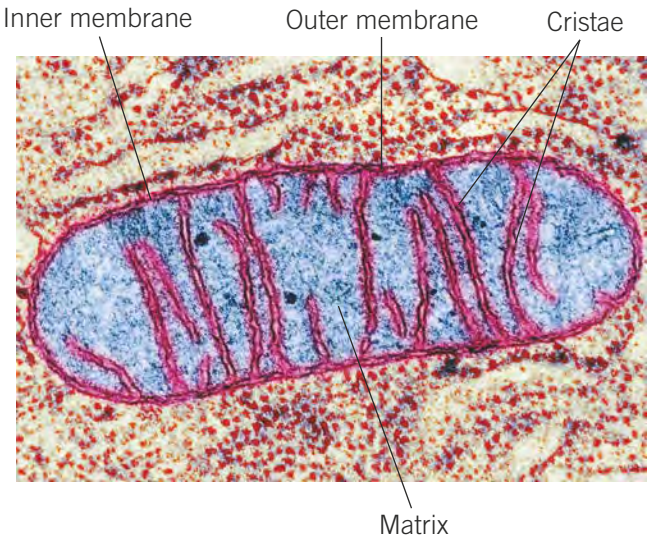


Figure 5C–8 Transmission electron microscope image of a mitochondrion

Crista
a fold in the inner membrane of a mitochondrion and site of the third stage of aerobic cellular respiration, the electron transport chain

Matrix
the fluid component of a mitochondrion and site of the second stage of aerobic cellular respiration, the Krebs Cycle

LINK 2D GENE STRUCTURE AND EXPRESSION

Krebs Cycle

The second stage of aerobic cellular respiration, the Krebs Cycle (or citric acid cycle) occurs in the fluid matrix of the mitochondrion and produces 2 ATP. Eight different reactions occur, each catalysed by a different enzyme. You are not expected to know all these reactions, just the main inputs, outputs and locations for the overall process, including ATP yield.

LINK 4A THE ROLE OF ENZYMES

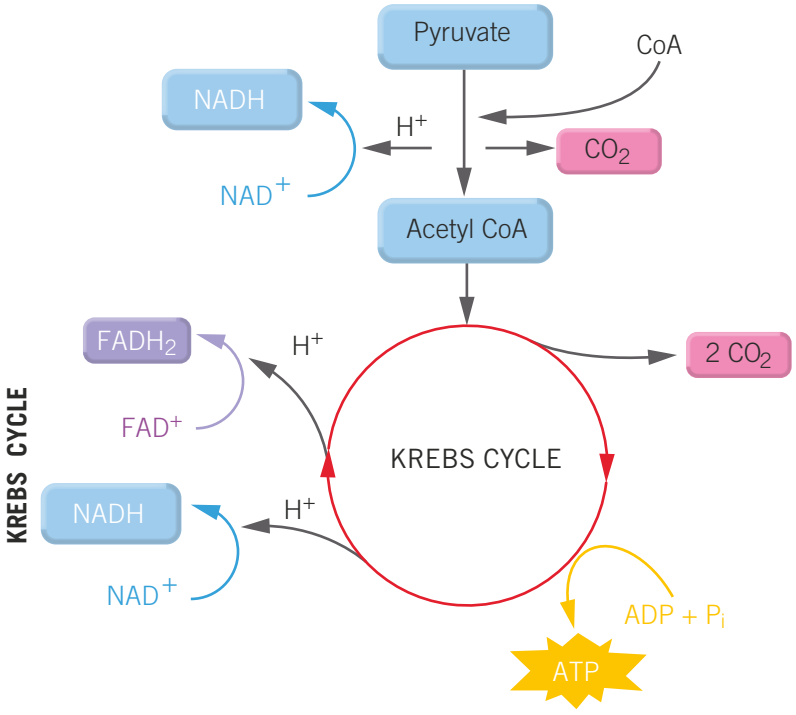


Figure 5C–9 A summary of the main inputs and outputs for one cycle (of the two cycles that occur) of the Krebs Cycle, including ATP yield, which occurs in the fluid matrix of the mitochondrion.

Figure 5C–10 summarises these processes:

1 Glycolysis in the cytosol produces two pyruvate molecules from one glucose molecule.
2 The two pyruvate molecules move by active transport into the fluid matrix of the mitochondrion where they are converted, using CoA (coenzyme A), into two acetyl CoA molecules, also producing two carbon dioxide molecules. NAD^+ forms NADH at this time.
3 The two acetyl CoA molecules enter the Krebs Cycle and more NADH, CO_2 and ATP are produced, plus $FADH_2$, another hydrogen ion-carrying coenzyme.

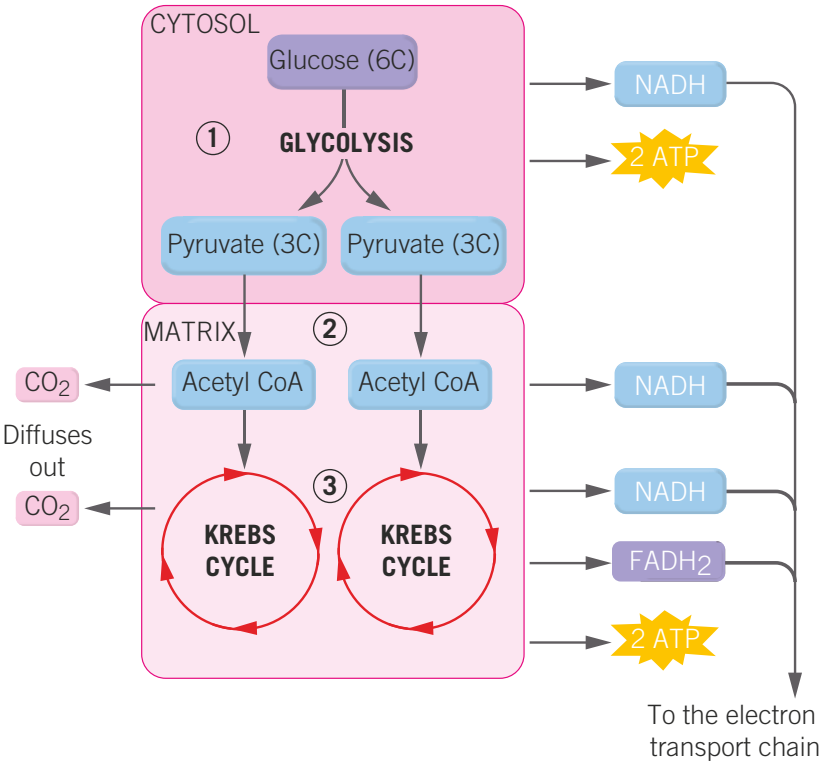


Figure 5C–10 Aerobic cellular respiration: an overview of the story so far.

The overall outputs are, in two cycles: four carbon dioxide molecules, NADH molecules, $FADH_2$ molecules (another hydrogen ion carrying coenzyme) and two ATP molecules.

Note that the carbon dioxide produced diffuses out of the mitochondria, and out of the cell, and is then released into the atmosphere, while the NADH, $FADH_2$ and ATP are used within the mitochondria or cell.

Electron transport chain

The electron transport chain involves making ATP in the inner membranes of the mitochondria, called the cristae. It is during this stage that the majority of ATP is made. The chain is a series of protein complexes (enzymes and peptides as well as other molecules) in the mitochondrial membrane, which are also called the cytochrome system. Their function is to pass electrons through a series of reactions, releasing energy that drives the transfer of H^+ hydrogen ions (protons) across a membrane. This creates an H^+ gradient across the membrane which drives the synthesis of ATP as the H^+ ions flow back. Figure 5C–11 summarises this process:

1 The starting point is the supply of hydrogen ions brought by coenzymes NADH and $FADH_2$, produced during glycolysis and the Krebs Cycle. The first protein complex transfers the hydrogen ions across the membrane and delivers electrons to the start of the chain.
2 The electrons are transported through a series of proteins on the inner membrane, called cytochromes, forming the cytochrome system. Along the way more hydrogen ions from $FADH_2$ are pumped across the membrane.
3 The higher concentration of hydrogen ions that have built up in the intermembrane space represents considerable chemical energy that is released to **ATP synthase**. This enzyme uses the energy to produce 32 or 34 molecules of ATP from the joining of ADP + P_i.
4 At the end, the electrons from the transfer chain and hydrogen ions flowing back across the membrane combine with oxygen from the atmosphere (or from photosynthesis), forming water.

ATP synthase an enzyme responsible for catalysing the formation of ATP from ADP and P_i

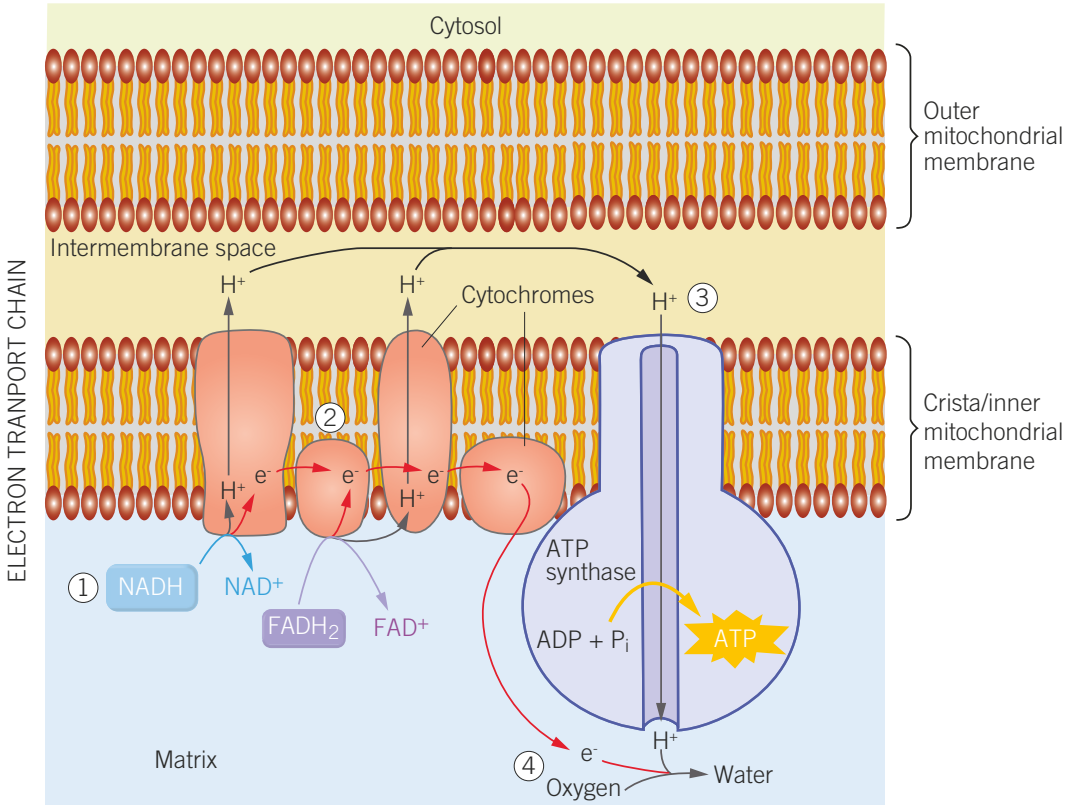
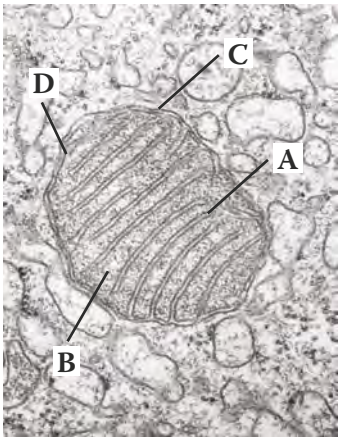


Figure 5C–11 The electron transport chain occurs in protein complexes (coloured orange) located in the cristae of the mitochondria. The coenzymes NADH and $FADH_2$ supply electrons and hydrogen ions to the chain. Electrons passing down the chain provide energy to pump hydrogen ions across the membrane; when these pass back through the enzyme ATP synthase they release the energy to enable the enzyme to make ATP. The numbers refer to the steps described in the text.

Check-in questions – Set 3

1 Shown at right is a TEM (transmission electron microscope) image of a mitochondrion.

- **a** Identify which of A–D is the:
  - **i** inner membrane
  - **ii** outer membrane
  - **iii** matrix
  - **iv** cristae.
- **b** Explain why a mitochondrion contains so many ribosomes.
- **c** How is the structure of the inner membrane of the mitochondrion beneficial to the cell?



2 Complete the table below, summarising the three stages of aerobic cellular respiration and the inputs, outputs, ATP yield and where in the cell they occur.

Stage	Inputs	Outputs	Location	ATP yield
Glycolysis				
Krebs Cycle				
Electron transport chain				

NOTE

Hydrogen has one electron and one proton, so it is the electron and proton in each hydrogen that are transported along the 'chain', thus forming the electron transport chain.

WORKSHEET 5C–1 SUMMARISING AEROBIC RESPIRATION

Anaerobic cellular respiration

Fermentation the process by which glucose is broken down in the absence of oxygen to produce 2 ATP; also called anaerobic cellular respiration

All organisms can metabolise glucose without oxygen. However, for a cell, this process is far less efficient than with oxygen (aerobic cellular respiration), as it produces only two ATP molecules for every glucose molecule that is broken down. Anaerobic cellular respiration, or **fermentation**, begins with the breakdown of glucose to pyruvate (glycolysis), forming two ATP. The next stage continues in the cytosol but depends on the organism.

The purpose of anaerobic cellular respiration is to restore levels of the coenzyme NAD^+ in the cell. Remember that NAD^+ is needed for glycolysis and therefore is necessary for ATP production. So the NADH produced by glycolysis must be 'unloaded' and restored to NAD^+. Figure 5C–12 illustrates the relationship between the coenzyme NAD^+ and the processes of glycolysis and fermentation.

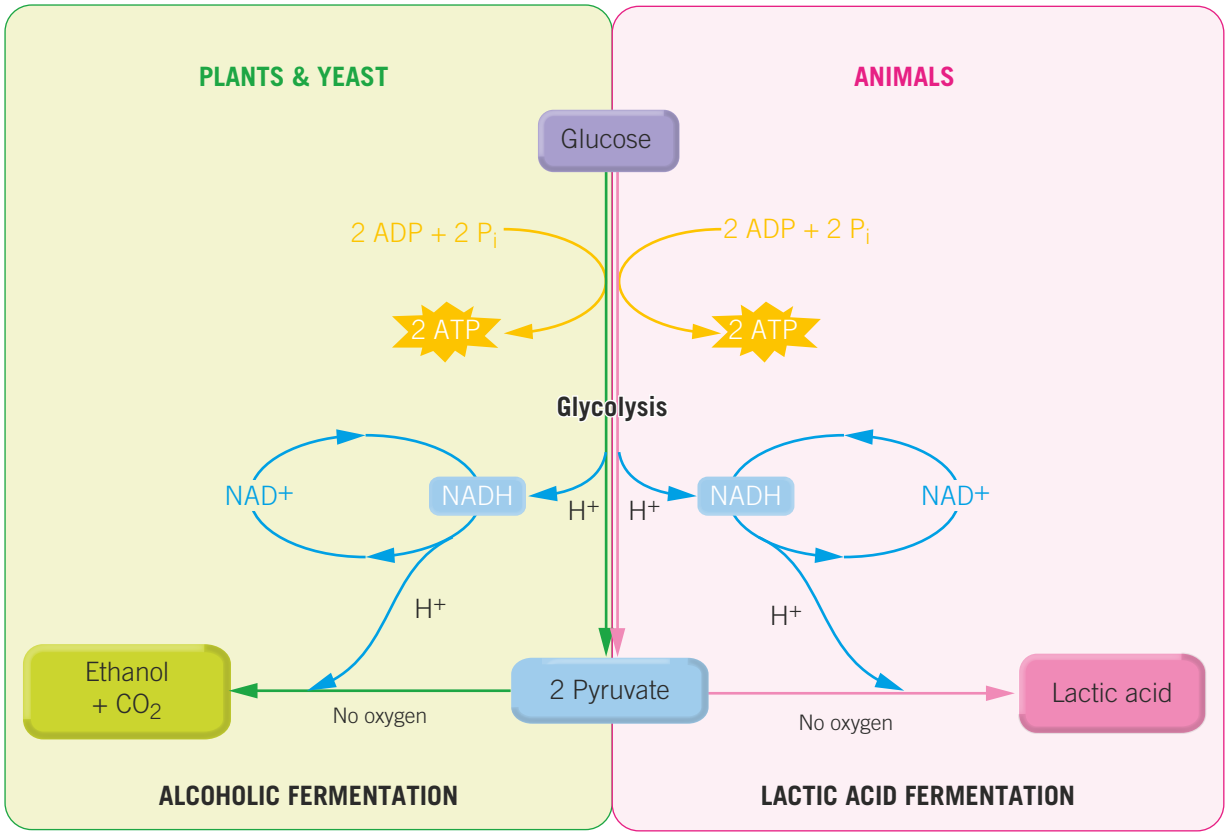


Figure 5C–12 Anaerobic cellular respiration or fermentation begins with the process of glycolysis, forming 2 ATP and NADH. The next stage differs between plant/yeast and animal cells. However, in both cases, the levels of NAD^+ are restored by unloading the hydrogen ions from NADH produced during glycolysis.

VIDEO 5C–2 ANAEROBIC RESPIRATION

In summary:

In plants and yeast, alcoholic fermentation occurs:

glucose → ethanol + carbon dioxide + 2 ATP

In animals, lactic acid fermentation occurs:

glucose → lactic acid + 2 ATP

Check-in questions – Set 4

1 Complete the table below, which summarises anaerobic cellular respiration and the inputs, outputs, ATP yield and where it occurs.

Stage	Inputs	Outputs	Location	ATP yield
Lactic acid fermentation				
Alcoholic fermentation				

Comparing aerobic and anaerobic cellular respiration

A comparison of the processes of aerobic and anaerobic cellular respiration is given in Table 5C–1.

Table 5C–1 Comparison of aerobic and anaerobic cellular respiration

	Aerobic cellular respiration	Anaerobic cellular respiration
Location	Cytosol and mitochondria	Cytosol
Oxygen required	Yes	No
ATP yield	36 or 38	2
Energy production speed	Slow ATP production	Rapid ATP production
Reactants	Glucose + oxygen	Glucose
Products	Carbon dioxide + water + ATP	Ethanol + carbon dioxide + ATP (plants / yeast) Lactic acid + ATP (animals)
Stages	Glycolysis Krebs Cycle Electron transport chain	Glycolysis Fermentation

WORKSHEET 5C–2 COMPARING AEROBIC AND ANAEROBIC RESPIRATION

Figure 5C–13 Anaerobic cellular respiration in yeast makes dough rise, as the carbon dioxide gas produced forms bubbles in it. The ethanol evaporates during baking, leaving trace amounts.

VIDEO 5C–3
SKILLS: UNDERSTANDING THE LINK BETWEEN PHOTOSYNTHESIS AND CELLULAR RESPIRATION

5C SKILLS

Understanding the link between photosynthesis and cellular respiration
An overview of the processes of photosynthesis and respiration is given in the diagram below.

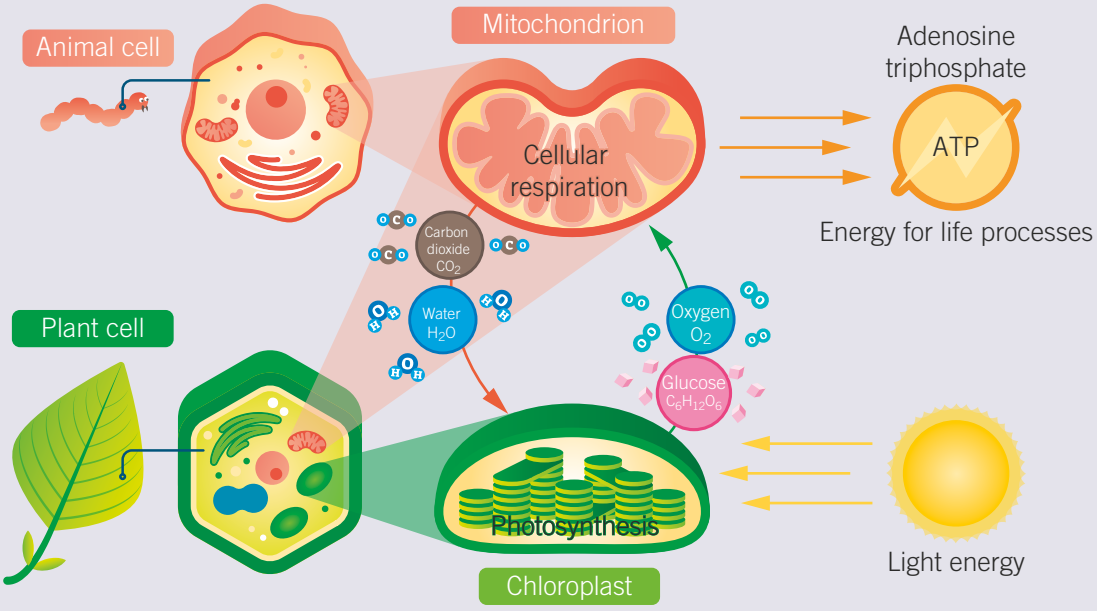


Figure 5C–14 The complementary nature of the photosynthesis and cellular respiration reactions means that carbon, hydrogen and oxygen cycle through organisms and their environments continually.

Referring to Figure 5C–14, note the following:

- Photosynthesis occurs in the chloroplast, an organelle found only in the cells of green plants and algae.
- Photosynthesis requires light energy, and so the light dependent stage can only occur during daylight hours.
- The products of photosynthesis (oxygen and glucose) are used as reactants in the process of aerobic cellular respiration.
- Aerobic cellular respiration occurs mostly in the mitochondrion, an organelle found in eukaryotic cells.
- Aerobic cellular respiration uses the products of photosynthesis to produce ATP, an energy storage molecule, which can then release energy to meet the cell's requirements.
- In plants, the products of aerobic cellular respiration (water and carbon dioxide) can then be used as reactants in the process of photosynthesis.
- Although photosynthesis appears to be the reverse of cellular respiration, remember that the processes are different and use different enzymes. Also, photosynthesis only occurs if light is present and only in cells with chlorophyll (or another photosynthetic pigment), while respiration occurs all the time (24 hours a day) and in (almost) every living cell.

VIDEO 5C–4
SKILLS: STUDY DESIGN TO GUIDE YOUR PREPARATION

Using the Study Design to guide your preparation

The most important way of determining the detail you need to know is by looking at the Study Design and completing many practice questions. Keep focused on the terms used in the Study Design dot points and, when making notes, always use the dot points, or part of the dot points, as your headings. The Study Design was written specifically to cover what you need to know, and the success criteria have been written to guide you through the necessary material.

For example, the Study Design requires you to know the inputs, outputs, location and ATP yield of glycolysis, the Krebs Cycle and the electron transport chain. This suggests that, if you know these things, you will be able to work through and determine the answers to assessment questions. Consider the following diagram and predict what the questions could be, based on what you know of the study design.

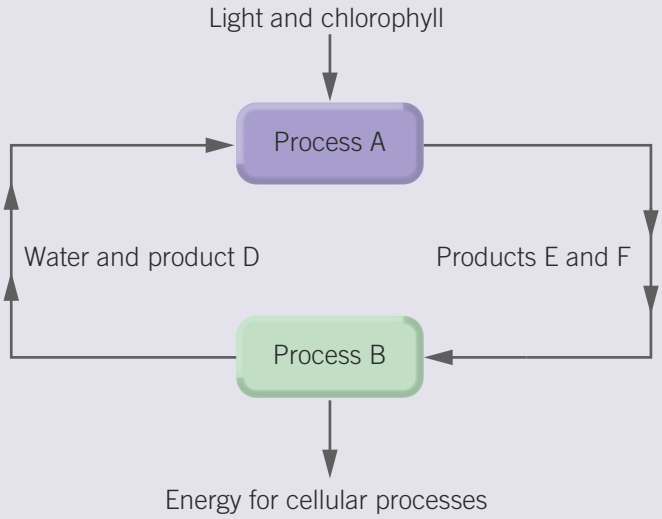


Figure 5C–15 Consider the diagram and predict possible questions that assessors may ask.

Possible questions:

- Determine what processes A and B are.
- Write word and balanced chemical equations for processes A and B.
- List where processes A and B occur.
- Outline the stages involved in processes A and B, including inputs, outputs, ATP yield and location.
- Identify products D, E and F.

If you know the inputs, outputs and location details, you will be able to identify the missing information in the diagram. Begin with the inputs of light and chlorophyll – this suggests that process A must be photosynthesis, as this biochemical process cannot occur without them. Can you answer the rest of the possible questions above? Remember, assessment questions often link the concepts to real-world examples, so the above diagram may be in the context of a particular organism. Don't let this scare you, as you are not expected to know a whole range of organisms. The assessors want to see that you can apply what you have learned to a new situation, and they will only expect you to know what the Study Design has outlined.

CHAPTER 4 LINK

Also keep in mind the interrelationships between concepts. Recall that Chapter 4 looked at enzymes: the action of enzymes and the factors that affect enzymes. Remember these two Study Design dot points:

- the general role of enzymes and coenzymes in facilitating steps in *photosynthesis* and *cellular respiration*
- the general factors that affect enzyme function in relation to *photosynthesis* and *cellular respiration*: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors.

This means that, while revising photosynthesis and cellular respiration, ensure you summarise the general involvement of enzymes and coenzymes in biochemical pathways, and include specific examples. For example: enzymes ATP synthase in cellular respiration and Rubisco in photosynthesis; coenzymes NAD^+ and FAD^+ in cellular respiration; and $NADP^+$ in photosynthesis. In the same way, you will need to reflect on the different factors that affect enzyme activity, keeping in mind that enzymes regulate biochemical pathways, so these factors will also affect the efficiency of cellular respiration and photosynthesis.

Here is an example of how this may appear in a question.

Question

ATP synthase is an enzyme that catalyses the production of ATP in a cell.

a Identify the substrate that ATP synthase binds to.
b Define the term catalyses.
c Give reasons why it is important for cells that ATP synthase is functioning under optimal conditions.
d What is the name of the biochemical pathway this enzyme is active in?
e What is ATP synthase made of and where is it produced?
f Outline the consequence for the cell if ATP synthase is exposed to high temperatures.

Section 5C questions

1 A student stated that photosynthesis happens in plants and cellular respiration happens in animals. Is this student correct? Give reasons for your answer.

2 Imagine that an animal cell is undergoing aerobic cellular respiration when suddenly there is not enough oxygen available to complete the process.
   a Name the stage of aerobic respiration where oxygen is an input.
   b Where does this stage occur?
   c If this stage cannot occur, the inputs of this stage will build up. What are the inputs of this stage?
   d Identify which of the inputs you listed in part **c** are coenzymes.
   e The Krebs Cycle will also slow down as a consequence, and a build-up of pyruvate and a slowing of glycolysis will follow. Write the word equation for the biochemical pathway that will occur instead of aerobic respiration, in this anoxic (or low-oxygen) environment.

3 Both lactic acid fermentation and alcoholic fermentation are reversible. This means that, as oxygen becomes available, pyruvate can be remade and aerobic cellular respiration can proceed. Suggest why a cell would benefit more from carrying out aerobic cellular respiration than from carrying out anaerobic cellular respiration.

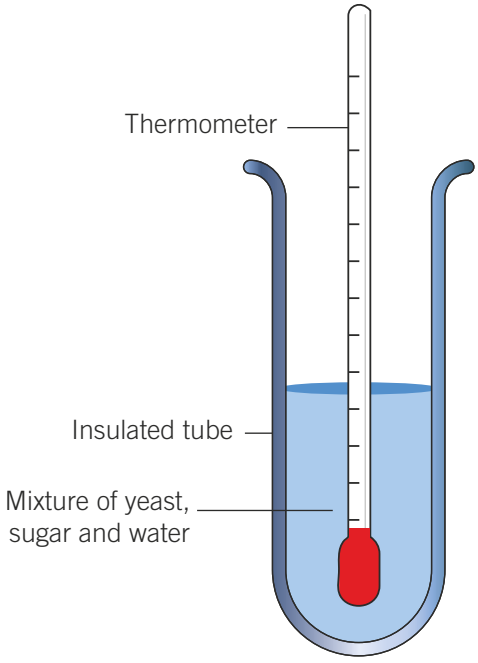
4 The energy stored in ATP is needed for numerous biochemical processes in cells, including the movement of molecules by active transport.

- **a** What organelle would you expect to find in large numbers in cells that do a lot of active transport? Justify your answer.
- **b** Outline the advantage of the cristae to a cell when carrying out aerobic cellular respiration.
- **c** What is the name of the process that produces 2 ATP regardless of whether oxygen is present or absent?
- **d** Name the input for the process you named in part **c**.
- **e** Give reasons why glucose is broken down to form ATP in numerous reactions instead of just one.
- **f** In the absence of oxygen, pyruvate can be converted into lactic acid. Explain the advantage for a cell in carrying out this transformation.

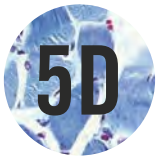
5 Students carried out an experiment where they mixed yeast cells with sugar and water, as shown in the diagram below. Air was able to move freely between the inside and the outside of the tube.

The temperature of the mixture was seen to increase, and bubbles formed. The students' results, comparing carbon dioxide production and temperature change using different sugar sources, are given in the table.

Time (minutes)	Height of bubbles (cm)		
	Tube 1: No sugar	Tube 2: White sugar	Tube 3: Natural sugar
0	4	4	5
180	4	18	15
Temperature change (°C)	0	+8	+4



- **a** State the independent variable in this experiment.
- **b** What evidence is there that CO_2 and ATP have been produced?
- **c** Name the process occurring and write the balanced chemical equation for the process.
- **d** State the stage and location where most of the CO_2 is produced.
- **e** Explain why tube 1 was included in the experiment, when it doesn't contain a sugar source.
- **f** If the experiment was repeated and the tube was sealed, what products would you expect and why?



Factors affecting the rate of cellular respiration

Study Design:
The factors that affect the rate of cellular respiration: temperature, glucose availability and oxygen concentration

ENGAGE

The respirometer

As you know, living organisms take up oxygen from the atmosphere and use it for aerobic cellular respiration, producing carbon dioxide in the process. The uptake of oxygen can be measured, and thus the rate of aerobic cellular respiration can be determined. A respirometer is the tool used to do this, as it measures the rate of exchange of oxygen and carbon dioxide.

- A respirometer (Figure 5D–1) consists of two sealed containers: one containing the living organism (for example, insects, woodlice or germinating peas) and one containing glass beads with the same mass (acting as a control).
- As the organism respires, carbon dioxide is produced. Sodium hydroxide is placed in the containers to absorb the carbon dioxide.
- Oxygen consumption is measured as a change in pressure within the system. A liquid-filled glass U-tube called a manometer allows this change in pressure to be visualised.
- Once the equipment has been set up, the movement of the liquid towards the organism demonstrates the volume of oxygen taken up by the organism for aerobic cellular respiration.
- The distance the liquid moves in a given time represents the volume of oxygen taken in by the organism per minute.

In this section, you will investigate factors that affect the rate of cellular respiration. Although you may not have access to a respirometer, other ways in which rate can be determined in the laboratory will be explored.

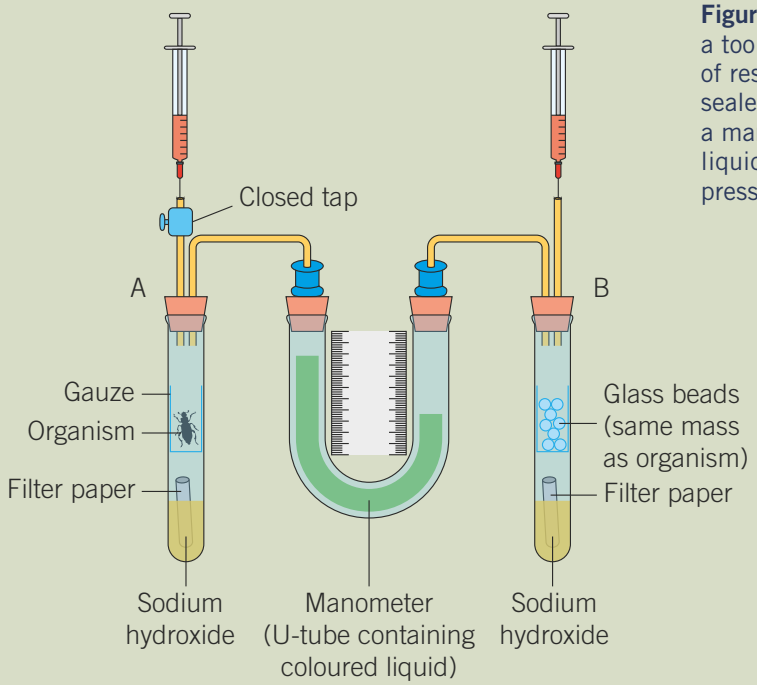


Figure 5D–1 A respirometer is a tool used to measure the rate of respiration. It consists of two sealed containers connected by a manometer, which contains a liquid that moves in response to pressure changes.

EXPLAIN

Factors affecting the rate of cellular respiration

VIDEO 5D–1 FACTORS AFFECTING THE RATE OF CELLULAR RESPIRATION

As you saw with photosynthesis, many factors can affect the rate of cellular respiration. The concentration of reactants can limit the reaction, or increase it to the point where the enzymes involved are saturated and cannot work any faster. Three factors that affect the rate of cellular respiration are:

- temperature
- glucose availability
- oxygen concentration.

Temperature

Temperature affects the rate of cellular respiration in the same way that it affects enzyme activity. This is because cellular respiration is a complex biochemical pathway consisting of many reactions, all regulated by specific enzymes.

- The rate of cellular respiration increases as temperature increases, but only up to the optimum. Beyond this point, the enzymes become denatured, their active site changes shape and they are unable to bind to the substrate. Consequently, the process of cellular respiration slows down.
- At low temperatures, the rate of cellular respiration decreases as the kinetic energy of the molecules involved decreases and there are fewer successful collisions between reactants (substrates) and enzyme active sites.

LINK 4A THE ROLE OF ENZYMES

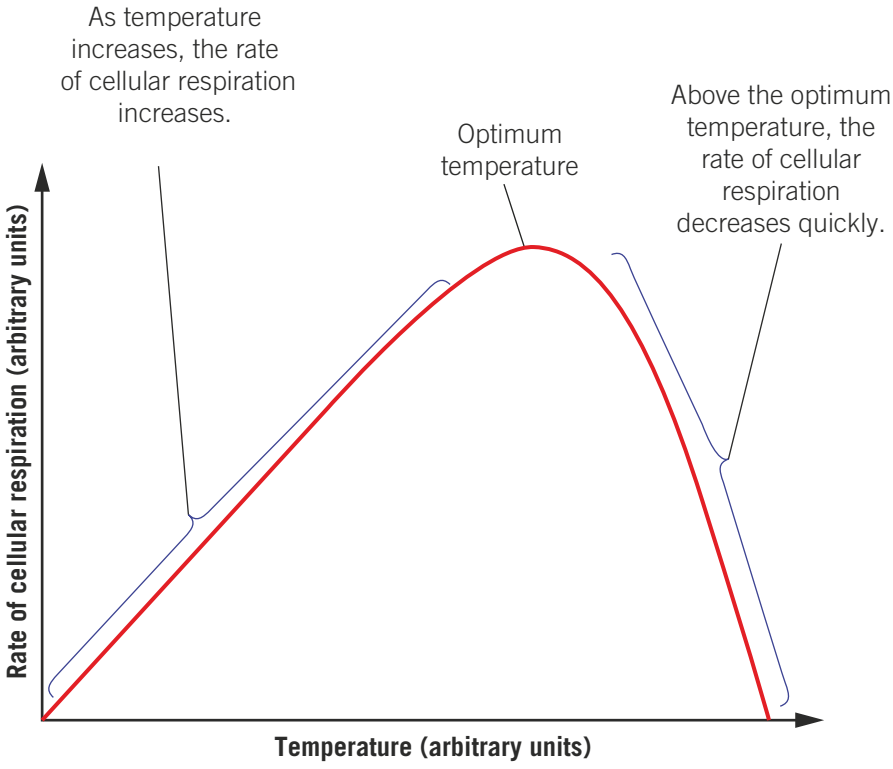
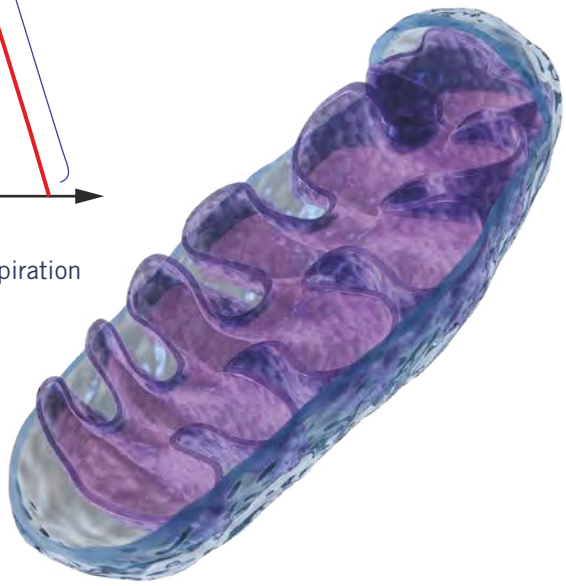


Figure 5D–2 The effect of temperature on the rate of cellular respiration



Glucose availability

As with all reactions, cellular respiration depends on a continual supply of its reactant, glucose. The availability of glucose will affect the speed or rate of the reaction, but where does glucose come from?

5B FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS LINK

- Photosynthetic green plants and algae make their own glucose via the process of photosynthesis.
- Simple or unicellular organisms obtain glucose from their environment or the food they eat.
- Multicellular organisms import extracellular glucose or use a stored form of glucose, called glycogen. If glucose stores become low, the body draws on alternative sources. For example, pyruvate, lactic acid and lipids may be recycled to make the glucose required for cellular respiration.

As with other reactions, the maximum rate of reaction will depend on the number or concentration of enzymes and coenzymes available to assist this biochemical pathway.

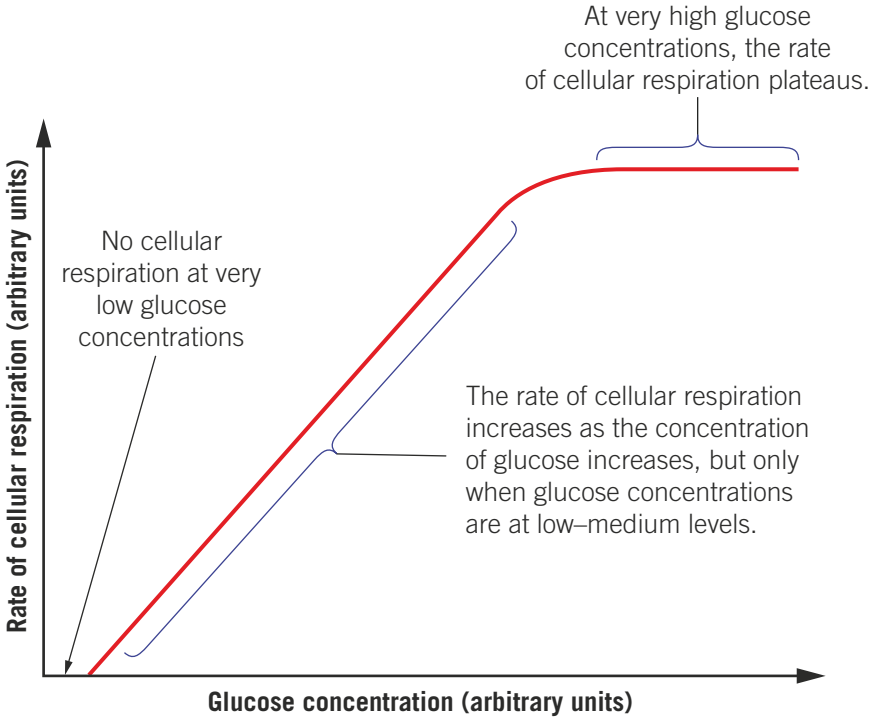


Figure 5D–3 The effect of glucose concentration on the rate of cellular respiration

Oxygen concentration

5C CELLULAR RESPIRATION LINK

As you know, oxygen is an input of the electron transport chain, which is the third stage of aerobic cellular respiration. Therefore, if oxygen availability is low, the progress of this stage will be limited and its rate will decrease. Also recall that when oxygen is low, cells stop aerobic cellular respiration and begin another biochemical pathway that doesn't rely on oxygen to proceed: anaerobic cellular respiration, or fermentation. Consequently, less ATP will be produced per glucose molecule (2 ATP) than when aerobic cellular respiration was occurring (36 or 38 ATP). Due to fermentation, pyruvate will not accumulate, glycolysis can continue, 2 ATP are made and levels of NAD^+ are restored. In this way the cells will remain alive.

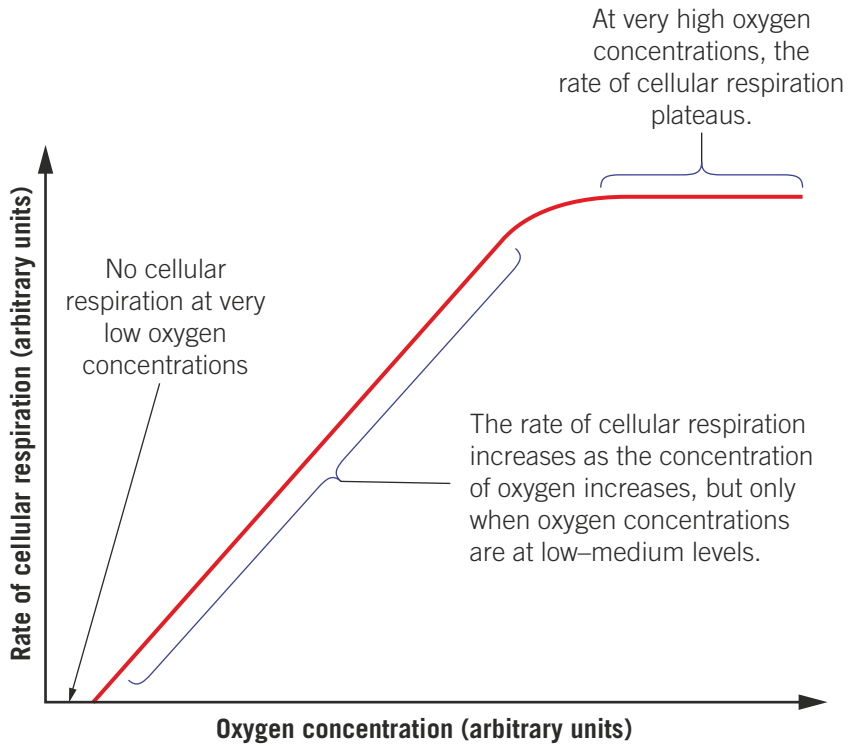


Figure 5D–4 The effect of oxygen concentration on the rate of cellular respiration

Check-in questions – Set 1

1 State three factors that affect the rate of cellular respiration.
2 Outline how temperature affects the rate of cellular respiration.
3 Outline why glucose and oxygen are able to affect the rate of cellular respiration.

WORKSHEET 5D–1 RATE OF CELLULAR RESPIRATION

5D SKILLS

VIDEO 5D–2 SKILLS: LINKING AREA OF STUDY – MIND MAPPING OR CONCEPT MAPPING

Linking areas of study – mind mapping or concept mapping

The links to other chapters you see in the margins of this resource are there to help you make connections between ideas and concepts. By making these links, not only are you giving the information more meaning, which increases the chance that the information will be retained in your long-term memory, but you are also preparing for assessment questions that cover multiple dot points and areas of study.

In assessment situations, assessors will try to identify the depth of your understanding and your ability to see the interrelationships between ideas. Consider some of the content you have covered so far in Unit 3: from DNA to proteins (Chapter 2) and enzymes (Chapter 4). The relationships between ideas can be predicted and therefore prepared for. For example, a question may address the following ideas using a scenario:

- Cellular respiration is a process that is regulated by enzymes.
- Enzymes are proteins.
- Factors affect protein structure and therefore enzyme activity.
- Proteins are synthesised at the ribosomes.
- Proteins are built according to the code in genes.
- Genes are sections of DNA.
- Mitochondria contain DNA and ribosomes.

LINK CHAPTER 2

LINK CHAPTER 4

These links are represented in Figure 5D–5.

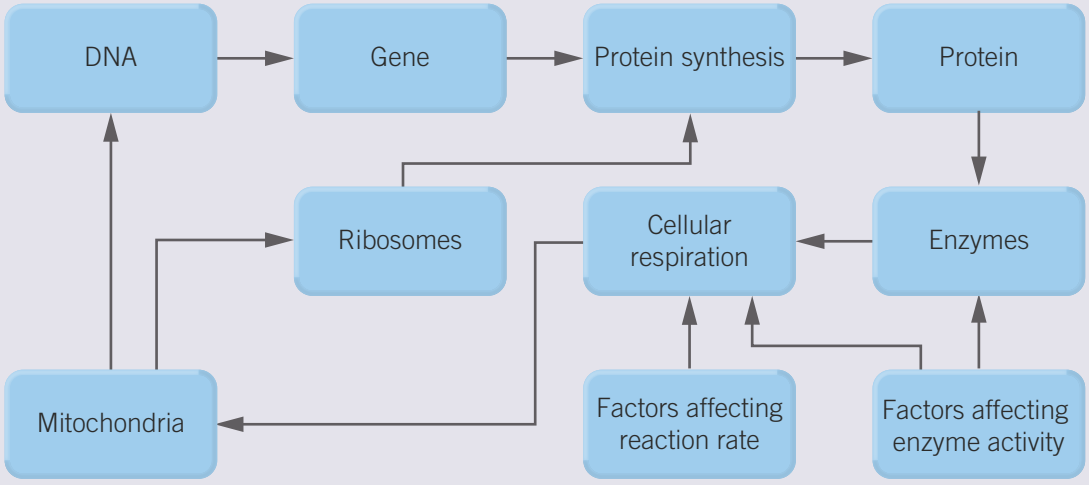


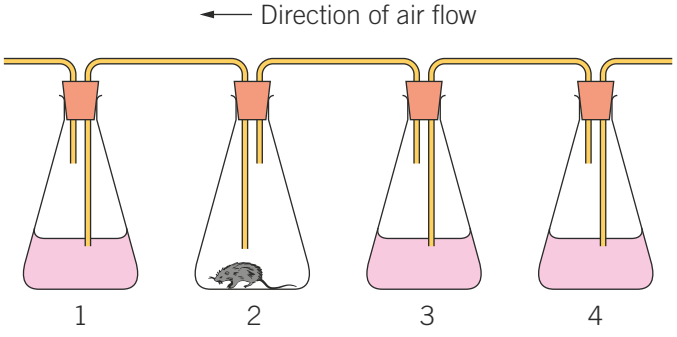
Figure 5D–5 Mind maps and concept maps allow you to link ideas so they make more sense to you. Including definitions and connections between terms can enhance your visual reminder of key content.

Drawing a mind map or concept map, and building on it as you learn more information, can help you illustrate and comprehend the links between ideas. You may like to include definitions as well. For example, where would you add the coenzyme NAD^+? And where would anaerobic respiration fit in? How could you include the different stages of aerobic respiration?

The Section 5D questions are an example of what a question may look like when different concepts are integrated.

Section 5D questions

1 Two Biology students conducted an experiment investigating the rate of cellular respiration. Their set-up is shown below.



The students used sealed flasks, as shown in the figure. Flasks 1, 3 and 4 contained distilled water, two drops of ammonia and phenol red indicator. Flask 2 contained a mouse. Phenol red is an indicator that is yellow under acidic conditions and pink under basic conditions. Initially, flasks 1, 3 and 4 were pink in colour.
Note: $H_2O + CO_2 \rightarrow$ carbonic acid

a After 24 hours, the solution in flasks 3 and 4 had remained pink, while flask 1 had turned yellow. Explain why this occurred.

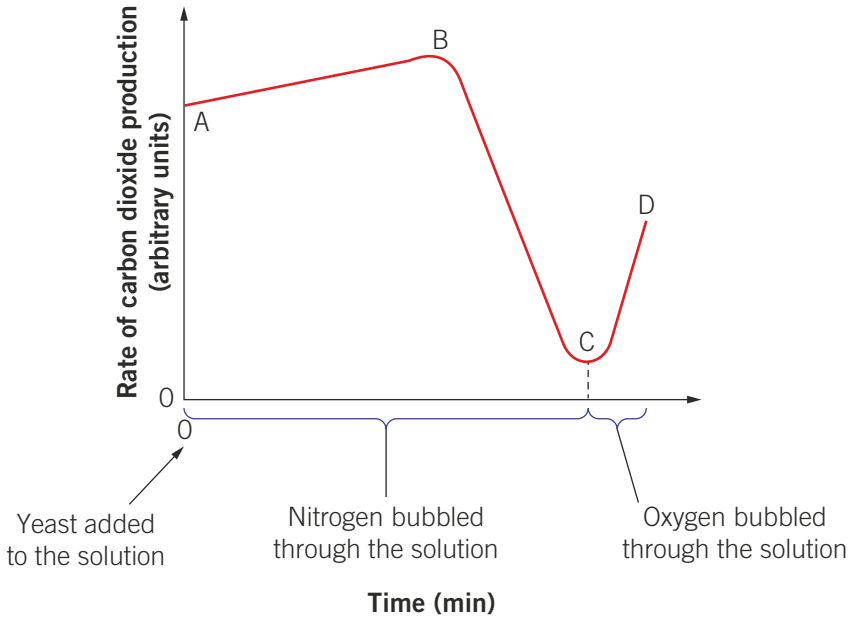
b Outline why the extent of the colour change in flask 1 could be used to indicate the rate of aerobic cellular respiration.

c Aerobic cellular respiration is a process that consists of many stages and reactions.
- **i** Which stage of this biochemical pathway produces CO_2?
- **ii** Which stage produces the most ATP?

d Enzymes are responsible for catalysing each reaction in aerobic cellular respiration. The enzymes therefore need to be specific for the substrate they need to bind to.
- **i** Define what specificity means in terms of enzymes.
- **ii** Summarise the advantage of an enzyme being specific.

e The experiment was repeated but this time the mouse was provided with a mini treadmill. Outline the consequence for cellular respiration in the mouse muscle cells if they experience a reduction in:
- **i** glucose availability
- **ii** access to oxygen.

2 Yeast is a unicellular fungus that undergoes aerobic and anaerobic cellular respiration in much the same way as plants. A group of students decided to investigate the rate of production of carbon dioxide by a particular yeast species.
They began with a solution of glucose, which they bubbled nitrogen gas through for 12 hours to remove all oxygen from the solution. At time = 0, they added yeast to the glucose solution. Nitrogen gas was then bubbled through the solution, again for a certain time, and then the nitrogen gas was removed and oxygen gas was bubbled through the solution instead.
The production of carbon dioxide was measured the entire time, and the students graphed their results at the conclusion of the experiment.



a By measuring the rate of carbon dioxide production, what biochemical process were the students investigating?

b At which of the four points marked was the rate of carbon dioxide production the greatest?

c Between points B and C, the rate of carbon dioxide production decreased rapidly. Explain why this would be the case.

d At point C, oxygen was bubbled into the solution. Write the balanced chemical equation for the biochemical process that is occurring between points C and D.

e Yeast cells are able to break down both glucose and alcohol in the presence of oxygen. Suggest at what point the levels of alcohol would be highest, and give reasons for your answer.

Biotechnological applications of biochemical pathways

Study Design:
- Potential uses and applications of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields
- Uses and applications of anaerobic fermentation of biomass for biofuel production

Glossary:
Biofuel
Biomass
Cas9
CRISPR-Cas9
First-generation biofuels
Gene editing
Second-generation biofuels

ENGAGE

The carbon economy

Have you heard of carbon offsetting, having a carbon footprint, carbon budgeting or even the carbon economy? All these terms relate to carbon dioxide emissions. As you know, carbon dioxide emissions (a type of greenhouse gas) are primarily responsible for global warming and climate change. Carbon dioxide is released naturally through processes like cellular respiration, but human activities, such as deforestation and the burning of fossil fuels, have been the main producers of the gas. For decades, efforts have been made to monitor and lower these emissions.

Governments and industries are trying to improve the efficiency of photosynthesis, which is the way carbon dioxide is naturally removed from the atmosphere by carbon fixation. The benefits of this improvement will be twofold. The world's population is growing rapidly and greater amounts of food will be needed to support future generations. Not only will there be less carbon dioxide in the atmosphere, but it is thought that increasing the efficiency of photosynthesis will mean more nutrient-rich crops and increased crop yields. Governments and industries also want to increase the production of biomass, a sustainable and renewable energy resource, in the hope that this will reduce our reliance on fossil fuels, and again reduce the amount of carbon dioxide in the atmosphere.

In this section you will explore the possible application of CRISPR-Cas9 technologies to improve the efficiency of photosynthesis and crop yields, as well as looking at the use of biomass to produce biofuel through anaerobic cellular respiration.

Figure 5E–1 A rise in carbon dioxide levels in the atmosphere is responsible for global warming and climate change. What can we do to turn things around?

EXPLAIN

CRISPR-Cas9 technology

The technology

In Section 3D, you learned about the function of **CRISPR-Cas9** in bacteria and its application in editing an organism's genome. Before investigating how this technology can be used to improve photosynthetic efficiencies and crop yields, let's revisit the CRISPR-Cas9 system and its role in gene editing.

LINK 3D THE FUTURE OF GENOME EDITING

Recall that CRISPR is a naturally occurring 'immune system' in bacteria. When a virus tries to take over a cell, its goal is to use the cell to replicate by inserting its genetic material. Scientists have found that certain bacteria have evolved a way to fight back – they use DNA-cutting proteins, for example **Cas9**, to chop up any viral genetic information floating around in their cell. The outcome is that the invading virus can no longer infect, replicate and damage the bacterial cell, or go on to infect other cells.

LINK 6C NON-CELLULAR PATHOGENS

Scientists can now use the CRISPR-Cas9 system, believed to be the cheapest, fastest and easiest form of genetic engineering, to edit genes. **Gene editing** involves the insertion, removal or replacement of DNA within the genome of a living cell. Figure 3D–3 summarises the technical steps, while Figure 5E–2 summarises the process.

CRISPR-Cas9 an immune system in bacteria that uses CRISPR nucleotide sequences and the Cas9 DNA-cutting enzyme, also modified for use as a genome editing tool

Cas9 an endonuclease (enzyme) that cuts DNA at a specific point determined by guide RNA (gRNA)

Gene editing the insertion, removal or replacement of DNA within the genome of a living organism

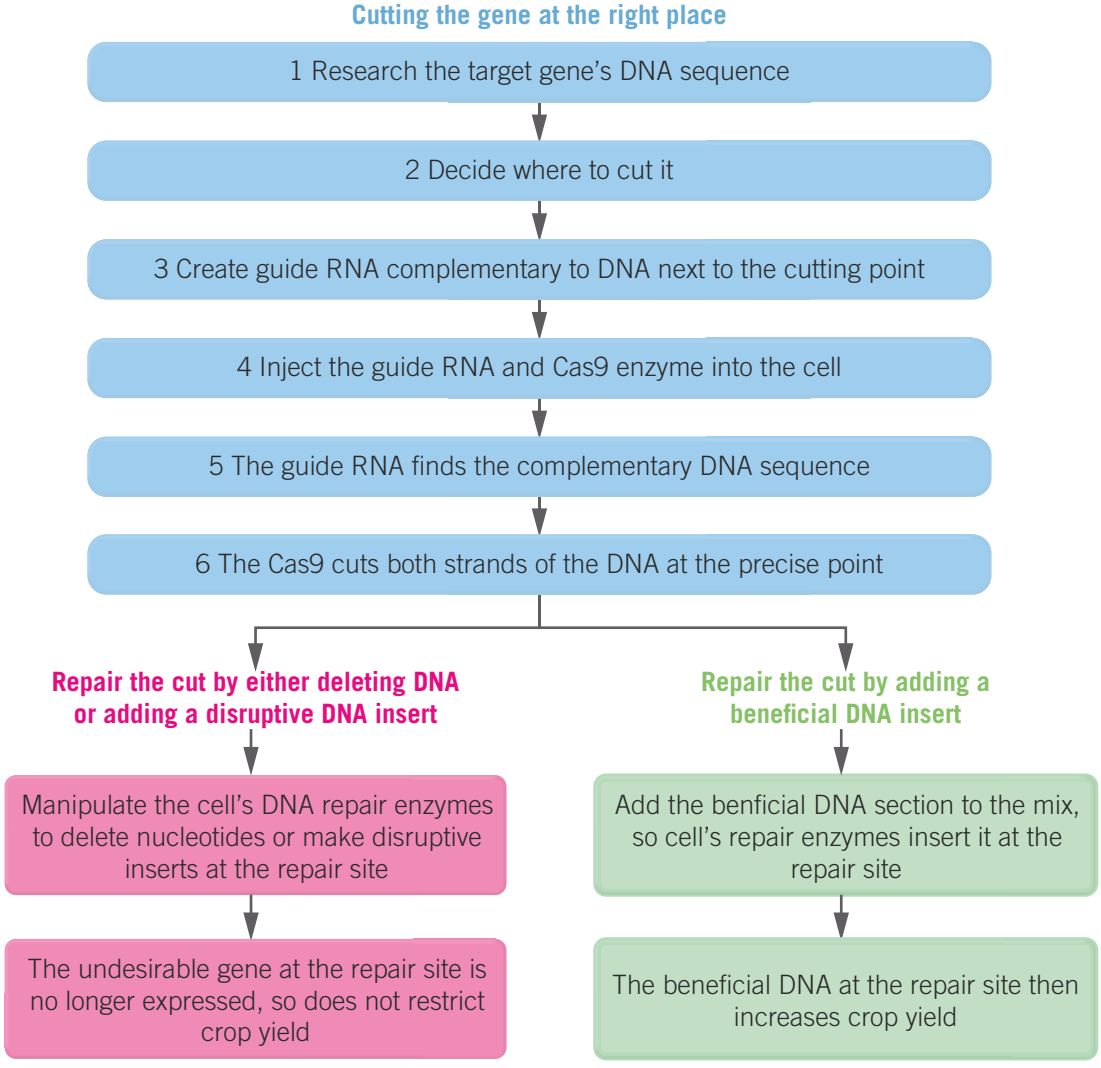


Figure 5E–2 Flow chart showing the process of genome editing using CRISPR-Cas9 applied to increase crop production. Once the DNA has been cut, the process diverges depending on whether the aim is to stop a gene from being expressed or to improve it.

5B FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS LINK

VIDEO 5E–1 BIOTECHNOLOGICAL APPLICATIONS OF BIOCHEMICAL PATHWAYS: CRISPR-CAS9

Application: improving photosynthetic efficiencies and crop yields

You know that plants convert light energy into chemical energy (glucose) by photosynthesis. You also know from Section 5B that most crops on Earth (C_3 plants) have evolved an energy-wasting process called photorespiration that drastically reduces their growth and yield potential. Recall that it is Rubisco, the plants' most abundant protein, that binds to oxygen instead of carbon dioxide, meaning that glucose isn't made by plants around 25% of the time.

By 2050, it is estimated that food production will need to double in order to meet the demands of the world's growing population. Global warming will also have an impact on food production, undoubtedly a negative one. Clearing more land to grow more crops is not an option, as this would worsen global warming. Researchers have suggested that photosynthesis needs to be engineered to make it more efficient. Specifically, we need to engineer crops that can bypass photorespiration so that photosynthetic efficiency will improve. Crops would therefore be at least 25% more productive.

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

Selective plant breeding over hundreds of years has already increased crop yields, but such an approach involves a lot of work and can take decades to develop improved varieties.

3C GENETICALLY MODIFIED AND TRANSGENIC ORGANISMS LINK

More recently, as you learned in Chapter 3C, scientists have been transplanting genes from one organism into another, producing GM (genetically modified) crops with increased yield.

3D THE FUTURE OF GENOME EDITING LINK

Now scientists are looking to use genome editing, like the CRISPR-Cas9 system, as its outcome is much the same as selective breeding: there is no introduction of foreign DNA into a plant (unless a gene is being inserted from another species), and the process is faster, cheaper, more precise and relatively easy for those with the appropriate training.

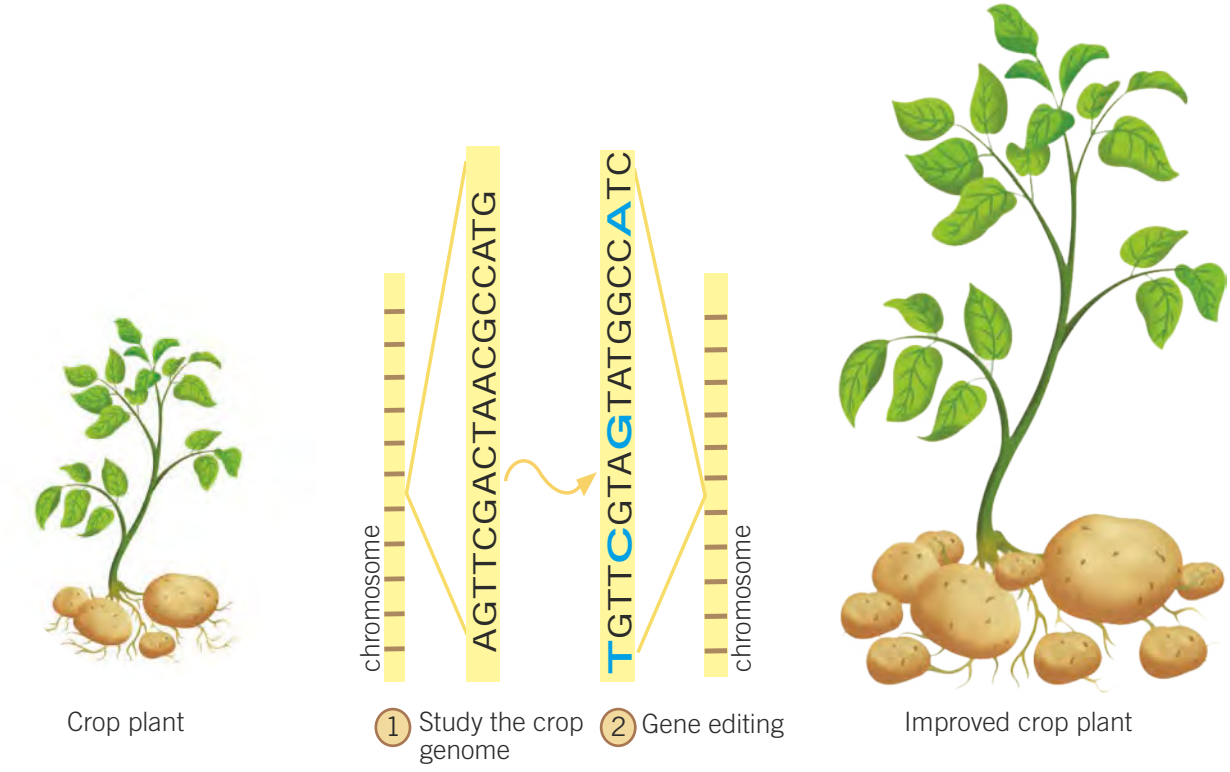


Figure 5E–3 Scientists hope that by understanding the role of the different genes in a genome, they can disable the genes that reduce the efficiency of photosynthesis, thus increasing the amount of glucose produced by the plant.

In 2019, scientists in the United Kingdom reviewed numerous studies and a number of approaches that focused on manipulating photosynthesis, including enhancing a plant's ability to capture light, reducing the occurrence of photorespiration, altering electron-transport and increasing the flow of carbon dioxide through the Calvin Cycle. Other scientists have investigated how CRISPR-Cas9 can be used to increase the stress tolerance, drought tolerance and insect resistance of plants in order to increase crop yields.

In terms of your Study Design, remember that this dot point is asking you to make links between two concepts. Exposing yourself to current research would be advantageous to your understanding as it is a relatively new area, although remember that recalling specific details of the studies is not required. Here are some key findings from researchers over the last 5 or so years, that highlight the potential uses and applications of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields:

- Modifying the activity of Rubisco, photorespiratory enzymes, and increasing the activity of other Calvin Cycle enzymes, can increase photosynthesis efficiency.
- More than one individual step in the Calvin Cycle needs manipulating using CRISPR-Cas9 in order to increase the assimilation of carbon and enhance growth.
- There are over 38 enzymes involved in C_3 plants' carbon fixation and their photorespiratory pathway, and therefore there are many possible targets for gene editing using CRISPR-Cas9.
- Interestingly, one approach to enhancing crop yields comes from the introduction of alternative routes to photorespiration, not by knocking out the genes responsible for the synthesis of photorespiratory enzymes.
- Many agriculturally important traits, like improved photosynthetic efficiency, require gene 'overexpression', meaning making the gene produce far more copies of the protein it codes for than normal. CRISPR-mediated gene regulation can do this.
- CRISPR technology can manipulate multiple genes simultaneously. This is important as, for example, to increase Rubisco content in maize, three genes must be edited at the same time.
- One suggested approach to increasing photosynthetic efficiency in C_3 crops, such as rice, is to convert them to C_4 plants.

Figure 5E–4 *Arabidopsis thaliana* is believed to be a useful model plant when screening approaches to improve photosynthesis and crop yield using CRISPR-Cas9 systems.

LINK **9B** EVOLVING AND NON-EVOLVING POPULATIONS

Essentially, three steps are necessary for any research in this field.

1. Understand the entire photosynthetic process including the regulation and diversity of other pathways related to photosynthesis (photorespiration, CAM pathways, C_4 pathways and so on), and gain a specific understanding of the genomes of the target crops, so that each gene's role in growth and yield is clear.
2. Use high-performance computing to model the photosynthetic pathway and identify bottlenecks, like photosynthetic inefficiency due to photorespiration.
3. Utilise CRISPR-Cas9 to target and edit the identified bottlenecks.

Check-in questions – Set 1

1. Define the following key terms: CRISPR, Cas9, gene editing.
2. State the advantages of gene editing over genetic engineering.
3. Explain how CRISPR-Cas9 can be applied to improve photosynthetic efficiency and therefore crop yield.

Anaerobic fermentation

The need for biofuels

Humans use enormous amounts of energy all day, every day – to heat homes, keep lights on, drive cars and so on. Currently we use fossil fuels, like coal, oil and natural gas (for example, petroleum), which are non-renewable energy sources as they take several million years to form. This, and concerns about global warming, have led scientists to search for alternative sources of energy and more efficient and sustainable ways to produce energy.

5C CELLULAR RESPIRATION LINK

In Section 5C, you learned about the process of fermentation, or anaerobic cellular respiration. Recall that fermentation is a metabolic process in which glucose is converted into lactic acid in animals, or ethanol and carbon dioxide in plants. Scientists have spent a lot of time investigating the process of alcoholic fermentation, as ethanol can be used as a **biofuel**. The term 'biofuel' comes from the fact that it is a (usually liquid) fuel produced from organic material, called **biomass**. Biomass includes plant material, animal by-products, microbes and waste material. It is produced by many different industries, including agriculture, fisheries, food manufacturing, forestry and even municipal waste.

Biofuel
fuel produced from biomass; usually liquid

Biomass
organic material, including plant material, animal by-products, microbes and waste material; produced by many different industries

If biofuels are created from biomass by biological processes like fermentation, then that means they are renewable (assuming there is a constant supply of reactants) and carbon neutral. That is, biofuels release no net amount of carbon dioxide into the atmosphere. The carbon dioxide that is released during the combustion of the biofuels was originally absorbed from the atmosphere during photosynthesis.

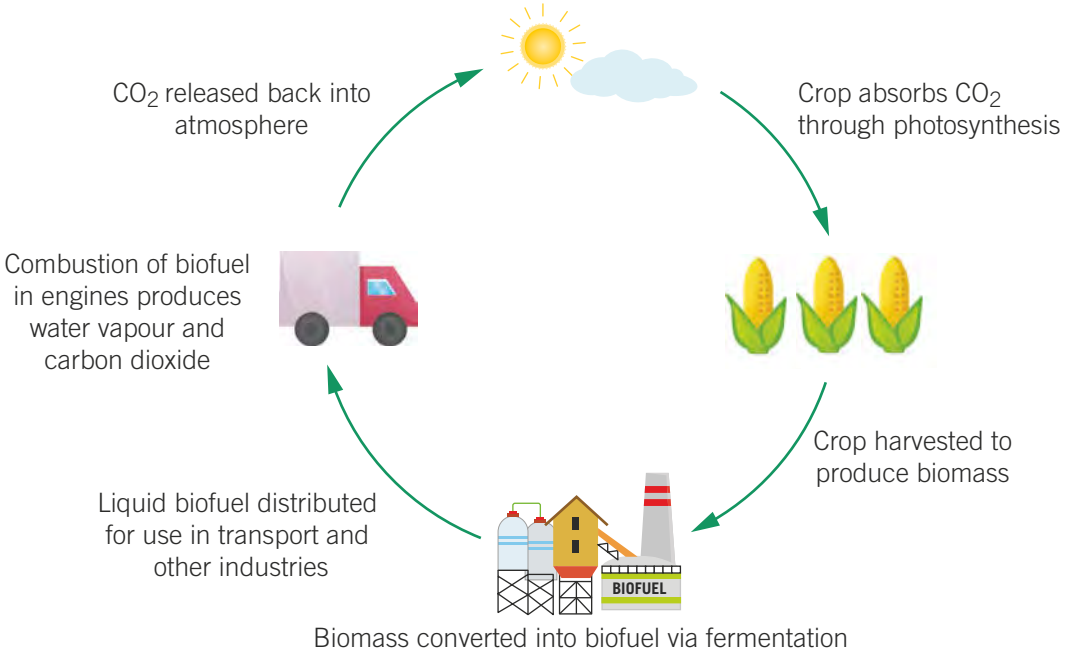


Figure 5E–5 Carbon-neutral cycle: biofuels are carbon neutral, as the carbon dioxide released during their combustion was originally absorbed by plants during photosynthesis.

Application: producing biofuels from biomass

Biofuels are categorised by the type of feedstock (raw material) that is used to create them.

- **First-generation biofuels**: produced directly from food such as corn and sugar cane.
- **Second-generation biofuels**: produced from cellulose-based non-food crops not suitable for human consumption. An example is lignocellulose biomass such as leftovers from a corn harvest (called corn stover), straw, wastepaper and other municipal waste. These feedstocks require several processing steps before they can undergo fermentation.

First-generation biofuel
a biofuel produced from edible feedstocks, e.g. starch and glucose from plants like corn and sugar cane

Second-generation biofuel
a biofuel produced from non-edible feedstocks, e.g. cellulose and other fibrous plant materials derived from crop residues, straw and municipal waste

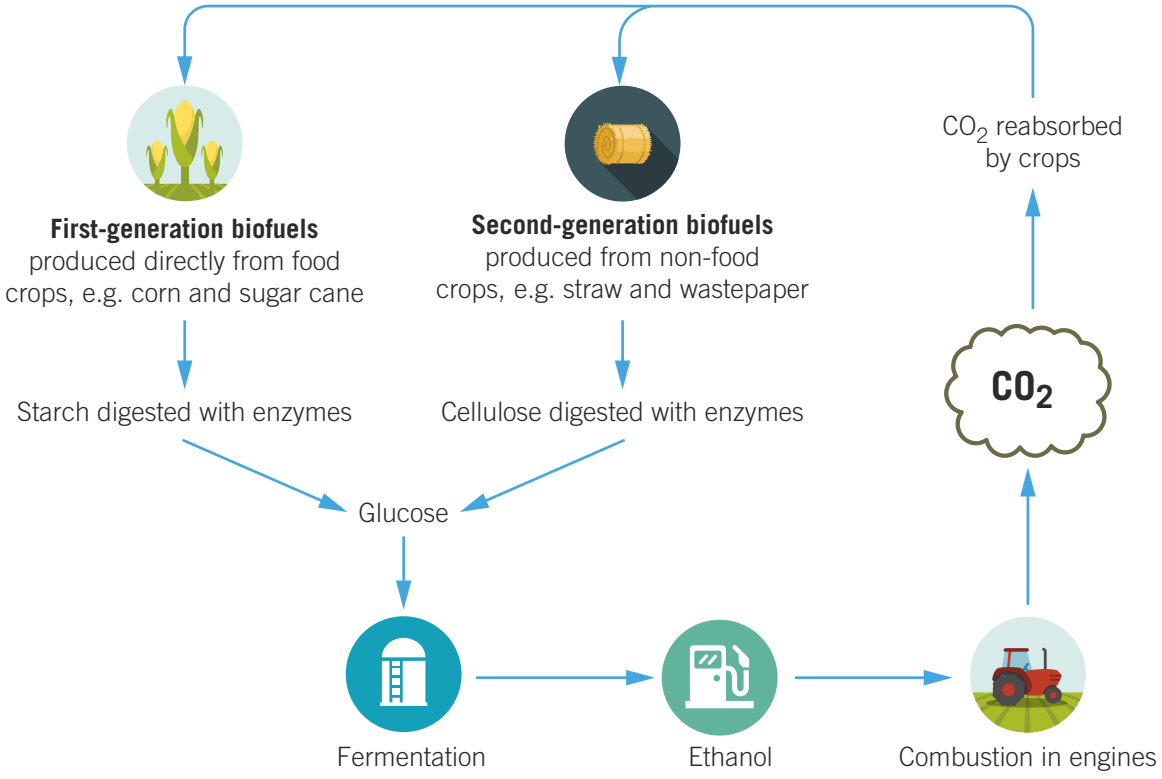


Figure 5E–6 The biofuel ethanol is both a first- and second-generation biofuel, as it can be produced directly from food crops and also from non-food crops.

The process

Bioethanol and biodiesel are the two main biofuels currently produced from biomass. Because bioethanol is the only one primarily produced via fermentation, we will focus on that. However, its production is not as simple as just fermenting sugars. The basic steps for the production of bioethanol are outlined below.

1 *Pre-treatment:* used to help break down the biomass to increase its surface area and therefore its accessibility by enzymes. Pre-treatment options include:
   - biological approaches – enzymes
   - chemical approaches – liquefaction, acids and alkalis
   - physical approaches – milling, mashing and grinding
   - physiochemical approaches – cooking/heating and steam.

2 *Enzymatic hydrolysis:* hydrolysis is the opposite of a condensation reaction (recall this term from Section 2C). This means water is used to help enzymes (including amylase and cellulase) break the glycosidic linkages between glucose molecules in the polysaccharides starch and cellulose (including hemicellulose and lignocellulose).

3 *Fermentation:* the anaerobic cellular respiration of glucose using a microorganism that is selected based on its efficiency at ethanol production. The yeast *Saccharomyces cerevisiae* is most commonly used, and the bacterium *Zymomonas mobilis* is an alternative, due to its greater sugar uptake, higher yields and greater resistance to high ethanol concentrations.

4 *Distillation and purification:* water is removed from the ethanol so that it is in a useable form. Following distillation, purification further removes water using desiccants and dehydration techniques, and filtering with a molecular sieve.

VIDEO 5E–2 BIOTECHNOLOGICAL APPLICATIONS OF BIOCHEMICAL PATHWAYS: BIOFUEL PRODUCTION

LINK 4A THE ROLE OF ENZYMES

LINK 2C PROTEINS

LINK 5C CELLULAR RESPIRATION

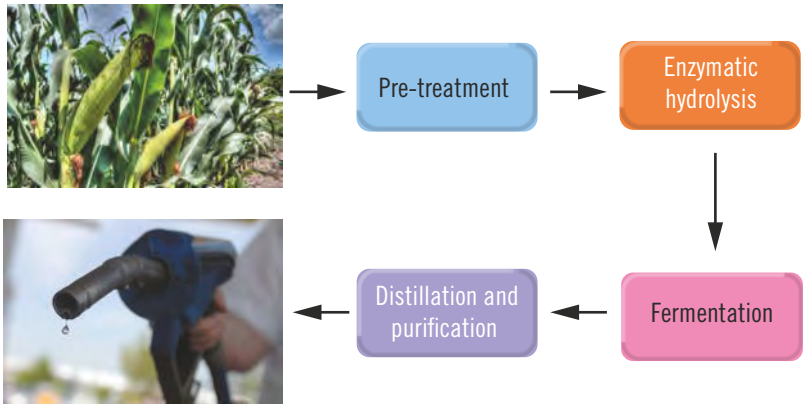


Figure 5E–7 Producing bioethanol from biomass using fermentation is a complex process.

Most bioethanol is made in this way primarily from corn or maize grain, but scientists are continuing to develop the pre-treatment technologies that will allow more efficient use of cellulose, the non-edible fibrous material that constitutes the bulk of plant matter. By using non-edible materials, industries will not need to use food crops to make biofuel, and therefore food crops could be used to support our increasing global population. The question of whether to allocate land for growing crops for food or for fuel production is known as the 'food versus fuel debate'.

Conditions

4B FACTORS IMPACTING ON ENZYME FUNCTION LINK

As with any biochemical process, there are factors that can affect the efficiency of the pathway. In the production of bioethanol, certain conditions need to be met if the process is to be successful.

- *Temperature:* you learned in Section 4B about how temperature affects enzyme activity. Recall that, if the temperature is too low, the activity of enzymes decreases, and the reaction slows down. As the temperature increases to the optimum, so does the kinetic energy of the enzymes, causing more successful collisions between the enzyme active site and the substrate. However, when the temperature is too high, the enzyme denatures and the active site can no longer bind to the substrate, so the reaction no longer occurs.
- *Substrate:* as you know, every reaction needs an ample supply of its reactants, or substrate, if it is to proceed. In the case of biofuel production, there needs to be a high glucose concentration so that the enzymes involved can work most efficiently. If too little substrate is available, the rate of the reaction will decrease.

5D FACTORS AFFECTING THE RATE OF CELLULAR RESPIRATION LINK

- *Absence of oxygen:* as you learned in Section 5D, fermentation is a biochemical pathway that occurs in the absence of oxygen. Therefore, oxygen must be excluded from the vessel in which fermentation is being carried out.
- *Yeast:* without yeast, the fermentation of the glucose solution to ethanol cannot occur. Yeast contains the enzyme 'zymase', which acts as a catalyst for the reaction.

Check-in questions – Set 2

WORKSHEET 5E–1 BIOTECHNOLOGICAL APPLICATIONS OF BIOCHEMICAL PATHWAYS

1 Define the two key terms: biofuel, biomass.
2 Write the word equation for anaerobic cellular respiration resulting in alcohol production.
3 Explain the role of anaerobic cellular respiration in producing the biofuel ethanol.

5E SKILLS

VIDEO 5E–3
SKILLS: UNDERSTANDING SOCIAL, ETHICAL, ENVIRONMENTAL AND ECONOMIC IMPLICATIONS

Understanding social, ethical, environmental and economic implications
Sometimes in assessment situations you may be required to answer questions that ask for the social, ethical, environmental or economic implications of a concept you have covered in class. In some cases, you will have covered issues and implications explicitly as part of your course as the Study Design demands it, but sometimes you will need to apply what you have learned on the spot.

First, consider the terms that you may come across:

- *Social*: Does your answer show an impact on society – for example, more than two people?
- *Ethical*: Does your answer show an impact on a moral code – for example, unknown impacts on future generations?
- *Environmental*: Does your answer show an impact on the environment – for example, living organisms and/or their habitat?
- *Economic*: Does your answer show an impact on income/wealth – for example, are there costs involved?

Imagine you have been asked about the implications of using the CRISPR-Cas9 system to minimise the impact of photorespiration on plants, to increase crop yields. The implications can be positive and/or negative.

- *Social implications:* greater crop yields, more food available, more jobs in food processing
- *Ethical implications:* no introduction of DNA into an organism from a different species, long-term effects on future generations unknown (i.e. genome instability)
- *Economic implications:* simple and efficient once protocols are established, cost effective once set up, fewer regulations, cost of setting up is large, increased income from crop sales
- *Environmental implications:* much like selective breeding in that no GMO is involved, impacts on non-target DNA are unknown.

Section 5E questions

1 Outline the process of gene editing using the CRISPR-Cas9 system.
2 Describe the role of Cas9 in gene editing.
3 Scientists would like to increase the yield of corn crops, as corn is one of the most widely used plants in the world. Corn is used for food, and also for fuel, and in pharmaceuticals, bioplastics, packaging, beverages, insecticides and more. Scientists have identified a gene that produces a protein that slows down the process of photosynthesis. Explain why it is necessary that scientists know part of the base sequence of the target gene in order to use CRISPR-Cas9 to inactivate the gene.
4 Bioethanol is currently a major biofuel being produced in the United States and Brazil. With the help of an annotated diagram, summarise the key steps involved in the production of corn bioethanol.
5 Give reasons why the process of producing bioethanol from biomass using fermentation needs to be carefully monitored and controlled.
6 Describe two social, ethical, environmental or economic implications of producing bioethanol from biomass using fermentation.

Chapter 5 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
5A.1	State the purpose of photosynthesis	12c☐
5A.2	Explain the general function of the chloroplasts	5☐
5A.3	Draw the chloroplast, label and recall the function of key structures	3☐, 10☐
5A.4	State the word equation and balanced chemical equation for photosynthesis	12a☐
5A.5	Summarise the inputs, outputs and location of the two stages of photosynthesis in C_3 plants	8☐, 10☐, 12c☐
5A.6	Outline what occurs during the light dependent and light independent stages of photosynthesis.	10☐, 12g☐
5A.7	Describe the role of Rubisco and ATP synthase in photosynthesis (C_3 plants)	12g☐
5B.1	List the factors that affect the rate of photosynthesis	12e☐
5B.2	Explain why and how each of these factors may affect the rate of photosynthesis	12b☐, f☐, h☐, 13b☐
5B.3	Describe the role of Rubisco in maximising the efficiency of photosynthesis, including a definition of photorespiration	13a☐, b☐
5B.4	Summarise the adaptations of C_3, C_4 and CAM plants that help to maximise the efficiency of photosynthesis	13c☐
5B.5	Draw, recognise and interpret graphical representations of rates of photosynthesis	12f☐
5C.1	State the purpose of cellular respiration	11a☐
5C.2	Explain the function of the mitochondria	11a☐
5C.3	Draw a mitochondrion label and recall the function of key structures	3☐
5C.4	Demonstrate an understanding of the energy shuttle and uses of ATP	11b☐
5C.5	Tabulate the inputs, outputs and location of glycolysis, Krebs Cycle and electron transport chain in aerobic cellular respiration, including ATP yields	4☐, 6☐, 9☐
5C.6	Outline what occurs during glycolysis, Krebs Cycle and electron transport chain in aerobic cellular respiration	1☐, 9☐, 12d☐

Success criteria – I am now able to:		Linked question
5C.7	State the word equation and balanced chemical equation for aerobic cellular respiration	12d☐
5C.8	Tabulate the inputs, outputs and difference in outputs of anaerobic fermentation in animals, plants and yeasts	6☐
5C.9	State the word equation for anaerobic cellular respiration	14a☐
5D.1	List the factors that affect the rate of cellular respiration	11c☐
5D.2	Explain why and how each of these factors may affect the rate of cellular respiration	7☐
5E.1	Define and explain the process involved in CRISPR-Cas9 technologies	2☐, 13d☐
5E.2	Summarise the potential applications and uses of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields	13e☐
5E.3	Define the terms biofuel and biomass	14b☐
5E.4	Summarise the uses and applications of anaerobic fermentation of biomass for biofuel production	14c☐

Multiple-choice questions

1 The correct order of stages in aerobic cellular respiration is

A glycolysis → Calvin Cycle → electron transport chain.

B light dependent stage → glycolysis→ Krebs Cycle.

C glycolysis → Krebs Cycle → electron transport chain.

D electron transport chain → Krebs Cycle → glycolysis.

2 The CRISPR-Cas9 system is like the human body's

A digestive system.

B respiratory system.

C immune system.

D reproductive system.

3 Which of the following statements is correct?

A Mitochondria are found only in eukaryotic animal cells, while chloroplasts are found only in eukaryotic plant cells.

B Chloroplasts contain stacks of thylakoid membranes called grana and a gel-like substance called the matrix.

C Mitochondria are the site of anaerobic cellular respiration.

D Mitochondria and chloroplasts both contain a double membrane and many ribosomes.

4 A reaction that occurs in a living cell has the following products: pyruvate, NADH and ATP. The name of this reaction is

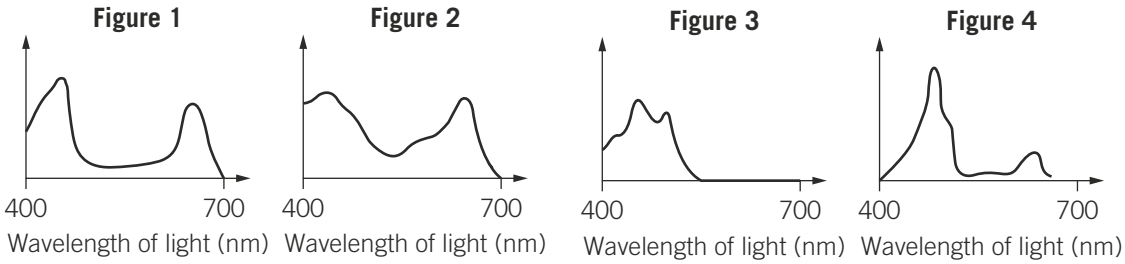
A glycolysis.

B aerobic respiration.

C the light independent reaction.

D the Krebs Cycle.

5 Three of the following figures show the absorption spectrum of chlorophyll a, chlorophyll b and the carotenoids. One of the figures shows the action spectrum of a plant that contains all three of these pigments.



Which of the following correctly identifies two of the four graphs?

A Chlorophyll a is Figure 1; action spectrum is Figure 4.

B Chlorophyll b is Figure 4; action spectrum is Figure 2.

C Carotenoids is Figure 1; action spectrum is Figure 4.

D Carotenoids is Figure 3; action spectrum is Figure 1.

6 Two different species of bacteria were investigated by scientists. The scientists found that species P always produces CO_2 and H_2O during cellular respiration. Species Q always produces alcohol and CO_2. Which one of the following conclusions can be made from these observations?

A Only species Q is aerobic.

B Only species Q is anaerobic.

C Both species P and Q are aerobic.

D Both species P and Q are anaerobic.

7 A student is conducting an experiment to determine the effect of temperature on the cellular respiration of yeast. Yeast and glucose are first added to water, and then the gas produced is captured and its volume measured. Which variables should the student ensure are held constant during the experiment?

A the mass of glucose and the solution temperature

B the mass of yeast and the mass of glucose

C the volume of gas and the mass of yeast

D the volume of gas and the solution temperature

8 Anabolic reactions are also referred to as being endergonic. Which of the following is an example of an anabolic reaction?

A Krebs Cycle

B glycolysis

C photosynthesis

D cellular respiration

9 Which of the following statements correctly represents what happens to NADH during cellular respiration?

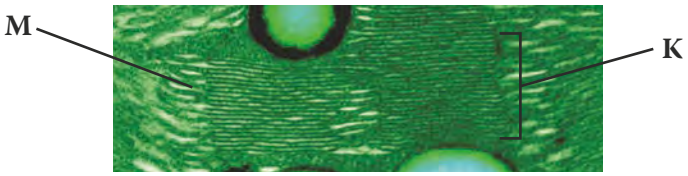
A It is broken down into ATP.

B It transfers hydrogen ions to the electron transport chain.

C It moves between the cristae and the matrix of the mitochondria.

D It is broken down by enzymes to form NAD^+.

10 The following image shows a section from a chloroplast, taken using a transmission electron microscope.



The light dependent reactions occur in the area labelled K, while the light independent reactions occur in the area labelled M. Considering the inputs and outputs of the different stages of photosynthesis, it is reasonable to state that

A reactions occurring at M require an input of chlorophyll.
B carbon dioxide is an input for reactions occurring at M.
C ADP produced during reactions at K are used by reactions at M.
D reactions occurring at K require an input of oxygen.

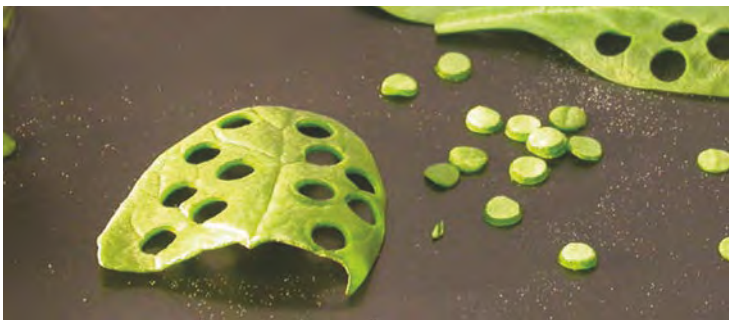
Short-answer questions

11 Melissa Breen holds the record for Australia's fastest female sprinter, with a time of 11.11 seconds over 100 metres.

a Name the organelle her muscles would contain large numbers of. Give reasons for your answer, referring to the function of this organelle. (2 marks)
b Draw an annotated diagram of the energy shuttle. (1 mark)
c List factors that can affect the rate of the biochemical pathway that makes energy for muscle cells to use. (1 mark)

12 Students Salima and Rashid were investigating how temperature affects the rate of photosynthesis.

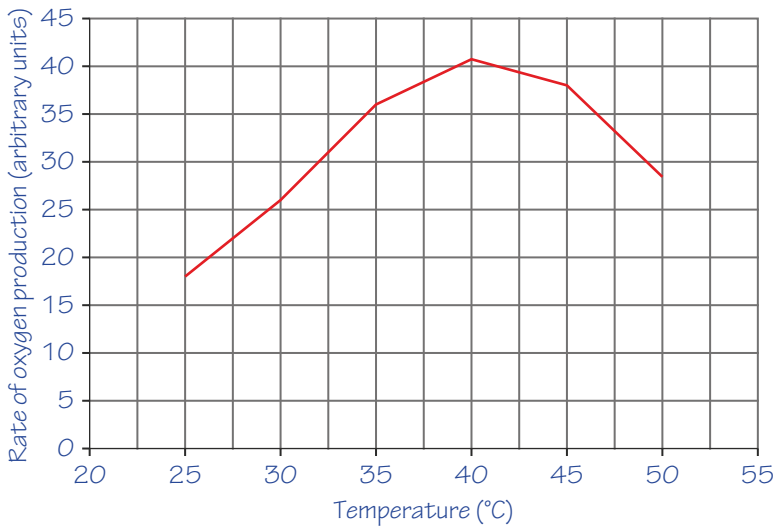
a Write the balanced chemical equation for photosynthesis. (1 mark)
b Write a possible hypothesis for their experiment. (1 mark)
c They began by visiting a friend's greenhouse, cutting discs from the leaves of a spinach plant and then measuring the rate of oxygen production by the leaf discs.



Rashid claimed that the purpose of photosynthesis was to make oxygen and that was why they were measuring the rate of oxygen production in this experiment. Give reasons why he is incorrect. (2 marks)

d They placed the leaf discs in the dark and found that the discs did not produce oxygen but took in oxygen. Explain why this is, and write the balanced chemical equation for the biochemical process that uses oxygen. (2 marks)

The students then repeated their experiment in the light at a range of temperatures, measuring the rate of oxygen produced. Their results are shown below.



e State the independent and dependent variables. (2 marks)

f Describe the relationship between temperature and oxygen production in the light. (3 marks)

g Summarise what occurs during the stage in photosynthesis where oxygen is produced. Include a reference to the role of ATP synthase. (2 marks)

h State two variables that should remain constant during the students' investigation and why each should be controlled. (4 marks)

13 Rubisco is an enzyme whose normal function is to fix CO_2 from the atmosphere to a five-carbon molecule called RuBP during the light independent stage of photosynthesis.

a When the level of carbon dioxide is low in the leaf, Rubisco switches to use oxygen as the substrate, beginning photorespiration. Define photorespiration. (1 mark)

b Give reasons why it is beneficial for a plant to maintain a high level of carbon dioxide inside its leaves. (2 marks)

c Explain how the adaptations of C_4 and CAM plants help to maximise the efficiency of photosynthesis. (2 marks)

d The CRISPR-Cas9 system of gene editing is believed to have a potential use in agriculture by increasing the efficiency of photosynthesis and minimising the occurrence of photorespiration. This gene editing technique involves several molecules: the Cas9 protein, the target DNA strand and guide RNA. What is the difference between the structure of DNA and RNA? (2 marks)

e Describe a consequence of disabling a gene using CRISPR-Cas9, when that gene codes for an enzyme that adversely affects photosynthetic efficiency. (1 mark)

14 Bioethanol is primarily produced by the fermentation of simple monosaccharide and disaccharide sugars by yeast such as *Saccharomyces cerevisiae* or bacteria such as *Escherichia coli.*

a Define fermentation and include a word equation illustrating the type of fermentation *Saccharomyces cerevisiae* would carry out. (2 marks)

b Bioethanol production can also use polysaccharides in the form of starch from corn. However, biomass of this sort must be pre-treated prior to fermentation. Define biomass. (1 mark)

c Outline one pre-treatment method and how it aids in the breakdown of biomass. (1 mark)

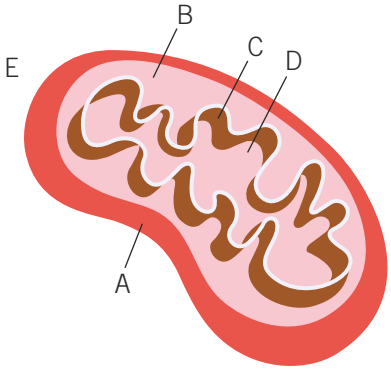
Unit 3 Revision exercise

Multiple-choice questions

1 Coenzymes are organic compounds involved in photosynthesis and cellular respiration. Which of the following is a coenzyme?
- **A** water
- **B** CO_2
- **C** potassium ions (K^+)
- **D** $NADP^+$

2 By the end of the light dependent stage of photosynthesis, light energy
- **A** has been stored in the chemical bonds of NADPH and ATP.
- **B** has entered the stroma ready for the Calvin cycle.
- **C** can be used by the cell.
- **D** has been transformed into the chemical energy in glucose.

3 Read each of the following statements about the following diagram and then select the best answer.



Statement 1: Glycolysis occurs at location E.
Statement 2: The enzymes needed for the Krebs Cycle are at location D.
Statement 3: ADP + $P_i \rightarrow$ ATP occurs at location B.
Statement 4 Oxygen acts as the final electron acceptor to make water at location C.

- **A** Statements 1, 2 and 3 are correct.
- **B** Statements 1, 3 and 4 are correct.
- **C** Statements 1, 2 and 4 are correct.
- **D** Statements 2, 3 and 4 are correct.

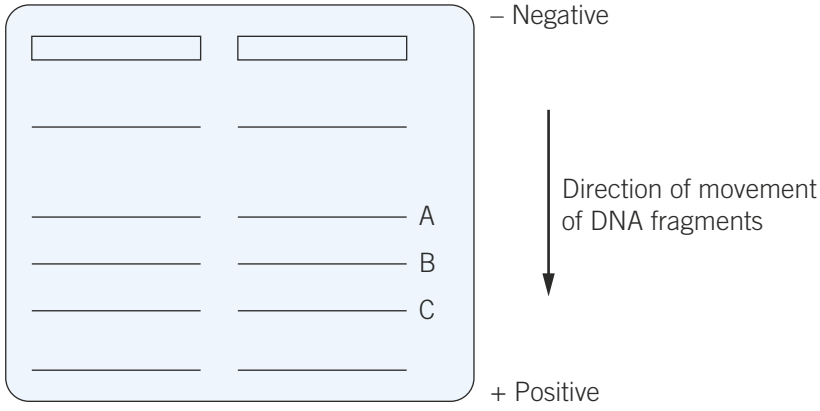
4 Which of the following organisms carries out alcoholic fermentation?
- **A** bacteria
- **B** bacteria, yeast and humans
- **C** bacteria and yeast
- **D** yeast

5 A DNA sample taken from an individual will produce a distinctive DNA profile. This is because each individual has a unique set of
- **A** amino acids in their proteins.
- **B** blood proteins.
- **C** variations in their DNA sequence.
- **D** genes in their chromosomes.

6 The process of inducing a bacterial cell to take up a recombinant plasmid is called
- **A** transduction.
- **B** transformation.
- **C** translocation.
- **D** heat shock.

7 Which of the following statements is true in relation to DNA recombinant technology or genetic engineering?
- **A** Restriction enzymes join different pieces of DNA.
- **B** DNA ligase splits different pieces of DNA, leaving sticky ends.
- **C** Gel electrophoresis is used to determine the sequence of nucleotides in DNA, but not the size of DNA.
- **D** Bacterial plasmids can be used to carry non-bacterial pieces of DNA.

8 The figure below represents the movement of DNA fragments during gel electrophoresis.



From the information provided in the figure, it is reasonable to conclude that
- **A** fragment C is larger than fragment A.
- **B** fragment B is larger than fragment A.
- **C** fragments B and C are each the same size as fragment A.
- **D** fragment B is larger than fragment C, but smaller than fragment A.

9 Which of the following statements is true regarding genetically modified organisms (GMOs) and transgenically modified organisms (TMOs)?
- **A** All GMOs are also TMOs.
- **B** All TMOs are also GMOs.
- **C** An organism cannot be both a GMO and a TMO.
- **D** Simple organisms, such as prokaryotes, can be genetically modified but cannot have DNA from eukaryotes inserted into them because they have circular DNA.

10 The level of protein structure that is formed due to hydrogen bonds alone is the
- **A** primary structure.
- **B** secondary structure.
- **C** tertiary structure.
- **D** quaternary structure.

11 A biochemical pathway
- **A** is made up of many reactions catalysed by one enzyme.
- **B** is made up of one reaction catalysed by many enzymes.
- **C** contains reactions where the substrate of one reaction is also the product of the reaction after it.
- **D** contains reactions where the substrate of one reaction is also the product of the reaction occurring before it.

12 A difference between pre-mRNA and mRNA is that
- **A** pre-mRNA contains introns and mRNA doesn't.
- **B** pre-mRNA is made in the nucleus and mRNA is made in the cytoplasm.
- **C** pre-mRNA has thymine and mRNA has uracil.
- **D** pre-mRNA has a poly-C tail and mRNA doesn't.

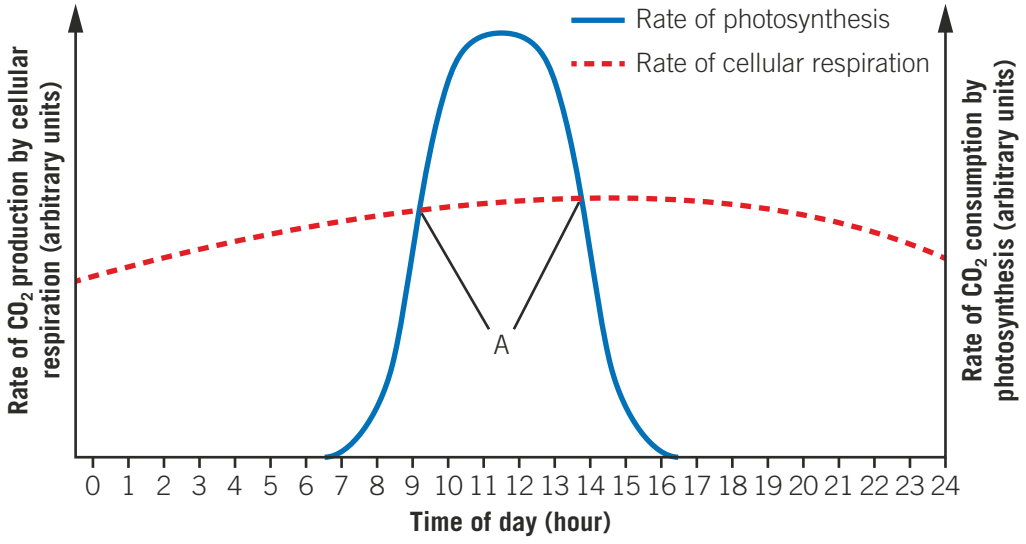
13 Which of the following is most true about an enzyme at a pH lower than its optimal?
- **A** All enzyme molecules will be completely denatured.
- **B** The binding of substrate will be unaffected.
- **C** The enzyme will function at less than 100%.
- **D** The enzyme will have an altered primary structure.

14 A repressor protein will most likely be coded for by
- **A** a structural gene.
- **B** a regulatory gene.
- **C** an operon.
- **D** an intron.

15 Which of the following is true about enzymes?
- **A** They lower the rate of biological reactions.
- **B** They all contain a quaternary structure.
- **C** They work more efficiently at higher temperatures.
- **D** They are all proteins.

Short-answer questions

16 Green plants undergo both photosynthesis and cellular respiration. The graph shows the rate of CO_2 consumption in photosynthesis and the rate of CO_2 production by cellular respiration in a green plant over 24 hours.



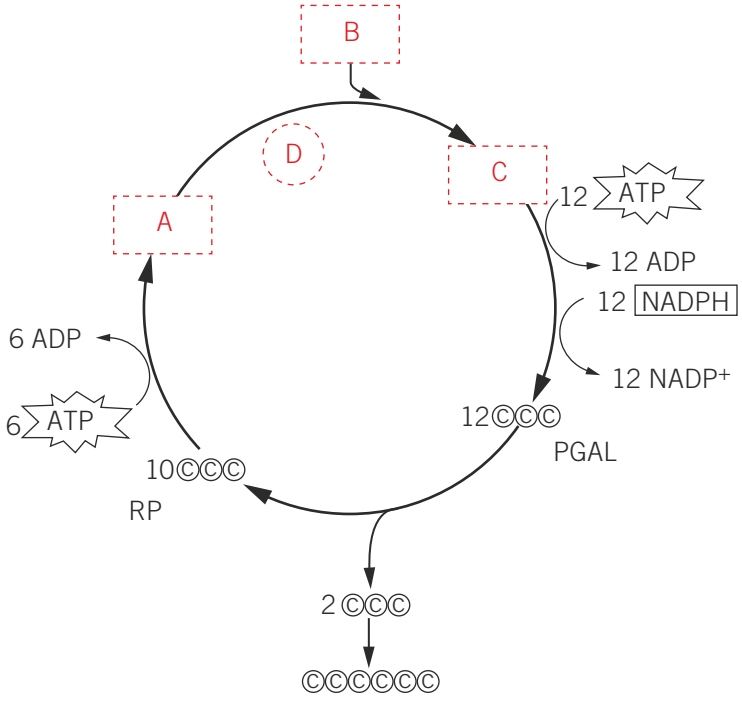
a Using data from the graph, explain the shape of the curve for the rate of photosynthesis. (3 marks)

b Using data from the graph, explain the shape of the curve for the rate of cellular respiration. (2 marks)

c Describe what is happening at the points labelled A. (1 mark)

17 Carbon fixation is the first part of the Calvin cycle.

a The Calvin cycle of a C_3 plant is shown below. Name compounds A, B and C and enzyme D. (2 marks)



b NADPH is a coenzyme involved in the Calvin cycle. Explain the role of NADPH. (1 mark)

c Where does the Calvin cycle occur? (1 mark)

d The most common type of plants are C_3 plants, which produce a three-carbon compound as the first product of carbon fixation. However, plants are adapted to grow in temperatures that suit their metabolism, so not all plants are C_3 plants. Some plants are adapted to be most efficient in cool climates, like the C_3 plants, while others are adapted to be most efficient in hot sunny climates. Outline why C_3 plants are not as efficient at photosynthesis in hot dry environments. (3 marks)

e Summarise how a C_4 plant is able to maximise the efficiency of photosynthesis in a hot environment. (3 marks)

f Scientists have been investigating CRISPR-Cas9 as a gene editing tool. Define CRISPR-Cas9 and then summarise how this tool can be used to improve the photosynthetic efficiency of C_3 plants in hot environments. (3 marks)

18 Biofuels can help to reduce demand for fossil fuels. This would result in a reduction of greenhouse gas emissions and global warming.

a Define biofuel. (1 mark)

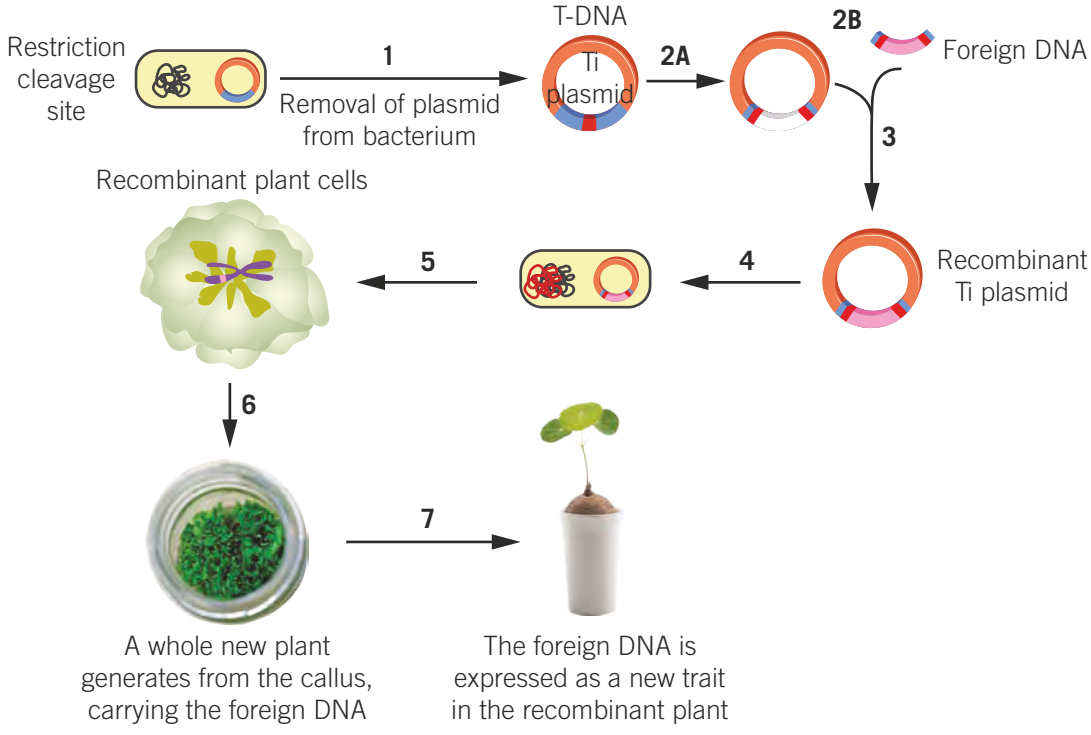
b Explain the relationship between biofuel production and anaerobic respiration. Include any relevant word equations. (3 marks)

c Distinguish between the anaerobic respiration mentioned in part **b** and the anaerobic respiration that occurs in animals. (2 marks)

19 Genetic modification in agriculture is a field of science that has grown over the past few decades. With rapidly growing populations, greater production of crops are required. Developing crops that are more resistant to disease can assist this greater production of unspoiled food. In Australia, cotton crops are typically predated on by the *Helicoverpa armigera* caterpillar, which chews the flower buds. These buds develop into cotton fruit, which contain the seeds that cotton fibre grows from. Historically, control of the caterpillar was maintained by spraying pesticides. However, now approximately 90% of the crop is genetically modified to resist the caterpillar.

This genetically modified cotton plant, called 'Bt cotton', produces a protein that is poisonous to the caterpillar but harmless to other insects and humans. The genetic information for making the protein is obtained from the bacterium *Bacillus thuringiensis* (hence the name 'Bt') and transferred to a plasmid, which is then inserted into a different bacterium, *Agrobacterium tumefaciens*. This bacterim is then mixed with the cotton plant cells to generate the growth of plant cells that carry DNA for resistance to the caterpillar.

a Using the figure below, outline what occurs at each of the stages numbered 2A to 5. (6 marks)



b Are the resulting recombinant plant cells genetically or transgenically modified? Explain by clearly describing the difference between the two terms. (2 marks)

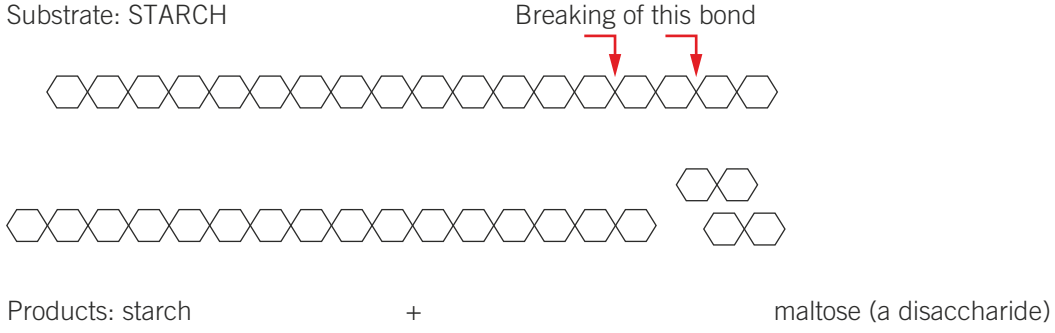
c Usually in the process of gene cloning, plasmids also contain an antibiotic resistance gene. Explain the benefit of these genes being present in the plasmid. (3 marks)

Prokaryotic cells, like the bacteria from which the plasmid and insect resistance gene have been obtained, do not contain introns.

d Identify the difference between introns and exons. (1 mark)

e What would the problem be if the insect resistance gene taken from the *Bacillus thuringiensis* contained introns? (1 mark)

20 A plant enzyme, amylase, catalyses the breakdown of starch, a complex carbohydrate, into simpler sugar molecules called maltose. This is then further broken down into an even simpler sugar, glucose. The rate of this reaction is dependent on a variety of factors, one being temperature. Amylase activity can be investigated by measuring the breakdown of starch. In this experiment, iodine turns purple in the presence of starch and colourless in the presence of simple sugars.
The reaction is shown below.



The experiment was conducted at four different temperatures: 25°C, 40°C, 70°C and 100°C. Each test was conducted once, and the time taken (in minutes) for the iodine to change colour was recorded.

a Formulate a hypothesis regarding the effect of temperature on the rate of amylase activity. (2 marks)

The method was conducted as follows:

1. *Label four test tubes 1–4.*
2. *Using a pipette, add 2 mL of 1% amylase solution to each of the test tubes. Place them in water baths at the following temperatures:*
   - *room temperature (25°C)*
   - *40°C*
   - *70°C*
   - *100°C (boiling water).*
3. *Stand the tubes at these temperatures for 5 minutes to allow them to come to the appropriate temperature.*
4. *Remove test tubes from their respective temperatures and, using a new pipette, add 2 mL of 0.7% starch solution to each of these tubes. Do not return the test tubes to the water baths; leave them at room temperature.*
5. *Using a pipette, add 2 drops of iodine to each solution. Mix thoroughly by swirling the contents of the test tube.*
6. *Compare the colour intensity of each tube. The more intensely coloured the solution is, the more starch is present; the less intensely coloured the solution is, the more simple sugars are present.*
7. *Time (minutes) how long each tube takes for the solution to turn colourless and record any other observations made.*
8. *Work out an estimated rate of reaction for each of the temperatures, by using the formula:*

$$\text{rate} \quad \frac{1}{\text{time}} \quad (\text{unit: } \text{min}^{-1})$$

9 *Draw a table of results showing time taken for the solutions to turn colourless and the individually calculated rates.*

10 *Draw a graph of rate against temperature.*

The table of results (from step 9 in the method) is provided below.

Temperature	Time	Rate
25	3	0.3
40	7	0.15
70	5	0.2
100	Does not change colour	0

b Identify an error with the construction of the table of results above. (1 mark)

c Using the results in the table, construct an appropriate graph to represent the results for each temperature. (4 marks)

d Using the results from the table (or your graph from part **c**), summarise the effect of the independent variable on the dependent variable. (3 marks)

e What is an example of a controlled variable in this experiment? Explain why it was necessary to keep this variable constant throughout the experiment. (2 marks)

f Considering the set-up of the experiment, what would be a suitable control group? Explain your answer. (2 marks)

g Is the experiment valid? Explain, using information given in the method. (1 mark)

h With reference to the results and the levels of protein structure, explain the effect of temperature on the action of amylase. (4 marks)

i Explain how the effect of high temperatures on enzyme activity is similar to enzyme inhibition. (3 marks)

21 Eric is a detective and is investigating a robbery. Camera footage has helped him identify the thief, but he has one problem. The thief is an identical twin, and both twins claim that the other one is responsible. A small spot of blood was found at the scene, but Eric isn't sure that DNA evidence is going to be helpful in this situation. However, genetic testing of the twins done on their 18th birthday showed that one had a single base mutation in the 'good behaviour' gene.

a Name and describe the technique through which you would amplify the DNA from the blood sample to allow you to analyse the 'good behaviour' gene. (4 marks)

b A small section of the 'good behaviour' gene containing the mutation is shown below.
Twin 1 (normal): GAATTC
Twin 2 (mutated): GGATTC
This sequence appears to align with a known recognition site. It appears towards the end of the overall gene sequence. The sites for three enzymes are shown below.

EcoRI	**XbaI**	**BamHI**
5'...G|AATTC...3'	5'...T|CTAGA...3'	5'...G|GATCC...3'
3'...CTTAA|G...5'	3'...AGATC|T...5'	3'...CCTAG|G...5'

Using the information provided above, explain which restriction enzyme would be the best to use to digest the DNA obtained from the crime scene. (3 marks)

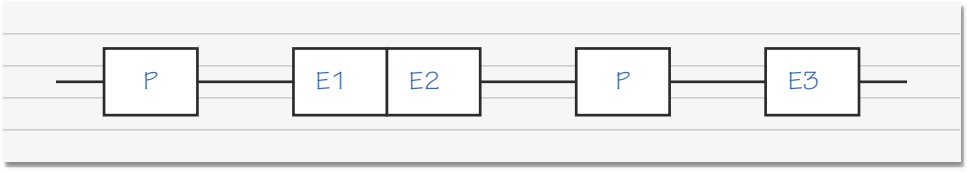
c Using the three restriction enzymes described above, provide a representative image of what the restriction digests (when each person's DNA was mixed with each restriction enzyme separately) would look like for each twin after gel electrophoresis. The exact sizes of fragments are not important. If the blood sample from the crime scene produced a single band with all three enzymes, which twin committed the robbery? (6 marks)

d Using the information provided in part **b** of this question and the codon table provided below, determine what the amino acid change is between Twin 1 and Twin 2. The DNA six-nucleotide sequence provided is in the correct reading frame. (2 marks)

First base	Second base: U		Second base: C		Second base: A		Second base: G		Third base
U	UUU	Phenyl-alanine phe	UCU	Serine ser	UAU	Tyrosine tyr	UGU	Cysteine cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine leu	UCA		UAA	STOP CODON	UGA	STOP CODON	A
	UUG		UCG		UAG		UGG	Tryptophan trp	G
C	CUU	Leucine leu	CCU	Proline pro	CAU	Histidine his	CGU	Arginine arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Glutamine gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Isoleucine ile	ACU	Threonine thr	AAU	Asparagine asn	AGU	Serine ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lysine lys	AGA	Arginine arg	A
	AUG	met (START CODON)	ACG		AAG		AGG		G
G	GUU	Valine val	GCU	Alanine ala	GAU	Aspartic acid asp	GGU	Glycine gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glutamic acid glu	GGA		A
	GUG		GCG		GAG		GGG		G

e The protein coded for by the 'good behaviour' gene is an enzyme. Describe what the possible effect of this mutation might be if it occurred in the active site. (2 marks)

22 The following figure depicting an operon was produced by a Biology student for an assessment task.



An operon containing promoters (P) and exons (E1-E3) found in a section of DNA

a List three things that are incorrect in the operon figure drawn by the student. (3 marks)

b The bacterium *E. coli* contains an operon known as the *lac* operon. It contains the genes necessary for the metabolism of the sugar, lactose. *E. coli* needs to break down lactose when other sugars, such as glucose, are not present. In the context of this example, explain the role of the repressor in the following situations:

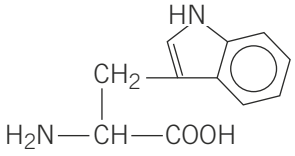
i High glucose, high lactose (1 mark)

ii No glucose, high lactose (1 mark)

iii No glucose, no lactose (1 mark)

c Explain the role of RNA polymerase in instances when the operon is turned on. (3 marks)

d The *trp* operon is a separate operon that is involved in the production of tryptophan (an amino acid). The structure of tryptophan is shown below. Draw a dipeptide (the joining of two amino acids) formed by two tryptophan residues. (2 marks)



Tryptophan

e What type of reaction is involved in the formation of the dipeptide? Use evidence from your diagram in part **d** to explain how this reaction occurs. (3 marks)

f Amino acids are the monomers that are joined together to create polypeptide chains and proteins. The process of protein synthesis is known as translation, and this process is outlined in the steps below. These steps contain errors. Find these errors and explain what the correct process should be. (5 marks)

Step 1 *Messenger RNA (mRNA) exits the nucleus and travels to the ribosome.*
Step 2 *mRNA binds to the ribosome.*
Step 3 *mRNA is read two base pairs at a time. These are referred to as codons.*
Step 4 *A ribosomal RNA (rRNA) binds to the codon via an anticodon.*
Step 5 *The codon is specific for an amino acid, which joins to the opposite end of the tRNA after binding via the anticodon.*
Step 6 *After two tRNA molecules are bound, one has to depart before the next one in the chain can bind. Before this happens, the amino acids must link together. This occurs via a hydrogen bond.*
Step 7 *This process is repeated until a tRNA attaches to a codon called a terminator, causing the polypeptide chain to break away.*

g Once a polypeptide is created in eukaryotic cells, it then requires the assistance of other organelles for its release from the cell. Identify three organelles that are involved in this process and explain their individual functions. (3 marks)

UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES?

CHAPTER 6

FOREIGN INVADERS: SELF VERSUS NON-SELF

Introduction

In this chapter you will begin your exploration of disease by examining the key differences between cellular and non-cellular pathogens. Key examples of each class of pathogen are investigated, including bacteria and fungi (cellular), and viruses (non-cellular). The various modes of transmission used by these pathogens to gain entry into a host and bring about the onset of disease within the infected organism are also discussed.

The fundamental principle your immune system relies upon to protect you is its ability to recognise self from non-self. You will learn how unique surface molecules, known as antigens, can be classified as either self-antigens or non-self antigens, and how the detection of non-self antigens present on invading pathogens leads to the initiation of an immune response. Finally, the role of allergens in eliciting an immune response is considered.

Curriculum

Area of study 1 Outcome 1
Responding to antigens

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens	**6A Recognising self from non-self** **6A.1** Distinguish between infectious and non-infectious disease and give examples of each **6A.2** Explain the relationship between disease, pathogen and antigen **6A.3** Recognise that pathogens can be transmitted in a variety of ways **6A.4** Define vector and explain the role of this type of organism in the transmission of infectious disease **6A.5** Describe the typical course of disease **6A.6** Discuss the nature of antigens in terms of self and non-self **6A.7** Explain the role of MHC markers in an organism **6A.8** Distinguish between MHC Class I and MHC Class II markers in terms of location and function **6A.9** Define allergen and explain how an allergen differs from a pathogen

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Initiation of an immune esponse, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens	**6B Cellular pathogens** **6B.1** Explain the features of a pathogen that must be present for it to be classed as cellular **6B.2** Identify the key structural features of a bacterial cell **6B.3** Describe the ways in which bacteria can cause disease **6B.4** Explain the key structural features of fungi **6B.5** Describe the ways in which fungi can cause disease **6B.6** Identify the key structural features of protozoa and describe the ways in which they contribute to disease
• Initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens	**6C Non-cellular pathogens** **6C.1** Explain the key differences between cellular and non-cellular agents of disease **6C.2** Describe the key structural features of a virus (nucleic acid, capsid and envelope) **6C.3** Discuss the ways in which viruses are able to cause disease **6C.4** Explain each stage of the viral life cycle in detail (attachment, entry, replication, assembly and release) **6C.5** Distinguish between the target host of a typical virus and a bacteriophage **6C.6** Identify the key structural features of a typical bacteriophage **6C.7** Explain the difference between a typical protein and a disease-causing prion

Glossary

Allergen
Antigen
Antigen-presenting cell (APC)
Bacteria
Bacteriophage
Capsid
Cellular pathogen
Cilia
Disease
Dormant
Epidemic
Endemic
Epidemiology
Epitope
Fungi
Haemagglutinin
Heterotrophic
Host
Hyphae
Infectious (communicable) disease
Malaria
MHC (major histocompatibility complex) marker
MHC Class I marker
MCH Class II marker
Mycelium
Neuraminidase
Non-cellular pathogen
Non-infectious (non-communicable) disease
Non-self antigen
Pandemic
Pathogen
Plasmodesmata
Prion
Protozoa
PrP^{c}
PrP^{Sc}
Rice blast disease
Self-antigen
Spores (bacterial)
Terrestrial
Tetanus
Tinea
Vector
Viral envelope
Virion
Virus

Concept map

6A Recognising self from non-self

How identifying self from non-self allows our immune system to identify pathogens and recover from disease

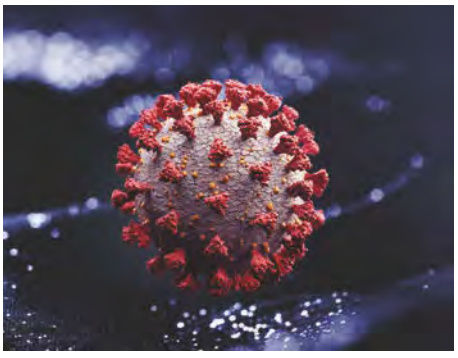
Explore the major classes of cellular pathogens (bacteria, protozoans and fungi) and the diseases they cause

Explore the major classes of non-cellular pathogens (viruses and prions) and the diseases they cause

6B Cellular pathogens

Case Study 1: Tetanus (bacterial)
Case Study 2: Tinea (fungal)
Case Study 3: Rice blast (fungal plant disease)
Case Study 4: Malaria (protozoan)
(Online) Case Study 6: Meningococcal disease (bacterial)
(Online) Case Study 7: Giardia (protozoan)

6C Non-cellular pathogens



Case Study 5: Influenza (viral)

See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

Recognising self from non-self

Study Design:
Initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens

Glossary:
Allergen
Antigen
Antigen-presenting cell (APC)
Disease
Epidemic
Epitope
Host
Infectious (communicable) disease
MHC (major histocompatibility complex) marker
MHC Class I marker
MHC Class II marker
Non-infectious (non-communicable) disease
Non-self antigen
Pandemic
Pathogen
Self-antigen
Vector

ENGAGE

Disease outbreaks

Throughout history, the world's population has been faced with the challenge of overcoming disease outbreaks. The earliest recorded pandemic occurred in Ancient Greece in 430 BC. This was the time of a great war between the Athenians and the Spartans. The disease, now suspected to be typhoid fever, caused fever, skin lesions and a bloody throat. The disease wiped out two-thirds of the population, significantly weakened the Athenians and is thought to be the reason that Athens fell to the Spartans.

Between 1347 and 1351, the Black Death wreaked havoc throughout Europe. Thought to be the result of a bacterial infection known as the bubonic plague, it would turn the skin black in patches. It killed 25 million Europeans and 75 million people worldwide, effectively wiping out 50% of the world's population. Bodies littered the streets and rotted on the ground, filling cities with the constant stench of death.

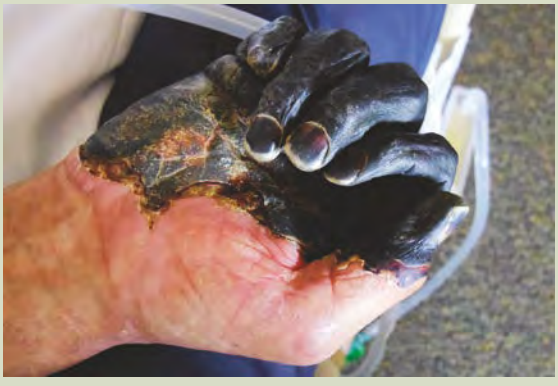


Figure 6A–1 Black patches of skin was a common symptom of bubonic plague, and the disease still appears today, such as this case in the USA.

Figure 6A–2 A mask worn by a plague doctor. Herbs were placed in the beak of the mask, in an attempt to purify the air being breathed in by the doctor.

8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT LINK

In 1918 the deadliest natural disaster in human history began its rampage. Between March 1918 and June 1920, an influenza pandemic known as the Spanish flu killed approximately 100 million people around the world. The pandemic occurred at the time of World War I and was given its name as a result of Spain being a neutral country during the war and accurately reporting its death rate. Germany, the United States, Britain and France were heavily engaged in combat and saw it as a sign of weakness to announce their death toll. In the end, influenza may have tipped the balance of power in the Allies' favour, as the mortality rate was higher in Germany and Austria than in Britain and France. Modern transport made it easy for troops to spread the disease, and malnourishment and stress as a result of war would have increased the lethality of the virus in soldiers.

Figure 6A–3 Victims of the Spanish flu being transported by nurses

8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT LINK

In more recent times, an outbreak of SARS (severe acute respiratory syndrome) in 2003 infected more than 8000 people in 26 countries and resulted in the deaths of 774 people. Lessons learnt from this outbreak led to many improvements in disease control practices and, as a result, diseases like influenza (swine and avian), Ebola and Zika have been brought under control, preventing further pandemics. Unfortunately, the same cannot be said for COVID-19, which spread to 114 countries in just three months. By the end of 2020 it had infected over 50 million people worldwide and caused more than a million deaths. A pandemic was declared by the World Health Organization in March 2020, with the outbreak showing that, despite all the technology we have available, we are still vulnerable to the devastating effects of disease outbreaks. As history can attest, the capacity of diseases to cripple our way of life should not be underestimated.

Disease
any condition that affects the normal function of either a part of an organism or the complete organism

EXPLAIN

The nature of disease

Information collected by the World Health Organization (WHO) over a number of years shows that nine of the top 10 causes of death worldwide relate to a variety of diseases (see Figure 6A–4). For the past 15 years, the top two causes of death around the world have been heart disease and stroke. But what exactly is a disease? **Disease** refers to any condition that affects the normal function of either a part of an organism or the complete organism. Diseases can be classified into two broad categories.

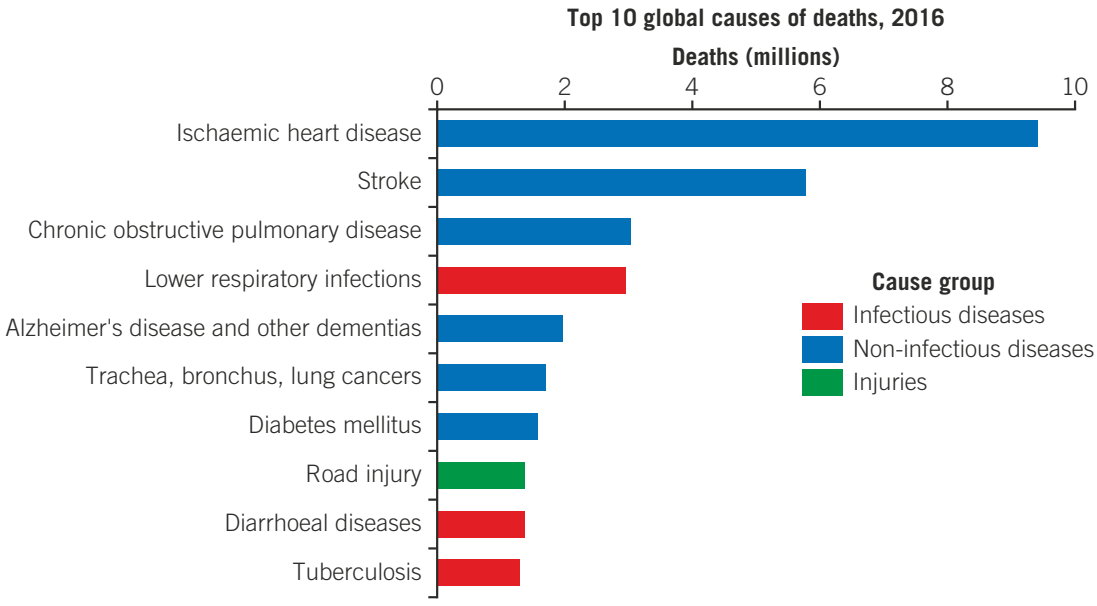
- **Non-infectious (non-communicable) diseases** are not contagious and therefore cannot be directly transmitted from one organism to another. Common examples of non-infectious diseases include cancer, heart disease, Alzheimer's and diabetes.
- **Infectious (communicable) diseases** are contagious, which means they can be transmitted from one organism to another. Common examples of infectious diseases include influenza, the common cold and chickenpox.

Non-infectious diseases result from genetics, poor lifestyle choices (diet, exercise) or physical/mental degeneration. Infectious diseases are caused by **pathogens**.

Non-infectious (non-communicable) disease
a disease that cannot be transmitted from one organism to another

Infectious (communicable) disease
a disease that can be transmitted from one organism to another

Pathogen
a disease-causing agent



Source: Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000–2016. Geneva, World Health Organization; 2018. Road injuries are the only cause of death in the top ten that do not relate to disease.

Figure 6A–4 The top 10 causes of death worldwide in 2016 (as determined by data collected by WHO)

Infectious disease and pathogens

Infectious diseases are responsible for the **epidemics** and, in some cases, **pandemics**, that you read about in the Engage box in this section. All infectious diseases are the direct result of a pathogen and its activity within the **host**. There are many types of pathogens, which cause a wide range of symptoms that result in the onset of infectious disease.

For example, recall the information you read earlier about the Spanish flu. This disease was the result of a viral pathogen known as influenza H1N1. When this virus gained entry to a host, it took effect through a range of symptoms, beginning with fever and fatigue, before running its course and ending with the sufferer's skin turning blue and their lungs filling with fluid, which led to suffocation and death.

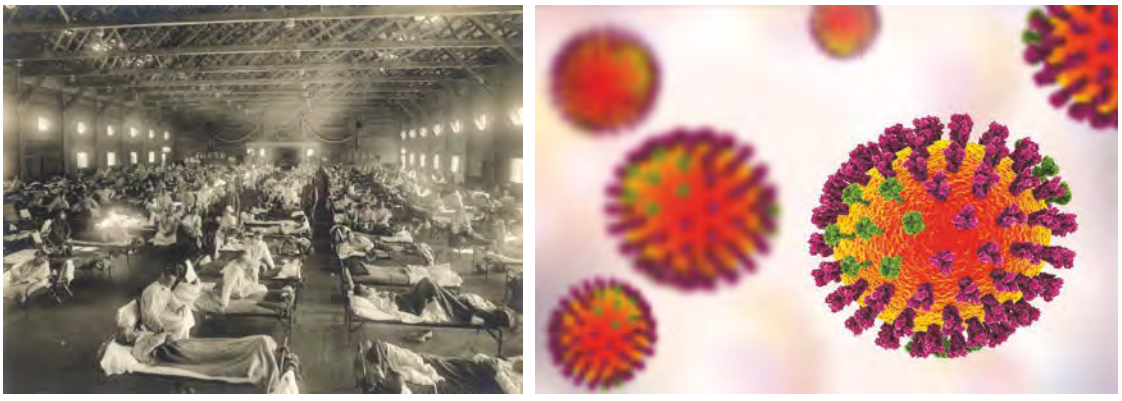


Figure 6A–5 Left: A hospital crowded with Spanish flu patients. Right: The H1N1 influenza virus

The influenza virus is just one example of many viruses that have had devastating effects on populations. Viruses are not the only form of pathogen. Other common examples of pathogens are bacteria, fungi and parasites. Major classes of pathogens are explored in detail in the remaining sections of this chapter.

Epidemic
the rapid spread of an infectious disease to a large number of people within a population

LINK **8A** EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT

Pandemic
an outbreak of infectious disease that occurs over a wide geographical area, affecting a large number of people

Host
an organism that has been infected by a pathogen

VIDEO 6A–1 INTRODUCTION TO DISEASE

LINK **6B** CELLULAR PATHOGENS

Modes of transmission

The transmission of infectious disease occurs through either direct contact with an infected host or indirect contact with an infected surface, or by breathing infected air and ingesting infected food or water. Direct contact allows the pathogen to easily transfer between organisms, where it can continue to exert its effects, resulting in the onset of disease. Modes of transmission by common pathogens are outlined in Table 6A–1. Note that, in many instances, pathogens are capable of gaining entry to a host through more than one transmission mode. An example is chickenpox, spread by direct contact with the blisters, and also through the air by coughs and sneezes.

Table 6A–1 Pathogen modes of transmission

Mode of transmission	Disease	Pathogen
Contact transmission		
Contact may be direct, e.g. handshaking, bodily fluid exchange (sexual intercourse) or indirect via something in between, e.g. infected blood or body fluids from drinking glasses, toothbrushes, etc.	Tinea	Fungus (dermatophyte)
	Herpes	Virus (HSV)
	AIDS	Virus (HIV)
	Tetanus	Bacterium (*Clostridium tetani*)
	Common cold	Virus (rhinovirus)
	Chickenpox	Virus (*Varicella zoster*)
Medium transmission		
Airborne Inhalation of infected droplets from sneezing, coughing or talking	Chickenpox	Virus (*Varicella zoster*)
	Whooping cough	Bacterium (*Bordetella pertussis*)
	Flu	Virus (influenza)
Waterborne Drinking contaminated water (streams, swimming pools, etc.)	Cholera	Bacterium (*Vibrio cholerae*)
	Gastroenteritis	Bacterium (e.g. *Escherichia coli*) and viruses
Foodborne Eating contaminated food	Food poisoning	Bacterium (e.g. *Salmonella*)
	Hepatitis A	Virus (HAV)

Figure 6A–6 From left to right: a cold sore caused by the herpes virus, sneezing infectious respiratory droplets, skin lesions associated with the fungal infection athlete's foot, and chickenpox

Vectors

As you read in the introduction to this section, a bacterial disease known as the bubonic plague was responsible for an outbreak known as the Black Death. The bubonic plague struck again with devastating effects in London between 1665 and 1666. Known as the Great Plague, it killed approximately 100 000 people, which was 20% of London's population at the time. It wasn't until the Great Fire of London in 1666, which burnt most of London to the ground, that the plague's death toll began to decrease, as it is thought that the fires killed the diseased rats carrying infected fleas. These infected fleas are an example of a **vector**, which is another mode of transmission by which pathogens can spread disease.

Vector
a living organism that carries and transmits a pathogen from one organism to another

Vectors are living organisms that carry and transmit a pathogen from the infected source to another living organism. The vector may transfer the pathogen and, as a consequence, the disease between humans, or it may be responsible for a cross-species transfer where the pathogen moves from animals to humans, as was the case with the Great Plague of London. The vectors themselves are usually not affected by the pathogen. This is in contrast to a carrier, who is infected by the pathogen and in most instances (unless asymptomatic) displays symptoms associated with the disease.

Each year, vector-borne diseases are responsible for 17% of all infectious disease and cause more than 700 000 deaths. Most of these deaths occur in poorer nations with subtropical climates. The two most common vector-borne diseases are malaria and dengue, both transmitted by mosquitoes, with annual death tolls of 400 000 and 40 000 respectively. Table 6A–2 lists some vectors and the diseases and pathogens they transmit.

Table 6A–2 Common vectors and the disease they transmit

Vector	Disease	Pathogen
Mosquito	Dengue	Virus (DENV)
	Yellow fever	Virus (Flavivirus)
	Malaria	Protozoan (*Plasmodium*)
	Zika	Virus (ZIKV)
Flea	Bubonic plague	Bacterium (*Yersinia pestis*)
Tick	Lyme disease	Bacterium (*Borrelia burgdorferi*)
	Spotted fever	Bacterium (*Rickettsia rickettsia*)
	Crimean-Congo haemorrhagic fever	Virus (Nairovirus)

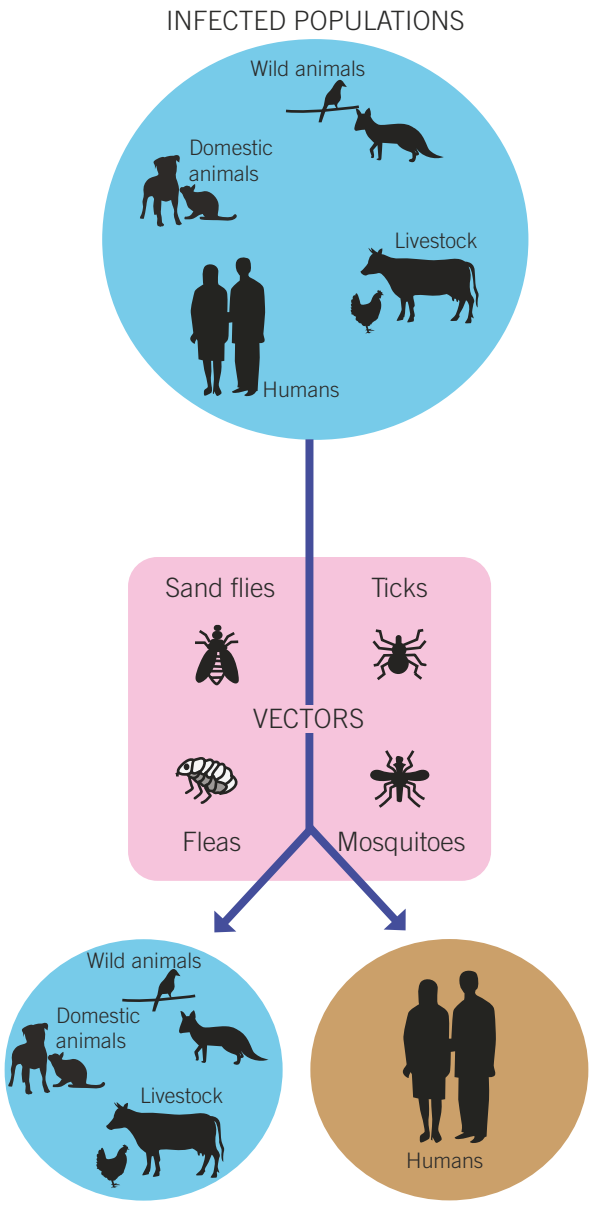


Figure 6A–7 Some common examples of vectors that transmit infectious disease

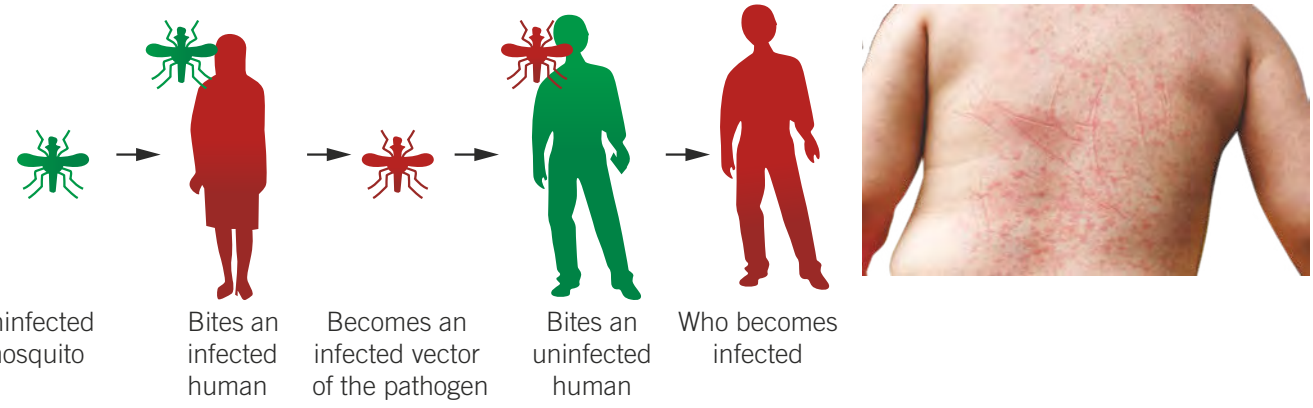


Figure 6A–8 How pathogens are transmitted via a mosquito vector (left). Skin rash caused by Dengue fever, a mosquito-borne vector disease (right).

Course of disease

All diseases, regardless of their pathogenic origin, follow a similar pattern of action. A general representation of the course of disease is shown in Figure 6A–9.

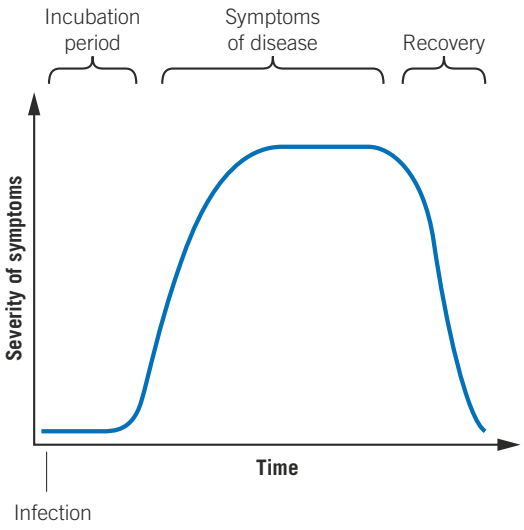


Figure 6A–9 The general course of disease

Upon infection by a pathogen, the progression of disease within a host has three stages:

1. *Incubation:* Initially there will likely be an incubation period, for various reasons. The pathogen may take time to multiply to a number sufficient to cause disease; it may take time to reach target tissue that is susceptible to its actions; or toxins released by the pathogen may take time to accumulate, before they cause disease.
2. *Symptoms of disease:* Symptoms result from the body's immune system trying to eliminate the infection, or they are the effect that the pathogen has on the body of the host (for example, runny nose, rash, coughing, sneezing). All diseases usually have characteristic symptoms that assist a doctor to make a correct diagnosis.
3. *Recovery:* The final stage in the course of disease is recovery. Usually the host's immune system will fight off the pathogen, either naturally or with the assistance of prescribed medication. If the pathogen cannot be eliminated, then disability or death is likely. Factors such as the infected person's age and state of health are important in determining whether the person makes a full recovery.

7D THIRD LINE OF DEFENCE LINK

Check-in questions – Set 1

1. Compare the main features of infectious and non-infectious diseases.
2. Distinguish between a pathogen and a disease.
3. How do vector-borne diseases differ from diseases caused by direct contact or medium-transmitted diseases?

WORKSHEET 6A–1 IDENTIFYING SELF FROM NON-SELF DOC

Antigen
a unique marker on the surface of cells or viruses that is used in the identification of self from non-self

Self-antigen
an antigen on the surface of cells of an organism that is identified by the immune system as belonging to the organism and therefore does not trigger an immune response

Determining self from non-self

Your body's ability to defend against pathogen invasion depends on the ability of your immune system to recognise what belongs to your body and what is foreign. In other words, your immune system must be able to identify 'self' from 'non-self'.

The cells of all organisms have unique molecules on their surface that distinguish them from the cells of other organisms. These unique molecules or markers are referred to as **antigens** and can be recognised by an organism's immune system as 'self' or 'non-self'.

Antigens

Antigens are most commonly made of protein and/or polysaccharides. They can be classified into two distinct groups, depending on their source of origin.

Self-antigens

- Originate from inside the body and are found on the surface of cells that make up the organism.
- The immune system recognises any object with these antigens as belonging to 'self' and no immune response is initiated.

Non-self antigens

- Originate outside the body and are found on the surface of cells that are foreign to the body.
- When the immune system recognises these antigens as '**non-self**', a complex immune response is activated to destroy the antigens and their source of production.
- There may be many different types of antigens on a pathogen's surface.

Non-self antigen an antigen on the surface of cells of an organism that is identified by the immune system as foreign to the organism and triggers an immune response when detected

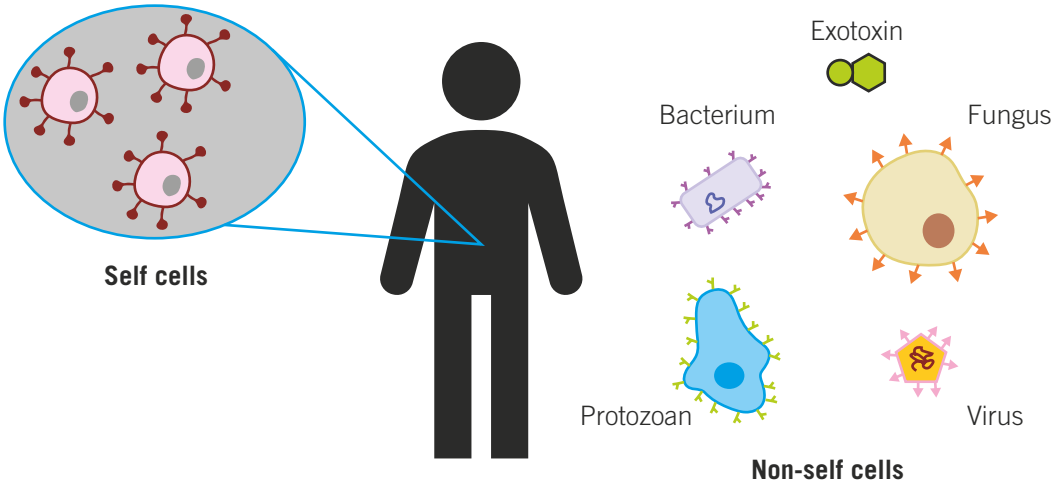


Figure 6A–10 Self-antigens are found on the surface of a host organism's own cells. Non-self antigens are found on the surface of all classes of pathogens and activate an immune response within the host.

LINK **7B** SECOND LINE OF DEFENCE

LINK **7D** THIRD LINE OF DEFENCE

MHC markers

MHC (major histocompatibility complex) markers are proteins found on the surface of cells and are unique to individual organisms. Their role is to identify the cell as 'self' if healthy, or 'non-self' if infected by a pathogen. To do this, they present small peptides to circulating immune cells (you will meet these cells in Chapter 7). These peptides (also referred to as **epitopes**) are mainly self-antigens except for when the cell is infected by a pathogen. In this case, non-self antigens are presented on the infected cell's MHC markers.

MHC (major histocompatibility complex) marker a protein that is found on the surface of cells and is used in the identification of pathogens in the immune response

Epitope the specific region of an antigen that is recognised by the immune system

As is the case with all proteins, the code for building MHC markers is located in the organism's DNA. The specific set of genes that codes for these protein markers is called the major histocompatibility complex (MHC), and this is the origin of the MHC markers' name.

There are two types of MHC markers on cells: Class I and Class II.

LINK **7B** SECOND LINE OF DEFENCE

LINK **CHAPTER 2**

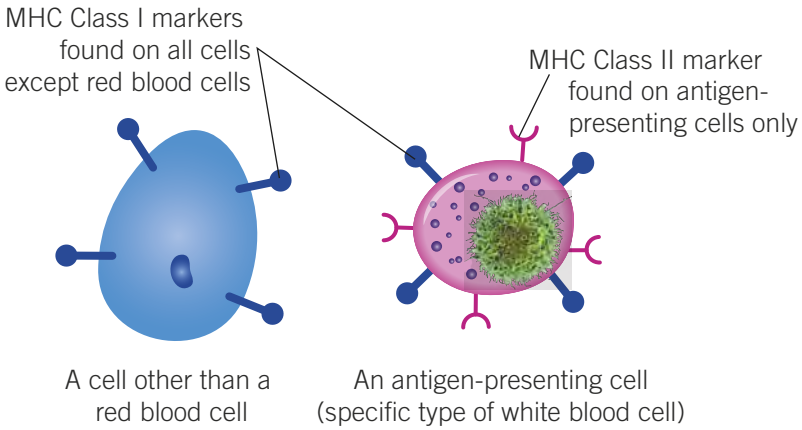


Figure 6A–11 MHC markers and the cells they are found on. Class I markers are binding sites for antigens that identify the cell as healthy or sick. Class II markers are used by specific white blood cells to present antigens to other immune cells to initiate and communicate an immune response.

MHC Class I marker
a type of protein marker on the surface of all nucleated cells that assists in the identification of self from non-self

MHC Class I

MCH Class I markers are made and found on all human cells except red blood cells, stem cells and some reproductive cells. You will learn in Sections 7C and 7D how MHC Class I markers assist the immune system to recognise cells that have been infected with a pathogen (bacterial or viral), a process known as the cell-mediated response.

CHAPTER 7 LINK

MHC Class II marker
a type of protein marker on antigen-presenting white blood cells that is used in the activation of a specific immune response

Antigen-presenting cell (APC)
a specific type of white blood cell that uses phagocytosis to engulf a pathogen before displaying peptide fragments (epitopes) on its MHC Class II markers for detection by white blood cells

MHC Class II

MHC Class II markers are found only on specific types of immune cells, white blood cells, known as **antigen-presenting cells (APC)**. Examples of APCs are macrophages, B cells and dendritic cells. You will learn in Section 7C how MHC Class II markers and APCs assist the immune system in defending against invading pathogens in the humoral response.

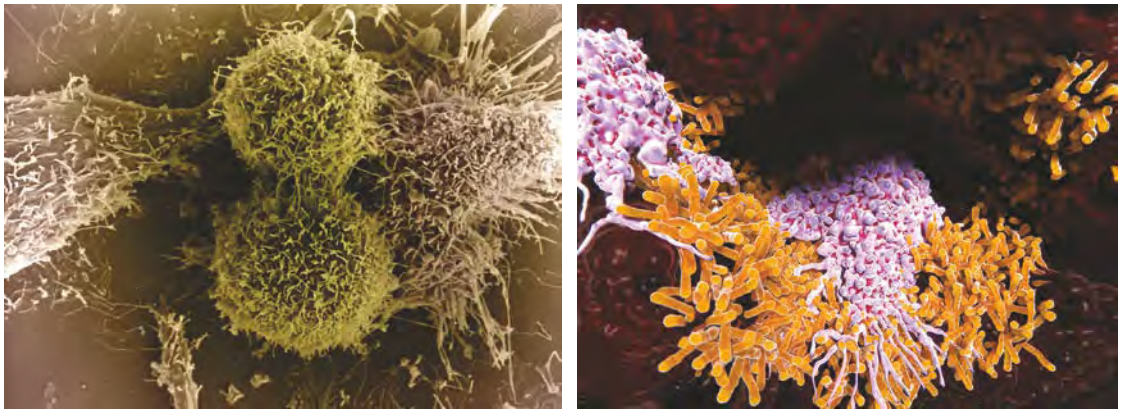


Figure 6A–12 Left: Scanning electron microscope image of two white blood cells (shown in the middle of the image) destroying viral-infected cells. Right: A white blood cell engulfing bacteria that cause tuberculosis; the white blood cell will then present the bacterial epitopes to immune cells on its MHC Class II markers.

Allergens

In Australia, allergies are common. Around one-third of the population will develop allergies at some point in their lifetime. Food allergies are some of the most common types of allergies, with 10% of children under the age of one, and 2% of adults, diagnosed with the condition. Other common triggers for allergic reactions are insect bites/stings, medications and environmental allergens (such as pollen and animal hair).

Figure 6A–13 Common allergens

An allergic reaction is the result of an overreaction by the body's immune system to a normally harmless substance. Any stimulus that triggers an allergic reaction is referred to as an **allergen**.

Allergen
any substance that causes an allergic reaction

Allergic reactions can result in a range of symptoms, from mild to severe.

Mild to moderate symptoms include:
- sneezing, with an itchy and runny nose
- red and watering eyes
- hives (red, itchy rash)
- vomiting/abdominal pain.

Severe symptoms (often associated with severe allergic reactions) include:
- difficulty breathing
- swelling of the lips and tongue
- dizziness
- loss of consciousness.

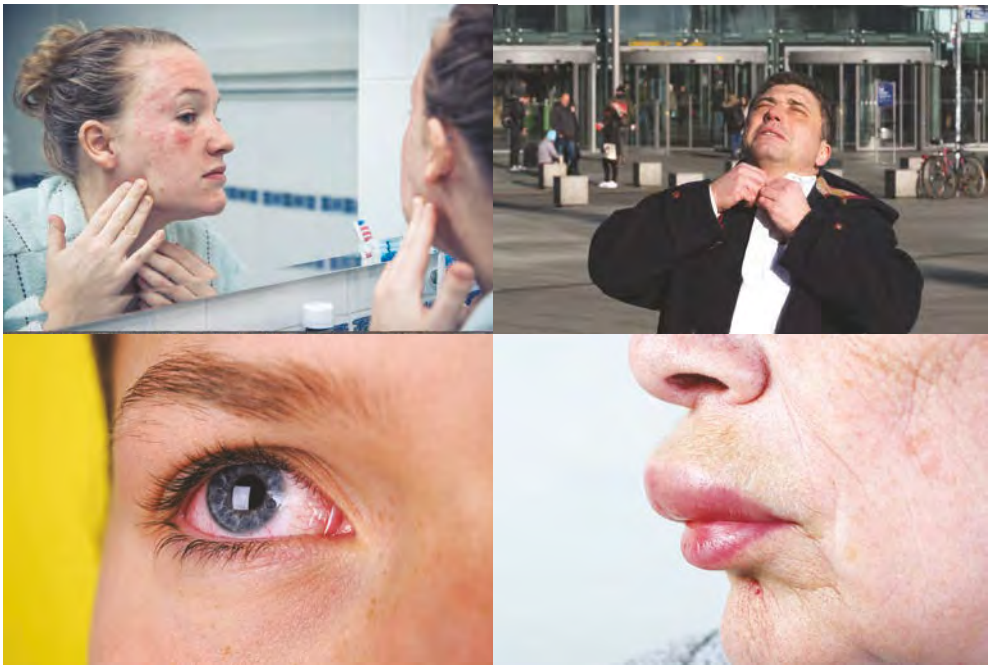


Figure 6A–14 Common allergic reactions: rash, breathing difficulty due to asthma or food allergy, red eyes, a swollen lip due to food allergy

While an allergic reaction is the result of the body's initiation of an immune response, allergens are not considered to be pathogens. A pathogen will elicit an immune response in all individuals (with or without symptoms), whereas an allergen affects only individuals who have a specific sensitivity to it. For example, consider food poisoning, which results from the direct ingestion of a bacterial pathogen (*Salmonella enterica*). All individuals who consume the infected food will fall ill as a result of the bacterial infection. On the other hand, of the many people who consume peanuts, only individuals who are allergic to this particular allergen will experience illness as a result.

LINK **7B** SECOND LINE OF DEFENCE

The symptoms associated with an allergic response are due to a process known as hypersensitivity. The development of hypersensitivity is a complex process that involves a variety of immune cells. These cells are explored in Sections 7B and 7D.

LINK **7D** THIRD LINE OF DEFENCE

Check-in questions – Set 2

1 Define antigen.
2 How do MHC markers differ from antigens?
3 What is the difference between MHC Class I and Class II markers?
4 Distinguish between an allergen and a pathogen.

DOC **WORKSHEET 6A–2** BLOOD ANTIGENS

VIDEO 6A–2
SKILLS: THE CONCEPT OF SELF VERSUS NON-SELF

6A SKILLS

The concept of self versus non-self

As you have read in this chapter, our immune system is programmed to initiate an immune response against any antigen that is not considered to be 'self'. The concept of self versus non-self underpins the way the immune system of any organism operates. For this reason, it is commonly assessed in multiple-choice questions in exams.

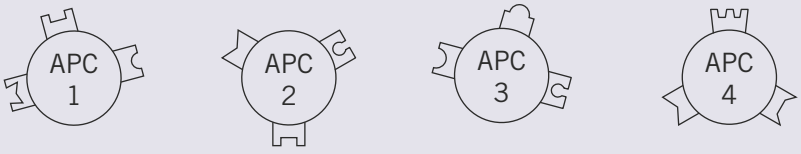
For example, the following question targets your knowledge of self-antigens.

Question:

Antigen-presenting cells (APCs) are a class of white blood cells that present antigens on their MHC Class II markers to other types of immune cells. The surface of APCs contains three main structures:

Structure 1	Identifies the cell's own self-antigens
Structure 2	Detects self-antigens present on any other type of cell
Structure 3	Recognises non-self antigens on foreign cells, e.g. pathogens

Examine the following APCs, paying particular attention to the various structures on their surface.



Which of the following statements is correct?
A Structure ⊓ is a self-antigen for APC 1.
B Structure ∪ is a self-antigen for APC 2.
C Structure ⌒ is a self-antigen for APC 3.
D Structure ⌒ is a self-antigen for APC 4.

The key information you need to answer to this question correctly is this:

Structure 1	Identifies the cell's own self-antigens

Structures 1, 2 and 3 are receptors, and these are what are shown on each APC. The information that there is an self-antigen receptor on the APC suggests that the correct statement will show a self-antigen that is complementary in shape, fitting together like jigsaw puzzle pieces. One of those pieces will be the self-antigen and the other will be the receptor that can detect it. Therefore:

- Option A is incorrect, as there are no complementary shapes for any structure on APC 1.
- Options B and D are also incorrect for the same reason.

This leaves Option C as the correct answer, due to the complementary pairing of ⌒ and ∩ on APC 3.

Section 6A questions

1 Classify each of the following diseases as infectious or non-infectious:
diabetes, cancer, AIDS, heart disease, common cold, influenza, anorexia, rabies, malaria

2 Explain why an incubation period is a common feature of a typical course of disease.

3 Distinguish between a pathogen and an antigen.

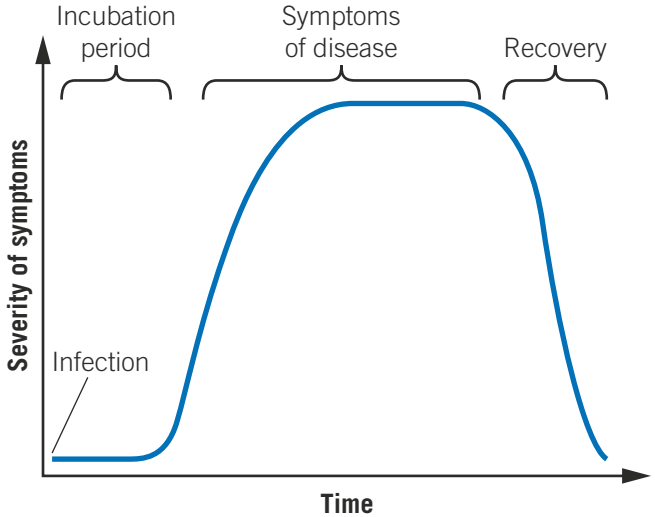
4 The Great Plague of London was the last epidemic of bubonic plague. Its mortality rate was significantly increased due to the transmission of the disease by flea-infested rats.
   a How does a pathogen-carrying vector differ from a carrier of disease?
   b Why was the flea, not the rat, classified as the pathogen vector?

5 Tissue typing, a procedure carried out prior to an organ transplant, is used to determine the compatibility of a prospective donor and the recipient. MHC markers, also known as human leucocyte antigens (HLA) can trigger organ rejection, so for a successful transplant, these antigens must be matched as closely as possible.
   A leukaemia patient requiring a bone marrow transplant had the following HLA antigens:
   A02 A03 B08 B40 C03 C07 DR07 DR15 DP10 DP11 DQ06 DQ08
   The patient's mother was tissue typed and found to have the following HLA antigens:
   A05 A06 B08 B40 C07 C09 DR07 DR21 DP10 DP12 DQ02 DQ06
   a Would you recommend the patient's mother as a suitable bone marrow donor for the leukaemia patient? Explain.
   b Give an example of the HLA antigens that would be present in the tissue of a more suitable donor. Explain your answer.

6 Kamal has adopted a dog from the local shelter. After living with his new dog for a week, Kamal notices that his eyes are becoming increasingly itchy.
   a What condition is Kamal most likely suffering from, and what name would be given to the dog's hair?
   b Explain the cause of this condition.
   c What other symptoms would need to be present for a doctor to confidently diagnose Kamal?

7 Using your knowledge of protein synthesis, explain why all MHC markers are unique to the individual. In your answer, clearly distinguish between the two stages of protein synthesis.

8 Experts believe that children who attend childcare centres experience up to 51% more episodes of infection than children who are cared for at home.
   a Identify three modes of transmission that would be common in childcare settings.
   b The graph shows the typical course of infection with the common cold. Using evidence from this graph, explain how the common cold can spread so successfully from child to child.



Cellular pathogens

Study Design:
Initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens

Glossary:
Bacteria
Cellular pathogen
Cilia
Endemic
Fungi
Heterotrophic
Hyphae
Malaria
Mycelium
Protozoa
Rice blast disease
Spores (bacterial)
Terrestrial
Tetanus
Tinea

ENGAGE

Elephantiasis

It is estimated that, in subtropical regions around the world (for example, Africa, Asia and South America), as many as 120 million people suffer from a condition known as elephantiasis. As you can see in Figure 6B–1, elephantiasis results in the swelling of body parts. Dry, thickened skin is also common, and together these symptoms cause the body parts of sufferers to look somewhat 'elephant-like' in appearance, hence the disease's name.

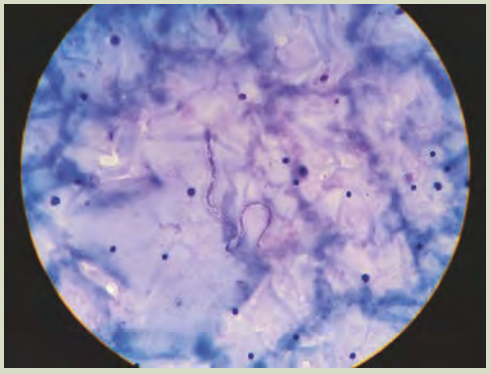


Figure 6B–1 Left: Elephantiasis symptoms caused by the parasitic worm *Wuchereria bancrofti*, which clogs the lymphatic system of the host, causing extreme swelling of body parts. Right: The worms in lymphatic tissue.

6A RECOGNISING SELF FROM NON-SELF LINK

Elephantiasis is also known as lymphatic filariasis and is caused by *Wuchereria bancrofti*, a pathogenic roundworm. A mosquito vector spreads the worms from person to person through its bite. When an infected mosquito bites an individual, the worms pass from the mosquito into the new host, where they make their way to the lymphatic system and become lodged in the lymph nodes. This results in blockages and prevents the proper circulation of lymph fluid. As lymph fluid builds up, swelling of the legs, arms and genitals occurs. The condition is not fatal.

7C THE LYMPHATIC SYSTEM LINK

8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT LINK

The main concern is that elephantiasis reduces the effectiveness of the immune system, making the sufferer susceptible to dangerous secondary infections. Prevention of disease is always preferable to treatment. Educating 'at-risk' communities to stop the transfer of disease through the use of mosquito nets and insect repellents is key to eliminating the disease.

EXPLAIN

What are cellular pathogens?

The pathogenic roundworm you have just read about is an example of a **cellular pathogen**. Cellular pathogens are living organisms that cause disease. This class of pathogen is capable of all activities associated with living independently: movement, reproduction, sensitivity, growth, respiration, excretion and nutrition. In Unit 1, you learnt that all living things are categorised as biotic and made up of at least one cell. All cells share four common factors regardless of their type (Table 6B–1) and it is these factors that allow cellular pathogens to live independently and not be reliant upon a host in order to survive.

Cellular pathogen living organism that causes disease within a host

LINK UNIT 1

Table 6B–1 The four characteristics of cells

Characteristic	Description
Genetic material	Hereditary information that contains genes that code for proteins
Cytosol	The liquid component of cells that contains water, salts and organic molecules
Ribosomes	Site of protein synthesis
Plasma membrane	Selectively permeable barrier that separates the intracellular from the extracellular environment

LINK 1A PLASMA MEMBRANE

In this section, you will learn about different classes of cellular pathogens, along with examples of diseases that they can cause.

Bacteria

Bacteria are prokaryotic, unicellular organisms. They are considered to have been the first life form on Earth, and are the most abundant and diverse of all the kingdoms of life. Bacteria come in a range of different shapes and forms. Regardless of their overall shape, all bacteria have the same basic structure.

Bacteria unicellular, prokaryotic organisms that lack membrane-bound organelles

VIDEO 6B–1 BACTERIA AS PATHOGENS

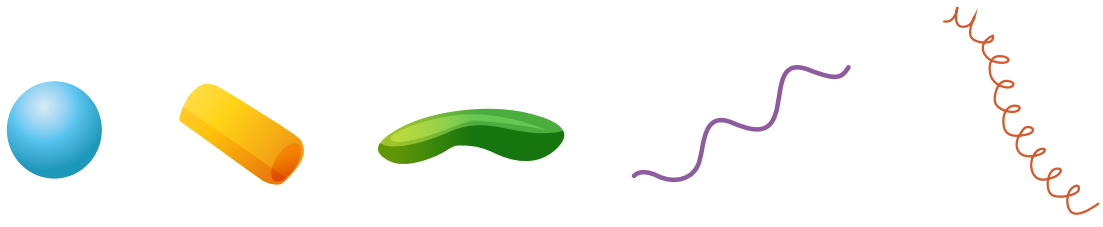


Figure 6B–2 The five main shapes of bacteria

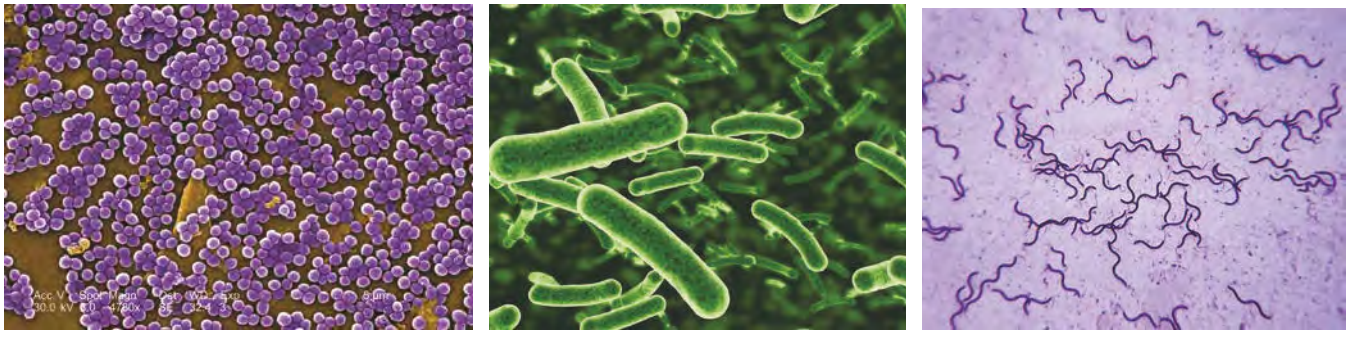


Figure 6B–3 Typical microscope views of (left to right) cocci, bacilli and spirochaete forms of bacteria

UNIT 1 LINK

Beneficial bacteria

Of the estimated millions of species of bacteria that inhabit Earth, less than 5% of these species are thought to be pathogenic. In fact, it is believed that only 10% of all the cells in and on our bodies are actually our own, with the rest being harmless and often useful bacteria that form what is referred to as our 'natural flora'. For example, our digestive system contains thousands of useful bacteria that not only assist in the breakdown of substances in food that are difficult to digest, but also occupy space within our bodies, making colonisation by harmful bacteria more difficult. They are an example of a microbiotic barrier, discussed in Section 7A.

7A FIRST LINE OF DEFENCE LINK

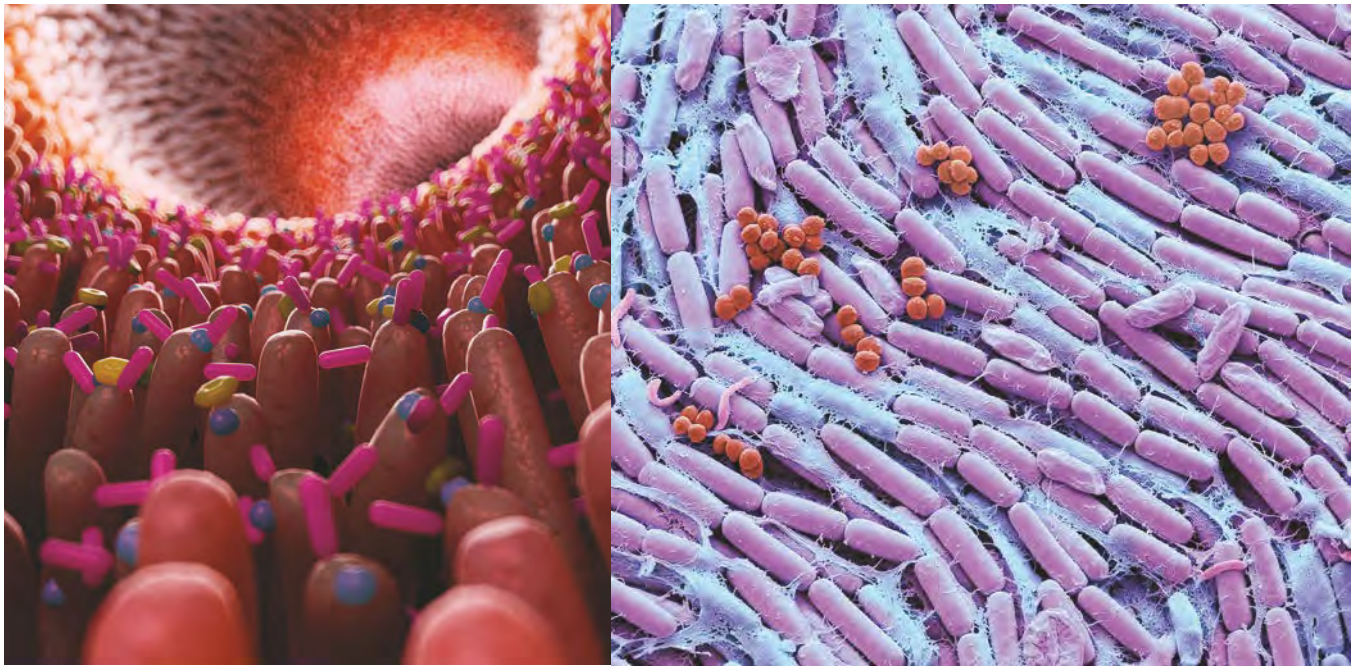


Figure 6B–4 Left: An illustration of bacteria among villi in the gut. More than 200 species of bacteria make up the natural flora of our gut, forming a mutualistic symbiotic relationship. Right: An scanning electron microscopy image of breast milk bacteria. Recent studies have shown that breast milk contains a healthy dose of good bacteria that, after birth, colonise the infant's intestine, helping digest food and training the baby's immune system to recognise the difference between bacterial allies and enemies.

Bacteria as pathogens

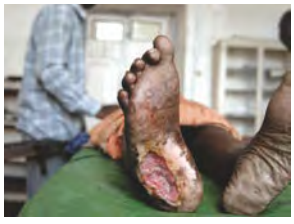
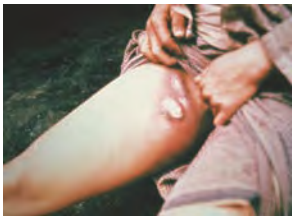
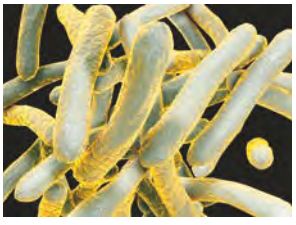
Pathogenic bacterial species are capable of causing disease in a variety of ways. Some produce toxins that build up and disrupt the normal functioning of cells, while others damage host cells directly, and some interfere with immune system cells that are working to defend the host. Most also produce and release enzymes that break down the connective tissues of the host, allowing the infection to spread faster.

WORKSHEET 6B–1 CELLULAR PATHOGENS: A CASE STUDY

As with all pathogens, bacteria must gain entry into the host to bring about the symptoms of disease. Bacteria use a variety of transmission methods to infect the host. Once inside the host's body, they are capable of dividing rapidly (once every 20 minutes) and can quickly compromise the health of the infected individual.

Table 6B–2 lists just a few examples of the many diseases caused by bacterial pathogens. Two case studies, one following Table 6B–2 and one in the interactive textbook, provide a more detailed insight into the mechanisms that bacteria can use to bring about the onset of the disease they are associated with.

Table 6B–2 Examples of diseases caused by various bacterial pathogens

Bacterial pathogen	Mode of transmission	Disease and symptoms
Salmonella enteritidis 	Ingestion of contaminated water or food	Gastroenteritis – diarrhoea, fever, chills and abdominal cramps
Streptococcus pyogenes 	Direct contact with infected respiratory droplets or carriers	Pharyngitis/tonsillitis – throat pain and difficulty swallowing
		Scarlet fever – fever, sore throat and bright red rash
		Flesh-eating disease (shown at left) – pain in skin, blisters, lesions and numbness
Vibrio cholerae 	Ingestion of contaminated water or food	Cholera – severe watery diarrhoea and dehydration
Salmonella typhi	Ingestion of contaminated water or food	Typhoid fever – fever, headache, abdominal pain, constipation or diarrhoea
Legionella pneumophilia	Direct inhalation from contaminated source of mist	Legionnaires' disease – severe form of pneumonia characterised by lung inflammation
Yersinia pestis 	Direct contact with vector (flea)	Bubonic plague – swollen lymph nodes, fever, chills, diarrhoea, vomiting and blackening of skin
Bacillus anthracis	Direct contact with sick animal or contaminated animal products such as hides or wool	Anthrax – fever, chills, shortness of breath, vomiting, drenching sweats
Mycobacterium tuberculosi 	Direct contact with infected respiratory droplets	Tuberculosis – often no symptoms. When present, symptoms include cough, weight loss, fever and night sweats

Case study 1: Tetanus (bacterial)

Pathogen: Clostridium tetani
Mode of transmission: Direct contact between contaminated environment and sufferer

Tetanus a bacterial disease characterised by muscle stiffness and spasms

Spores (bacterial) structures that bacteria form that aid in the survival of the organism under adverse environmental conditions

Tetanus is the name of a disease caused by the rod-shaped, bacterial pathogen *Clostridium tetani*. It is acquired through direct contact when **spores** of the bacterium, which are most commonly located in contaminated soil, the faeces or mouths of animals and the surface of rusty tools, enter an open skin wound. These spores are heat resistant and able to survive in the environment for as long as 40 years. When the spores enter a deep skin wound, they grow into bacteria that produce a strong toxin called tetanospasmin, which disables the motor neurons that control muscles. As the bacteria divide, more toxin is produced, leading to muscle stiffness (particularly in the jaw and neck) and painful body spasms, the major symptom of the disease. Other symptoms include difficulty swallowing, fever and sweating. In severe cases (10–20%), breathing can be compromised, inducing cardiac arrest and death.

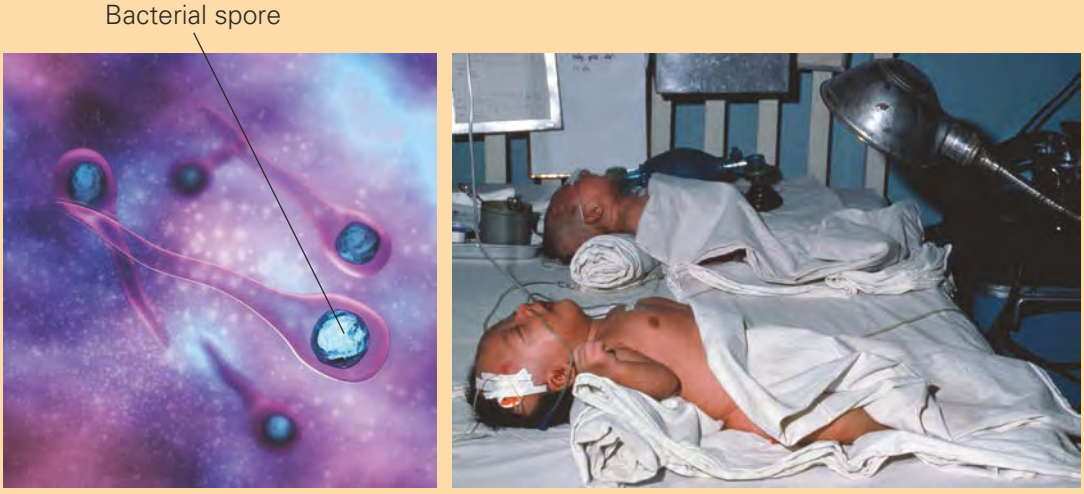


Figure 6B–5 Left: Tetanus caused by *Clostridium tetani* is the result of deep wound contamination by bacterial spores in soil. Right: Tetanus is commonly called 'lockjaw' due to the characteristic muscle stiffness associated with infection.

7E ACTIVE AND PASSIVE IMMUNITY LINK

Once the toxin has bound to the endings of motor neurons, it is impossible to remove. To completely recover from the infection, new nerve endings must grow, and this can take months. There is no cure, and treatment options centre around antibiotics to kill the *Clostridium tetani* bacteria and muscle relaxers to control the spasms. The most effective method to protect against tetanus is vaccination programs. In Victoria, the tetanus vaccine forms part of the state's immunisation program and is administered to babies from 6 weeks of age. Regular booster shots are also administered throughout childhood, and it is recommended that adults receive a booster dose every 10 years to ensure continued protection against the effect of *Clostridium tetani* toxin. You can read about the important role vaccinations play in protecting us against the effects of disease in Section 7E.

CASE STUDY 6: MENINGOCOCCAL DISEASE DOC

Superbugs

The overuse of antibiotics has led to many bacteria developing resistance. Infection with bacteria that have developed antibiotic resistance is difficult to control and treat. The term 'superbug' is used to describe any organism that has developed resistance to antibiotics. While superbugs are most commonly bacteria, fungi and other parasites are also capable of becoming resistant to antibiotics. You will learn more about how antibiotic resistance develops in microorganisms as well as the serious implications of this for the future treatment of infectious disease, in Section 9C.

LINK **9C** APPROACHES TO PATHOGENIC EVOLUTION

Check-in questions – Set 1

1 Recall the key cellular characteristics that an organism must possess to be considered 'living'.
2 With the aid of a diagram, identify the key features of a bacterial cell.
3 Name the pathogenic bacteria responsible for tetanus. Briefly explain how it brings about disease within the sufferer.

Eukaryotic pathogens

Fungi

Fungi are a group of eukaryotic organisms that include yeasts, moulds and mushrooms. Fungi can be simple unicellular organisms or highly complex and multicellular. They inhabit almost any environment but are primarily found in **terrestrial** environments that are high in moisture. There are over 100 000 known species of fungi; however, it is estimated that only about 100 of these species are capable of causing disease in humans. They are far more problematic when it comes to the health of plants, with thousands of species known to be pathogenic to plants.

Fungi
a wide variety of eukaryotic organisms that include mushrooms, mould and yeasts

Terrestrial
describes any living organism that lives or grows on land

Figure 6B–6 Kingdom Fungi includes organisms that come in a wide variety of shapes and sizes, like the porcelain fungus (top left), yeast (top right) and mould (bottom left).

Hyphae
long, branching filaments that extend off the main body of the fungus and secrete digestive enzymes

Mycelium
a collection of hyphae

Structure of fungi

The main body of a fungus has long filaments, known as **hyphae**, branching off it. Hyphae serve a variety of functions in a fungus. They contain the cytoplasm and nucleus and are collectively referred to as a **mycelium**. Importantly, hyphae are responsible for the growth of the fungus where it secretes digestive enzymes into its surroundings to help break down organic matter for nutrient absorption. Once absorbed into the hyphae, nutrients are transported to the main body of the fungus.

Figure 6B–7 Left: the fungal mycelium branches off the main fungal body and grows through the soil in search of nutrients. Right: fungal spores ready to be released and spread.

Fungi reproduce by spreading microscopic spores in a similar manner to the way in which plants produce seeds. These tiny spores form on special hyphae and are so lightweight that they are easily picked up by air currents and dispersed by the wind, where they are deposited into new areas, and then grow.

Figure 6B–8 Fungi have a great variety of so-called fruiting bodies, which produce and release spores.

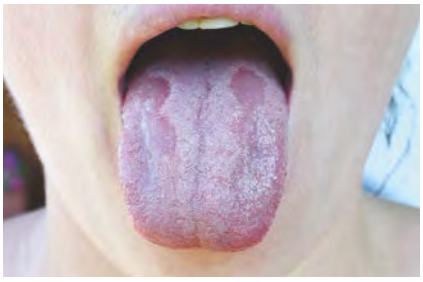
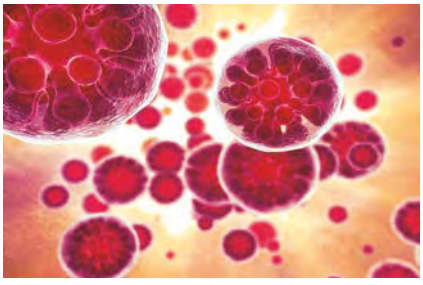
Fungi as pathogens

LINK 7B SECOND LINE OF DEFENCE

Most fungal infections are superficial, meaning they affect the external surfaces of individuals. A common cause of fungal disease in animals stems from the digestive enzymes released from the hyphae. Direct contact with these enzymes on the skin can cause irritation resulting in inflammation. Alternatively, symptoms of fungal disease may be the result of spore inhalation into the lungs or implantation into the skin.

In plants, it is mainly the parasitic nature of fungi that results in plant death. The fungal hyphae or spores make their way into the plant, where they live on the resources that the plant needs to survive. As the fungus grows, it causes the plant stress, resulting in the plant's eventual death. Some fungi penetrate the plant at the root hairs in the soil, blocking the plant's access to water, causing it to wilt and die.

Table 6B–3 Examples of diseases caused by fungi

Fungal pathogen	Mode of transmission	Disease and symptoms
In humans		
Candida (yeast) 	Direct contact with spores or infected source	Thrush – itching and inflammation at infected site (skin, nails, genitals or mouth) The image at left shows oral thrush on the tongue
Histoplasma capsulatum 	Direct inhalation of spores	Histoplasmosis – fever, dry cough and chest pain
In plants		
Diplocarpon rosae 	Direct contact with spores	Black spot – black, grey or brown spots on leaves of rose plants The image at left shows a rose plant infected with black spot disease

Case study 2: Tinea (fungal)

Pathogen: Three different species of fungi (*Trichophyton, Microsporum, Epidermophyton*)
Mode of transmission: Direct contact with infected host or fungal spores

Tinea
a common fungal infection that results in a red, flaky rash in the area of the body that is affected

Tinea is a common and contagious fungal infection that presents as a red, flaky rash. It can affect many areas of the body and is named according to the region of the body that is infected:

- athlete's foot – infection of the feet
- ringworm – infection of the body or scalp
- jock itch – infection of the groin.

In most cases, tinea is mild and no cause for concern. As the fungus that causes the infection thrives in moist, warm environments, it most commonly infects individuals who spend a lot of time in communal change rooms and showers, like people who play a lot of sport (hence the name, athlete's foot).

It is easily treated with anti-fungal cream or, in more persistent cases, prescription tablets. Good personal hygiene, drying susceptible areas thoroughly and wearing thongs in communal change rooms and showers will help to prevent infection.

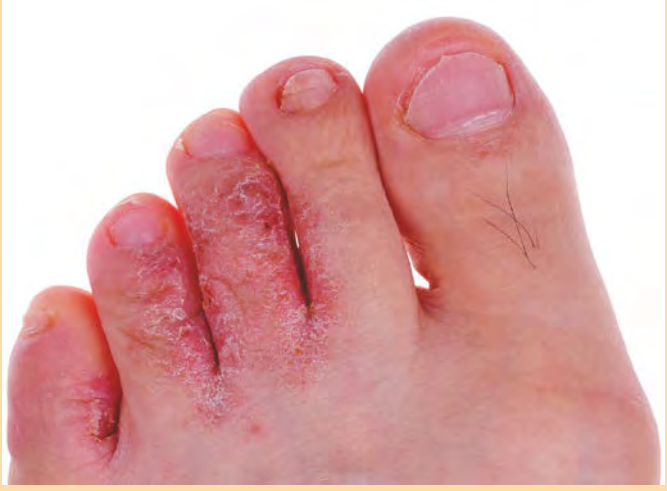


Figure 6B–9 Close-up view of a human foot infected by the athlete's foot fungus.

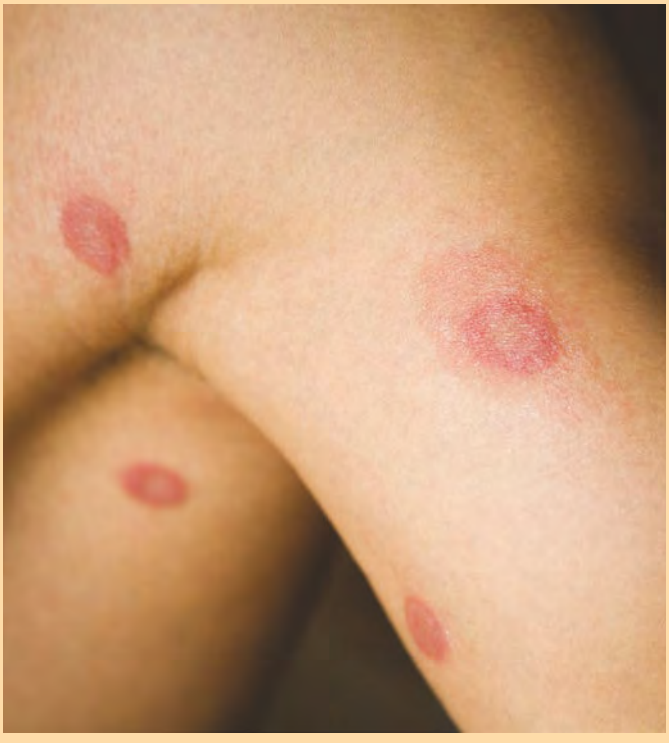


Figure 6B–10 Multiple ringworm infections characterised by ring-shaped scaly patches on the skin

Case study 3: Rice blast (fungal plant disease)

Pathogen: Magnaporthe oryzae
Mode of transmission: Direct contact with spores

Rice blast disease is a fungal infection of rice that occurs in about 80 countries around the world. The agricultural impact is devastating – an annual loss of 10–30% of the global rice harvest, enough to feed 60 million people, and costing the rice industry approximately $66 billion. For this reason, it is considered the most serious disease of rice worldwide.

Rice blast disease a fungal infection of rice that results in characteristic lesions and spots throughout the plant's shoot system

Figure 6B–11 Rice blast disease on the leaves of a rice plant

The pathogenic fungus can infect the rice plant at any stage of its growth and affects all areas except the roots. Common symptoms of infection are characteristic lesions or spots that appear on the diseased areas of the plant. The fungal spores undergo a series of developmental stages that enable them to break through the plant cell walls. From there, invasive hyphae develop and spread throughout the host cells, ultimately resulting in complete infection of the plant and total crop failure.

Figure 6B–12 Rice blast disease in the stem nodes

Effective fungicide protectants have been difficult to develop because of the multi-cycle developmental stages of the fungus. Cost and damage to ecosystems have also made protective measures against rice blast disease challenging. With recent evidence suggesting that the fungal pathogen is now infecting wheat crops, scientists are in a race against time to implement effective protective mechanisms.

Check-in questions – Set 2

1 Describe the type of environment that fungi are primarily found in.
2 What are hyphae and how are they responsible for the onset of fungal disease?
3 Briefly explain the two main ways that fungi cause disease.

Protozoa
unicellular, eukaryotic organisms that belong to the kingdom Protista; singular *protozoan*

Heterotrophic
describes any organism that obtains its nutrients by feeding on organic matter

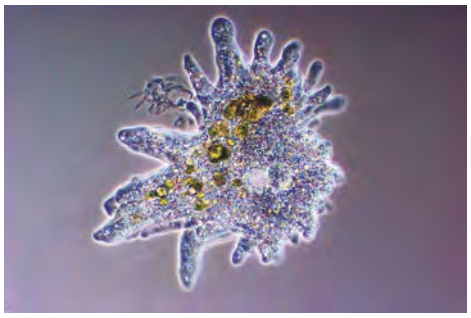
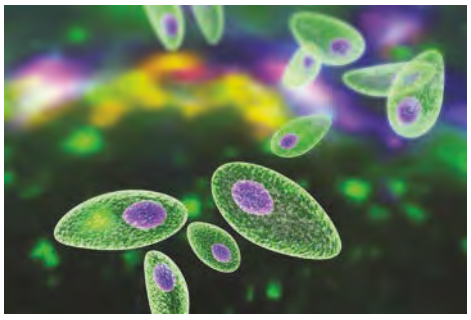
Cilia
short microtubule projections from a cell that move to provide motility (movement of the cell) or movement of fluid

Protozoa – unicellular parasites

Protozoa are unicellular, eukaryotic organisms that belong to the protist kingdom. They are **heterotrophic** and most range in size from 10 to 50 micrometres, although some are larger and visible to the naked eye. They have a wide variety of shapes and sizes, and favour moist environments such as waterways (fresh or salt) and soil. Like bacteria, some species are part of the natural flora within animals. Protozoa are classified into four groups, mainly according to how they move. They usually have features such as **cilia** or a flagellum, which allow them to be highly mobile.

Examples of each type of protozoan group are shown in Table 6B–4.

Table 6B–4 Classification of protozoa

Microscope view	Protozoan group name and key features
	**Amoeboid** Clear and jelly-like in appearance. Capable of changing shape by extending its cytoplasm into feet-like projections known as pseudopods. This feature assists in the movement of the organism.
	**Flagellates** Have a flagellum for locomotion. A whip-like motion of the flagellum propels the organism through its environment.
	**Ciliated** Body surface is covered in cilia. By beating in unison, the cilia help provide locomotion as well as feeding.
	**Sporozoans** Not mobile. Pathogenic varieties produce enzymes to help with host tissue entry, making them effective parasites.

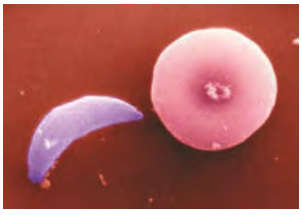
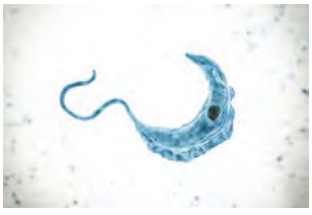
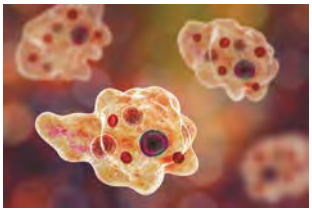
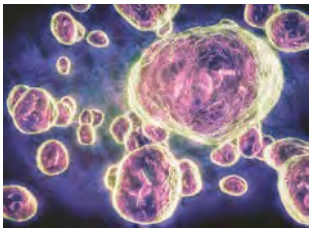
There are approximately 65 000 known species of protozoa, but fewer than 24 of these species are considered pathogenic. Despite this, they infect hundreds of millions of people worldwide annually and, with little in the way of effective drugs or vaccines against them, continue to be a worldwide health challenge.

Protozoa as pathogens

Like all pathogens, the success of protozoa in causing disease depends on their ability to reproduce and spread throughout the host. Pathogenic protozoa use a unique and complex reproductive strategy. In some stages of their life cycle, reproduction is sexual, and in other stages it is asexual. Overall, this complex reproductive strategy coupled with their use of multiple hosts makes the development of effective tools against the pathogen more difficult.

LINK UNIT 2

Table 6B–5 Examples of diseases caused by protozoa

Pathogenic protozoan	Group	Mode of transmission	Disease and symptoms
Toxoplasma gondii 	Sporozoan	Direct contact with cysts passed into cat faeces	Toxoplasmosis – muscle pain, fever and headaches
Trypanosoma 	Flagellate	Vector (tsetse fly)	African sleeping sickness – fever, headaches, joint pain, itching, confusion and decreased coordination
Entamoeba histolytica 	Amoeboid	Ingestion of infected food or water	Amoebic dysentery – abdominal cramp, diarrhoea, blood in faeces and fever
Pneumocystis carinii 	Sporozoan	Infected respiratory droplets	Pneumonia in individuals with compromised immune system – dry cough, wheezing, difficulty breathing

The most common modes of transmission for protozoa infection are direct ingestion of dormant cysts and bites from insect vectors. Protozoan cysts are resistant to adverse conditions and have the ability to survive stomach acid and other host defences.Infections can target many different areas of the body, initiating a wide variety of symptoms. Two case studies are provided, one on the next page and one in the Interactive Textbook, to highlight the different ways that protozoa can cause disease.

Case study 4: Malaria (*protozoan*)

Pathogen: Plasmodium
Mode of transmission: Bites of *Anopheles* mosquitoes (vector)

Malaria
a serious disease caused by the *Plasmodium* protozoan, which invades red blood cells when transmitted by mosquito vectors into the host

Malaria is a life-threatening disease caused by single-celled protozoan parasites in the *Plasmodium* genus. It is transmitted to humans through the bite of infected female *Anopheles* mosquitoes (vector). It infects over 200 million people annually (particularly young children) and results in approximately 400 000 deaths every year, with most cases occurring in Africa; however, more than 40% of the global population are at risk.

Five species of *Plasmodium* are responsible for malaria in humans, but two of these species – *P. vivax* and *P. falciparum* – pose a greater threat. Once bitten by an infected mosquito, sufferers will experience symptoms within 10–15 days. Early symptoms include fever, headache and chills. More severe symptoms include seizures, coma, anaemia, jaundice and enlargement of the liver and spleen. Severe symptoms require immediate medical attention; if left untreated, death will occur in a matter of days.

Malaria life cycle

The Plasmodium species responsible for malarial infection operate across two hosts according to their life cycle stage. Part of the life cycle occurs in the female *Anopheles* mosquito, with the remainder occurring in a human host. This cyclic nature of infection is outlined below. The stages are illustrated in Figure 6B–14.

Mosquito stage:

1. When a female *Anopheles* mosquito is ready to develop eggs, she seeks a blood meal.
2. When she feeds on an infected source, *Plasmodium* gametocytes (pre-gametes) are ingested.
3. The gametocytes fuse in the mosquito's gut.
4. After 10–18 days, a new form of the parasite, known as a sporozoite, moves to the mosquito's salivary glands.
5. When the *Anopheles* mosquito feeds again, the sporozoites move into the new host (human), where they travel to the liver and begin the next phase of the cycle.

Human liver stage:

6. Sporozoites infect liver cells and mature into the next developmental stage, known as schizonts.
7. The schizonts rupture and release merozoites, which infect red blood cells.

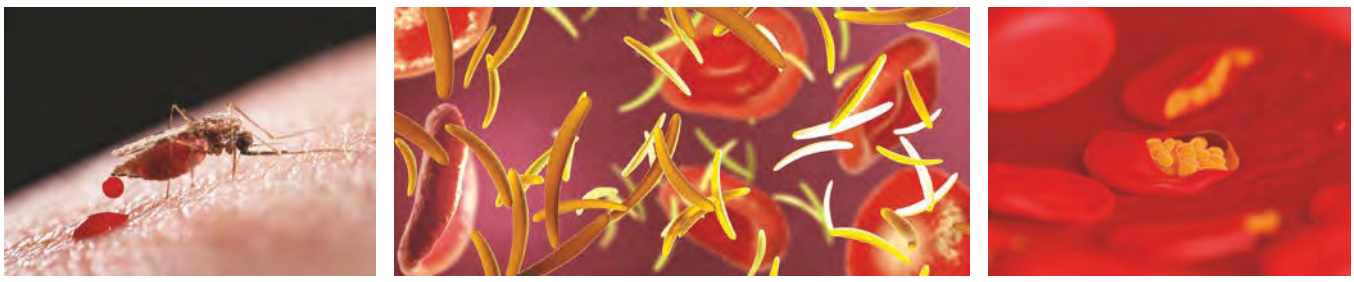


Figure 6B–13 Left: Infected female *Anopheles* mosquitoes transfer the *Plasmodium* parasite to the host. Middle: In the host's bloodstream, the parasite (yellow shapes) makes its way to the liver. Right: The parasite infects and bursts red blood cells.

Human blood stage:

8 The parasite continues to reproduce asexually, producing more schizonts, which rupture, releasing more merozoites, which target and infect other red blood cells within the host.

9 Release of the merozoites and the waste they produce in the blood results in the characteristic symptoms of shaking, chills and fever. Symptoms will usually subside but then reappear with the bursting of new cells.

10 Some of the parasites develop into male and female gametocytes which, when ingested by a feeding *Anopheles* mosquito, reproduce in its gut, ready to commence the cycle of infection in a new host.

CASE STUDY 7: GIARDIA ENTERITIS

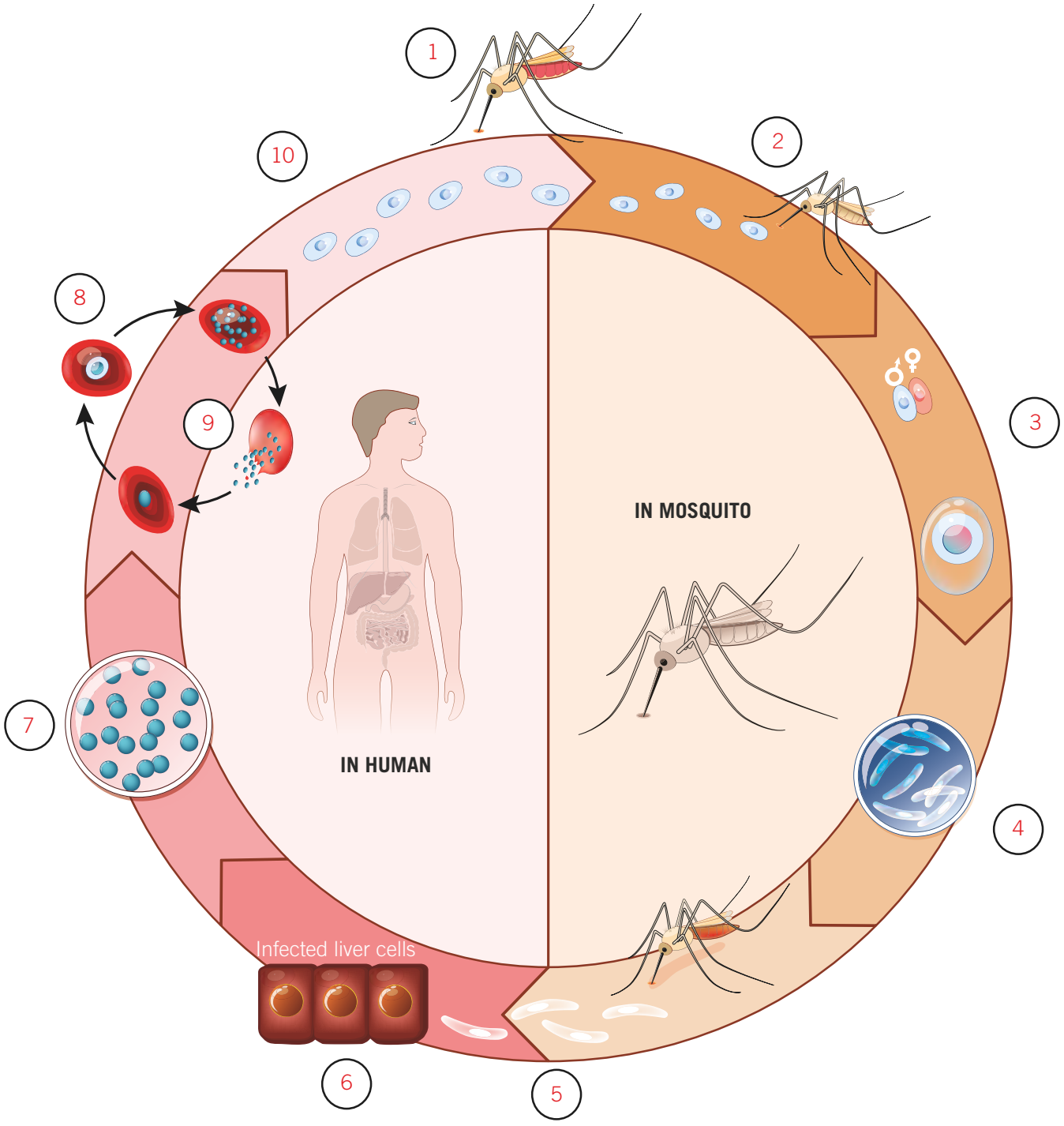


Figure 6B–14 The *Plasmodium* life cycle

Check-in questions – Set 3

VIDEO 6B–2 SKILLS: HOW TO IDENTIFY CELLULAR PATHOGENS BASED ON STRUCTURE

1 What kinds of features would you expect protozoa to possess that would make them eukaryotic?
2 Describe the key characteristics of pathogenic protozoa that make them successful at causing disease.
3 Describe *Plasmodium's* (malaria's) mode of transmission.

6B SKILLS

How to identify cellular pathogens based on structure

While you will not be required to memorise every detail of every disease caused by cellular pathogens, it is important that you have a clear understanding of each category's key structural features, so that you can identify them from an image.

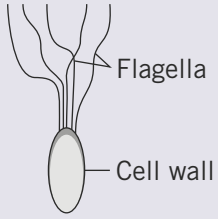
Consider the following example.

Question:

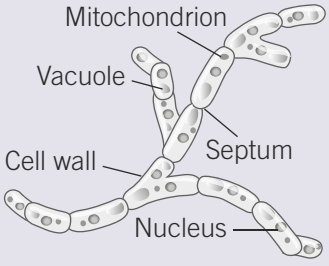
The following images represent a variety of cellular pathogens that cause disease in plants and animals.

Identify the class of cellular pathogen that each image belongs to and justify your choice.

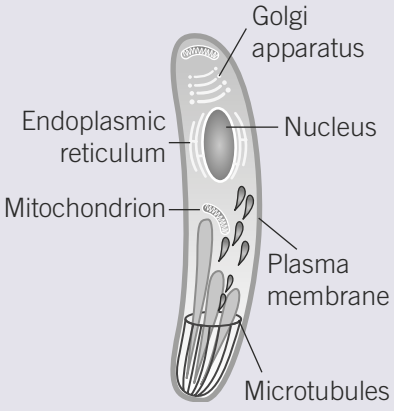
Pathogen A



Pathogen B



Pathogen C



Sample answer:

Pathogen A
- Has a cell wall, so cannot be a protozoan.
- Has flagella and is unicellular, so cannot be a fungus.
- Therefore, Pathogen A is a bacterial cell.

Pathogen B
- Contains a cell wall, so must be bacterial or fungal.
- Presence of membrane-bound organelles such as a nucleus and mitochondria means the organism is eukaryotic.
- Organism is also multicellular.
- Therefore, Pathogen B must be a fungus.

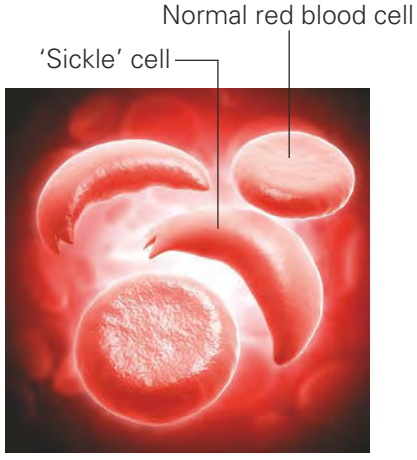
Pathogen C
- Contains membrane-bound organelles (nucleus, mitochondria, Golgi apparatus, endoplasmic reticulum), so cannot be a prokaryotic bacterial cell.
- Organism is unicellular, so cannot be a fungus.
- Therefore, Pathogen C is a protozoan.

Note that, in order to provide a clear justification of the identity of each pathogen, you must eliminate all other possibilities. Simply stating the 'Pathogen A is a bacterium because it is unicellular and contains a cell wall and flagella' is not enough to fully justify your response.

Section 6B questions

1 A deep cut from some rusty garden equipment will usually result in a trip to the doctor and a routine tetanus shot.
   a Why is this the case?
   b Tetanus is an unusual type of vaccine-preventable disease in that it is classified as non-communicable. What does this mean and why does it apply to tetanus?

2 Sickle cell anaemia is a condition that results in malformed red blood cells shaped like a crescent moon or 'sickle'.



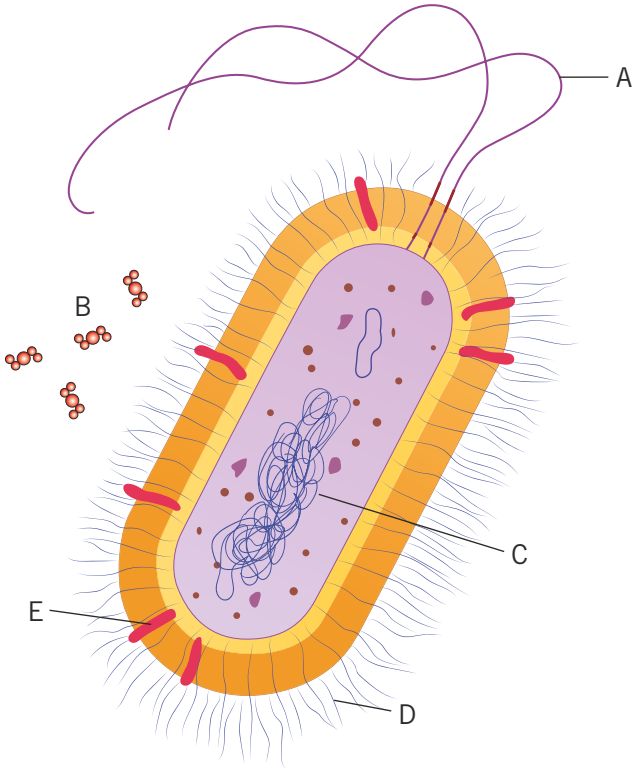
Research has shown that individuals who carry the sickle cell trait are highly protected against malaria. In fact, the mutation that results in the sickle cell trait is unusually prevalent where malaria is **endemic**.
Why do you think individuals with the sickle cell trait would be less susceptible to malaria?

Endemic the usual area where an organism is found

3 Explain why the fungal condition tinea is commonly referred to as athlete's foot.

4 In a bid to reduce the hundreds of thousands of deaths that malaria causes each year, scientists working for a biotechnology company have created a genetically modified (GM) adult male mosquito. Each male mosquito carries a bacterial gene that causes the death of its offspring before they become adults. The company is currently awaiting approval to release millions of these GM mosquitoes into areas where malaria is endemic.
   a Why have scientists chosen to genetically engineer and release millions of male mosquitoes instead of females?
   b Explain how scientists are hoping to reduce the onset of malaria.
   c Identify other actions that people living in malaria-infected areas could take to reduce their risk of contracting the disease.
   d Is the GM adult male mosquito an example of a GMO, a TMO or both? Explain.
   e Use your knowledge of gene technology techniques to explain how one of these techniques would have been used in the creation of the GM mosquito.

5 The diagram at right illustrates a typical bacterial pathogen.



- **a** Identify structures A–D and explain how these features assist bacteria in causing disease.
- **b** Structure E is a surface protein. In a host organism, what would this type of protein be referred to as?
- **c** Why is this type of surface protein a vital component of an effective immune response?

6 Fungal infections that occur deep inside the body and affect internal organs such as the lungs, lymph nodes and heart are referred to as systemic infections. Infections often start in the lungs and then spread to other parts of the body.

- **a** Explain the most likely mode of transmission that causes a systemic infection in the lungs.
- **b** Which groups of people are at greater risk of becoming infected with this type of fungal infection, and why?

7 Tapeworms are a class of parasitic worm that live in the intestines of some animals. They have been known to grow to 25 metres and survive within a host for almost 30 years.

- **a** Explain what is meant by the term parasitic.
- **b** Explain how the tapeworm meets the requirements of a parasite.
- **c** Tapeworms have no digestive system and no means of locomotion. Explain why these features are not necessary for survival of the tapeworm.

8 With the rise of antibiotic-resistant 'superbugs', scientists have been looking for alternative methods of treatment. Honey has been known to kill bacteria when placed over an infected area. A group of scientists were interested in testing the effectiveness of honey in overcoming infection. They recruited 100 participants who had all recently presented to a GP for treatment of a mildly infected cut. The details of the study are outlined below.

	Group A	Group B
Experimental method	Were told to treat the infected area with warm salty water twice a day. Treatment was to continue for 2 weeks or until cut had healed.	Were told to apply a thin layer of honey to the infected area twice a day. Treatment was to continue for 2 weeks or until cut had healed.
Results	Average healing time was 7–10 days.	Average healing time was 5–8 days.

- **a** Write a hypothesis for this study.
- **b** What conclusion can be drawn from the results of this study?
- **c** What are two factors in the experimental design that scientists would have to control?
- **d** How could the study be improved?

Non-cellular pathogens

Study Design:
Initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens and allergens

Glossary:

Bacteriophage	Plasmodesmata
Capsid	Prion
Dormant	PrP^{c}
Epidemiology	PrP^{Sc}
Haemagglutinin	Viral envelope
Neuraminidase	Virion
Non-cellular pathogen	Virus

ENGAGE

HIV

On 7 November 1991, prominent LA Laker, Magic Johnson, shocked the world by announcing that he had contracted HIV (human immunodeficiency virus). The diagnosis in such a prominent American sporting star was pivotal in changing the public's perception of the disease.

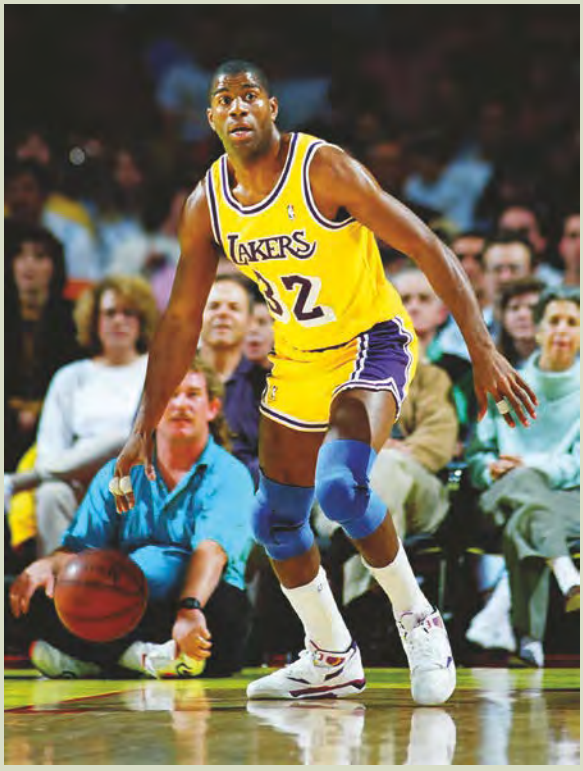


Figure 6C–1 US professional basketball player, Magic Johnson

HIV is thought to have originated in Africa, when the virus crossed the species barrier from chimpanzees into humans sometime in the 1920s. It wasn't until the 1980s that the disease became commonly reported by the media. In the 1980s and 1990s, the disease was reported as a condition that affected mostly homosexual men or drug addicts. Transmission of HIV was not well understood, and misleading information created a damaging stigma associated with these communities.

HIV is transmitted through direct contact with infected bodily fluids (for example, blood, sperm, breast milk). Upon infection, the virus is almost entirely killed off by the host's immune system, but a small number of viral particles remain and replicate over time. If untreated, the virus gradually (over many years) destroys the person's immune cells, making them vulnerable to opportunistic infections and cancers – this stage of the disease is known as AIDS (acquired immunodeficiency syndrome). These opportunistic infections would not cause illness in a person with a healthy immune system, but in someone who has HIV that has progressed to AIDS, they are lethal, and can cause death within months.

By the end of the 1990s, the World Health Organization reported that AIDS was the fourth-largest cause of death worldwide and the number one killer in Africa. Approximately 33 million people were living with HIV, and around 14 million people had died of AIDS-related complications since the first reporting of the disease in 1981.

Today, approximately 38 million people around the world are thought to be infected with HIV. The key to living with the virus is to stop it from developing into AIDS. Thankfully, advances in technology have enabled scientists to develop drugs that effectively prevent the progression of HIV into AIDS. The bravery of Magic Johnson in announcing to the world that he was HIV-positive played a crucial role in disease education and dispelling the negative stereotypes that plagued already vulnerable communities.

6B CELLULAR PATHOGENS LINK

1A PLASMA MEMBRANE LINK

Non-cellular pathogen
a disease-causing agent that lacks cellular structures and cannot replicate outside a host cell

Virus
a non-cellular pathogen that causes disease by taking over host cell machinery to rapidly produce identical virus copies, which further infect host cells, disrupting normal cellular function

EXPLAIN

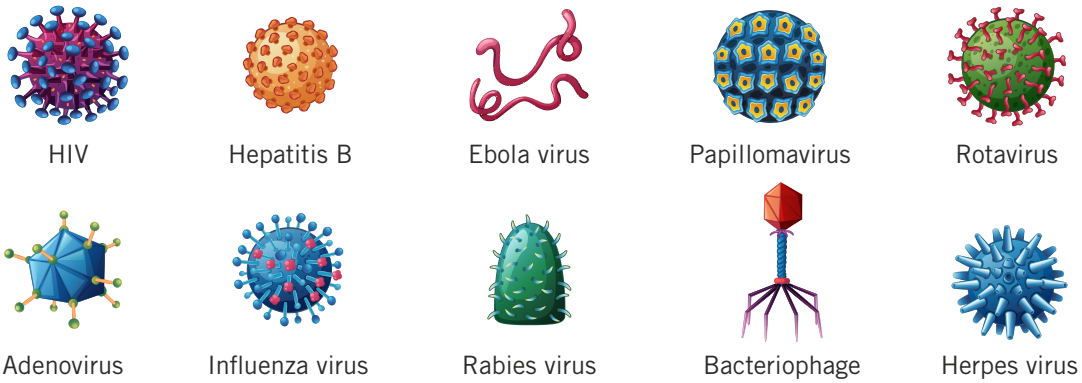
Non-cellular pathogens

In Section 6B, you read about the classification of cellular pathogens as living organisms that are made up of at least one type of cell. Recall that all cellular pathogens have four key characteristics: genetic material, cytosol, ribosomes and a plasma membrane (Table 6B–1). In this section, you will explore a different class of pathogen. **Non-cellular pathogens** are disease-causing agents that lack the cellular structures necessary for independent replication. Unlike their cellular counterparts, non-cellular pathogens are considered to be non-living. They do not possess ribosomes or a cytosol and, in order to cause disease, must gain entry into a host so they can replicate using the host cell's machinery. The onset of symptoms within the host depends on the pathogen's ability to increase its numbers within the host.

As you will see throughout this section, non-cellular pathogens have evolved to become very effective at exploiting host cells and causing disease. In fact, diseases associated with non-cellular pathogens have some of the highest mortality rates of all infectious disease.

Viruses

Viruses are non-cellular infectious agents that cause disease by forcing host cells to produce thousands of identical copies of the virus, which infect new host cells, disrupting normal cellular function. Viruses are often referred to as obligate, because they cannot survive for long outside a host cell and are therefore obligated to infect new cells as a means of survival.



VIDEO 6C–1 VIRAL STRUCTURE

Figure 6C–2 Some of the many shapes that viruses take. Because viruses can only be seen with an electron microscope, which only give grey images, the colours have been added to highlight the different parts.

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Virus structure

There are millions of types of viruses in our environment, in a wide range of shapes and sizes (Figure 6C–2). Outside of a cell, a virus is known as a **virion**, and it is about 20–250 nanometres in size, approximately 100 times smaller than a bacterial cell. All viruses contain the following two features:

- genetic material
- a protective protein shell known as a **capsid**.

Some viruses also contain a structure known as a **viral envelope** (Figure 6C–3). You can read about each of these key features in more detail below.

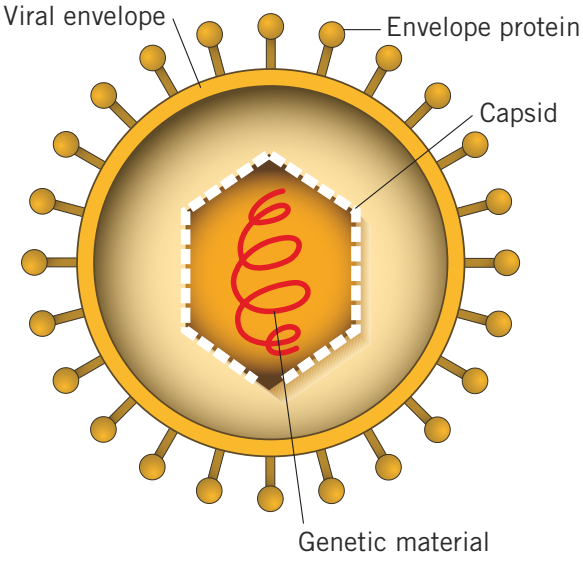

Figure 6C–3 The structure of a typical virus

Genetic material

All viruses contain genetic material that forms the viral genome. This genome is made up of nucleic acid, either DNA or RNA but never both. In other organisms, DNA is double-stranded and RNA is single-stranded. However, the viral genome does not follow these rules. Single- or double-stranded DNA, and single- or double-stranded RNA are all viral genome possibilities.

LINK 2A NUCLEIC ACIDS

The genetic code used by the viral genome is the same as that of living organisms (A, T, C, G and U), further emphasising the universal nature of genetic material. This ensures that the virus is able to take over host cell machinery and reprogram it to synthesise and assemble new viral particles.

Virion
a single virus particle existing outside a host cell

Capsid
protective protein coat that surrounds the genetic material of a virion

Viral envelope
the lipid-based, outermost layer of the capsid on some types of viruses

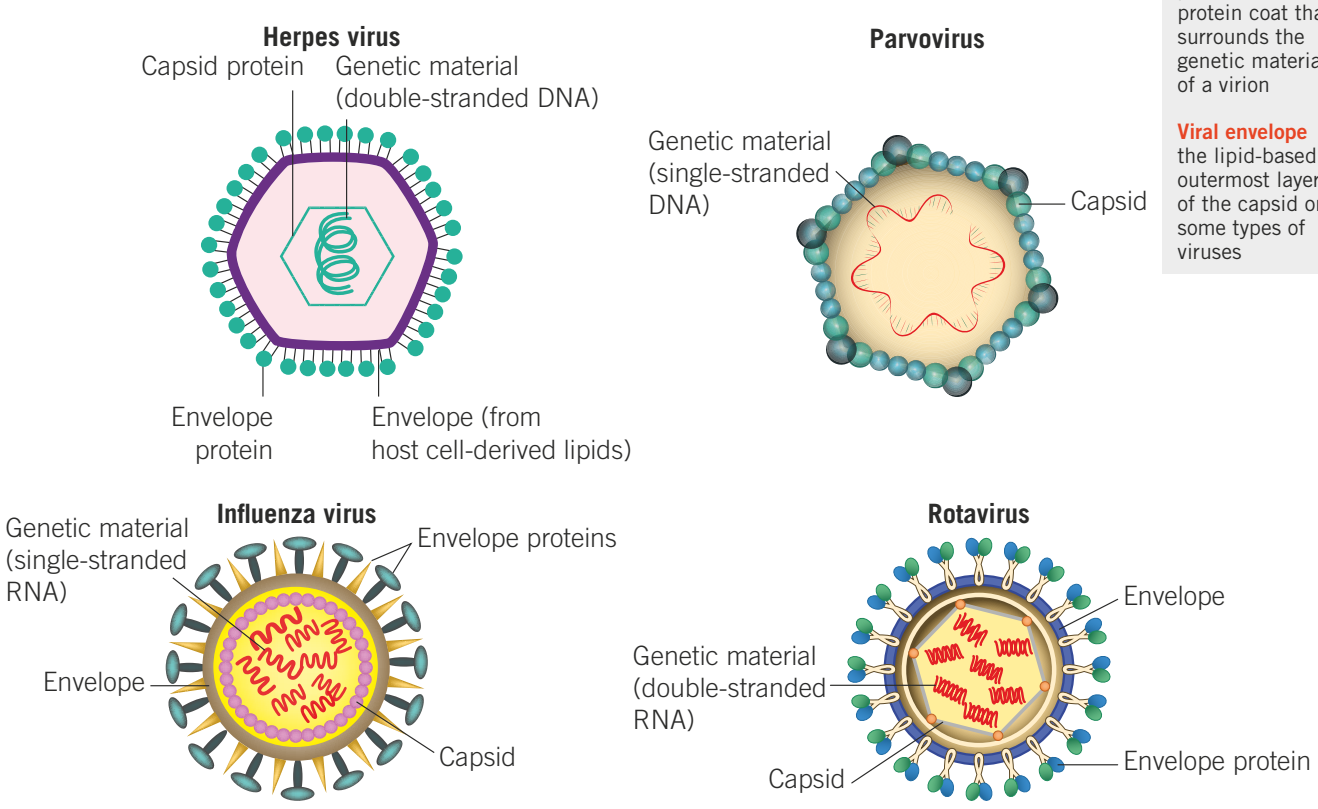

Figure 6C–4 The viral genome can be found in a variety of nucleic acid forms.

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Capsid

2C PROTEINS LINK

The viral capsid is a protein shell that surrounds the genetic material of the virus. It is found in all viruses and is made up of a series of polypeptide chains joined together to form the structure. The instructions for making these chains come from within the viral genome, so it is the virus, not the host cell, that provides the information for capsid production. The capsid provides protection for the genome and, in many cases, houses enzymes that assist the virus to enter its target host cell.

Viral envelope

1C MEMBRANE TRANSPORT LINK

Some viruses contain an external layer known as the envelope. The envelope is a lipid membrane that surrounds the capsid, encasing it and the viral genome inside. Unlike the capsid, the instructions for making the envelope do not come from within the viral genome. Instead, the virus 'borrows' a section of the host cell's membrane as it exits the cell.

9C APPROACHES TO PATHOGENIC EVOLUTION LINK

As you may have noticed in the previous images in this section, many viruses contain proteins that are embedded in the viral envelope. These are glycoproteins and are encoded by the virus itself. They play an important role in allowing the virus to bind and infect host cells. You will learn more about the specific role of these envelope proteins in Section 9C.

Note that while envelopes are a common viral feature, they are not found in every virus.

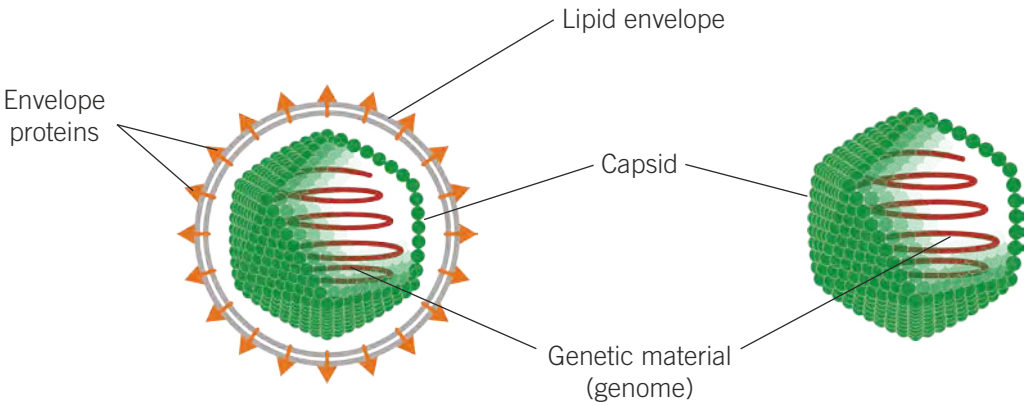
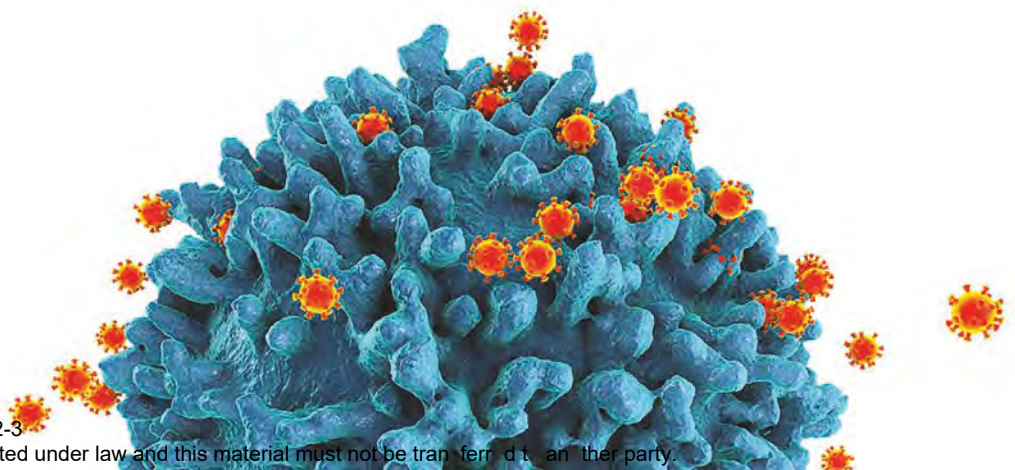


Figure 6C–5 Comparison of viral structure with an envelope (left) and without (right)

Check-in questions – Set 1

1 Define non-cellular pathogen.
2 Draw and label a typical virus.
3 Which component of viral structure would the immune system recognise as a non-self antigen?



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Mechanisms of disease

Viruses are capable of infecting all life forms and have even been found to infect each other. Virus virulence ranges from mild (for example, the common cold) to serious, with diseases such as influenza and HIV resulting in millions of deaths annually. Viruses are known causes of some types of cancer, and current research is investigating viruses as causative agents of other types of disease like multiple sclerosis and chronic fatigue syndrome. The emergence of new viral diseases and the re-emergence of old ones mean that scientists are constantly learning about the pathogenic capacity of viruses.

LINK **8A** EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT

Recall from Section 6A the many modes of transmission used by pathogens to gain entry into their host. Viruses are highly effective at breaching initial host defences and, once inside, generally result in disease through one (or more) of the following mechanisms:

LINK **6A** RECOGNISING SELF FROM NON-SELF

- cell lysis, by invading and destroying cells – when enough cells die, the organism will suffer as a result
- initiation of an immune response – this will bring on symptoms such as fever, inflammation and fatigue
- the onset of cancer – through direct manipulation of host cell DNA
- disabling of the host's immune system – through the destruction of white blood cells.

While some viruses can cause lifelong, chronic infections (for example, hepatitis viruses), others can exist harmlessly within the organism. The virus responsible for cold sores and chickenpox (*Varicella zoster*) can remain **dormant** within a host. Initial infection with *V. zoster* will result in chickenpox. Once the individual has recovered, the virus can lie dormant in nerve cells before reappearing many years later, causing a condition known as shingles.

Dormant when a virus is present within the host but is inactive and therefore not currently causing symptoms associated with the disease

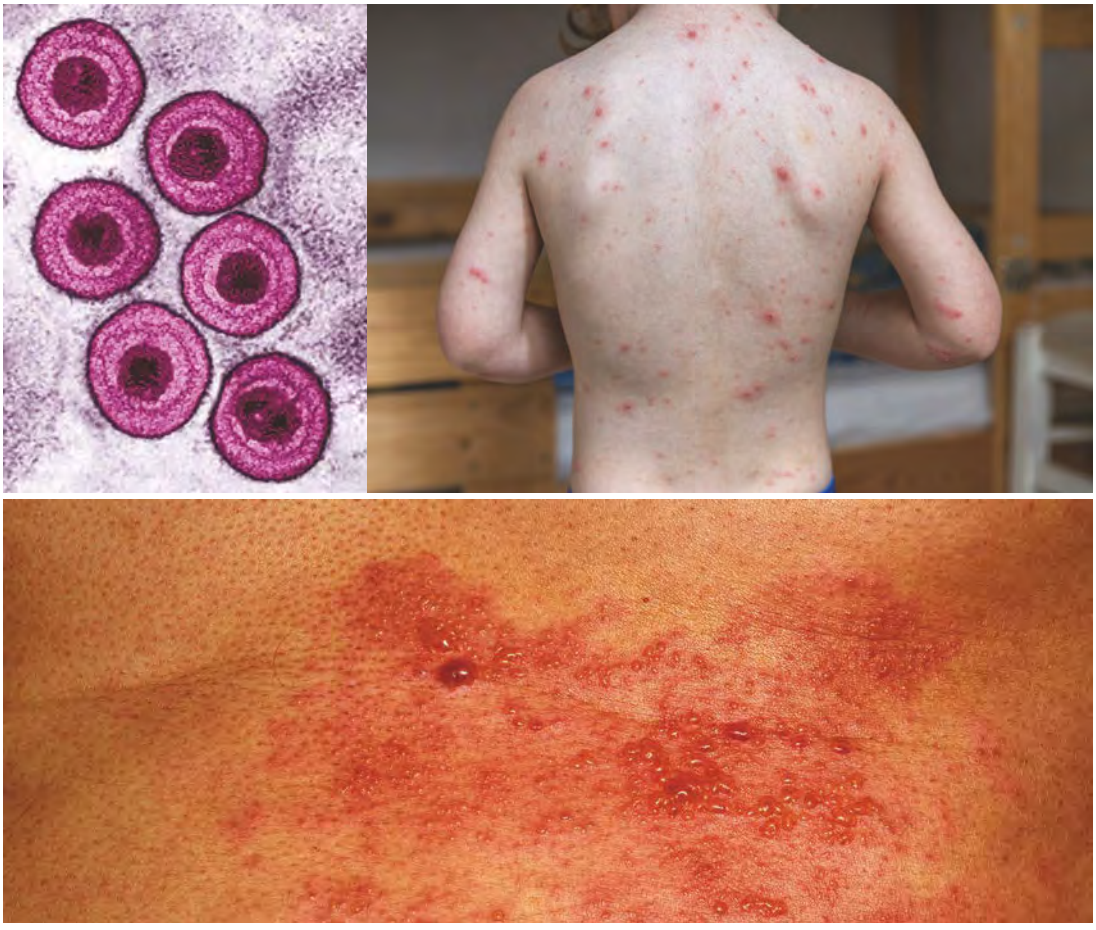

Figure 6C–6 Varicella zoster (top left), a type of herpes virus that causes chickenpox (top right) and shingles (bottom).

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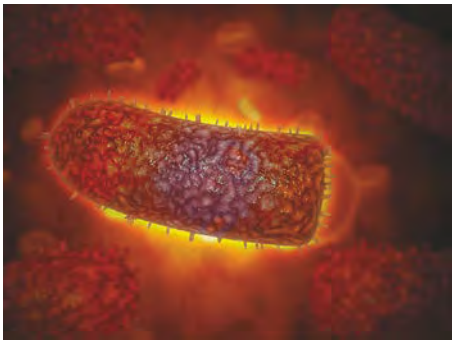
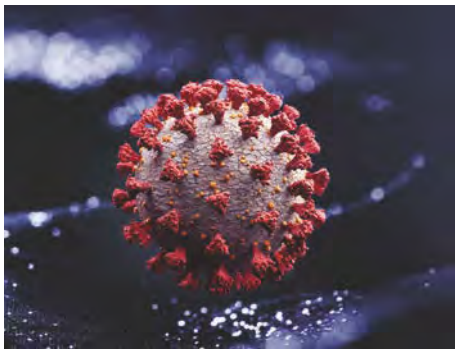
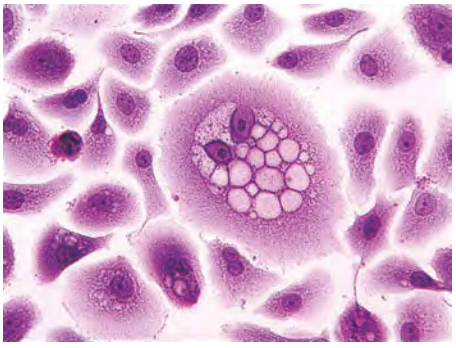
Table 6C–1 Examples of human diseases caused by viral pathogens

Disease and class of viral pathogen	Mode of transmission	Common symptoms
Rabies (Lyssavirus) 	Bite or scratch from infected animal, e.g. dog, fox, bat	Vomiting, hyperactivity, excessive salivation, hallucination and partial paralysis
SARS, COVID-19 (coronavirus) The protein projections on the surface of coronaviruses resemble crowns, hence the name 'corona', which is Latin for 'crown'. 	Inhalation of infected respiratory droplets, from the air or a contaminated surface	Fever, cough, fatigue and shortness of breath (COVID-19 only)
Common cold (Rhinovirus)	Inhalation of infected respiratory droplets, from the air or a contaminated surface	Runny nose, sneezing and congestion
Warts (Papillomavirus) (also linked to cervical cancer) Image shows an epithelial cell that has been altered by HPV. Note the irregular nucleus of the cell in the centre. 	Direct contact (usually sexual intercourse) with infected partner	Itching or warts in affected area. Can be asymptomatic
Cold sores, chickenpox, shingles, herpes (also linked to Burkitt's lymphoma) (herpes virus)	Direct, physical contact with an infected source.	Sores and blisters in affected areas
AIDS (HIV)	Direct contact with infected bodily fluids, usually through sexual intercourse or use of contaminated needle	Generally asymptomatic until infection progresses to AIDS, resulting in weight loss, fever and recurrent infections

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The viral life cycle

As you know, viruses are non-cellular pathogens that take over host cell machinery to synthesise and assemble multiple copies of themselves. While the exact details of viral life cycles differ according to the specific structure of the virus, there are five basic stages in all viral life cycles. These are shown in Figures 6C–7 and 6C–8.

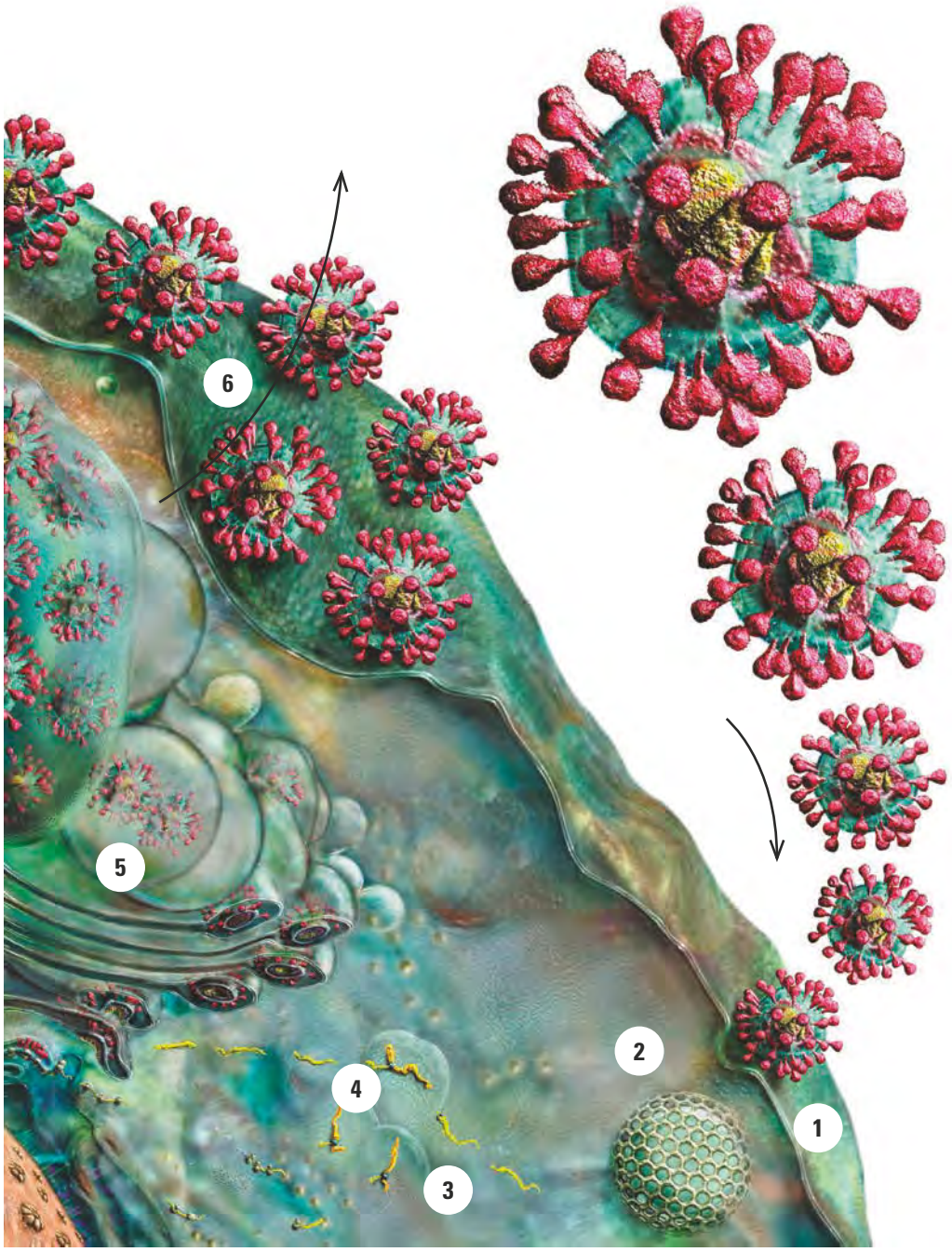

Figure 6C–7 The life cycle of a coronavirus. Coronaviruses cause the common cold, gastroenteritis, SARS (severe acute respiratory syndrome) and COVID-19. The virus (with red spikes) reproduces inside a host cell (green). It binds to the cell surface with its protein spikes (1) before entering the cell (2) and releasing its RNA (ribonucleic acid) genetic material (yellow strands, 3). This is copied by the cell's own genetic machinery (4). The new viral genetic material produces its own surface and spike proteins (5) in the host cell's ribosomes, and eventually new virions burst from the cell (6).

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Viral attachment and entry into the host cell

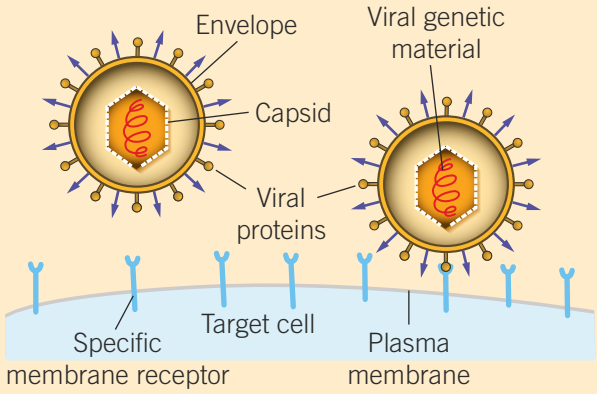
For eukaryotic host cells without a cell wall

1. Attachment

Occurs when specific viral proteins on the viral capsid or envelope detect and bind to specific receptor proteins on the membrane of the target host cell. The diagram shows how this happens in the case of a virus with an envelope.

The specific nature of the interaction between viral proteins and host cell membrane receptors determines the type of host cell a virus can infect.

Attachment must occur before the virus can enter the host cell.

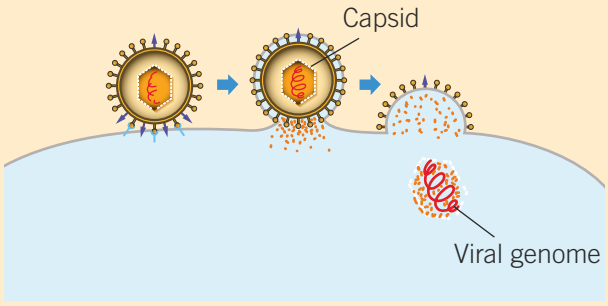


2. Entry

Upon attachment, the virus breaches the plasma membrane. There are three methods of entry.

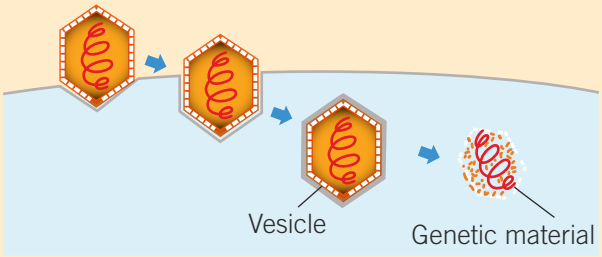
2.1 Membrane fusion

Viruses that possess a viral envelope use a secondary protein to puncture the host cell membrane. This allows the viral envelope to fuse with the the cell's plasma membrane, giving the capsid entry to the cell. The capsid breaks down by enzyme action, releasing the viral genome.



2.2 Endocytosis

Viruses without a viral envelope enter the host cell by endocytosis, inside a vesicle. Once inside the host cell, the virus exits the vesicle and gains access to the cytoplasm. The capsid breaks down by enzyme action and releases the virus's viral genome.



2.3 Genetic injection

Upon attachment, some viruses simply inject the viral genome into the cytoplasm of the cell and leave the rest of the viral structure on the external surface of the host cell.

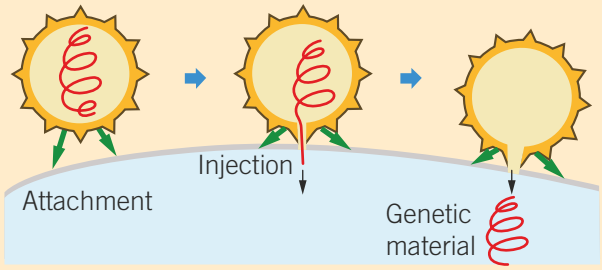


Figure 6C–8 Viral life cycle

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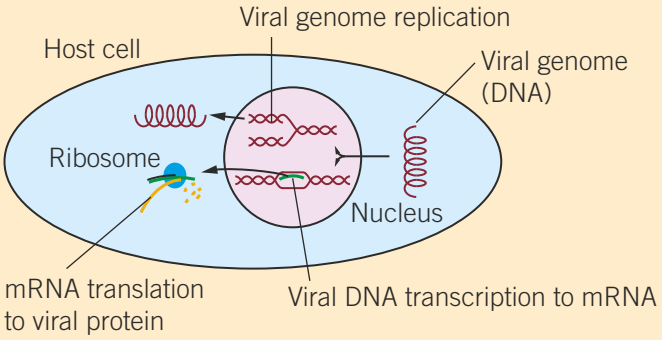
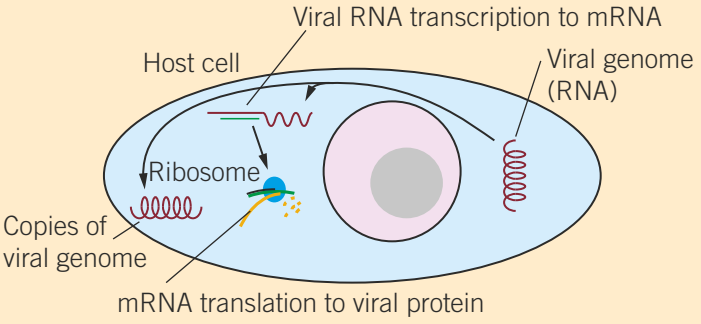
Methods of viral replication, assembly and release

3. Replication

The host cell machinery is taken over for viral genome replication and protein expression. This stage differs depending on the type of nucleic acid that makes up the viral genome.

- **DNA genome**
  Genome replication occurs primarily in the host cell's nucleus. The cell's normal transcription process is used to encode the viral genome into mRNA that will be translated into viral proteins using the cell's own ribosomes and amino acids.
- **RNA genome**
  Replication of the genome occurs predominantly in the cytoplasm of the host cell. Translation of viral RNA occurs on the host cell's ribosomes, resulting in production of viral proteins.





4. Assembly

Newly synthesised viral particles are assembled from the replicated genome and viral proteins.

Some of these viral proteins come together to build the complete capsid that usually forms around a newly replicated viral genome.

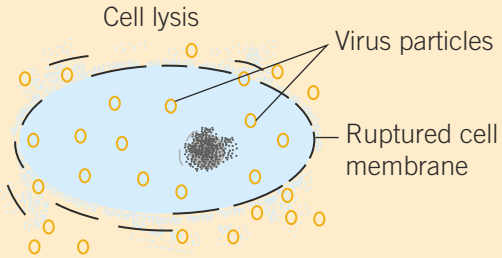
Other viral proteins embed into the envelope that forms as the virus leaves the host cell.

5. Release

Newly assembled viral particles exit the host cell in one of three ways, and when they meet a new host cell they commence the life cycle once again for further infection.

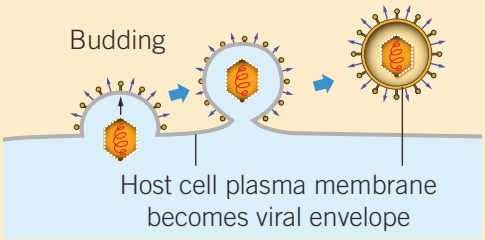
5.1 Cell lysis

The hostcell bursts, releasing all the newly created virus particles.



5.2 Budding

The virus pushes its way through the plasma membrane of the cell. This is how enveloped viruses acquire their lipid envelope – from the plasma membrane of the host cell.



5.3 Exocytosis

Viruses inside the host cell are packaged into a vesicle and transported to the plasma membrane, where they are released into the extracellular space. Here they are free to infect new host cells.

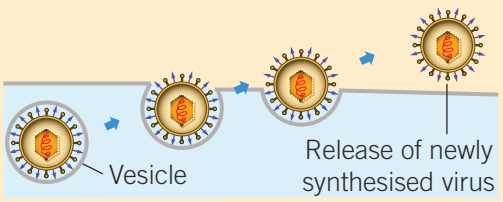


Figure 6C–8 *Continued*

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Case study 5: Influenza (viral)

Pathogen: Influenza virus
Mode of transmission: Direct inhalation of infected respiratory droplets or hand-to-mouth/eye contact from contaminated objects

The flu is a seasonal respiratory disease caused by the influenza virus. According to the WHO, it is responsible for 1 billion cases annually, with 3–5 million of these resulting in severe illness, and 250 000–500 000 deaths globally every year.

Most of us can relate to the typical symptoms of flu, including:

- fever and chills
- muscle aches
- headaches
- fatigue
- congestion (runny nose and cough).

Usually, the sufferer's immune system will fight off the infection. However, flu infection can progress to a more serious state. Flu-related complications can happen at any age, but young children, the elderly and anyone with pre-existing health ailments are more susceptible to these complications.

Haemagglutinin a glycoprotein embedded in the viral envelope of the influenza virus; plays an important role in the attachment and entry of the virus into the host cell

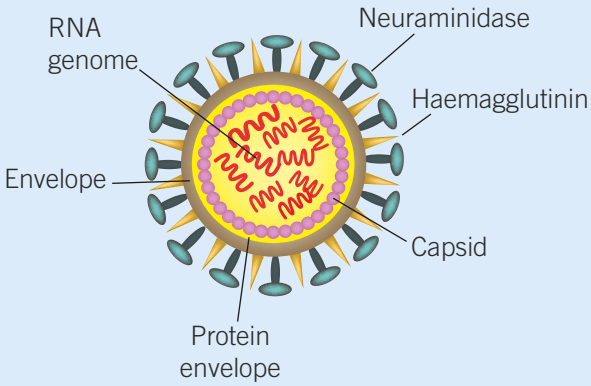

Figure 6C–9 The structure of the influenza virus

Influenza virus structure and life cycle

The influenza virus consists of a viral envelope surrounding a capsid that encloses an RNA genome. In most cases the genome consists of segments of single-stranded RNA.

Neuraminidase a glycoprotein embedded in the viral envelope of the influenza virus; plays an important role in the detachment of new viral particles from the host cell, freeing them to infect new host cells

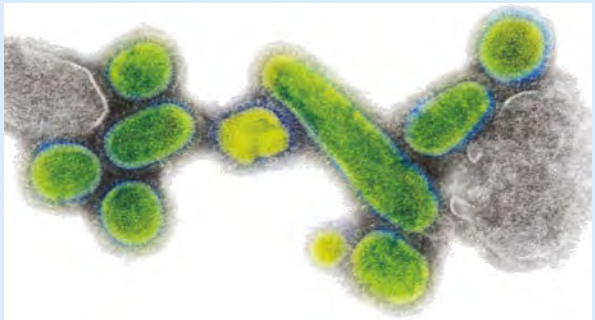

Figure 6C–10 A microscope image of influenza strain H1N1, responsible for the 1918 pandemic that killed 50 million people

Embedded into the viral envelope are two major glycoproteins, known as **haemagglutinin** (H) and **neuraminidase** (N). These glycoproteins differ between influenza strains and form the basis of subtype classification (for example, H5N1, H2N2). They are important in the 'entry' and 'release' phases of the viral life cycle. You will explore the differences between these glycoproteins in Section 9C.

9C APPROACHES TO PATHOGENIC EVOLUTION LINK

DOCUMENT 6C–1 LIFE CYCLE OF THE INFLUENZA VIRUS DOC

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The best form of defence against the influenza virus, particularly for those at high risk, is vaccination. Mutations in the viral genome result in different strains of the virus from season to season. As you read earlier, the different strains result in slight differences in the make-up of the haemagglutinin and neuraminidase glycoproteins that are embedded in the viral envelope. For this reason, an influenza vaccination does not give long-lasting protection. Each year the WHO predicts the strains of the influenza virus that are most likely to be prevalent the following year. Vaccinations that offer the best protection against these strains are developed and distributed. The ability of the influenza virus to mutate means that the threat of an influenza pandemic is ever-present. You will learn about vaccination and the effect of viral mutations in Sections 8A and 9A.

LINK 8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT

LINK 9A MUTATIONS

Check-in questions – Set 2

1 Draw a simple flow chart that shows the life cycle of a virus.
2 Distinguish between the methods used by viruses for host cell entry and for host cell exit.
3 How do the functions of the influenza glycoproteins haemagglutinin and neuraminidase differ?

WORKSHEET 6C–1 VIRAL STRUCTURE

Viruses and plants

Just like animals, plants are susceptible to viruses. Plant viruses are also obligate, intracellular parasites because, just like their animal virus counterparts, they lack the ability to replicate independently and must use host cell machinery to make copies of themselves.

Most plant viruses have RNA as their genome, and a capsid, but lack a viral envelope. The first virus discovered was tobacco mosaic virus in 1892. Plant viruses cause approximately 80 billion dollars worth of crop loss annually, but despite this, their **epidemiology** is not as well understood as that of animal viruses.

Epidemiology a branch of medicine based on the study of disease distribution and control

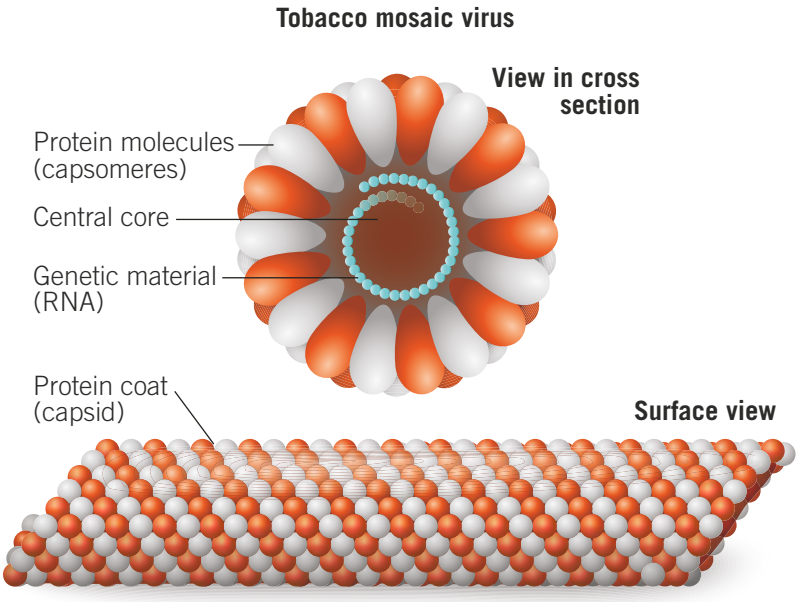

Figure 6C–11 Typical structure of a plant virus

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Plasmodesmata microscopic channels that connect the cell walls of plant cells, allowing communication and transport between the cells

As plants do not move, plant-to-plant transmission usually occurs through a vector (usually an insect). When the insect feeds on an infected plant, it can then carry the virus and transfer it to a healthy plant during a subsequent feed. As the insect feeds on sap, the virus can be transmitted into the phloem, giving it access to the plant. As plant cells contain a rigid cell wall, the virus will generally move through channels called **plasmodesmata**, allowing it to spread through the plant.

Other methods of transmission include the spread of infected pollen or seed, as well as agricultural practices that transfer infected sap of a wounded plant to a healthy one. This may be the result of tool use, manual handing or animal grazing.

Figure 6C–12 Plants infected with various viruses. From left to right: tobacco mosaic virus on a grapevine, tomato spotted wilt virus, cucumber mosaic virus, potato virus Y.

Bacteriophages

Bacteriophage a virus that specifically infects bacteria

Animals and plants are not the only organisms susceptible to viral infections. Bacteria can also be infected by a group of viruses known as **bacteriophages**. Bacteriophages are found wherever bacteria exist and are by the far the most abundant biological agent on the planet, outnumbering every other organism on Earth combined.

Like all viruses, bacteriophages contain a viral genome surrounded by a protein capsid. The genome may be DNA- or RNA-based, and single- or double-stranded. The structure of a typical bacteriophage is shown in Figure 6C–13.

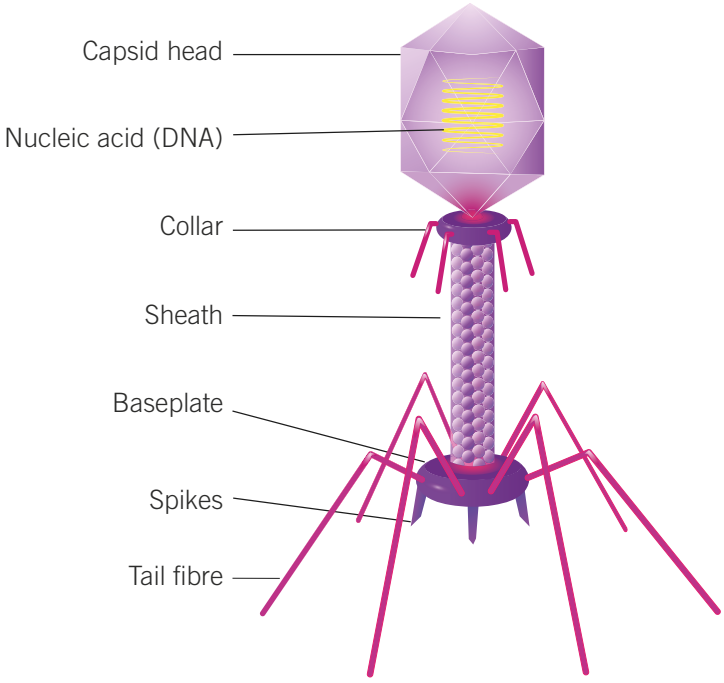


Figure 6C–13 Typical bacteriophage structure

As is the case with animal and plant viruses, bacteriophages are obligate and must enter a host cell before they can replicate. Upon attachment, the bacteriophage injects its genome into the bacterial host.

Prions

Prions are small, infectious proteins that are associated with a range of neurodegenerative diseases in humans and animals. They are unique in their ability to cause disease because, unlike other pathogens, they do not contain any form of genetic material.

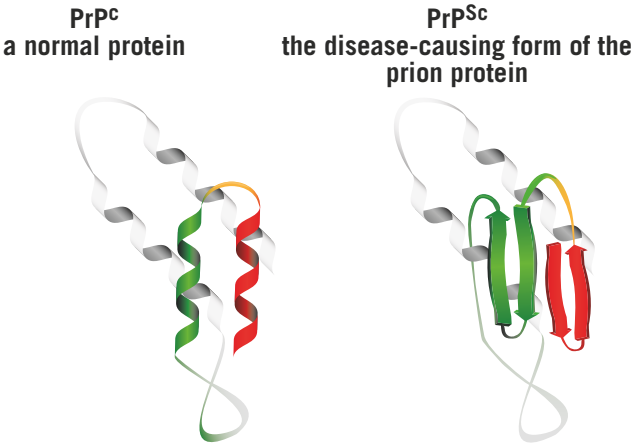


Figure 6C–14 A normal protein (left) versus the pathogenic form (right). Note the change from alpha helices to beta pleated sheets in the pathogenic form.

The protein that prions arise from exists naturally on the membrane of neurons in healthy humans and animals. In the case of prion disease, these proteins are misfolded, giving rise to a mutant shape. When a misfolded protein comes into contact with the normal form (**PrP^{C}**), it converts it into the mutated form (**PrP^{Sc}**). When enough pathogenic prions are produced, they collect together, forming plaques that damage neural tissue, affecting brain function as a consequence. The diseases associated with prions are all variants of a larger group of diseases known as transmissible spongiform encephalopathy (TSE).

The pathogenic form of the prion can arise spontaneously through mutation in the gene that codes for the normal prion protein. More commonly, the mutant form is passed on through the consumption of infected food. Mutant prions are resistant to high temperatures (they do not denature), strong enzymes and radiation. A treatment is yet to be found that is effective in destroying the prions without causing harm to the patient. For this reason, prion diseases are currently untreatable and fatal.

Prion
a pathogenic protein with a mutant structure that can trigger normal proteins to fold abnormally, resulting in disease

PrP^{C}
normal form of the protein associated with prions

PrP^{Sc}
disease-causing, mutant prion

LINK **2C** PROTEINS

LINK **9A** MUTATIONS

LINK **4A** THE ROLE OF ENZYMES

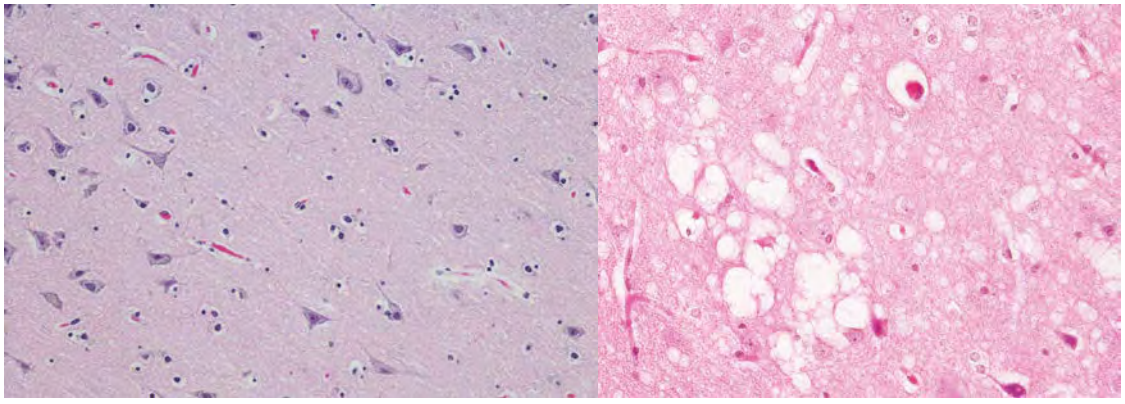


Figure 6C–15 Effect of TSE on the brain. Left: Normal brain tissue. Right: Brain tissue infected with TSE has a 'spongy' appearance with large intracellular vacuoles.

Check-in questions – Set 3

1 Identify the main characteristics of a plant virus.
2 Explain how plant viruses are most commonly transmitted.
3 How does the target cell of a bacteriophage differ from other types of viruses?
4 What is the difference between PrP^{C} and PrP^{Sc}?

WORKSHEET 6C–2 VENN DIAGRAM: CELLULAR VS NON-CELLULAR PATHOGENS

VIDEO 6C–2 SKILLS: DISTINGUISHING CELLULAR AND NON-CELLULAR PATHOGENS

6B CELLULAR PATHOGENS LINK

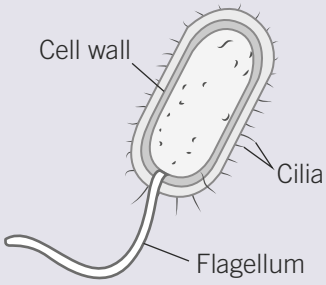
6C SKILLS

Identifying cellular from non-cellular pathogens

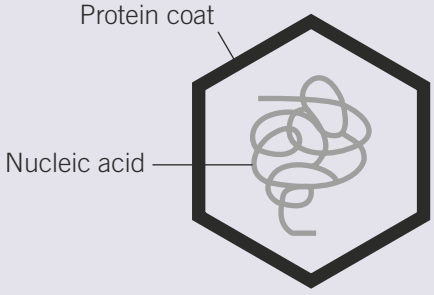
In the 6B Skills section, you learnt how to use the structural features shown in images to distinguish between different classes of cellular pathogens. Just as important is your ability to identify cellular from non-cellular pathogens using only the visual features shown in diagrams. This key skill is explored here.

The following diagrams represent a variety of pathogens capable of causing disease in plants and animals.

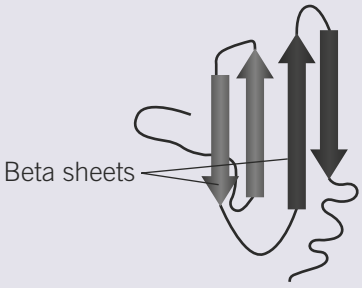
A



B



C



Classify each of the above images **A**–**C** as a cellular pathogen or a non-cellular pathogen. Justify your choice in each case.

Pathogen A

- Presence of a cell wall, flagellum and cilia indicate that this a bacterium.
- Bacteria are capable of dividing independently, which is a key trait of living organisms.
- Therefore, Pathogen A is a cellular pathogen.

Pathogen B

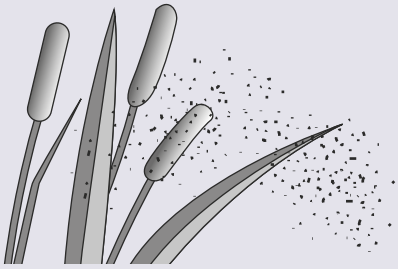
- Presence of nucleic acid surrounded by a protein coat (capsid) indicates that this is a virus.
- Viruses are not capable of replicating independently.
- Therefore, Pathogen B is a non-cellular pathogen.

Pathogen C

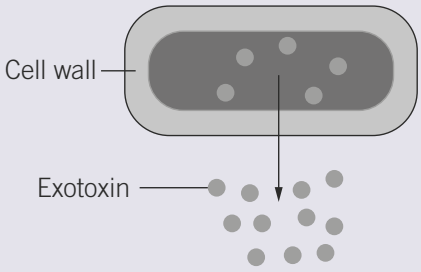
- Presence of beta sheets identifies this as a protein.
- Proteins do not replicate. They are synthesised according to instructions held in the genetic code of the organism.
- Therefore, Pathogen C is a non-cellular pathogen.

If the question had included the following two diagrams, how would you have classified these examples?

Pollen



Bacterial toxin



Pollen

- Pollen is an allergen, not a pathogen.
- Recall from Section 6A that a pathogen is a disease-causing agent and will result in the onset of disease in most hosts.
- Pollen will not result in the symptoms of hay fever in everyone, only in individuals who have a specific sensitivity to pollen.
- Therefore, pollen is neither a cellular nor a non-cellular pathogen.

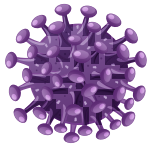
LINK 6A RECOGNISING SELF FROM NON-SELF

Bacterial toxins

- These toxins are produced by bacteria, which are an example of a cellular pathogen.
- The toxin itself, however, does not replicate independently and therefore cannot be classed as cellular.
- The toxin is therefore not considered to be a cellular pathogen. Rather, it is a method used by bacterial pathogens to cause disease.

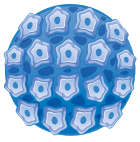
Section 6C questions

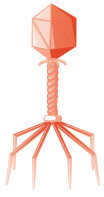
1 Examine the images of the virus examples shown here.


HIV


Rabies virus


Adenovirus


Papillomavirus


Bacteriophage

a What is the main difference between the bacteriophage and the four other viruses?

b Which part of the virus would be identified as non-self antigens by the immune system of a host?

2 Why are the pathogens discussed in this section classified as non-cellular?

3 Viruses can contain a genome made up of DNA or RNA.

a Evidence suggests that viruses with a DNA genome are able to avoid detection by the host's immune system for longer than viruses with an RNA genome. Suggest a reason for this.

b Using your knowledge of transcription and translation, explain how RNA viruses differ from DNA viruses in the way they infect host cells.

4 Why might the final step of 'release' be considered the most important step in the life cycle of a virus?

5 Compare a typical animal virus and a bacteriophage.

6 The range of possible host cells that a virus can attach to is known as the host range. In most cases, viruses show only a narrow host range due to the receptor sites on the plasma membrane of host cells. With the aid of a diagram, explain why this is the case.

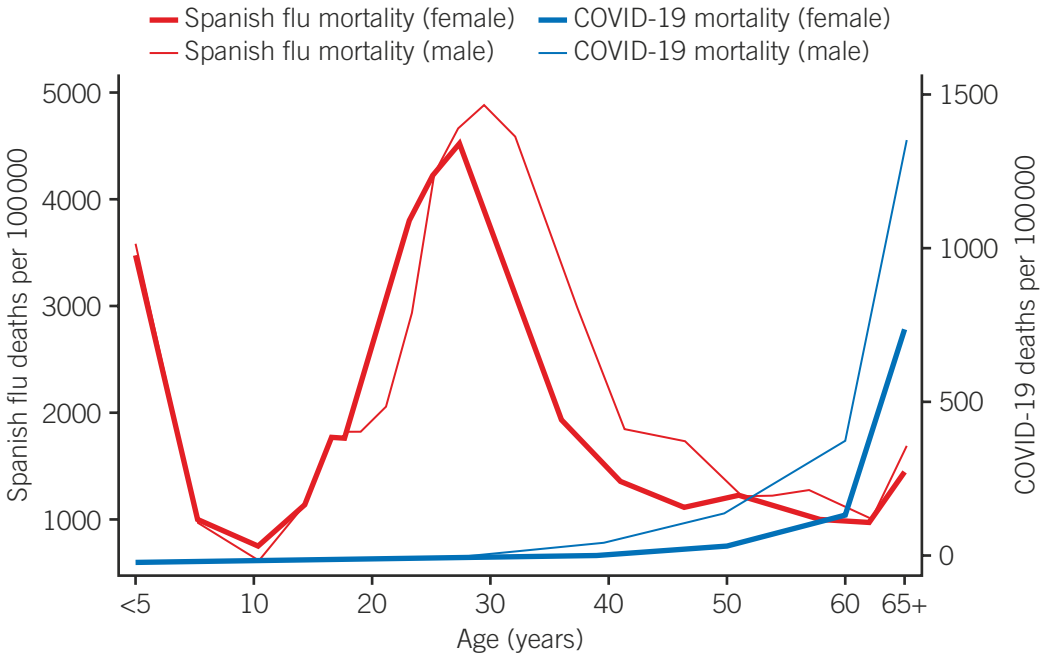
7 The outbreaks of mad cow disease in the United Kingdom during the 1980s and 1990s saw dramatic news stories of farms being evacuated and thousands of cows killed and their bodies burned. Even though it's been 20 years since the last major outbreak in the United Kingdom, the consequences of mad cow are still being felt, even in Australia.

People who lived in the United Kingdom for six months or more between 1980 and 1996 are still unable to donate bodily fluids and tissues, including blood and breast milk.

Explain why this is still the case despite the amount of time that has passed since the last outbreak.

8 Herpes viruses are known to cause cold sores, chickenpox, shingles and genital herpes. Explain how a virus is capable of causing a variety of symptoms and diseases despite belonging to the same class of viral pathogen.

9 The graph below shows the mortality rate of COVID-19 (New York City) and the Spanish flu (Philadelphia) according to age and gender. (Note that the scales for the Spanish flu and COVID-19 are different, the COVID-19 scale is enlarged three times more, for the purpose of illustrating the relative shapes of the graphs).



Use the information in the graph to answer the following questions.

a Were males or females more at risk of dying as a result of infection with either disease?

b Compare the age ranges that resulted in the highest mortality rates for both diseases.

c What strikes you as unusual about the most common mortality age of the Spanish flu?

d For each disease, describe the course of mortality across age and gender.

Chapter 6 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
6A.1	Distinguish between infectious and non-infectious disease and give examples of each	7☐
6A.2	Explain the relationship between disease, pathogen and antigen	17c☐, d☐
6A.3	Recognise that pathogens can be transmitted in a variety of ways	13☐
6A.4	Define vector and explain the role of this type of organism in the transmission of infectious disease	2☐, 14a☐
6A.5	Describe the typical course of disease	17e☐
6A.6	Discuss the nature of antigens in terms of self and non-self	6☐, 10☐, 17a☐
6A.7	Explain the role of MHC markers in an organism	17b☐
6A.8	Distinguish between MHC Class I and MHC Class II markers in terms of location and function	5☐
6A.9	Define allergen and explain how an allergen differs from a pathogen	4☐
6B.1	Explain the features of a pathogen that must be present for it to be classed as cellular	9☐, 11b☐
6B.2	Identify the key structural features of a bacterial cell	1☐, 11a☐
6B.3	Describe the ways in which bacteria can cause disease	11c☐
6B.4	Explain the key structural features of fungi	16a☐
6B.5	Describe the ways in which fungi can cause disease	16b☐
6B.6	Identify the key structural features of a protozoa and describe the ways in which they contribute to disease	14b☐
6C.1	Explain the key differences between cellular and non-cellular agents of disease	3☐, 8☐
6C.2	Describe the key structural features of a virus (nucleic acid, capsid and envelope)	1☐, 12a☐, b☐, 14b☐
6C.3	Discuss the ways in which viruses are able to cause disease	15a☐
6C.4	Explain each stage of the viral life cycle in detail (attachment, entry, replication, assembly and release)	15b☐

Success criteria – I am now able to:		Linked question
6C.5	Distinguish between the target host of a typical virus and bacteriophage	15b☐
6C.6	Identify the key structural features of a typical bacteriophage	15b☐
6C.7	Explain the difference between a typical protein and a disease-causing prion	17a☐, b☐, c☐

Multiple-choice questions

1 A true structural difference between a virus and a bacterium is that
- **A** a virus is prokaryotic and a bacterium is eukaryotic.
- **B** a virus is much smaller than a bacterium.
- **C** a virus has antigens but a bacterium does not.
- **D** a virus contains only RNA, while a bacterium contains only DNA.

2 Which of these statements best describes a vector in the transmission of infectious disease?
- **A** A vector is the process by which a disease is spread.
- **B** A vector is an agent that carries and spreads a disease.
- **C** A vector refers to the infection of a pathogen through a contaminated surface.
- **D** A vector is a pathogenic virus that is spread through ingestion.

3 Non-cellular agents capable of causing infectious disease include
- **A** prions and vectors.
- **B** bacteriophages and bacteria.
- **C** protists and pathogens.
- **D** viruses and bacteriophages.

4 Which of the following is true of allergens?
- **A** They trigger the onset of disease in the same manner as pathogens.
- **B** They are the result of an overreaction of the immune system.
- **C** A consistent level of response is produced in all affected individuals.
- **D** Their effects are never life threatening.

5 Major histocompatibility complex (MHC) Class II markers are
- **A** found only on somatic cells.
- **B** identical in all organisms.
- **C** located on the surface of antigen-presenting white blood cells.
- **D** lipid-based in structure.

6 An example of 'self' material in an adult human is
- **A** symbiotic bacteria living in the gut and aiding digestion.
- **B** adult stem cells implanted successfully from a donor program.
- **C** a healthy cell presenting a peptide on its MHC Class I marker.
- **D** virions using host cell machinery inside somatic cells.

7 Which of the following is *not* true of infectious diseases?
- **A** They can be transmitted through a range of mediums.
- **B** Typically, they begin with an incubation period, where symptoms are mild or absent.
- **C** Meningococcal, tinea, heart disease and influenza are all examples.
- **D** They can be classed as communicable, but not non-communicable.

8 Pathogens 1–4 are examples of common types. From these diagrams, it is correct to state that

A Pathogen 4 is a prokaryotic organism.

B Pathogen 3 and Pathogen 4 are both non-cellular.

C Pathogen 2 is a bacterium.

D Pathogen 1 and Pathogen 2 are not always pathogenic.

9 Most foods left out of the refrigerator will rapidly become spoiled, with growth of bacteria and fungi. However, most microorganisms will not grow on the surface of honey, which is a mixture of fructose and glucose. The most likely reason for this is that

A the sugars in honey are toxic to most microorganisms.

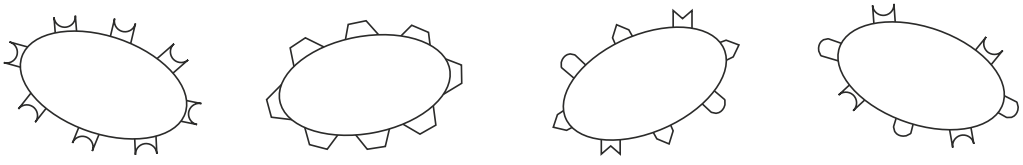
B microorganisms do not use sugars as an energy source.

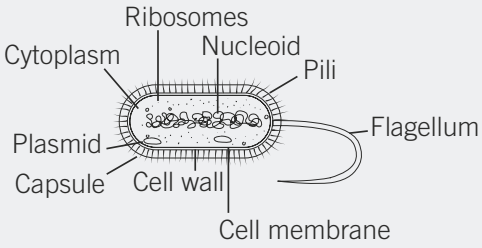
C honey is hypotonic to the cells of the microorganisms, causing them to take up water and burst.

D honey is hypertonic to the cells of the microorganisms, causing them to lose water and dehydrate.

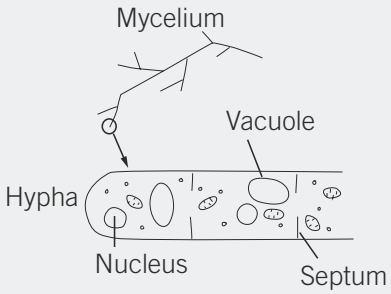
10 The diagram shows antigens that were present in a sample of tissue from a wound showing signs of a bacterial infection.

Which of the following cells would be most likely to detect the bacteria as non-self?

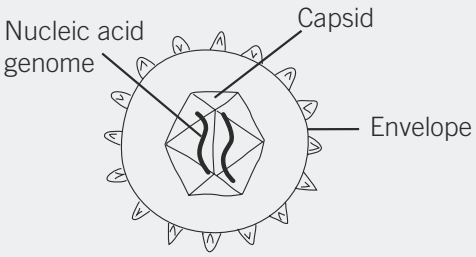




Pathogen 1



Pathogen 2



Pathogen 3

Pathogen 4

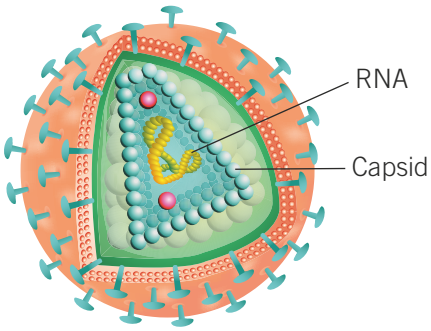
Short-answer questions

11 **a** Draw and label the structure of a typical bacterial cell. (2 marks)

b Bacterial pathogens are classed as 'cellular' pathogens. Describe the features a pathogen must have for it to be considered 'cellular'. (1 mark)

c The virulence of a bacterium is the degree to which it may cause disease. What features may a bacterium have that increase its virulence? Explain. (2 marks)

12 Pictured here is a typical virus.



a What features does this virus have in common with all other viruses? (1 mark)

b What features may differ in other types of viruses? (1 mark)

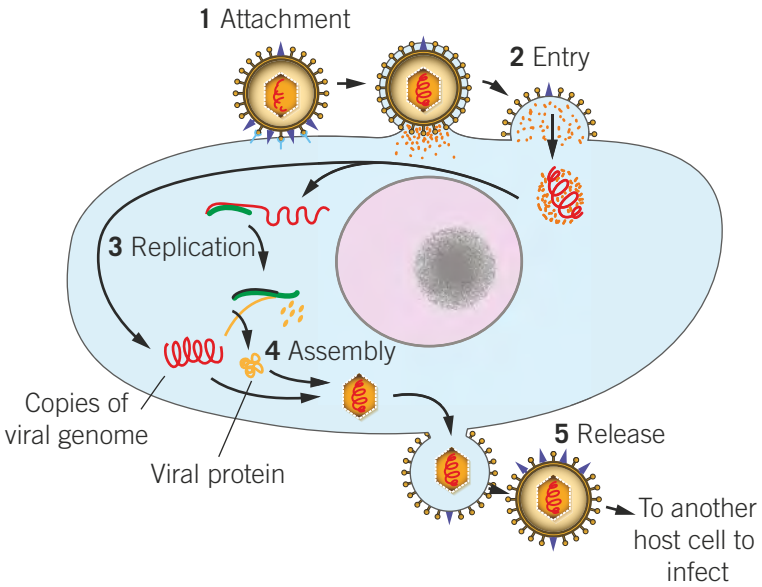
13 Identify three common modes of transmission for viral pathogens. (1 mark)

14 The Zika virus is transmitted by the *Aedes* mosquito.

a Two students were discussing the transmission of the Zika virus and both agreed that the virus is a cellular pathogen because it is transmitted through a biological organism. Do you agree with the students? Explain. (1 mark)

b Another type of infectious disease carried by mosquitoes is malaria. This is caused by a pathogen known as *Plasmodium*. How would the structure of *Plasmodium* differ from that of the pathogen responsible for Zika? (2 marks)

15 The following diagram represents the reproductive process of a virus.



a Referring to the diagram, describe how the virus reproduces. (3 marks)

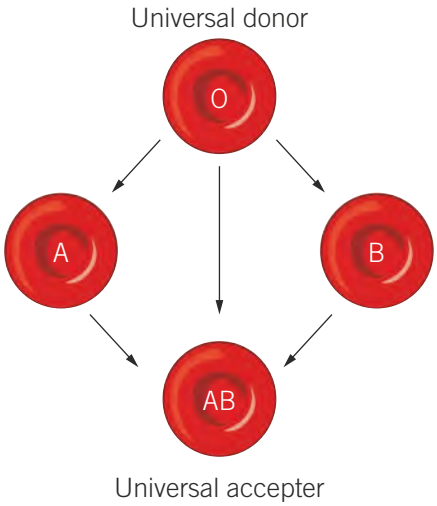
b Bacteriophages are a class of viruses that specifically infect bacterial cells. How does the process of infection of a bacterial cell through bacteriophage action differ from that of this virus? (1 mark)

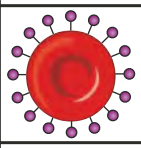
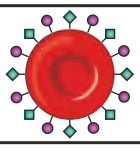
16 Histoplasmosis is a soil-based fungus that causes lung disease as a result of spore inhalation.

a Describe the key structural components of fungal pathogens and explain how they contribute to the onset of diseases like histoplasmosis in an infected host. (2 marks)

b Fungi are often described as 'parasitic'. Define this term and explain how the characteristics of fungal disease make the classification of these fungi as parasites appropriate. (2 marks)

17 Our immune system is programmed to initiate an immune response against any red blood cell antigen that is not considered to be 'self'. When this occurs, clumping or agglutination of blood occurs, resulting in death. For this reason, very strict protocols must be followed in blood donation programs.
Below is a diagram that represents the blood donation pathway between the four human blood types.



ABO blood group system				
Group	A	B	AB	O
Red blood cell type				
Self-antigen present	 Antigen A	 Antigen B	 Antigen A&B	None
Antibodies produced by immune system	 Anti-B	 Anti-A	None	 Anti-A & Anti-B

a Using your knowledge of antigens, along with the information presented above, explain why blood type O is considered the universal donor and blood type AB the universal recipient. (2 marks)

b Explain the role of MHC markers in blood transfusion recipients. (1 mark)

Antigen recognition on donor blood is not the only job of our immune cells. Antigens also play an important role in the recognition of pathogens.

c Use a diagram to demonstrate the difference between an antigen and a pathogen. (1 mark)

d Explain why it is so important that our immune system is able to identify pathogens. (1 mark)

e Describe, with the aid of a diagram, the typical course of disease that results from pathogen infection. (2 marks)

18 **a** What kind of substance is a prion? (1 mark)

b The image at right shows a normal cellular prion (PrP^{c}). The infectious mutant form of a prion (PrP^{Sc}) is described as having a 'decrease in alpha helical content with an increase in its beta sheet content'.
Draw a diagram that shows the secondary structure of the disease-causing prion in comparison to the normal one pictured here. (1 mark)

c Prions are believed to change their conformation from normal to infectious without altering their primary sequence.
Identify the type of disease caused by prions, and explain whether there would be any differences in the production of normal proteins and the infectious prions responsible for the disease. (1 mark)

UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES?

CHAPTER 7

IMMUNITY: LINES OF DEFENCE

Introduction

The presence of pathogens with non-self antigens presents a problem, and organisms need to be able to defend against them – they need immunity. Immunity has two components: the innate response and the adaptive response. The innate response has two levels of defence, and the adaptive response has a further level. A key difference between the two is that the innate response involves no 'memory' of the pathogen but is rapid, whereas the adaptive response is slower but once it has memory of a pathogen is quick to respond upon subsequent exposure.

Three lines of defence make up the innate and adaptive responses. The first line of defence includes physical, chemical and microbiota barriers to prevent the pathogen from entering. The second line of defence involves immune cells, complement proteins and signalling molecules. The third line of defence comprises the humoral response and cell-mediated immunity. The inflammatory response supports the second line of defence, while the lymphatic system transports the immune system components and detects the presence of antigens at lymph nodes.

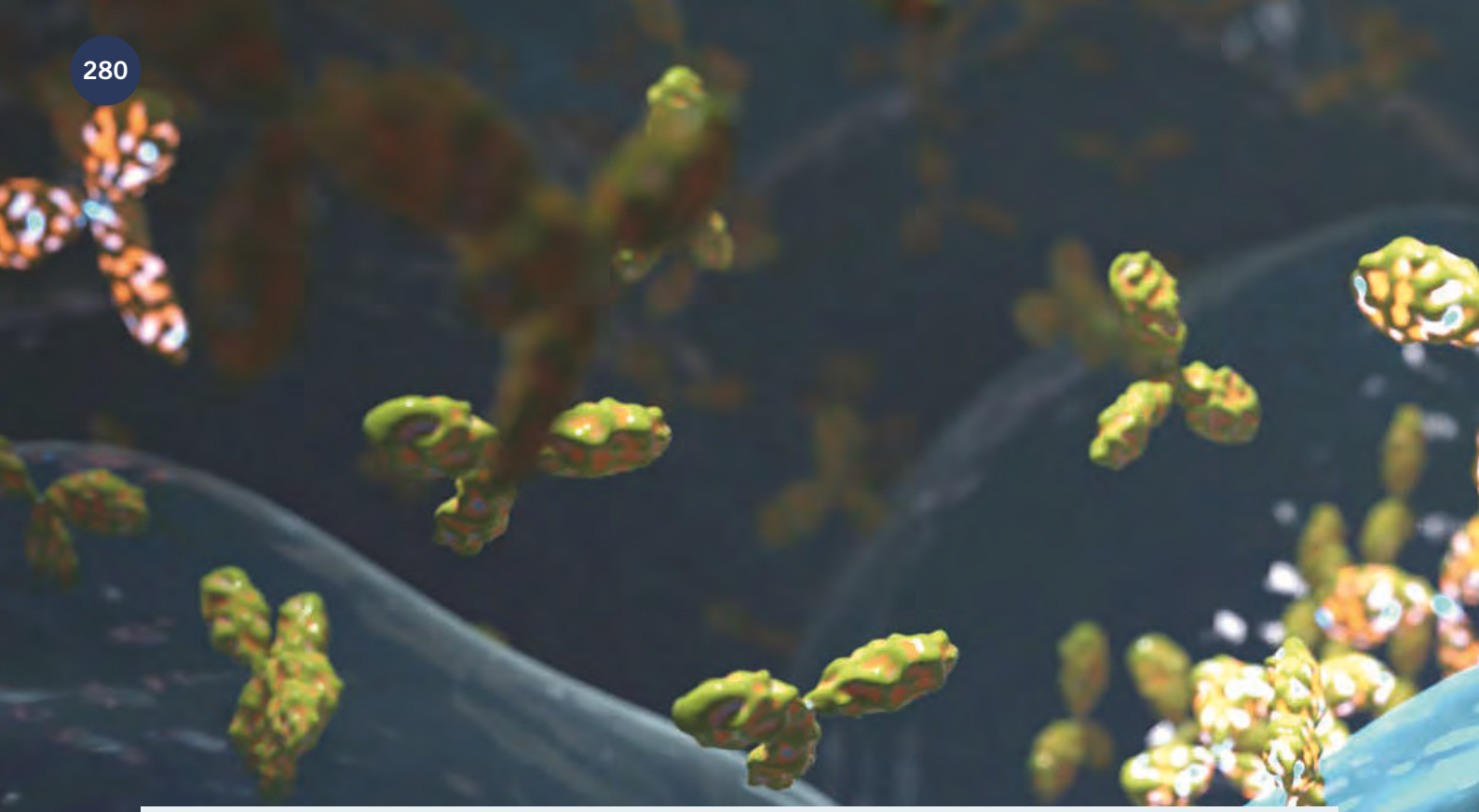
Immunity can be acquired by active or passive means. Active immunity initiates the third line of defence to cause an adaptive response, whereas a passive response acts like the third line of defence, except that the individual does not form any long-term immunity.

Curriculum

Area of Study 1 Outcome 1
Responding to antigens

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Physical, chemical and microbiota barriers as preventative mechanisms of pathogenic infection in animals and plants	**7A First line of defence** **7A.1** Recall and define terms to do with the first line of defence **7A.2** Describe what physical, chemical and microbiota barriers are, and recall that they make up the first line of defence **7A.3** Provide examples of physical and chemical barriers in plants and animals **7A.4** Identify where examples of physical, chemical and microbiota barriers would be on a body

Study Design	Learning intentions – at the end of this chapter I will be able to:
	7A.5 Compare pathogenic bacteria to normal flora **7A.6** Explain the role that normal flora have for animals as a microbiota barrier and part of the first line of defence **7A.7** Explain how examples of physical and chemical barriers work
• The innate immune response including the steps in an inflammatory response and the characteristics and roles of macrophages, neutrophils, dendritic cells, eosinophils, natural killer cells, mast cells, complement proteins and interferons	**7B Second line of defence** **7B.1** Recall and define terms that relate to the specialised cells, molecules and physiological responses of the second line of defence **7B.2** Identify and describe the function of the different specialised cells and molecules of the second line of defence **7B.3** Compare the functions of the different specialised cells and molecules of the second line of defence **7B.4** Explain the function of the specialised cells and molecules of the second line of defence **7B.5** Describe the steps of phagocytosis **7B.6** Link different chemicals to specialised cell types, in terms of what chemical is released from what cell and what chemical the cells respond to **7B.7** Identify the different components of the inflammatory response **7B.8** Apply the role of the different specialised cells and molecules in the context of an inflammatory response **7B.9** Explain the role of the different components of the inflammatory response
Acquiring immunity • The role of the lymphatic system in the immune response as a transport network and the role of lymph nodes as sites for antigen recognition by T and B lymphocytes	**7C The lymphatic system** **7C.1** Recall and define the terms associated with the lymphatic system **7C.2** Describe the function of the different sections of the lymphatic system: lymphatic capillary, lymph node and lymphatic vessel **7C.3** Describe and explain the function of the lymph node **7C.4** Describe and explain the role of the lymphatic system in connecting the second and third lines of defence
• The characteristics and roles of the components of the adaptive immune response against both extracellular and intracellular threats, including the actions of B lymphocytes and their antibodies, helper T and cytotoxic T cells	**7D Third line of defence** **7D.1** Recall and define the terms associated with the third line of defence **7D.2** Outline the functions of the different components of the cell-mediated and humoral immune responses **7D.3** Describe the process of the cell-mediated and humoral immune responses **7D.4** Compare and contrast the cell-mediated and humoral immune responses **7D.5** Compare the adaptive immune response to the innate immune response **7D.6** Explain how B and T lymphocytes are activated **7D.7** Draw appropriate antibodies for a given antigen and label the different components of an antibody **7D.8** Explain the different roles of antibodies and how they assist with the adaptive immune response

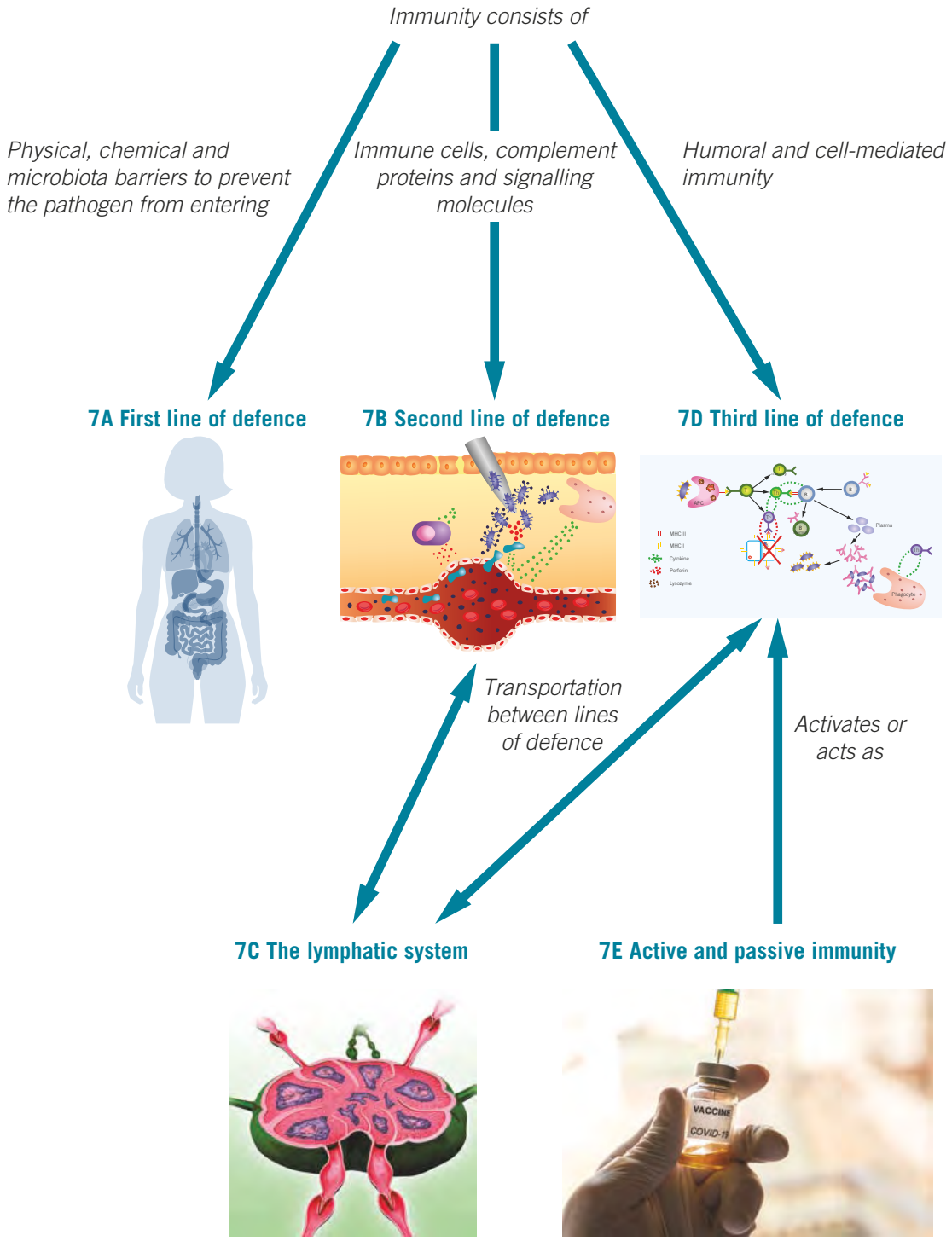


Study Design	Learning intentions – at the end of this chapter I will be able to:
• The difference between natural and artificial immunity and active and passive strategies for acquiring immunity **Disease challenges and strategies** • Vaccination programs and their role in maintaining herd immunity for a specific disease in a human population	**7E Active and passive immunity** **7E.1** Recall and define the different terms associated with active and passive immunity **7E.2** Provide examples of natural active, artificial active, natural passive and artificial passive forms of acquiring immunity **7E.3** Compare the different forms of acquiring immunity **7E.4** Explain the benefits of both passive and active forms of acquiring immunity **7E.5** Describe and compare the different forms of vaccines **7E.6** Define and explain the importance of herd immunity

Glossary

Active immunity
Adaptive immune response
Agglutination
Antibody
Antigen-presenting cell (APC)
Apoptosis
Attenuated
Blood–brain barrier
Clonal expansion
Clonal selection theory
Cytokines
Defensins
Dendritic cell
Effector cell
Eosinophil
Fever
First line of defence
Herd immunity
Histamine
Immunological memory
Inflammatory response
Innate response
Interstitial fluid
Lymph
Lymphocyte
Lymphoid organ
Lysis
Macrophage
Mast cell
Naive
Natural killer (NK) cell
Neutrophil
Normal flora
Passive immunity
Pathogenic bacteria
Perforin
Phagocytosis
Phagosome
Saponin
Surfactants
Vaccination
Vaccine
Vasodilation

Concept map



See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

First line of defence

Study Design:
Physical, chemical and microbiota barriers as preventative mechanisms of pathogenic infection in animals and plants

Glossary:
Blood–brain barrier
Defensins
First line of defence
Innate response
Normal flora
Pathogenic bacteria
Saponin
Surfactants

ENGAGE

Plants contend with pathogens

Plants, just like animals, come under attack from pathogens. As a result, plants have mainly physical and chemical barriers, and microbiotic barriers to a limited extent, to help protect them (these are explored in the Explain section). These barriers are vital for a plant's survival, as plants lack the cellular defence mechanisms that animals have in their second and third lines of defence. Therefore, each plant cell effectively has to defend itself. An example of a bacterial infection that affects plants is crown gall disease (Figure 7A–1). Crown gall is caused by the bacterium *Agrobacterium tumefaciens*, which enters via damaged roots or stems and causes growths known as galls. The galls prevent the plant from transporting water and nutrients, which causes the plant to become weak and stunted. An example of a viral infection in plants is tobacco mosaic virus (Figure 7A–2), which causes a distinct 'mosaic' pattern of discolouration on the leaves. This affects the chlorophyll content of the leaves and reduces the amount of photosynthesis that is able to occur, resulting in stunted growth. An example of a fungal infection in plants is rose black spot (Figure 7A–3). You may have seen rose black spot in gardens, where the rose leaves appear yellow with black spots on them. The infected leaves drop early, resulting in less photosynthesis, which causes stunted growth.

5A PHOTOSYNTHESIS LINK

Figure 7A–1 Crown gall disease caused by a bacterial infection

Figure 7A–2 A grape leaf affected by tobacco mosaic virus

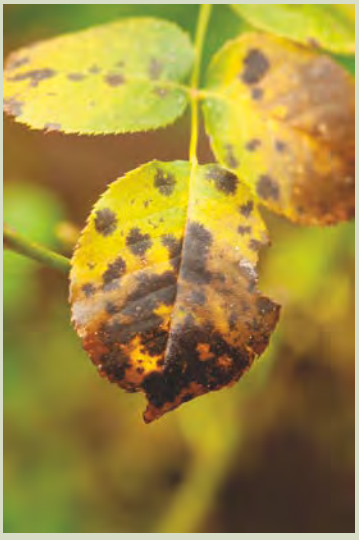


Figure 7A–3 The distinctive pattern of rose black spot, a fungal disease

EXPLAIN

Immunity structure

The immune system has three levels of defence against pathogens. You can think of the immune system as being like a series of three babushka dolls. The outer doll is the first line of defence and the first of two innate responses. The second line of defence, represented by the middle babushka doll, is the second innate response (covered in Section 7B) and the smallest babushka doll is the third line of defence, known as the adaptive response (covered in Section 7D).

Figure 7A–4 Three babushka dolls representing the three lines of defence: two innate responses and the adaptive response

LINK 7B SECOND LINE OF DEFENCE
LINK 7D THIRD LINE OF DEFENCE

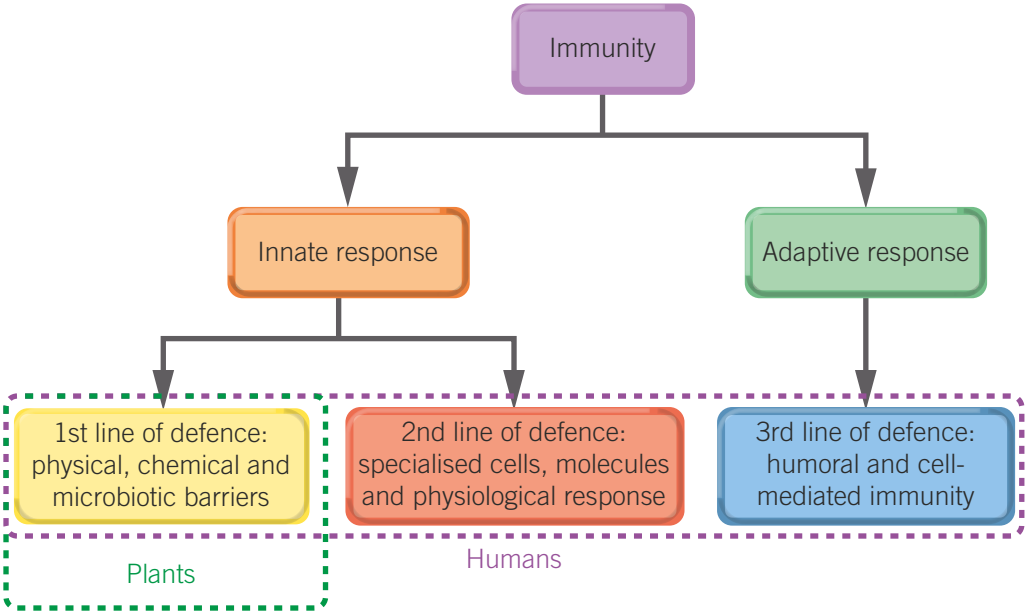


Figure 7A–5 Overview of immunity in humans and plants

A key feature of the **innate response** is that there is no memory of the pathogen. This means that the first and second lines of defence do not respond any faster on reinfection by the same pathogen. The adaptive response *does* have memory of a pathogen and results in a larger and faster response.

Innate response a non-specific defence against a pathogen

First line of defence the first innate response; consists of physical, chemical and microbiota barriers

Keeping the pathogens out: barriers

The **first line of defence** is the barrier defence. It is part of the innate response, as the barriers aim to keep all pathogens out, no matter what they are. There are three types of barriers: physical, chemical and microbiota. Table 7A–1 lists examples of barriers and Figure 7A–6 demonstrates them in the context of a human.

Table 7A–1 Barriers of plants and animals

	Physical	Chemical	Microbiota
Animals	• Intact skin • Mucous membranes • Mucus • Cilia • Hairs	• Lysozyme (an enzyme) in tears, saliva, sweat and other bodily fluids • Stomach acid and digestive enzymes • **Surfactants** in the lung	• **Normal flora**
Plants	• Thickened cell wall • Thick bark • Waxy cuticles • Stomata that close • Thorns and spines	• Resins • Toxins • **Saponin** • Oils • **Defensins**	• Normal flora present in roots

Normal flora naturally occurring microorganisms that live in or on animals and plants and do not cause harm or an immune response

Surfactants molecules that reduce the surface tension of water and aqueous solutions

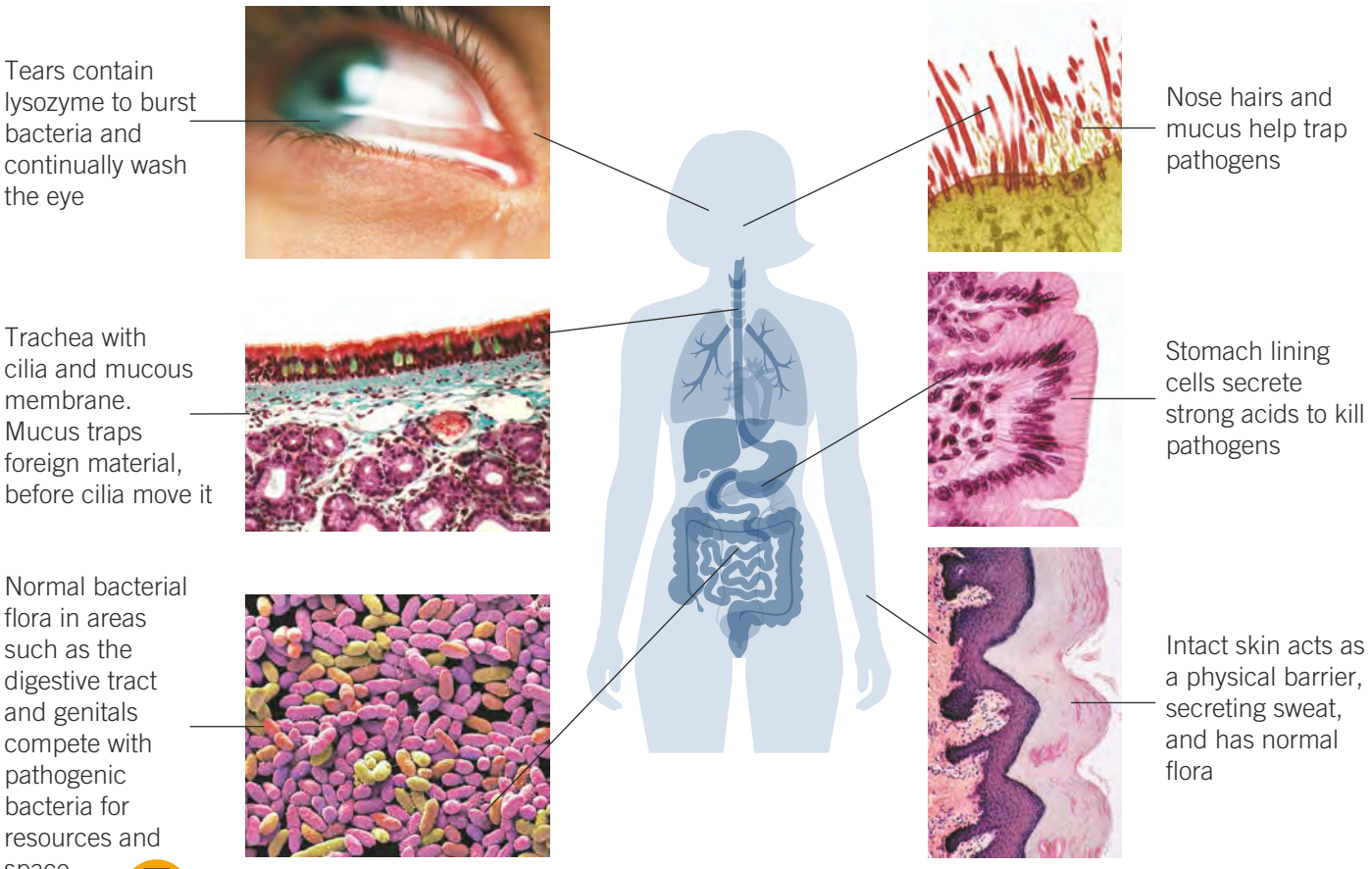


Figure 7A–6 Some of the physical, chemical and microbiota barriers in a human body, to prevent a pathogen from entering

Saponin soapy compound that occurs naturally in plants; has anti-fungal and antimicrobial properties

Defensins proteins that are toxic to microbes

The general role of a physical barrier is to prevent a pathogen from entering the organism, while the chemical barrier reduces the pathogen's ability to grow. The microbiota barrier competes with the pathogen for resources and space, preventing the pathogen from growing and reproducing.

It is important to be able to distinguish between the three types of barrier, identify examples and explain how each helps prevent a pathogen from entering the internal environment. Note that elements of the first line of defence have both physical and chemical features. For example, intact skin is a physical barrier and it contains cells that help to produce the

chemical barrier of sweat. An important adaptation in both animals and plants is to repair or replace the physical barrier when the organism suffers a penetrating injury, especially if blood (in animals) or phloem/xylem fluid (in plants) is being lost, both to prevent water and solute lost and also to prevent pathogens entering via the wound. In vertebrates this is done by blood clotting, which plugs the leak and allows new skin to grow. This is a complex process that interacts with the immune system. In plants, phloem sap may also coagulate to plug the wound, while new epithelial cells grow beneath it.

Barriers in animals

Physical and chemical

One of the main barriers for an animal is skin. To be effective as a barrier, preventing pathogens from entering the internal environment, skin must be intact. A breach in the skin is a site for pathogens to enter the body, where the second line of defence is activated (covered in Section 7B).

LINK 7B SECOND LINE OF DEFENCE

As well as being a physical barrier, the skin also has chemical barriers such as secretions that lower the pH to help prevent bacteria from growing. Skin also secretes sweat containing salt, which inhibits bacteria.

Tears are another example of a chemical barrier, as they help to wash or flood bacteria out of the eye, as well as containing lysozyme, which causes the bacteria to *lyse* (rupture). Lungs have surfactants (lipid-protein complexes) on their moist internal surfaces in contact with air to prevent the alveoli (tiny air sacs) collapsing under the surface tension of water. A protein component of surfactants also binds to pathogens, marking them for macrophages to engulf them.

The lining of the air-conducting part of the respiratory system, including the nasal cavity, trachea, bronchi and bronchioles, has mucus-secreting cells, along with cilia. If a pathogen enters the airways, the mucus acts as a physical barrier, as it aids in trapping the pathogen, while the cilia (also a physical barrier) assist in directing the mucus with the trapped pathogens towards the mouth to be swallowed. Swallowing the mucus sends the pathogen into the highly acidic environment of the stomach.

Neurons in the brains of vertebrates are highly vulnerable to pathogens and toxins, and a so-called **blood–brain barrier** has evolved to prevent pathogens getting out of the blood capillaries into the extracellular fluid that bathes the neurons. The barrier is formed by specialised epithelial cells that are highly selective, allowing only beneficial molecules through.

Blood–brain barrier a barrier of specialised epithelial cells in the brain and spinal cord that prevents pathogens and toxins reaching the neurons

Microbiota

Non-pathogenic bacteria that live in the body are known as normal flora, and form a microbiological barrier against pathogens. Normal flora live in many locations in the body, including the skin, digestive tract, mouth, nose and vagina. The presence of normal flora helps prevent **pathogenic bacteria** from being able to grow, as they compete with the pathogen for resources and space.

Pathogenic bacteria bacteria that cause harm and an immune response

Check-in questions – Set 1

1 Apart from physical barriers, what other forms of barriers aid the first line of defence?
2 Outline why it is important for skin to be intact.
3 What is the difference between pathogenic bacteria and normal flora?
4 What type of barrier is lysozyme?

WORKSHEET 7A–1 FIRST LINE OF DEFENCE ANIMALS

Barriers in plants

Physical

The main physical barrier for plants is a thickened cell wall. This acts as a thick barrier that is hard for pathogens to penetrate. The thicker the cell wall, the more effective it is at preventing pathogens from entering the plant. Covering the surface of the leaf is a waxy cuticle, which helps prevent water from pooling (collecting) on the surface of the leaf and ensures that the water runs off, reducing opportunities for any water-based pathogen to enter the leaf. It also prevents pathogens from coming into direct contact with epidermal cells.

The biggest weak point for a plant is its open stomata. These openings, which are essential for gas exchange by the plant during photosynthesis, can be closed to help prevent pathogens from entering.

5A PHOTOSYNTHESIS LINK

Some plants have thick bark, which acts as a thick physical barrier preventing the entry of pathogens. The bark in some plants also drops from the plant and in doing so can take the pathogen with it.

Figure 7A–7 The bark of a tree is a physical barrier to pathogens.

Chemical

Plant chemical barriers include enzymes that disrupt the cell walls of fungi or bacteria, preventing them from functioning normally. Plants can also produce antibacterial compounds to inhibit bacterial growth, and we use some of these to make antiseptics. More specific examples are given in Table 7A–1.

Figure 7A–8 Mint produces a chemical defence in the form of antibacterial chemicals.

Microbiota

Non-pathogenic bacteria that live in and around the roots of plants have a similar role to those that live in and on the body of humans. They form a microbiological barrier against pathogens. Similarly, they help prevent pathogens from growing, as they compete with the pathogens for resources and space.

The key thing to note is that plants have the first line of defence, but lack any further immune system responses like those of animals.

Check-in questions – Set 2

WORKSHEET 7A–2 FIRST LINE OF DEFENCE PLANTS DOC

1 What types of barriers do plants have to defend themselves against pathogens?
2 List three examples of barriers in plants.

7A SKILLS

VIDEO 7A–1 SKILLS: REMEMBERING HOW TO IDENTIFY THE FIRST LINE OF DEFENCE

Remembering how to identify the first line of defence

To help you remember and discern what the first line of defence is, compared to the other two lines of defence, think of all the entry points into the human body. Knowing these locations will help you identify the different barriers preventing pathogens from entering the internal environment. Noting the physical, chemical and microbiological features will help you identify, outline and explain how the first line of defence works.

Sketch a body shape and annotate the points on the body where a pathogen would be able to enter, as in Figure 7A–9.

From here, use Table 7A–1 and Figure 7A–9 to help you elaborate on each of the points of entry, to practise explaining how each barrier helps defend against pathogens. Enhance your diagram by colour coding the three different types of barriers.

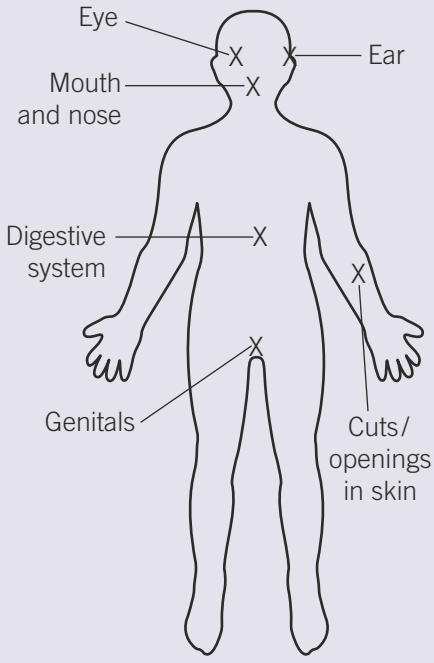


Figure 7A–9 The different sites of entry into the body have been identified.

Section 7A questions

1. How many lines of defence does a human have?
2. Which lines of defence make up the innate immune response?
3. Outline what the first line of defence is.
4. What is the difference between a plant's immune response and an animal's?
5. Describe how mucus and cilia work together to help defend against pathogens.
6. Describe how a plant's bark acts as a physical barrier.
7. Describe how natural flora assist in protecting a human and plants against pathogens.
8. What are two locations where you would expect to find normal flora in humans, and one location where you would expect to find them in plants?
9. Explain why it is important for skin to be intact when acting as a physical barrier for animals.
10. Provide a drawing of the different entry points where a pathogen could enter the body. Colour code to distinguish between physical, chemical and microbiota.

Second line of defence

Study Design:

The innate immune response including the steps in an inflammatory response and the characteristics and roles of macrophages, neutrophils, dendritic cells, eosinophils, natural killer cells, mast cells, complement proteins and interferons

Glossary:

Antigen-presenting cell (APC)
Apoptosis
Cytokines
Dendritic cell
Eosinophil
Fever
Histamine
Inflammatory response
Lysis
Macrophage
Mast cell
Natural killer (NK) cell
Neutrophil
Perforin
Phagocytosis
Phagosome

ENGAGE

Allergic response

6C NON-CELLULAR PATHOGENS LINK

An allergic response is a hypersensitive immune reaction to a substance that normally would be harmless and not cause an immune response. These substances could be things such as peanuts, pollen or cat hair, which are referred to as allergens. Recent Australian data has indicated that 10% of children under the age of one year have a food allergy, while 19% of all age groups self-report as having hay fever.

An allergic reaction can cause symptoms such as itching, swelling, rash (redness) and shortness of breath.

6A RECOGNISING SELF FROM NON-SELF LINK

A severe and potentially life-threatening allergic reaction is called anaphylaxis. The rate of new cases of anaphylaxis has been estimated to be one new case per 5000 people per year.

Figure 7B–1 Pollen being blown by wind

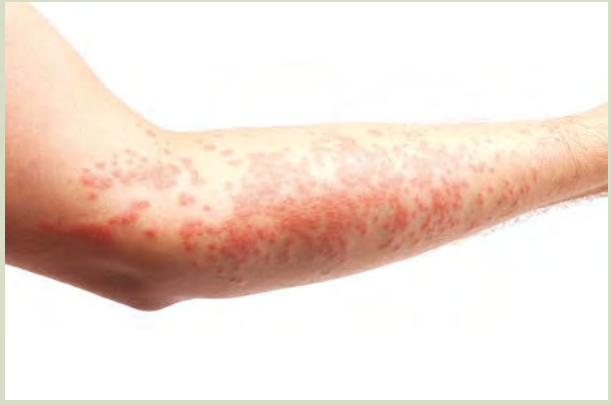


Figure 7B–2 An allergic reaction causing hives over an arm

Although the allergic response is not part of the course, it relates closely to the inflammatory and adaptive immune responses.

The key thing to note is that an allergic response is an abnormal process of something that would normally occur to protect you if you were ill due to a pathogenic infection.

EXPLAIN

Connection between the first and second lines of defence

A breach of the first line of defence will activate the second line of defence. A cut in the skin allows bacteria to enter the body, or a virus can be transmitted in droplets from someone sneezing and enter via the respiratory system, nose or eyes. The second line of defence is vital for the survival of an organism, as it can take days for the adaptive (third line) immune response to become activated.

The second line of defence, much like the first, is non-specific (innate) and responds the same way each time. This means that the response is fixed: it does not change the next time the same pathogen enters the body, and it has no memory and therefore is not faster the next time.

Figure 7B–3 A sneeze can have up to 30 000 droplets, while a cough contains 3000 droplets.

Figure 7B–4 is a summary of the second line of defence, which consists of specialised cells and molecules, which act together to produce the inflammatory response, and which also interact with the third line of defence.

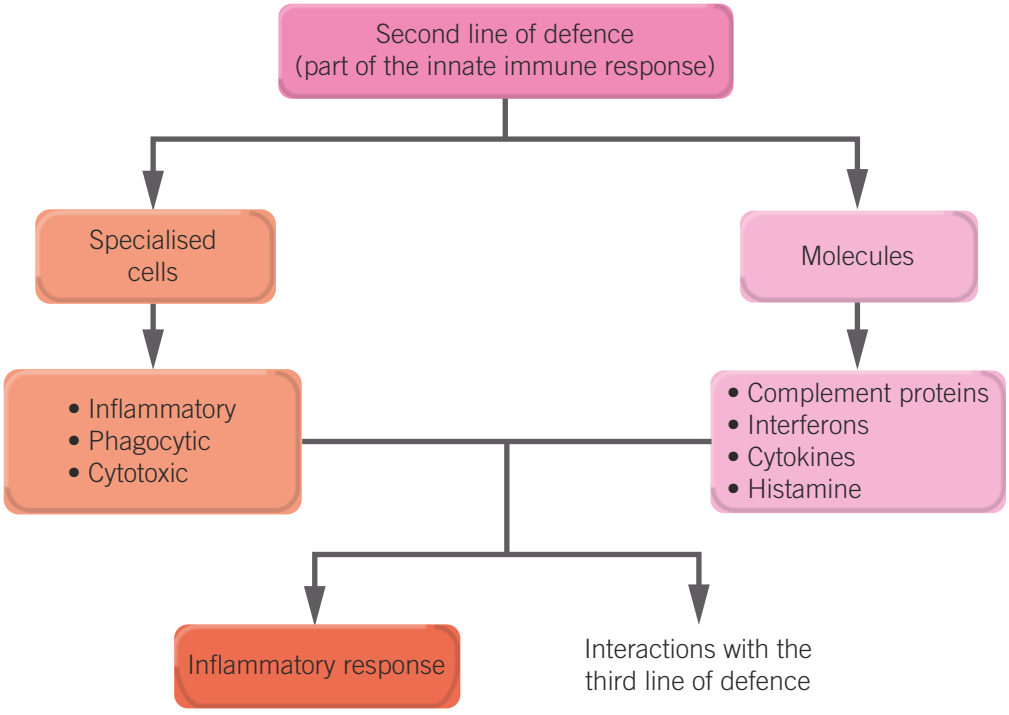


Figure 7B–4 Overview of the second line of defence, which is part of the innate immune response

Cells of the second line of defence in the innate immune response

Inflammatory, phagocytic and cytotoxic cells are the three main categories of specialised cells that make up the second line of defence. It is important that you are able to distinguish between these specialised cells and outline their functions (Table 7B–1). The main area where you will need to be able to apply your understanding of these cells is during the **inflammatory response**.

Inflammatory response heat, pain, redness, swelling and loss of function as part of the innate immune response to harmful stimuli

Mast cell
white blood cell involved in inflammatory response, releasing histamine, triggering inflammation

Histamine
compound released by cells to start an inflammatory response

Macrophage
large white blood cell that carries out phagocytosis and may act as an antigen-presenting cell

Cytokines
compounds released by cells as chemical signals to other cells

Table 7B–1 Cells involved in the second line of defence

Type of cells	Cell type	Function
Inflammatory	**Mast cells**	• Involved in the inflammatory response through the release of **histamine**
Phagocytic	**Macrophages**	• Engulf pathogens via phagocytosis • Break down pathogens, retaining their antigens • Can act as antigen-presenting cells • Release **cytokines** to attract more immune cells to the area of infection
	Neutrophils	• Engulf pathogen via phagocytosis • Release defensins that are toxic to bacteria and fungi
	Dendritic cells	• Engulf pathogen via phagocytosis • Break down pathogens and retain antigens • Act as antigen-presenting cells
Cytotoxic	**Natural killer (NK) cells**	• Destroy virally infected or damaged host cells and cancerous cells through recognition of an absent or damaged MHC I marker • Release cytotoxic chemicals (such as **perforin**) that cause holes in plasma membranes and trigger cell death (apoptosis) • Release cytokines to attract other specific adaptive immune cells to the area of infection
	Eosinophils	• Contain granules (which contain cytotoxic chemicals) that target parasites • Secrete cytotoxic chemicals

VIDEO 7B–1 THE INNATE IMMUNE RESPONSE

7A FIRST LINE OF DEFENCE LINK

Phagocytes perform phagocytosis

Phagocytes, such as macrophages and neutrophils, are immune cells that perform **phagocytosis** (Figure 7B–6). Their role is to seek, engulf and destroy pathogens. The pathogens are engulfed to form **phagosomes** inside the phagocyte.

Macrophages are found throughout the body and are particularly abundant in sites of the body that are prone to infection. They are one of the first cells to recognise pathogens that have managed to breach the first line of defence.

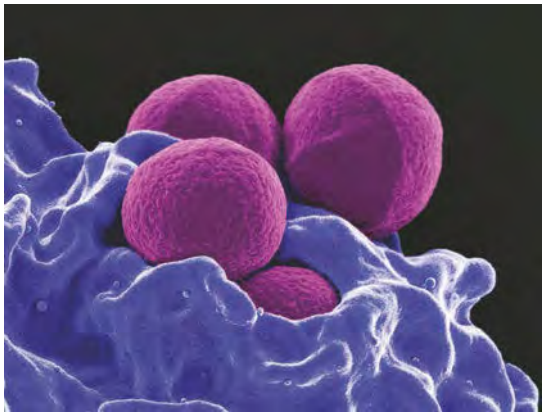


Figure 7B–5 A neutrophil phagocytosing bacteria

Neutrophil
white blood cell that carries out phagocytosis and kills pathogens with defensins

Dendritic cell
white blood cell with many folds and projections in its membrane, carries out phagocytosis and acts as an antigen-presenting cell to the adaptive immune system

Natural killer (NK) cell
white blood cell involved in innate immune response; kills infected host cells and cancer cells

Perforin
a protein that kills cells by making holes in their plasma membranes

Eosinophil
white blood cell that targets parasites

Phagocytosis
a type of endocytosis in which a solid substance enters a cell via vesicle mediated transport

Phagosome
a vesicle that engulfs a pathogen during phagocytosis

Neutrophils are short-lived cells in the bloodstream. They are attracted to sites of infection through chemical signalling, which is part of the inflammatory response.

Dendritic cells, like macrophages, are key phagocytes that act as **antigen-presenting cells (APC)**. The APC is the bridge between the second and third lines of defence, where they play a role in activating the humoral and cell-mediated responses.

Phagocytes are efficient cells that are able to destroy an invading pathogen. This is due to the presence of the highly toxic lysosomes. These contain lysozyme, which has the capability to drastically lower the pH of the phagosome, assisting in the degrading of the pathogenic contents.

LINK **6A** RECOGNISING SELF FROM NON-SELF

LINK **7D** THIRD LINE OF DEFENCE

Antigen-presenting cell (APC) a specific type of white blood cell that uses phagocytosis to engulf a pathogen

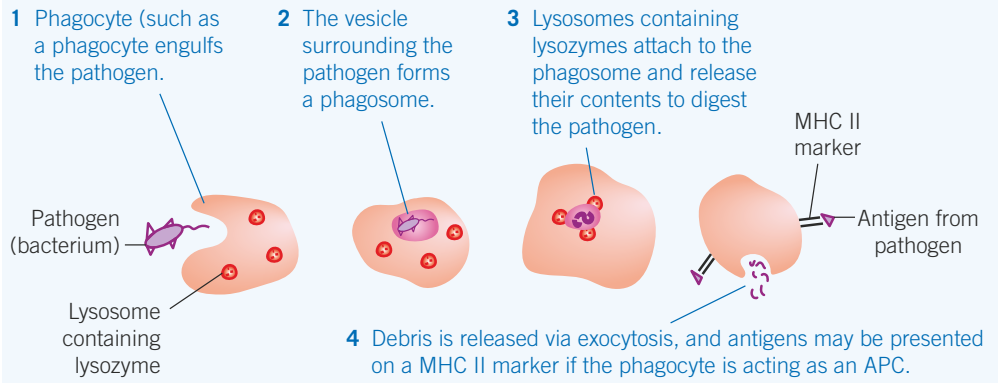


Figure 7B–6 The process of phagocytosis. If the phagocyte acts as an APC, it now goes to the third line of defence.

Natural killer cells

Natural killer (NK) cells are cytotoxic innate immune response cells that kill unhealthy host cells (see Figure 7B–7). The following numbered points refer to those marked on the diagram.

1 A NK cell has two types of receptor on its surface.
2 An inhibitory receptor will bind to a *natural killer ligand* (a kind of signalling molecule) on 'self' cells. An activating receptor will bind to an MHC I marker. When both receptors bind, the cell is recognised as healthy and no activation occurs.
3 An unhealthy cell lacks the MHC I marker and also releases interferon, which attracts NK cells.
4 The activating receptor doesn't find an MHC I marker, so it identifies the unhealthy cell as 'non-self' and releases cytotoxins, such as perforin, to cause **apoptosis** of the cell.
5 The NK cell also releases cytokines to signal the infection to other immune cells.

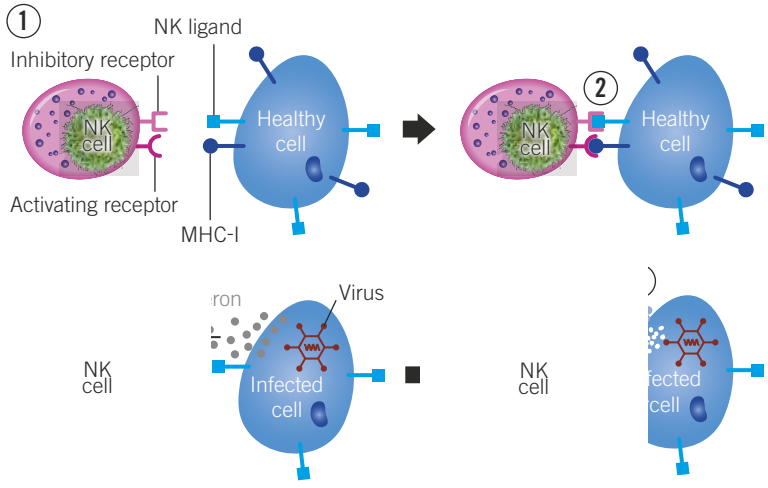


Figure 7B–7 Action of NK cells in killing unhealthy cells. The numbers refer to steps explained in the text.

Apoptosis death and disintegration of a cell through a controlled process

Check-in questions – Set 1

1 What type of pathogen do eosinophils target?
2 What would be found within a phagosome?
3 Which specialised cell releases defensins?
4 What are two functions of a macrophage?

WORKSHEET **7B–1** CELLS OF THE INNATE IMMUNE RESPONSE

Molecule defence

Molecules that assist the body in defending against pathogens that have breached the first line of defence are summarised in Table 7B–2.

Table 7B–2 Molecules involved in the innate immune response

Molecule	Description/function
Complement proteins	• Circulate in the blood • Can cause **lysis** by puncturing the pathogen's plasma membrane • Coat pathogens to make them more identifiable for phagocytes and to reduce the pathogen's mobility
Cytokine	• A signalling molecule of the immune system • Released by immune cells and acts on other specific immune cells (lymphocytes) to activate further adaptive immune responses • Examples include interleukins and interferons
Interferon	• A type of cytokine released by an infected host cell that defends against further viral infections • Aids in inhibiting the ability of a virus to synthesise new viral proteins within a host cell • Attracts NK cells to assist in killing virus-infected host cells
Histamine	• A signalling molecule released by mast cells that initiates the inflammatory response

Lysis
breakdown of the cell membrane

The complement protein system is complex, with many cascades of events, which you do not need to understand. The important functions during the inflammatory response that you need to be able to recall and apply are summarised in Table 7B–2 and Figure 7B–8. An important point is that lysis is not the same as apoptosis. In lysis, holes are punched in the surface of a cell, causing the cell to rupture, whereas apoptosis is a series of controlled steps that lead to the death of a cell.

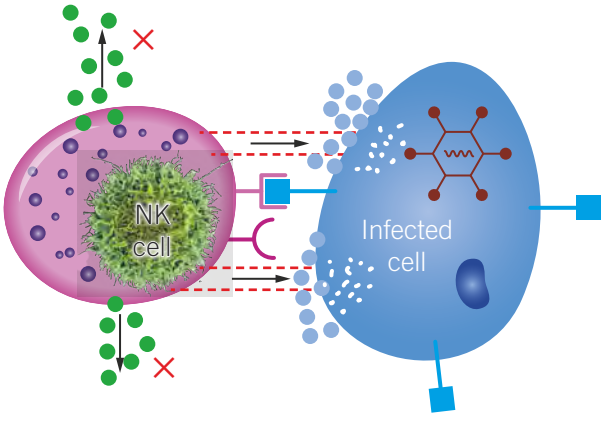


Figure 7B–8 Natural killer cell releasing cytotoxins to destroy the infected cell, and cytokines to signal the infection to other immune cells

Interferon is important in defence against viral infections. A host cell that has been invaded by a virus will release interferon in an *autocrine* and *paracrine* manner: an autocrine signal acts on the cell itself, whereas a paracrine signal acts on nearby cells. The autocrine signal from the virus-infected host cell triggers the production of a protein that will inhibit viral proteins from being synthesised within it. The paracrine signal allows uninfected host cells nearby to prepare for the production of the inhibitory proteins, in case they are invaded by the virus. Interferon also aids in attracting NK cells, which will destroy virus-infected host cells.

Check-in questions – Set 2

1 Where would complement proteins be found, most of the time?
2 What specialised type of cell do complement proteins help to attract to the pathogen?
3 What type of pathogen would cause a host cell to release interferon?
4 What type of chemical is interferon and what is its role?

The inflammatory response

The inflammatory response is a physiological response to a pathogen breaching the first line of defence. This response is characterised by redness, heat, pain and swelling. **Vasodilation** causes an increase in blood flow at the site of inflammation, which results in redness and heat. Pain and swelling are associated with the capillaries becoming more permeable and allowing fluid to enter the site of inflammation. The fluid build-up results in swelling, while the extra pressure due to the excess fluid applies pressure to nerve endings, causing pain.

Vasodilation the widening of a blood vessel (especially capillaries), to increase blood flow

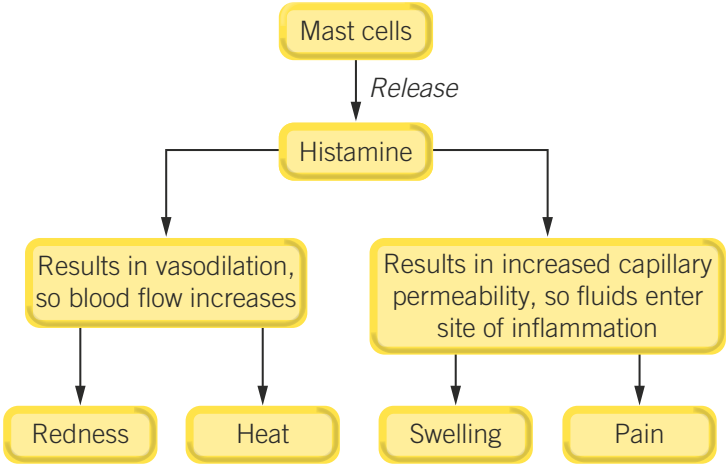


Figure 7B–9 Summary of the characteristics of the inflammatory response

Table 7B–3 Key components of the inflammatory response

Component	Function
Cytokines	• Damaged cells from the breached skin release cytokines to attract second line of defence cells (neutrophils and macrophages) and molecules to site of infection
Platelets	• Release clotting factors to prevent further bleeding at site of breach • Block blood vessels, which can help prevent the pathogen from entering the bloodstream and spreading infection to other parts of the body • Also assist in re-establishing a physical barrier to aid the first line of defence
Mast cells	• Release histamine to initiate vasodilation of local blood vessels
Vasodilation	• Increases blood flow and permeability of blood vessels • This enables more second line molecules and cells to come to the site of infection and leave the blood vessel
Complement proteins	• Are activated and attach to the pathogen to cause lysis, attract phagocytes and aid in immobilising the pathogen
Macrophages	• Are attracted to the site of infection by the presence of cytokines • Assist by engulfing pathogen via phagocytosis and breaking it down, retaining the antigens • Can act as an APC and be the link to the activation of the cell-mediated response in the third line of defence
Neutrophils	• Leave the blood vessel and are attracted to the site of infection by the presence of cytokines • Assist by engulfing pathogen via phagocytosis • Release defensins, which target and disrupt bacterial and fungal membranes • Release further cytokines to attract more immune cells

LINK 7A FIRST LINE OF DEFENCE

LINK 7D THIRD LINE OF DEFENCE

The inflammatory response is a key concept that is used to test your application skills in regards to the second line of defence. Table 7B–3 outlines the key functions of the different components of the response, while Figure 7B–10 provides a step-by-step account of the connections between the different components.

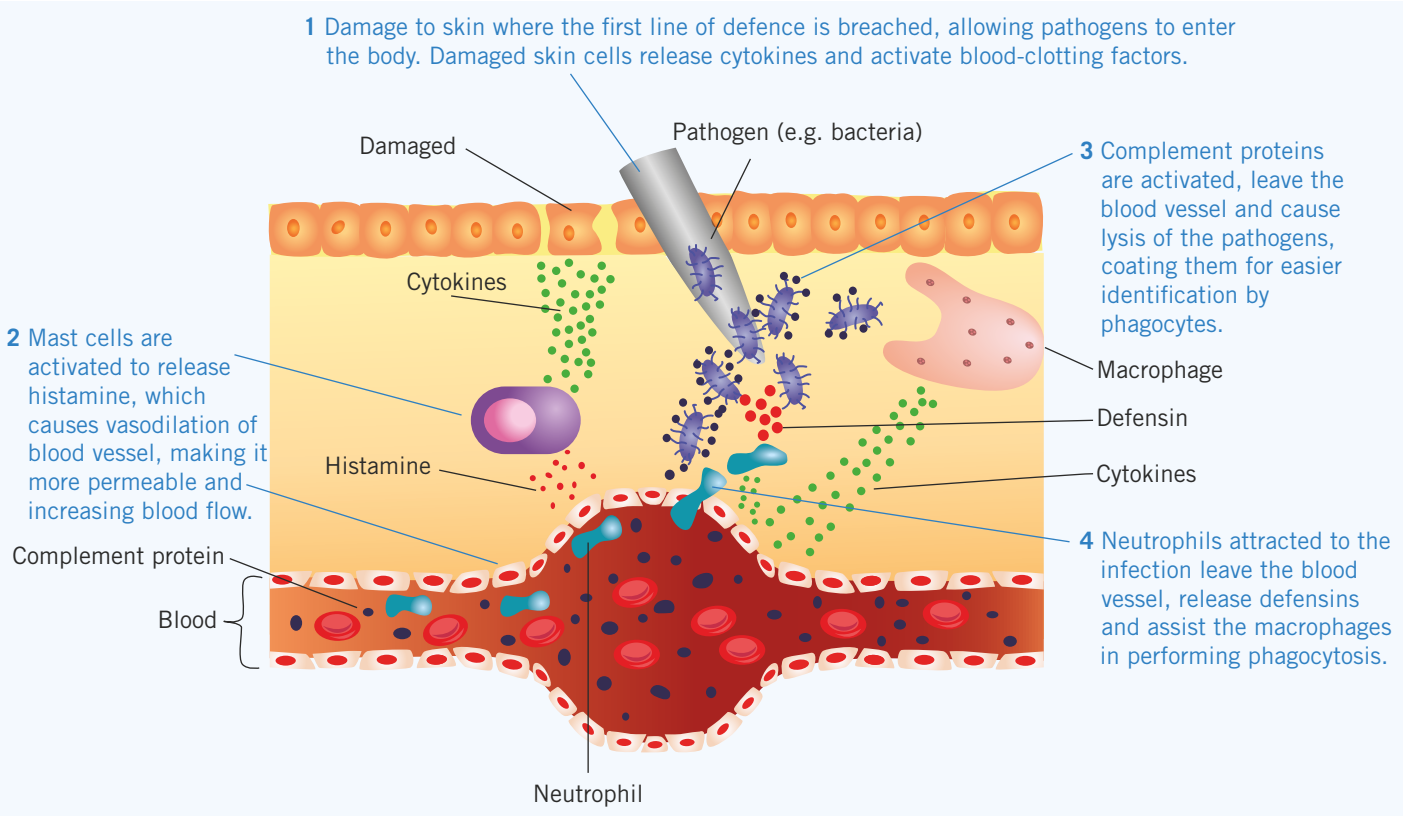


Figure 7B–10 Inflammatory response sequence

Fever
a rise in body temperature caused by infection

Cytokines from the inflammatory response can also cause **fever**. The rise in body temperature helps the immune system to fight the infection. Most bacteria and viruses prefer a lower body temperature in order to replicate more efficiently, while the immune cells perform better at slightly higher temperatures.

Check-in question – Set 3

WORKSHEET 7B–2 INFLAMMATORY RESPONSE SEQUENCE

1 What do platelets assist with?
2 How does vasodilation assist the inflammatory response?
3 What chemical triggers vasodilation?
4 Which cell releases the chemical that triggers vasodilation?
5 What function do neutrophils and macrophages have in common during the inflammatory response?
6 How do neutrophils and macrophages compare?

VIDEO 7B–2 SKILLS: GENERATING FLIP CARDS

Using flip cards, as detailed in the Skills box on the following page, could be a useful tool to help you revise your learning on the lines of defence.

7B SKILLS

Generating flip cards

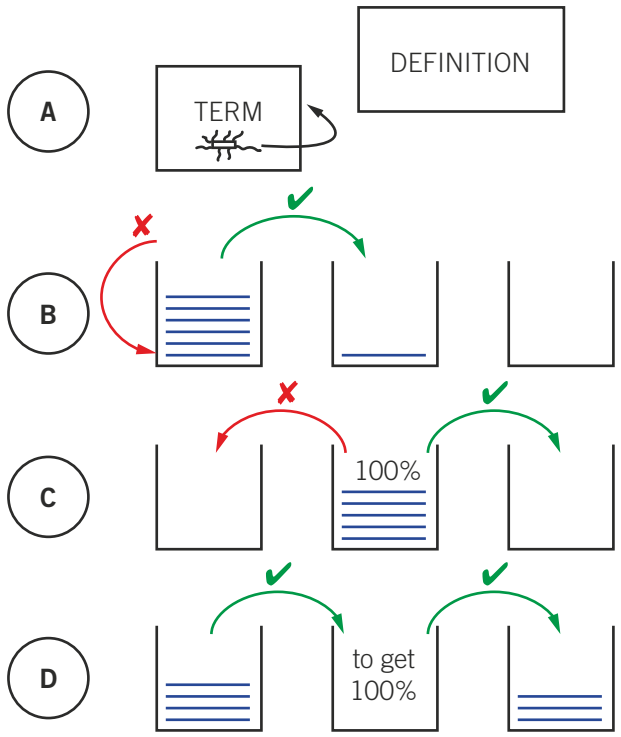
Flip cards are a great tool to use to increase your ability to recall information and test your understanding. The technique outlined here can help increase your long-term memory. This can be used for all topics, not just this section.

A Identify the key word, term, phrase or process and write it on one side of a card. On the other side, write the explanation or definition. You can mix this up by using images instead of words so you start to dual code by associating images with definitions, which also helps to increase your long-term memory.

B Collect three boxes. Put all the cards in box 1. Read the cards one by one and as you get a term correct, place the card in box 2. Return cards you did not get correct to box 1. Continue to go through all the cards until they have all been placed in box 2

C Go through the cards in box 2 again. As you get a term correct, place the card in box 3. Place cards you did not get correct in box 1. Continue until there are no cards left in box 2.

D If there are cards in box 1, continue testing yourself until all the cards are in box 2. Then, try to get the all in box 3 without making a mistake.

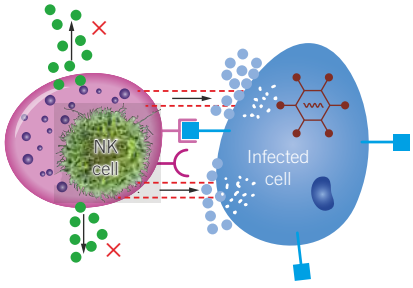


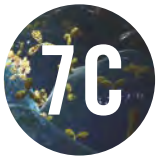
The goal is to get all the cards into the third box. When you are able to do that, start the process over again, going back to stage B. To increase the difficulty, put breaks in between your attempts to move from box to box, by coming back to it a day later or a week later.

A final note: doing this cannot be a one-off event. You need to continue to practise this process throughout, so that you are continually forcing yourself to recall content on an ongoing basis.

Section 7B questions

1 Apart from specialised cells, what is the other component of the second line of defence?
2 What are the three categories of specialised cells?
3 Outline the role of a natural killer cell.
4 What is the name of the enzyme within the lysosome of a macrophage?
5 Compare the function of a mast cell to that of a dendritic cell.
6 Describe how complement proteins assist the body in defending itself against pathogens.
7 In the diagram, identify what molecule X would be if a cell has been infected by a virus.
8 In the diagram, explain what role molecule X plays in the second line of defence.
9 List the different components of the inflammatory response.
10 Explain how vasodilation assists with the inflammatory response.





The lymphatic system

Study Design:
The role of the lymphatic system in the immune response as a transport network and the role of lymph nodes as sites for antigen recognition by T and B lymphocytes.

Glossary:
Adaptive immune response
Interstitial fluid
Lymph
Lymphocyte
Lymphoid organ

ENGAGE

Hodgkin lymphoma

Hodgkin lymphoma is a blood cancer that begins in a type of white blood cell known as a lymphocyte, in the lymphatic system. This cancer reduces the individual's ability to fight infections, which reflects the important role the lymphatic system plays in defending the body against pathogens. Hodgkin lymphoma accounts for 0.5% of all cancers diagnosed in Australia and is most common in people aged 15–29 or over 65 years old.

Symptoms include swollen lymph nodes in the neck, armpits or groin, and fatigue, fever and chills. Common treatments for Hodgkin lymphoma include chemotherapy and radiation.

You may have also heard of non-Hodgkin lymphoma. The identification of lymphoma as either Hodgkin or non-Hodgkin is based on the presence of Reed-Sternberg cells. If these cells are present, the cancer is Hodgkin lymphoma. Reed-Sternberg cells are large, abnormal lymphocytes that may contain more than one nucleus.

Figure 7C–1 Potential site of swollen lymph nodes in the neck

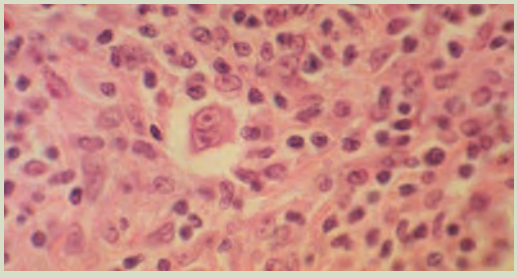


Figure 7C–2 Reed-Sternberg cell (located in the middle) with two nuclei, indicative of Hodgkin lymphoma

Lymphocyte
a type of white blood cell; includes B and T cells

Adaptive immune response
response of the vertebrate immune system to a specific antigen, which typically results in immunological memory

EXPLAIN

Role of the lymphatic system

The lymphatic system plays a vital role during an immune response, including:

- providing a location for **lymphocytes** to mature
- transporting lymphocytes and antigen-presenting cells (APCs) to the lymph nodes
- transporting pathogens and antigen fragments from the **interstitial fluid** to the lymph nodes
- providing a site for antigen recognition by lymphocytes to trigger an **adaptive immune response**.
- providing a site for clonal selection and expansion, and subsequent storage of specific memory cells.

VIDEO 7C–1
THE LYMPHATIC SYSTEM

Interstitial fluid
fluid that collects in spaces between cells and tissues

The lymphatic system also does the following:

- returns interstitial fluid that has been lost from cells or blood vessels to the circulatory system
- absorbs and transports fatty acids and fats that have been digested.

The fluid that flows through the lymphatic system and originates as interstitial fluid is called **lymph** and it is similar to blood plasma, the fluid in the blood minus the red blood cells.

Lymph colourless fluid that flows through the lymphatic system

Lymphoid organ organ involved in the production or function of lymphocytes

Structure of the lymphatic system

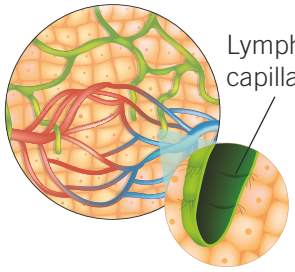
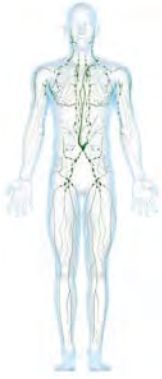
The lymphatic system consists of lymphatic capillaries, lymph, lymphatic vessels and primary and secondary **lymphoid organs**. The primary lymphoid organs include the bone marrow and the thymus. Bone marrow is where B and T lymphocytes originate. These two types of white blood cells are important in the adaptive immune response, which is covered in Section 7D. Immature T lymphocytes travel to the thymus, where they mature before ending up in the lymph node.

LINK 7D THIRD LINE OF DEFENCE

Lymph nodes are an example of a secondary lymphoid organ. This is where the B and T lymphocytes are activated and perform their roles in responding to non-self antigens in the adaptive immune response (Figure 7C–3).

Some of the key lymphatic system structures are outlined in Table 7C–1.

Table 7C–1 Structures of the lymphatic system

Name, description, diagram	Function
Lymphatic capillaries Collection of interstitial fluid from spaces between the cells begins here 	• Closed-ended tubes where interstitial fluid that is bathing the tissue enters through mini-valves of the capillary • The interstitial fluid could contain: immune cells, pathogens or fragments from pathogens (antigens) • Once in the lymphatic capillary, the fluid is known as lymph
Lymphatic vessels Follow a similar pattern to the venous part of the vascular system. Collect interstitial fluid to filter and return to the bloodstream 	• Lymphatic vessels bring lymph to the lymph node and return lymph to the blood through a lymphatic duct into the vein located near the clavicle/collarbone • The movement of the lymph is largely due to external pressure applied to the lymph vessels through muscle contractions • One-way valves ensure that the flow of the lymph occurs in a single direction (just like the venous system)
Lymph node A major site of filtration for the identification of non-self antigens to trigger the adaptive immune response 	• The site where lymph is filtered • Any pathogen or pathogen fragments would encounter phagocytes and lymphocytes • The phagocytes can present the pathogen's antigen to a helper T lymphocyte • APCs can also be present in lymph that has come from a site of infection to present the non-self antigen to a helper T lymphocyte (Figure 7C–3)

7D THIRD LINE OF DEFENCE LINK

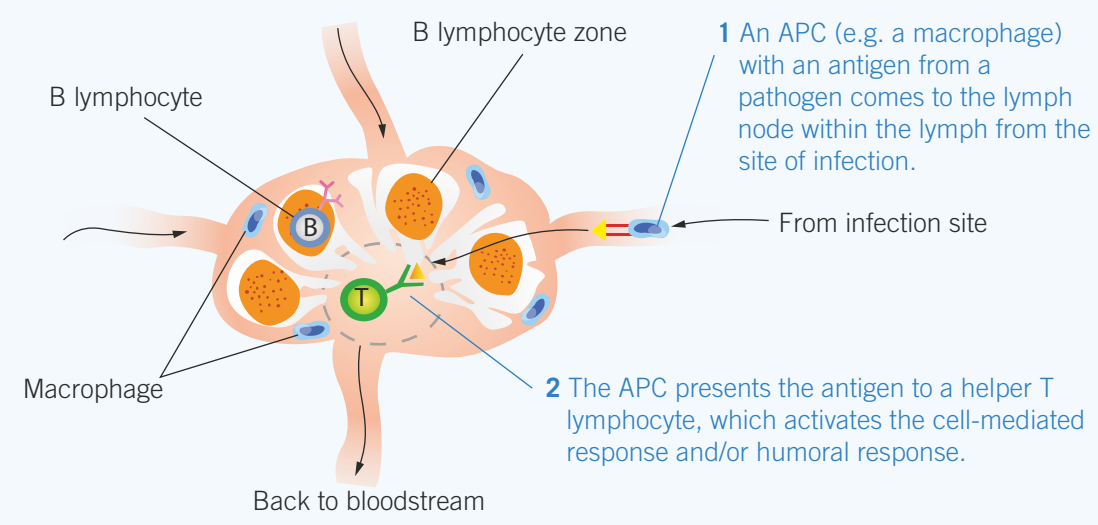


Figure 7C–3 Antigen recognition by T lymphocytes in a lymph node

Check-in questions – Set 1

WORKSHEET 7C–1 STRUCTURE OF THE LYMPHATIC SYSTEM

1 Where does fluid entering the lymphatic capillary come from?
2 What enables the lymph to flow in one direction?
3 What would an antigen or pathogen encounter if it were to enter the lymph node?

7C SKILLS

VIDEO 7C–2 SKILLS: CREATING MIND MAPS WITH FLIP CARDS

Creating mind maps with flip cards

In the 7B Skills section, you learnt a technique for increasing long-term memory using flip cards. To build on that, this Skills section focuses on how to use the flip cards to construct mind maps to make connections. It is vital that you are able to make connections in Biology while responding to explain-style questions, as these can require you to incorporate information from multiple areas.

UNIT 2 LINK

To think about building a mind map, remember junior science and/or Unit 2 Biology, when you explored food chains and food webs. Start with a series of connections, just like a food chain. Another way to think of it is like a flow chart, where you make connections from one card to the next.

To do this, place your flip card on a large piece of paper, then draw an arrow on the paper to connect to your next flip card. Continue to do this until you have run

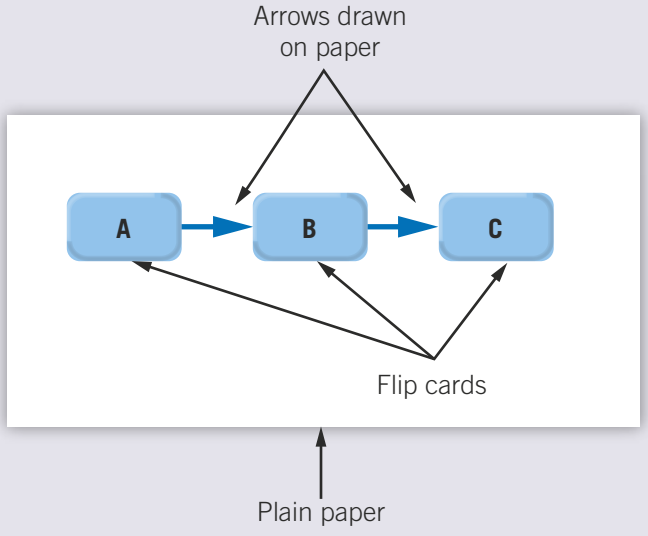


Figure 7C–4 Set up the first flow chart of connections between flip cards

out of single connections. The benefit of using flip cards on the paper is that is easy to start again and try different connections.

You can use this first step when practising with explain-style questions. This will give you the order in which to present your response, so your ideas are in a logical order. (This is explored further in the 8B Skills section.)

The next step is to bring in other connections coming off the original flow chart. Continue to build until you have made connections between multiple concepts.

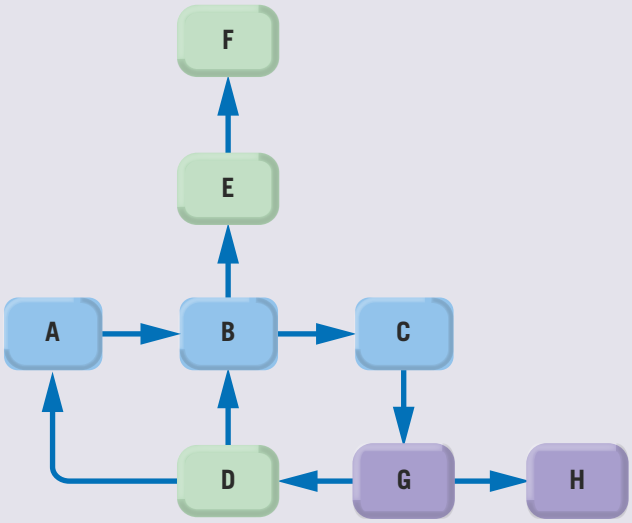
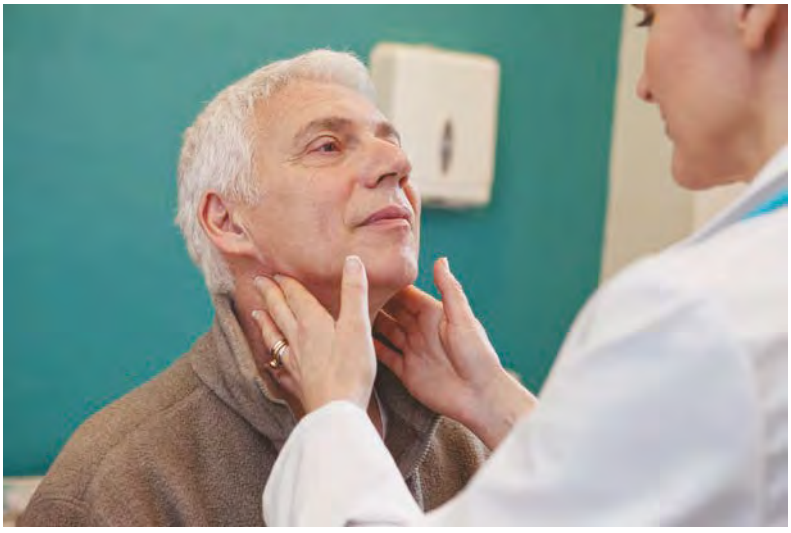


Figure 7C–5 Build up the mind map by making connections with other flip cards

The key is to continue to repeat this process. There will be more than one way to make the different connections. This approach aims to increase your memory capacity even further while also increasing your efficiency in recalling and applying your understanding. This technique requires you to think and reorganise the information from your notes or this textbook. The more you actively make connections, the deeper your understanding will be.

Section 7C questions

1 Where does interstitial fluid eventually end up?
2 List two functions of the lymphatic system in relation to an immune response.
3 Explain why valves are important in the lymphatic vessels.
4 Outline the function of the lymph node in assisting in an immune response to a pathogen. Include what cellular structures would be present to assist this function.
5 Your grandfather goes to see his GP because he is feeling unwell. Explain why the doctor would feel under your grandfather's jaw.
6 Explain why the lymphatic system is an important part of the adaptive immune response.



Third line of defence

Study Design:
The characteristics and roles of the components of the adaptive immune response against both extracellular and intracellular threats, including the actions of B lymphocytes and their antibodies, helper T and cytotoxic T cells

Glossary:
Agglutination
Antibody
Clonal expansion
Clonal selection theory
Effector cell
Immunological memory
Naive

ENGAGE

Autoimmune disease

As you will discover in this section, the third line of defence targets non-self antigens, to cause an immune response – that is, to fight foreign pathogens and build immunity. An autoimmune disease is one in which the adaptive immune response targets specific self-antigens on host (self) cells and causes damage via cytotoxic T cells and antibodies from B lymphocytes.

There are a broad range of autoimmune diseases, more than 80 disorders that affect around 5% of Australians. The causes of these autoimmune diseases are unknown, but there appears to be a genetic link. Autoimmune diseases can be grouped into two categories: localised (organ specific) or systemic. Some common examples of autoimmune diseases are rheumatoid arthritis, type I diabetes and multiple sclerosis.

Rheumatoid arthritis is an autoimmune disease that targets joints, causing painful swelling. Over time, the inflammation can cause bone erosion and joint deformity. There is currently no cure, but physiotherapy and medication can be used to slow the progression of the disease.

Multiple sclerosis is an autoimmune disease that targets the myelin sheath of nerves in the central nervous system, disrupting communication between the brain and the body. Symptoms include vision loss, pain, fatigue and impaired coordination.

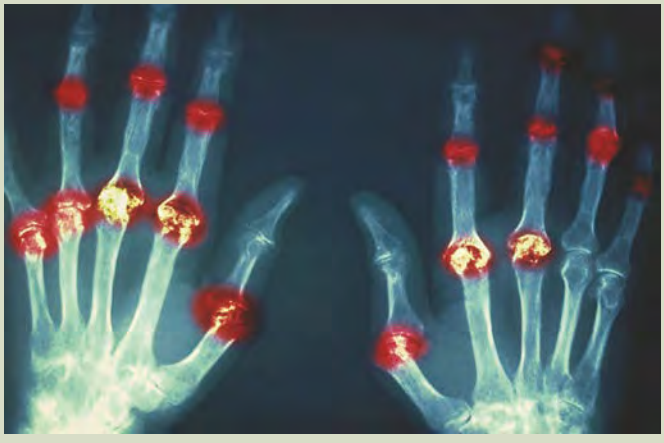


Figure 7D–1 X-ray of the hands of a person with rheumatoid arthritis, showing inflamed finger joints

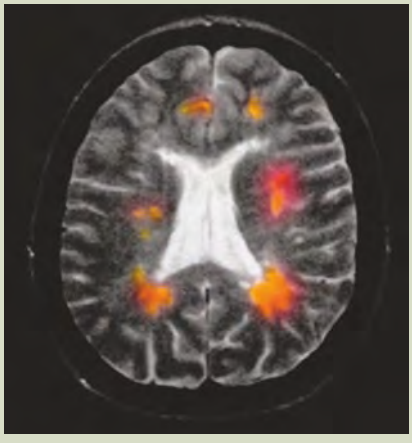


Figure 7D–2 MRI scan of a human brain affected by multiple sclerosis.

EXPLAIN

Adaptive immune response

The discussion in this chapter has until now been about the innate immune response, although Section 7C gave some information about the adaptive immune response. Unlike the innate immune response, the adaptive immune response is highly specific to antigens and forms **immunological memory**. The immune system's ability to remember the antigen, and therefore the pathogen, means that upon reinfection by the same pathogen, the immune response will be faster and stronger. This results in immunity against a pathogen, which is the basis of vaccination programs.

The adaptive immune response is carried out by white blood cells called lymphocytes, which consist of two main groups of cells: B cells and T cells.

VIDEO 7D–1 ADAPTIVE IMMUNE RESPONSE SUMMARY

LINK 7C THE LYMPHATIC SYSTEM

LINK 7E ACTIVE AND PASSIVE IMMUNITY

Immunological memory the ability of the immune system to quickly and specifically recognise an antigen that the body has previously encountered and initiate a corresponding immune response

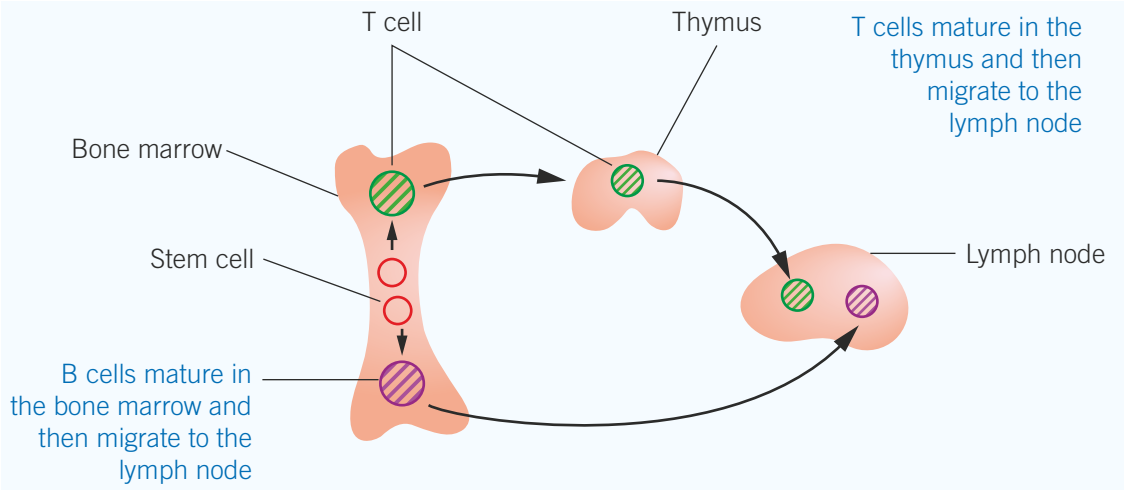


Figure 7D–3 Production and maturation sites of B and T lymphocytes

B cells help perform the main role of the humoral immune response, and T cells are associated with the cell-mediated immune response.

Humoral immunity involves B cells being activated and secreting specific antibodies, a special protein called immunoglobulins. The antibodies that have been secreted circulate in the bloodstream and target non-self antigens that are in the extracellular space.

In contrast, in the cell-mediated immune response, T cells target host cells that have been infected by a pathogen, such as a virus. The antigen from the virus is presented by the infected host cell to the T cell, and this instructs the T cell to induce the virus-infected cell to die. This helps prevent the virus replicating and invading more cells. T cells also assist in killing cancerous cells or cells from transplanted tissue organs (leading to transplant rejection).

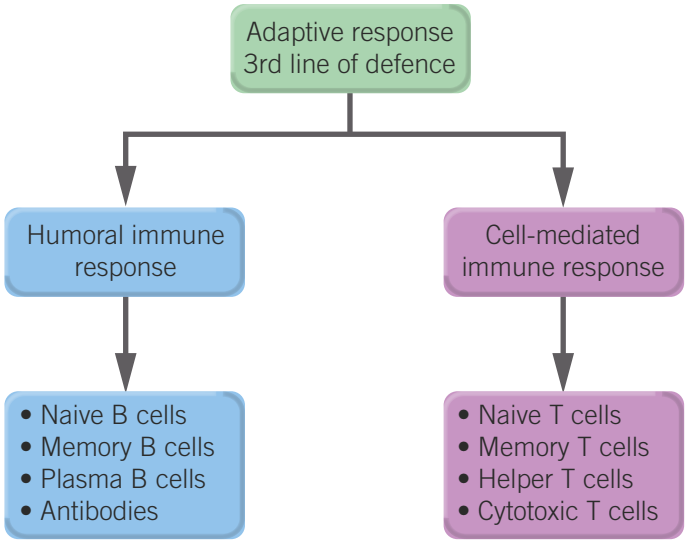


Figure 7D–4 Overview of the adaptive immune response

7B SECOND LINE OF DEFENCE LINK

The adaptive immune system does not work in isolation from the innate immune system. There is crossover between the two systems, as was mentioned in Section 7B. Helper T cells are activated by cytokines released from an APC (antigen presenting cell) and naive B cells are activated by cytokines released from a helper T cell.

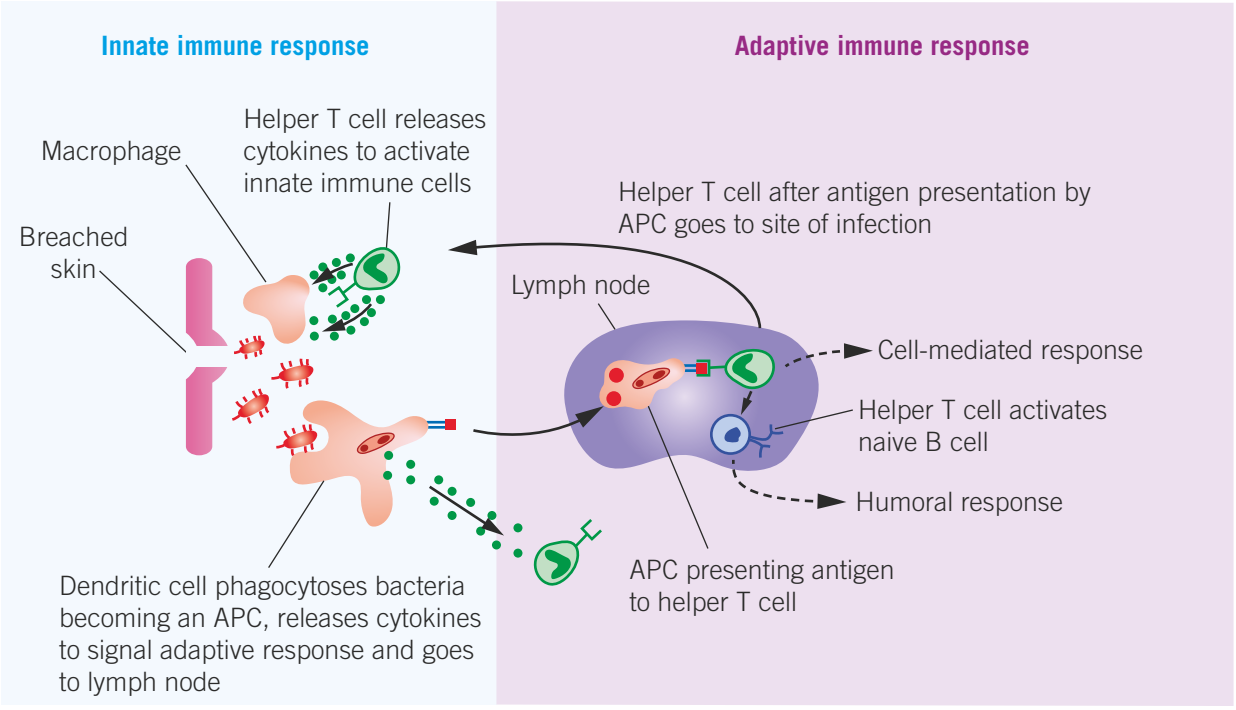


Figure 7D–5 Interaction between the innate and adaptive immune responses

Clonal selection theory the scientific theory that a specific antigen activates a specific lymphocyte that has a complementary receptor

Naive not yet activated

Antibody a protein that has a Y shape containing two identical arms with an antigen-binding site specific to a antigen (or allergen); also referred to as immuno-globulins (Ig)

Clonal expansion the proliferation of a lymphocyte that has been selected by an antigen

In the rest of this section, the humoral and cell-mediated immune responses are explored in more detail, and the connection between the innate and adaptive immune responses is demonstrated.

Check-in questions – Set 1

1 Which two immune responses make up adaptive immunity?
2 Where do B and T cells mature?
3 Which immune responses are B and T cells associated with?

Humoral immunity

The remarkable thing about the adaptive immune system is its ability to recognise and respond specifically to millions of different non-self antigens. The non-self antigen acts as the selecting agent, activating the correct naive B cell, which initiates the humoral immune response. This theory is known as **clonal selection theory** (Figure 7D–6).

Within the lymph node are millions of **naive** B cells with unique **antibody** receptors (about 10^5 antibody receptors on each B cell) that have complementary antigen-binding sites to different antigens. When a non-self antigen enters a lymph node and selects the corresponding naive B cell (assisted by a helper T cell), the naive B cell proliferates, making many clones of itself. This **clonal expansion** results in identical copies of the naive B cell that have the same genetic material and antibody receptor as the original naive B cell. The clones then differentiate into memory B and plasma B cells.

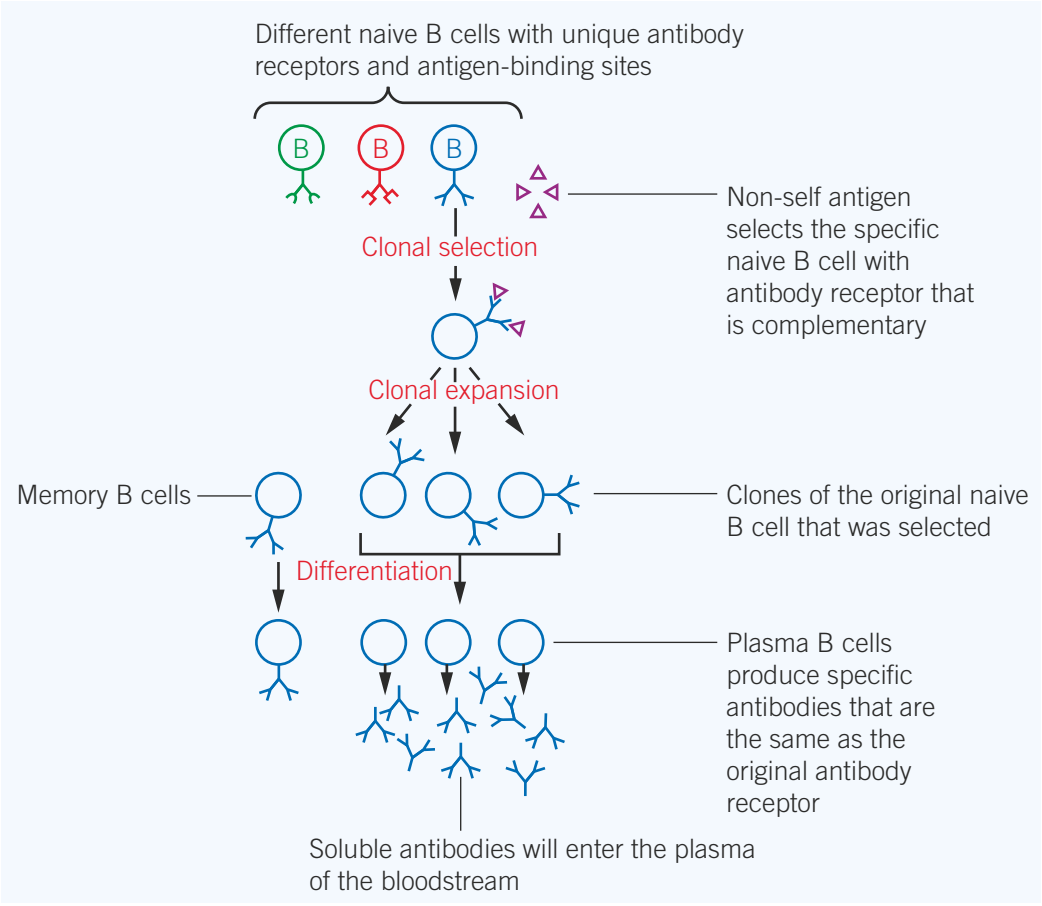


Figure 7D–6 Clonal selection and clonal expansion of a naive B cell

The plasma B cells secrete specific antibodies with the same antigen-binding site as the original cell surface antibody receptors on the original naive B cell. The specific role of plasma B cells is to produce these antibodies, which they do at a rate of 2000 a second. The specific antibodies flood the bloodstream and perform their function at the site of infection in the extracellular space.

The memory B cells have the same antibody surface receptors as the original naive B cell. Unlike the plasma cells, which survive for a few days, the memory cells can last a long time (occasionally for life). This is what leads to long-term immunity. If the same pathogen with the same antigen invades again, the memory cells still in the bloodstream will recognise it and the immune response will be much faster and stronger (Figure 7D–7).

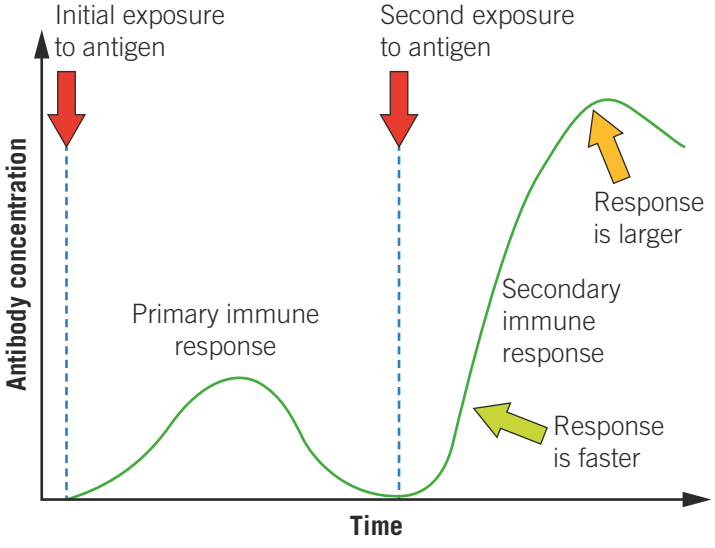


Figure 7D–7 Difference between first and second immune response to the same pathogen

Table 7D–1 Components of the humoral response

Component	Function
Naive B cells	• Have immunoglobulin (Ig)-like receptors (similar structure to antibodies) • Identify antigens through their Ig-like receptors binding to them • There are specific naive B cells for different antigens (therefore different pathogens)
Plasma B cells	• Result from naive B cell being activated and going through clonal expansion • Produce specific antibodies against antigen that activated the naive B cell • Antibodies are released into the plasma of the circulatory system
Memory B cells	• Result from naive B cell being activated and going through clonal expansion • Remain in the lymph tissue for long periods, aiding in providing long-term immunity • Can be activated to proliferate into plasma B cells if secondary exposure to the same antigen occurs, resulting in faster and stronger secondary immune response
Antibodies	• Also known as immunoglobulins (Ig), made up of protein • Produced by plasma B cells • Bind to specific antigens (function is outlined in Table 7D–2).

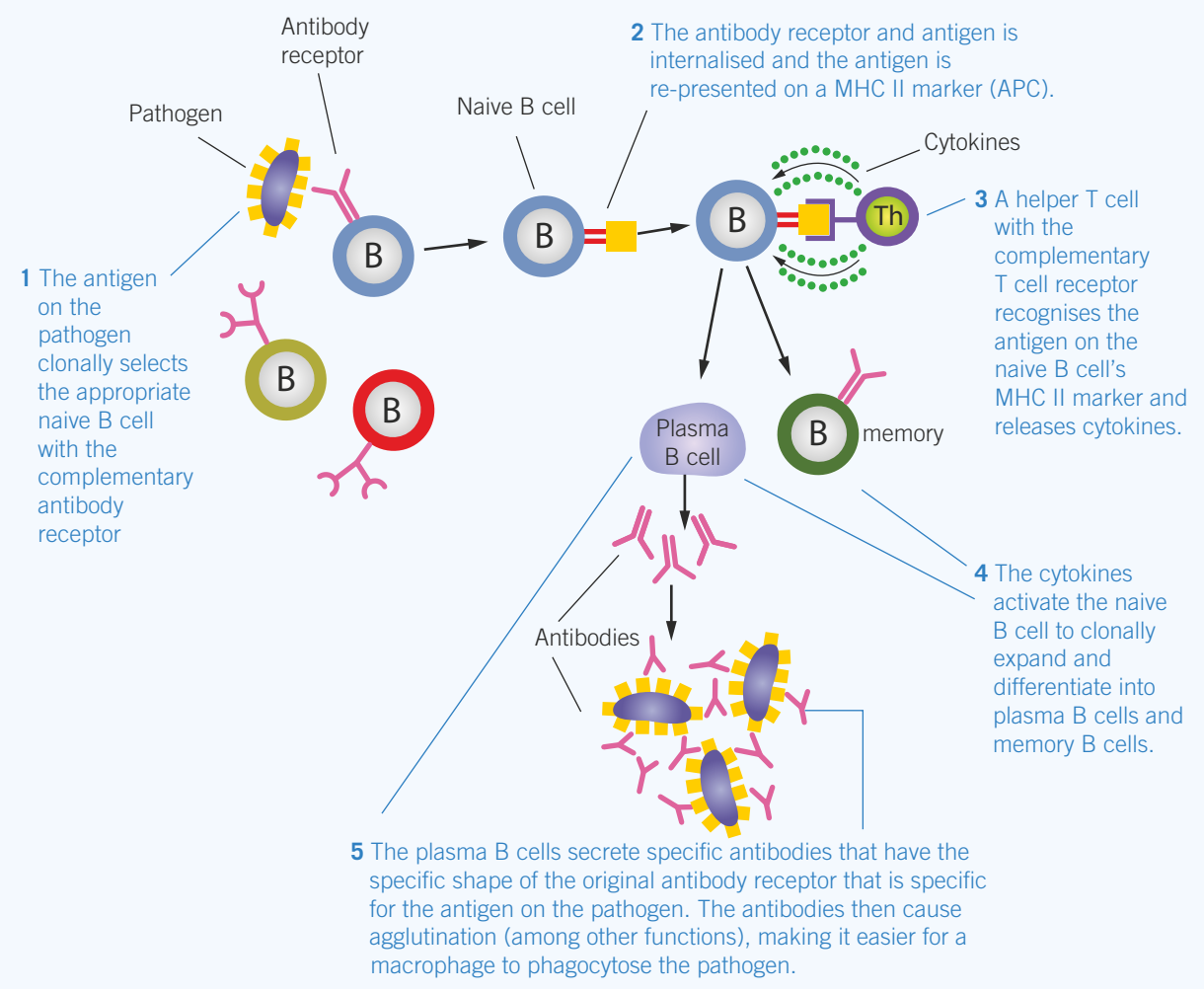


Figure 7D–8 The humoral response

Check-in questions – Set 2

1 What marker type does the naive B cell present the antigen on, to the helper T cell?
2 What chemicals does the helper T cell release to activate the naive B cell before clonal expansion can occur?
3 Define clonal selection theory.
4 Which cell produces antibodies after clonal selection has occurred?
5 Which cell(s) aid the development of long-term immunity against a pathogen?

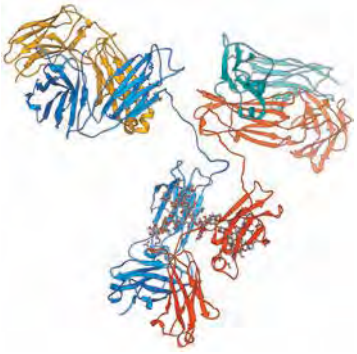
WORKSHEET 7D–1 COMPONENTS OF THE HUMORAL RESPONSE

Antibody structure

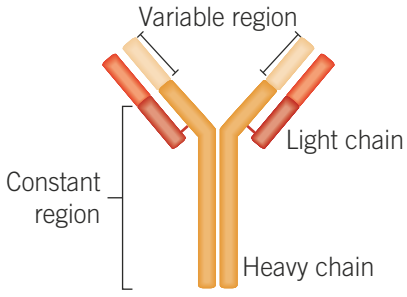
An antibody is composed of four polypeptide chains: two heavy chains and two light chains, forming a Y shape (Figure 7D–9). At the end of each arm of the Y shape is the same antigen-binding site, which is made up of both a heavy chain and light chain. The two chains that make up this binding site are known as the variable region. This is how each antibody is unique for the different naive B cells present and therefore specific to only one type of antigen.

Below the variable region is the constant region. The constant region is the same for all the antibodies and is important in functions such as activating complement proteins or binding to phagocytes (Table 7D–2). The hinge region of the antibody increases its flexibility, which improves its efficiency in antigen binding and cross-linking during agglutination (Table 7D–3).

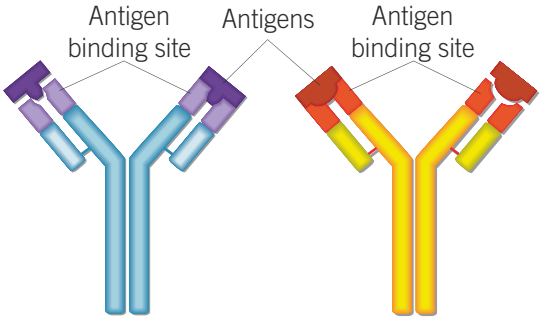
LINK 2C PROTEINS



Antibody molecular model highlighting protein structure with distinctive beta pleated sheets (shown as ribbons) and alpha helices (shown as thin strands)



Antibody with four polypeptide chains (two heavy and two light). The lighter section of the chains represents the variable region and the darker sections are the constant region.



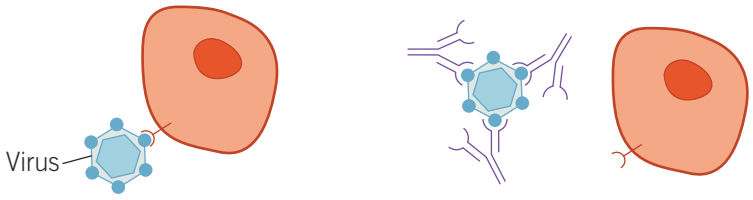
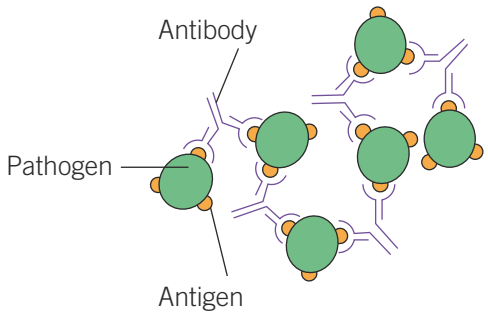
Different antibodies have different antigen-binding sites for specific antigens, but are the same on each arm of an antibody.

Figure 7D–9 Antibody structure

Table 7D–2 Antibody structure and function

Structural component	Function
Variable region	• Has a specific shape to be able to select a specific antigen • Is the antigen-binding site between the antigen and the antibody • The two binding sites are identical to each other on each arm of the Y shape
Constant region	• The same in all antibodies • Aids in recruiting other components of the immune system and acting as binding sites (e.g. with a macrophage)

Table 7D–3 Two functions of an antibody: neutralisation and agglutination

Function	Description
Neutralisation	• Blocks the action of toxins • Prevents pathogen from entering a host cell (see diagram below)  Virus is able to enter the host cell on the left, whereas on the right it has been neutralised by the antibody and is not able to enter the host cell.
Agglutination	• Grouping or clumping of pathogens together through the cross-linking of antibodies joining with the surface antigens on the pathogen • Enhances the ability of phagocytes to engulf more pathogens at once 

Agglutination where antibodies join to the pathogen's antigens, joining more than one pathogen together

Antigen-binding site of an antibody

When an antigen binds with an antibody, it does so at the end of the arms of the Y shape, as shown in outcome A of Figure 7D–10. It is a common mistake and incorrect representation to show the antigen binding at the base of the fork of the Y, as shown in part B in Figure 7D–10. Also, the antigen-binding sites on both sides of the antibody are the same, which is not the case in part C in Figure 7D–10.

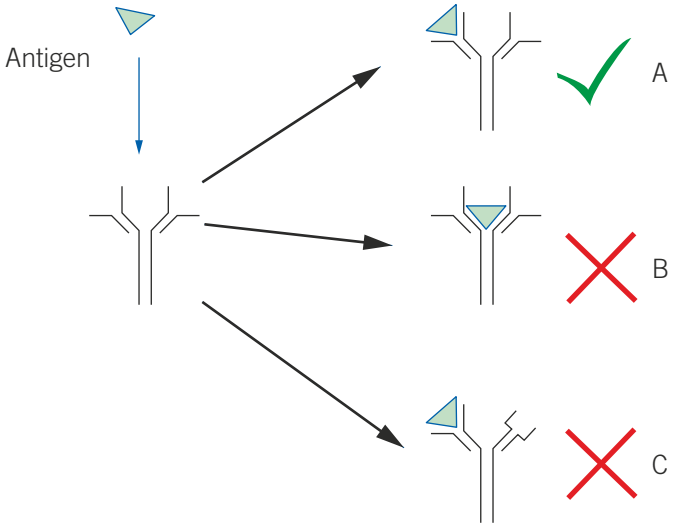


Figure 7D–10 Site of antigen binding on an antibody

Types of antibodies

There are five types of antibodies (Figure 7D–11). IgE antibodies act as receptors for mast cells during an allergic response, as outlined in Section 7B. IgG are the main antibody that assists phagocytes to perform phagocytosis. The tail end of the Y shape binds to receptors on the macrophage. This makes it easier for the macrophage to engulf the pathogen, as it has been immobilised by the antibodies (Figure 7D–12).

LINK 7B SECOND LINE OF DEFENCE

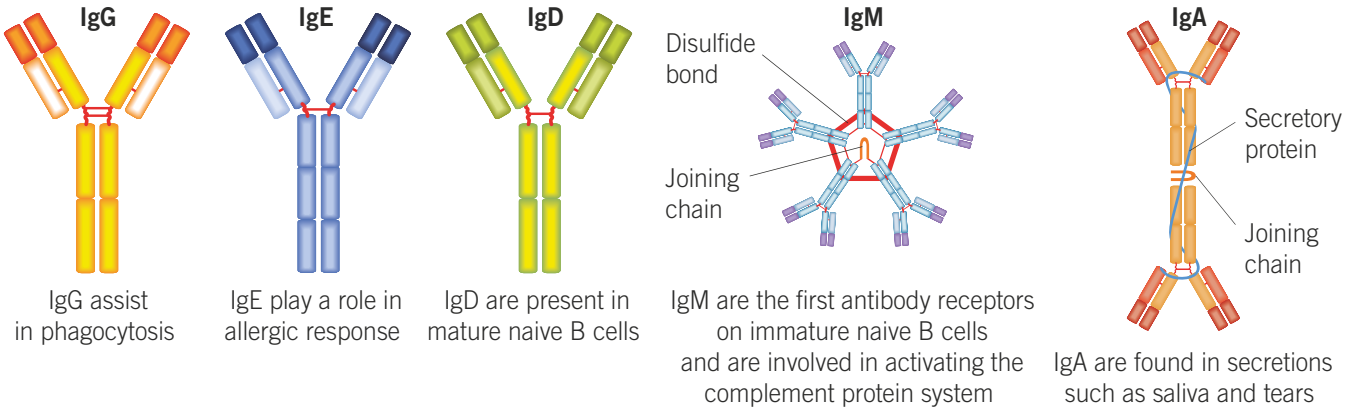


Figure 7D–11 The five types of antibodies: IgG, IgE, IgD, IgM and IgA.

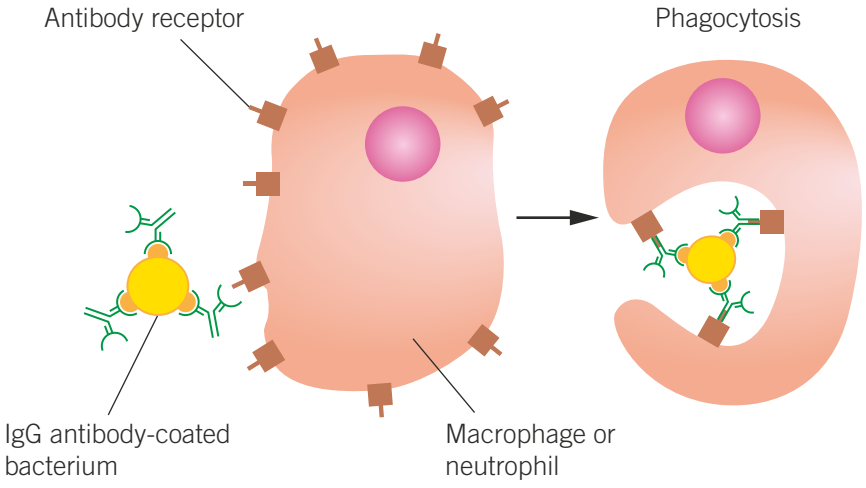


Figure 7D–12 A phagocyte engulfing a bacterium with the assistance of IgG antibodies

Check-in questions – Set 3

1 How many polypeptide chains make up an antibody, and what are the names of these chains?
2 Draw an antibody with labels indicating where the antigen-binding sites are.
3 What are two significant features of the antigen-binding sites?
4 Outline what agglutination is.

WORKSHEET 7D–2 ANTIBODY STRUCTURE AND FUNCTION

Cell-mediated immunity

Just like B cells, T cells are highly specific to non-self antigens, due to the presence of their T cell receptors (TCRs). T cells respond to antigen fragments, whereas B cells respond to whole antigens. Another difference between the cell-mediated response and the humoral response is that T cells need to be presented with the antigen fragment from an APC and respond by targeting host cells that have been infected by a pathogen, whereas in the humoral response, antibodies target extracellular pathogens (i.e. pathogens outside a host cell).

Table 7D–4 Components of the cell-mediated response

Component	Function
Naive T cells	• Have T cell receptors (TCRs) that recognise specific antigens • Unable to recognise antigens without the assistance of an APC
Helper T cells (T_h)	• With their TCR will recognise specific complementary antigens presented to them on the MHC II marker of an APC • After being activated by cytokines released from the APC, they undergo clonal expansion, producing effector T_h and memory T_h cells • Effector T_h cells release cytokines • This assists the activation of cytotoxic T cells and naive B cells • The cytokines also assist with activating macrophages and promote inflammation at the site of infection
Cytotoxic T cells (T_c)	• Directly attack pathogen-infected host cells by recognising antigen fragments presented on MHC I of the infected host cells • Cells are killed through the release of perforins, which puncture the plasma membrane of the cell, assisting the initiation of apoptosis
Memory T cells	• Remain present after the primary infection has been completed • Assists in long-term immunity, generating a faster and stronger response upon subsequent exposure to the same pathogen and antigen

7B SECOND LINE OF DEFENCE LINK

Figure 7D–13 shows the steps of how the cell mediated response is activated. These steps are also described in detail here. The numbers refer to the steps on the diagram.

1 Activation of a naive T cell requires antigen fragments from a pathogen to be presented to it by an APC. The APC can be a macrophage, dendritic cell or naive B cell. The APC comes from the site of infection (not a B cell, which is located in the lymph node with the T cells) and travels to the lymph node. The APC has phagocytosed the pathogen and presents a fragment of the antigen on its MHC II marker.

Effector cell a cell that has been activated to perform its role

2 The APC needs to find the complementary TCR on a naive T cell. Once located, the naive T cell proliferates into **effector cells**.

3 Effector cells include helper T cells (T_h) and cytotoxic T cells (T_c). Memory T cells are also produced and aid in long-term immunity, just like memory B cells from the humoral response.

4 The effector T_h activates macrophages at the site of infection to carry out further phagocytosis, and they also activate T_c cells to kill infected host cells.

5 T_c cells recognise host cells that have been infected by a pathogen through the identification of antigen fragments being presented on a MHC I marker of the infected cell. Once a T_c has recognised an infected cell via the MHC I marker, a T_c will induce the cell to undergo apoptosis in one of two ways. The first way is to release perforin. Perforin is a protein that forms pores in the cell's membrane. A further protease (an enzyme that targets proteins) within the same vesicle that contains the perforin enters the cell and triggers caspase (protein that mediates the apoptosis process). The second way is to activate a death receptor on the cell, which will then undergo apoptosis. The T_c cell kills infected host cells with the aim of killing the pathogen that is within the cell, preventing spread of the pathogen to other cells.

LINK 1C MEMBRANE TRANSPORT

6 The effector T_h also activate the naive B cell, which can go on to produce specific antibodies after going through clonal expansion and differentiation. Importantly, the naive B cell also needs to have encountered the same antigen itself and be presenting the fragment on its MHC II marker before the T_h can activate it with cytokines.

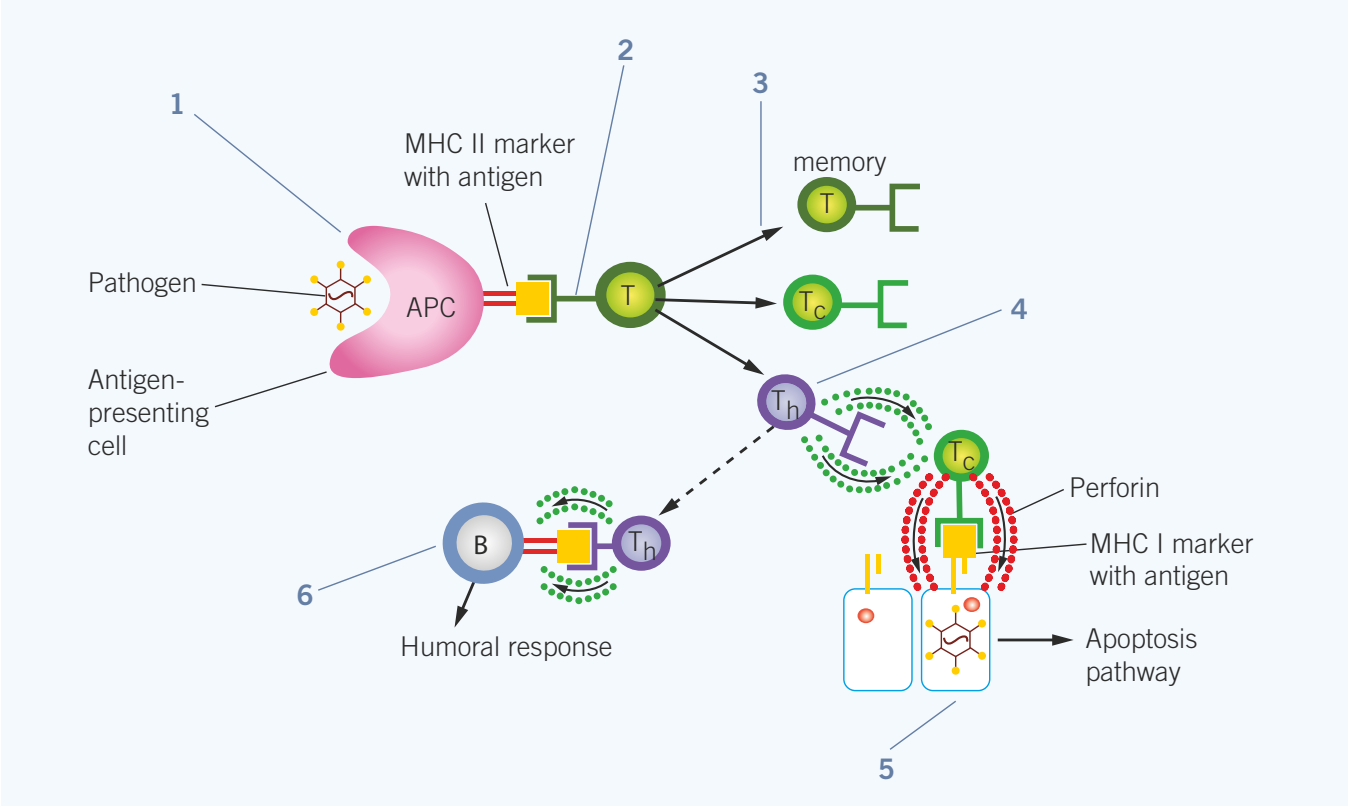


Figure 7D–13 Process of activating the cell-mediated response. Numbers refer to steps described in the text.

Check-in questions – Set 4

1 What are the two T cells other than cytotoxic T cells?
2 Describe how a naive T cell is activated.
3 What marker assists a cytotoxic T cell to recognise an infected cell?
4 What chemical does a cytotoxic T cell release to help cause apoptosis of an infected cell?

WORKSHEET 7D–3 COMPONENTS OF THE CELL MEDIATED RESPONSE

Connecting the humoral and cell-mediated immune response

Figure 7D–14 gives an overview of how the humoral response and the cell-mediated response work together.

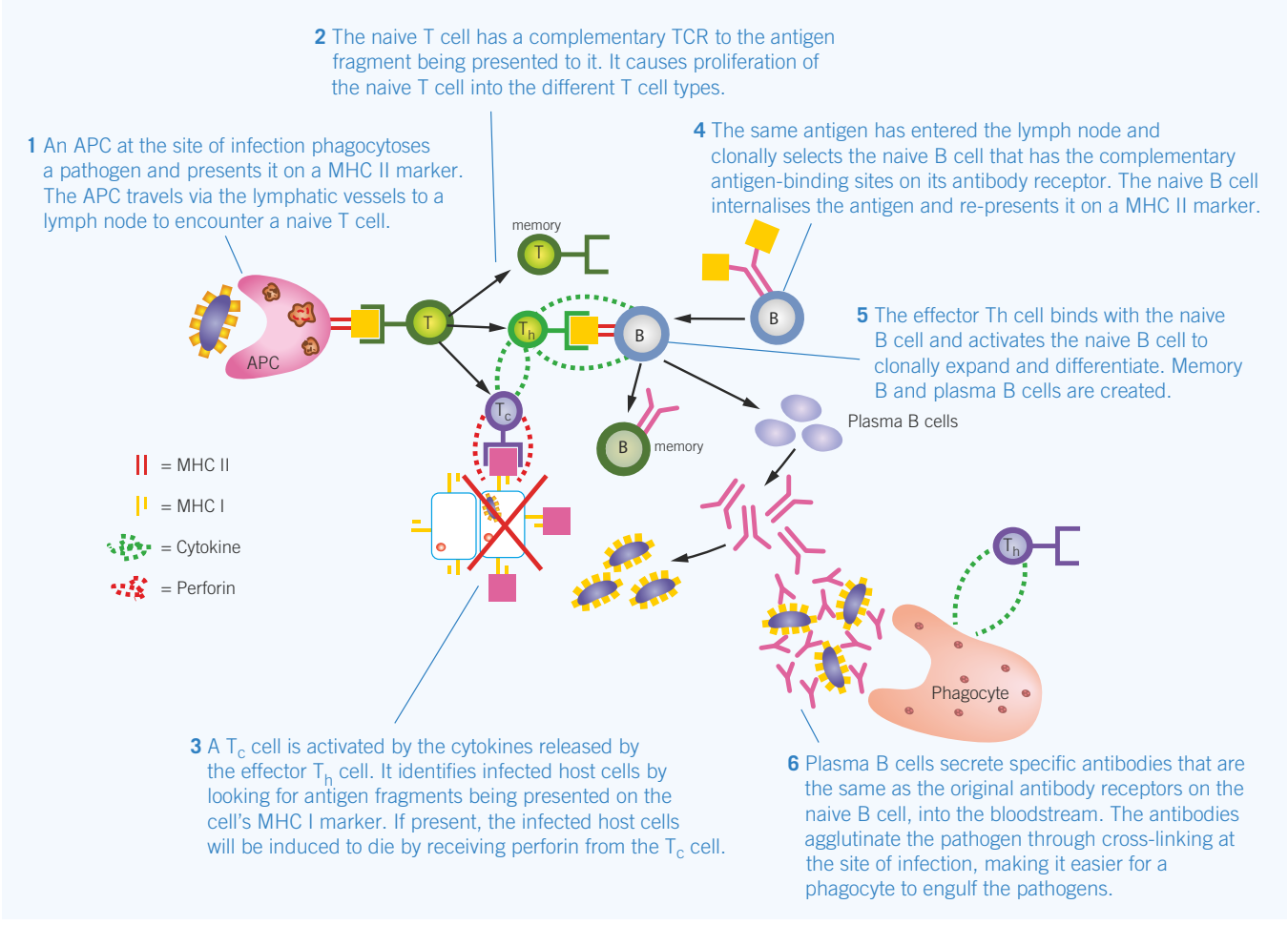


Figure 7D–14 Overview of the connection between the humoral and cell-mediated immune responses

VIDEO 7D–2
SKILLS: USING A COMPARATIVE ORGANISER

7D SKILLS

Using a comparative organiser

For the immunity component of the study design, it is important that you are able to distinguish between the different responses of the body in relation to invading pathogens. You need to be able to categorise the different immune responses and then place those components into a sequential order. You also need to be able to compare the different responses. For example, you might be required to compare a virus to a bacterial infection, a humoral response to a cell-mediated response, the second line to the third line, or physical barriers to chemical barriers.

A useful strategy is to create a simple comparative graphic organiser. This is a tool that you can use to directly identify similarities and differences. It is best to do this after you have organised your understanding using the mind map technique outlined in the 7C Skills section.

Topic		
Sub-topic	vs	Sub-topic
Similarities		
Differences		

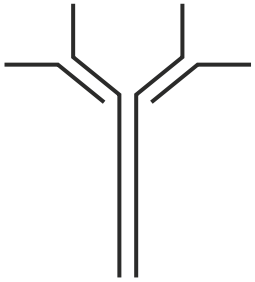
Figure 7D–15 Comparative graphic organiser to identify similarities and differences between concepts

Topic *Antigen-presenting cells*		
Sub-topic *Cell-mediated*	vs	Sub-topic *Humoral*
Similarities		
Both involve connection between the APC and a T lymphocyte.		
Differences		
A phagocyte such as a macrophage will travel to the lymph node with the antigen on a MHC II marker to activate a naive T cell.		*Antigen fragment travels to lymph node via lymphatic vessel and encounters a naive B cell, which then acts as an APC to be activated by an effector T cell.*

Figure 7D–16 A simple example of how the graphic organiser can be used. It is important to continue to match differences directly at the same point with each other. This is to ensure that you are making direct comparisons.

Section 7D questions

1 What are three examples of antigen-presenting cells?
2 List the cell components of the humoral immune response.
3 Which cell from the cell-mediated immune response needs to assist the activation of a naive B cell?
4 Outline what needs to happen to the naive B cell before it can be activated by the cell from the cell-mediated immune response.
5 Explain what a B plasma cell does.
6 Draw an antigen that would be complementary to the antibody shown on the right.
7 Explain how agglutination assists with phagocytosis.
8 Describe how a cytotoxic T cell induces apoptosis of an infected cell.
9 What are two differences between the humoral and cell-mediated immune responses?
10 List the steps that would occur during an adaptive immune response to a viral infection.



Active and passive immunity

Study Design:
- The difference between natural and artificial immunity and active and passive strategies for acquiring immunity
- Vaccination programs and their role in maintaining herd immunity for a specific disease in a human population

Glossary:
Active immunity
Attenuated
Herd immunity
Passive immunity
Vaccination
Vaccine

ENGAGE

An attempt to gain herd immunity

COVID-19 was first detected in the city of Wuhan in China around 30 December 2019. On 11 March 2020, the World Health Organization declared COVID-19 a pandemic; this was due to the global spread of the disease – 118 000 cases over 110 countries, at the time of the announcement. By April 2021, Australia had recorded 30 000 cases with over 900 deaths, and worldwide there had been more than 132 million cases with more than 2.86 million deaths.

This novel virus has caused governments around the world to implement a wide range of strategies to keep people safe and reduce the number of cases. One country, Sweden, took a very different course of action, with the intention of generating herd immunity within the population as a tool to battle the virus. This was in contrast to the more common strategy of lockdowns implemented elsewhere. The reasoning behind the herd immunity approach is that, if there were to be a second wave of the virus, a large proportion of the population would already be immune and therefore the virus would not spread. Time will tell, as the world looks back and reflects on which strategies have been most effective in reducing the impact of COVID-19.

EXPLAIN

Acquiring immunity

7D THIRD LINE OF DEFENCE LINK

Section 7D explored how the adaptive immune response results in immunity through the establishment of memory B and T cells. This section examines two ways of acquiring immunity: active and passive. Each of these has a natural and an artificial way of acquiring immunity. The terms 'natural' and 'artificial' have particular connotations – that natural is organic and therefore better for you, and artificial is chemically engineered or genetically modified, and causes harm. This is not the case. A key skill for you is to be able to identify the differences between the ways of acquiring immunity as either active or passive.

Active immunity when an individual's adaptive immune response is activated

Passive immunity short-term immunity resulting from a person receiving antibodies from another person or animal; no memory

Types of immunity

Active immunity occurs when an individual's adaptive immune system responds to an antigen, producing not only specific antibodies but also memory B and T cells. The individual gains immunological memory, which can last a lifetime.

When an individual is provided with antibodies that have been made by another organism, this results in **passive immunity**. This type of immunity is immediate but does not result in any long-term memory of the pathogen that causes a disease and therefore no immunological memory. This is because the individual's adaptive immune response has not been activated, and hence they have no specific antibodies or memory B and T cells.

Both the active and passive forms of immunity can be induced by natural or artificial means. This is summarised in Table 7E–1 and Figure 7E–1.

Table 7E–1 Active and passive forms of immunity

	Active immunity	Passive immunity
Natural means	 *Natural active immunity* is an individual's response to a pathogen (through production of specific antibodies) that they have been exposed to. If the individual were to be exposed to the same pathogen in the future, they would be immune to that pathogen due to the presence of memory B and T cells.	 *Natural passive immunity* is the transfer of antibodies from a mother to the foetus through the placenta prior to birth or to the baby through breastfeeding after birth. The maternal antibodies provide immune protection for the baby, whose immune system is still developing.
Artificial means	 *Artificial active immunity* is created when antigens or whole pathogens are deliberately introduced to induce an adaptive immune response (production of specific antibodies and memory B and T cells) through a vaccination, for example.	 *Artificial passive immunity* is created when an individual receives antibodies made by another organism. This is useful against things such as bites and stings that could cause death before an individual's own adaptive immune response could act.

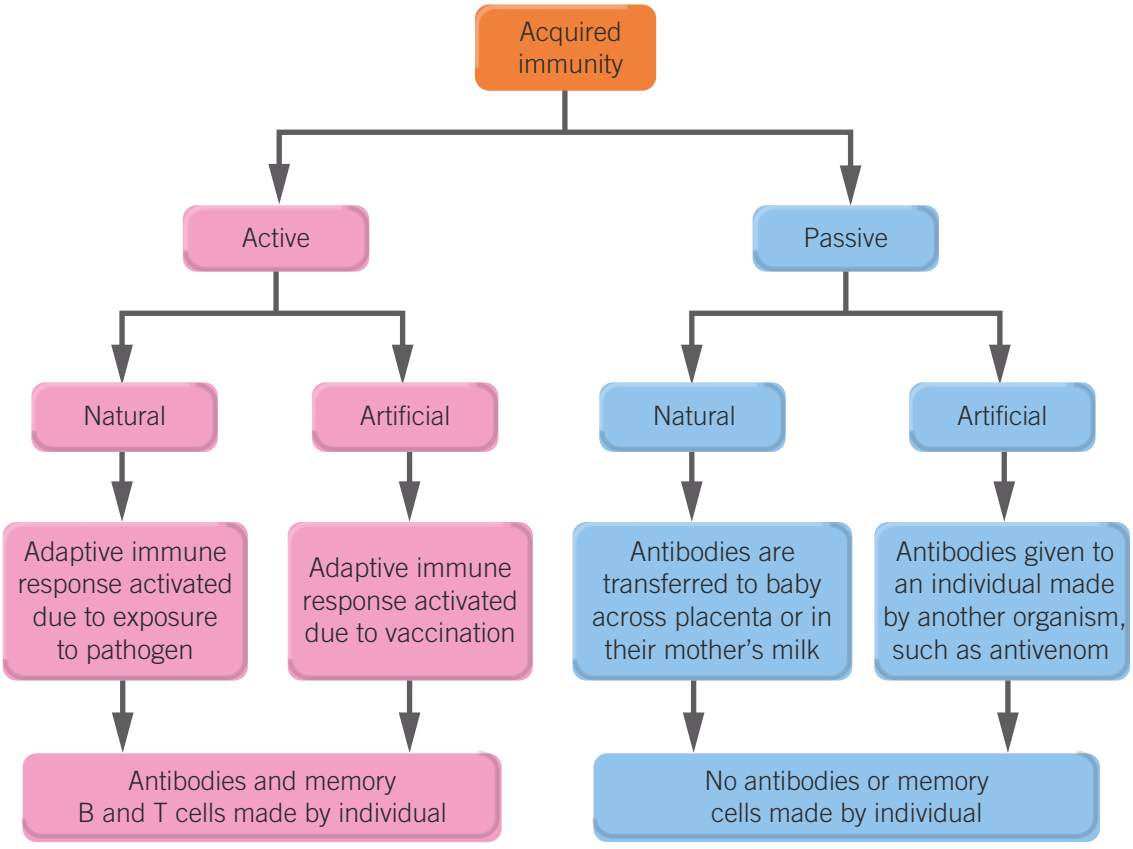


Figure 7E–1 Summary of acquiring immunity

Check-in question – Set 1

1 List the four ways in which individuals can acquire immunity.
2 Which strategies lead to long-term immunity?
3 If someone were to be vaccinated against COVID-19, which type of strategy for acquiring immunity would this be?

Vaccination programs and herd immunity

Vaccination the administration of a vaccine to cause an adaptive immune response

Vaccine substance that contains an agent (usually an antigen or an attenuated version of the pathogen) that will induce an adaptive immune response when administered

Attenuated describes a pathogen that has been altered to remove its harmfulness

Vaccinations involve the use of a **vaccine** to induce artificial active immunity. To induce an adaptive immune response, the vaccine needs to be specific to the pathogen. Three types of vaccines can be used to do this: live attenuated, inactivated and subunit.

A live **attenuated** vaccine contains a weakened form of the pathogen that causes the disease the vaccine is intended to give immunity against. Because of the similarity between the vaccine and the natural pathogen, there is a strong adaptive immune response. This can result in lifetime protection against that pathogen. There is a limitation with this type of vaccine: a person who has a weakened immune system (e.g. elderly, babies, immune-deficient individuals, cancer patients) may contract the disease, and so people in this group need to speak with their health-care provider before considering this type of vaccine. Some examples of live attenuated vaccines are those against measles, mumps, rubella (MMR combined vaccine); smallpox; and chickenpox.

An inactivated vaccine uses a killed version of the pathogen that causes the disease. (Note that technically viruses are not living so it is better to talk about them being either viable or inactivated.) Nothing in the inactivated vaccine is harmful, so it is safe for people with weakened immune systems to receive. A disadvantage of this type of vaccine is that there is no lifelong immunity, so booster vaccinations are required to maintain long-term immunity, by increasing the number of memory B and T cells. Examples of inactivated vaccines are those against hepatitis A and influenza.

A subunit vaccine uses specific pieces of the pathogen, such as parts of antigens, whole antigens, or a capsid (protein coat of viruses). As with inactivated vaccines, people with weakened immune systems can safely be adminstered a subunit vaccine, because it does not contain any viable components of the pathogen. It has the same disadvantage as an inactivated vaccine – booster injections are required to maintain the required levels of memory B and T cells. Examples of subunit vaccines are those against hepatitis B, human papillomavirus and whooping cough.

Herd immunity

Herd immunity when a large percentage of a population is immune to a disease (through vaccination), slowing the spread of the disease and protecting those who are not immune

Herd immunity exists when a large percentage of the population has been immunised against a contagious disease. This is vital for any immunisation program to be successful. Herd immunity indirectly provides protection to individuals in the community who are not immune. This includes those with suppressed or weakened immune systems, people with immune diseases, babies, the elderly and individuals taking immunosuppressant medications. Herd immunity can be likened to trying to find a needle in a haystack: if there is widespread immunity in a population (that is, herd immunity), then there is very little chance that an infected person will encounter someone who is not immune, and so the pathogen has very little opportunity to invade a new host, and the disease is not able to spread (Figure 7E–2).

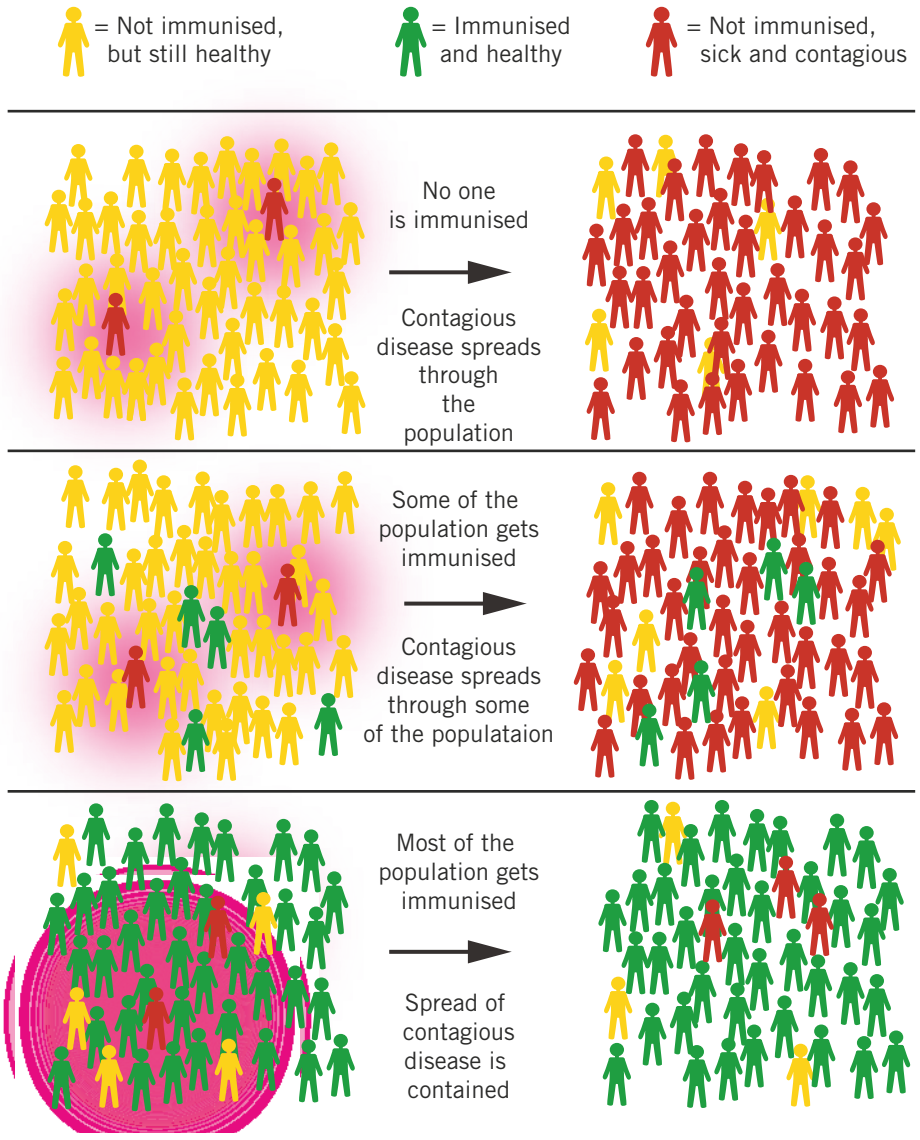


Figure 7E–2 How herd immunity reduces the spread of a disease

7E SKILLS

Picture representation: dual coding

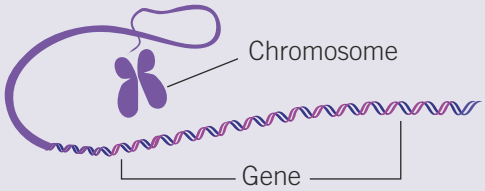
Many of the Skills sections in this chapter focus on how to increase your capacity to memorise and organise information. A further strategy to increase your memory is dual coding.

Dual coding means making an association between written information and an image. Using images helps to build your understanding of a concept while also retrieving information about the concept. When you are making flip cards, draw an image on one side and write the definition on the other side. When building a mind map, instead of using words you can use images or diagrams to highlight connections between concepts.

The images you use can be abstract or concrete. An abstract image does not have complete connection with the concept being covered, whereas a concrete image is an actual (realistic) representation of the concept or term. Table 7E–2 is an example of dual coding, where the images can help you remember and understand a concept.

VIDEO 7E–1
SKILLS: PICTURE REPRESENTATION: DUAL CODING

Table 7E–2 Example of dual-coding images for the concept 'gene'

	Abstract image	Concrete image
Image		
Concept	A pair of jeans represents a gene.	The image shows a gene in relation to a section of DNA and a chromosome.

In the example in Table 7E–2, the abstract image is more of a stimulus to think and remember an association that has already been made. By contrast, the concrete image can be helpful in developing an understanding of the concept being covered.

The images you saw in Table 7E–1 are another example of using an image as a stimulus to remember a concept.

	Active immunity	Passive immunity
Natural means		
Artificial means		

Have a go at dual coding. As suggested already, you can use images in your flip cards or mind maps, as well as in your class notes and chapter summary notes.

Section 7E questions

1 Outline the difference between active and passive immunity.
2 Provide an example of passive natural immunity.
3 Explain how active immunity leads to long-term immunity.
4 What is a vaccine?
5 Outline an advantage and a disadvantage of using live attenuated vaccines.
6 What are the other two forms of vaccines?
7 Who is protected by herd immunity?
8 Draw a graph to show the number of antibodies after a first vaccination and a booster vaccination.

Chapter 7 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
7A.1	Recall and define terms to do with the first line of defence	11a☐
7A.2	Describe what physical, chemical and microbiota barriers are and recall that they make up the first line of defence	1☐
7A.3	Provide examples of physical and chemical barriers in plants and animals	2☐, 12☐
7A.4	Identify where examples of physical, chemical and microbiota barriers would be on a body	11a☐
7A.5	Compare pathogenic bacteria to natural flora	11b☐
7A.6	Explain the role that natural flora have for animals as a a microbiota barrier and part of the first line of defence	11b☐
7A.7	Explain how examples of physical and chemical barriers work	11b☐
7B.1	Recall and define terms that relate to the specialised cells, molecules and physiological responses of the second line of defence	13☐
7B.2	Identify and describe the function of the different specialised cells and molecules of the second line of defence	13c☐, 14a☐
7B.3	Compare the functions of the different specialised cells and molecules of the second line of defence	14b☐
7B.4	Explain the function of the specialised cells and molecules of the second line of defence	14b☐
7B.5	Describe the steps of phagocytosis	3☐, 15f☐
7B.6	Link different chemicals to specialised cell types, in terms of what chemical is released from what cell and what chemical the cells respond to.	15g☐
7B.7	Identify the different components of the inflammatory response	14e☐
7B.8	Apply the role of the different specialised cells and molecules in the context of an inflammatory response	14e☐
7B.9	Explain the role of the different components of the inflammatory response	4☐, 14e☐
7C.1	Recall and define the terms associated with the lymphatic system	14c☐
7C.2	Describe the function of the different sections of the lymphatic system: lymphatic capillary, lymph node and lymphatic vessel	6☐
7C.3	Describe and explain the function of the lymph node	14d☐

Success criteria – I am now able to:		Linked question
7C.4	Describe and explain the role of the lymphatic system in connecting the second and third lines of defence	14c☐
7D.1	Recall and define the terms associated with the third line of defence	15a☐
7D.2	Outline the functions of the different components of the cell-mediated and humoral immune responses	5☐
7D.3	Describe the process of the cell-mediated and humoral immune responses	7☐, 15b☐, d☐
7D.4	Compare and contrast the cell-mediated and humoral immune responses	8☐
7D.5	Compare the adaptive immune response to the innate immune response	16☐
7D.6	Explain how B and T lymphocytes are activated	15b☐
7D.7	Draw appropriate antibodies for a given antigen and label the different components of an antibody	15e☐
7D.8	Explain the different roles of antibodies and how they assist with the adaptive immune response	15c☐
7E.1	Recall and define the different terms associated with active and passive immunity	10☐
7E.2	Provide examples of natural active, artificial active, natural passive and artificial passive forms of acquiring immunity	17a☐
7E.3	Compare the different forms of acquiring immunity	9☐
7E.4	Explain the benefits of both passive and active forms of acquiring immunity	17b☐
7E.5	Describe and compare the different forms of vaccines	17c☐
7E.6	Define and explain the importance of herd immunity	17d☐

Multiple-choice questions

1 How does the stomach help to defend the body against pathogens?
- **A** It contains hairs, which trap pathogens.
- **B** It secretes antibodies, which kill pathogens.
- **C** It secretes hydrochloric acid, which kills pathogens.
- **D** It has muscular walls, which contract and churn the pathogens.

2 Which of the following is a chemical defence for a plant?
- **A** waxy cuticle
- **B** thorns
- **C** bark
- **D** toxins

3 Phagocytosis is the process in which phagocytes
- **A** release antibodies.
- **B** release antitoxins.
- **C** engulf and digest.
- **D** form a vesicle around the antigen.

4 What is redness a result of during the inflammatory response?
- **A** having a high temperature
- **B** the fluid escaping the capillaries and flooding the site of inflammation
- **C** feeling the pain as a result of the inflammation
- **D** increased blood flow as a result of vasodilation

5 In what location does a T lymphocyte mature?
- **A** thymus
- **B** bone marrow
- **C** lymph node
- **D** bloodstream

6 Which is the best description of the role of the lymph node?
- **A** It is a close-ended tube that carries interstitial fluid.
- **B** It is the site where lymph is filtered.
- **C** It assists in sending signals as part of the inflammatory response to cause vasodilation.
- **D** It is where macrophages and other phagocytes encounter pathogens.

7 Which receptor of a compromised host cell does a non-self antigen need to be presented on, for cytotoxic T cells to recognise it?
- **A** TCR
- **B** MHC I
- **C** MHC II
- **D** antibody

8 What type of adaptive immune response would bacteria in the interstitial fluid cause?
- **A** inflammatory response
- **B** cell-mediated response
- **C** humoral response
- **D** allergic reaction

9 The difference between active and passive immunity is that active immunity
- **A** exists when an individual receives antibodies from an organism that has produced them.
- **B** exists when an individual activates their adaptive immune response to produce antibodies.
- **C** exists when the vaccine provided has been made with organic ingredients.
- **D** ensures that a population will experience herd immunity to help protect those who are unable to get a vaccine.

10 Passive immunity
- **A** only exists when you passively acquire immunity.
- **B** can induce an immune response; for example, when a vaccine is given.
- **C** does not provide long-term immunological memory.
- **D** is created when an individual fights off an infection on their own and becomes immune.

Short-answer questions

11 Sauerkraut and kimchi are two examples of probiotics (microbiota) in food. People who consume these foods claim that it helps with their digestive health.

a What line of defence would the consumption of kimchi or sauerkraut assist? (1 mark)

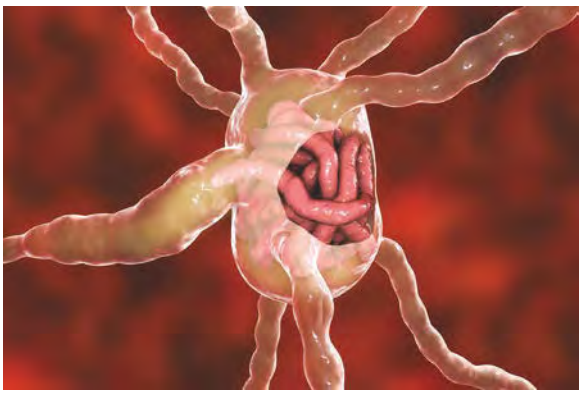
b Explain how eating these foods could potentially protect an individual against getting ill. (2 marks)

12 Annotate the diagram below, or draw your own diagram like this, with two examples of both physical and chemical defences against pathogens. ($4 \times \frac{1}{2} = 2$ marks)

13 Add the missing entries to the shaded cells. (5 marks, 1 for each missing entry)

Type of cell	Name of cell type	Function
Inflammatory		Involved in the inflammatory response through the release of histamine
	Macrophage	
	Neutrophil	Engulfs pathogen via phagocytosis Releases defensins that are toxic to bacteria and fungi
		Engulfs pathogen via phagocytosis Breaks down pathogen and retains antigen Acts as an antigen-presenting cell
Cytotoxic		Destroys virally infected or compromised host cells and cancerous cells Releases cytotoxic chemicals (such as perforin) that punch holes in plasma membrane and trigger apoptosis
	Eosinophil	Contains granules that target parasites Secretes cytotoxic chemicals

14 Your friend returned from a trip overseas with swelling in their legs that did not go away. They thought it was due to their lack of walking and stretching during the flight. However, it turned out they had been infected by filarial worms which are parasites spread by mosquito vectors, and the worms were blocking their lymph nodes.



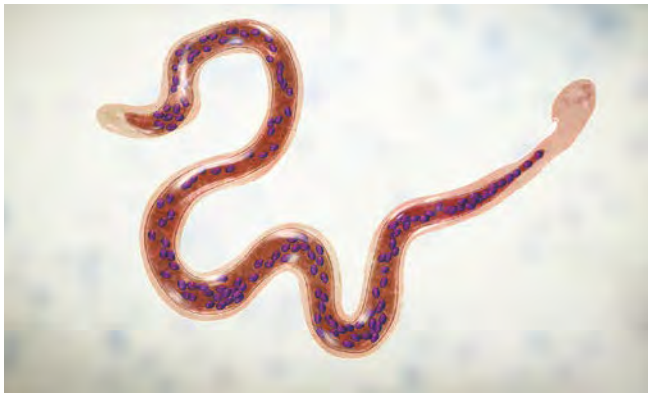
a What type of specific second line cell type would you expect to see high counts of as a result of the worms? (1 mark)

b Compare the function of the cell you identified in part **a** with the role of mast cells. (2 marks)

c Explain how the filarial worm managed to breach the first line of defence and make its way into your friend's lymph node. Be sure to include what vessels it would have travelled through to end up in the lymph node. (3 marks)

d Describe the function of the lymph node. (1 mark)

e Swelling can also occur at the site of the mosquito bite, where there is a raised bump. Outline why there would be isolated swelling at the site of the mosquito bite. (3 marks)



15 Antibodies are a key part of the adaptive immune response.

a What cell is responsible for releasing antibodies? (1 mark)

b Describe the steps of how the cell you identified in part **a** is activated. (3 marks)

c Explain why an antibody against COVID-19 would not be effective against a different type of virus. (2 marks)

d If the COVID-19 virus has invaded a host cell, explain what type of adaptive immune response would occur. (2 marks)

e Draw and label an antibody if the COVID-19 antigen was square in shape. (2 marks)

f Once antibodies against COVID-19 have been released, describe the connection between the function of the antibodies and the role of a macrophage. (3 marks)

g What chemical would a macrophage release and what would be attracted? (2 marks)

16 The immune system can be split into the innate and adaptive immune responses.

a What lines of defence are associated with both the innate and adaptive immune responses? (1 mark)

b Identify and explain a key difference between the innate and adaptive immune responses. (3 marks)

17 By the end of 2020 there were over 100 different vaccines in development, in the fight against COVID-19. The process of developing a vaccine usually takes a long time – for example, 15 years for the human papillomavirus vaccine to be developed.

a What type of immunity does a vaccine provide? (1 mark)

b What is the benefit of inducing this type of immunity? (1 mark)

c Suggest what type of vaccine you would propose to use, and justify the benefit. (2 marks)

d Vaccination programs are important for establishing herd immunity. Define herd immunity. (1 mark)

UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES?

CHAPTER 8

EMERGENCE AND TREATMENT OF NEW DISEASES

Introduction

The scope of this chapter is the emergence and control of new infectious diseases, and the use of the immune system in the treatment of autoimmune diseases and cancer.

If a pathogen gets through the first line of defence, it can cause disease. Over time, new diseases have emerged, and continue to emerge to this day, with COVID-19 an example. Not only do new diseases arise, but old diseases can re-emerge if the pathogen develops resistance to medications or if vaccination lapses.

It is important for governments and individuals to do their part in preventing a pathogen from spreading between individuals within the community. In order to put effective control measures in place, epidemiologists need to understand the infectious cycle of a disease. Considerations such as how transmission occurs, whether there are vectors involved and where the disease comes from all help the formulation of a plan to fight the disease.

A new strategy in fighting disease involves monoclonal antibodies. This strategy begins with the natural process of how the immune system works and manipulates it to our advantage, to create treatments. The ability to tailor treatments for cancers and autoimmune diseases is the future of medical research.

Curriculum

Area of Study 1 Outcome 1
Disease challenges and strategies

Study Design	Learning intentions – at the end of this chapter I will be able to:
• The emergence of new pathogens and re-emergence of known pathogens in a globally connected world, including the impact of European arrival on Aboriginal and Torres Strait Islander peoples • Scientific and social strategies employed to identify and control the spread of pathogens, including identification of the pathogen and host, modes of transmission and measures to control transmission	**8A Emergence, re-emergence and disease containment** **8A.1** Recall and define the terms associated with emerging and re-emerging diseases, and preventing the spread of pathogens **8A.2** Compare emerging and re-emerging infectious diseases with examples of the impact of European arrival on Aboriginal and Torres Strait Islander peoples **8A.3** Explain why diseases emerge or re-emerge **8A.4** Identify different scientific and social measures to control and prevent the spread of a pathogen **8A.5** Understand the infectious disease cycle as a relationship between pathogen, host and forms of transmission **8A.6** Understand, compare and explain the different types of transmission
• The development of immunotherapy strategies, including the use of monoclonal antibodies for the treatment of autoimmune diseases and cancer	**8B Treatment of disease** **8B.1** Recall and define the terms associated with immunotherapy and monoclonal antibodies **8B.2** Describe and explain the function of monoclonal antibodies **8B.3** Compare the different types of monoclonal antibodies **8B.4** Draw the different types of monoclonal antibodies **8B.5** Outline and explain the benefits of monoclonal antibodies

Glossary

Bispecific monoclonal antibody
Conjugated monoclonal antibody
Contagious
Disease
Emerging infectious disease
Epidemiologist
Fatality
Hybridoma
Immunotherapy
Indirect transmission
Infection
Infectious
Monoclonal antibody
Myeloma cell
Re-emerging infectious disease
Reservoir
Selection pressure
Transmission
Virulence

Concept map

Infectious disease

Understanding the infectious cycle of new diseases and how transmission occurs is the key

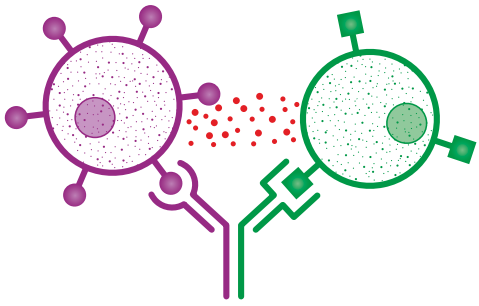
8A Emergence, re-emergence and disease containment

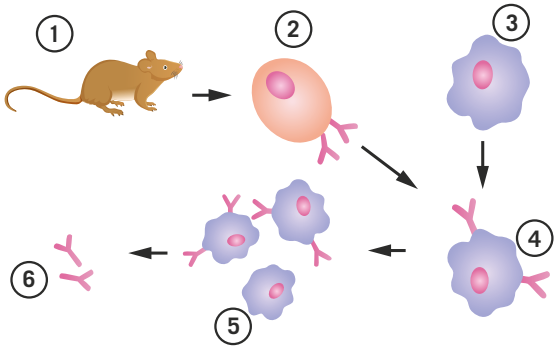
New pathogens and the re-emergence of known pathogens, and scientific and social strategies to control the spread

Prevent the spread

8B Treatment of disease

Use of the immune system in the treatment of cancer and autoimmune diseases





See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

Emergence, re-emergence and disease containment

Study Design:

- The emergence of new pathogens and re-emergence of known pathogens in a globally connected world, including the impact of European arrival on Aboriginal and Torres Strait Islander peoples
- Scientific and social strategies employed to identify and control the spread of pathogens, including identification of the pathogen and host, modes of transmission and measures to control transmission

Glossary:

Contagious
Disease
Emerging infectious disease
Epidemiologist
Fatality
Indirect transmission
Infection
Infectious
Re-emerging infectious disease
Reservoir
Selection pressure
Transmission
Virulence

ENGAGE

2020: the year that epidemiology became famous and controversial

At the height of the COVID-19 pandemic, medical officers' and politicians' daily press conferences announced controls. Harsh measures at times polarised the community. Epidemiology featured in everyday conversations. Experts were sometimes divided on the best strategies, while a minority of non-experts spread misinformation and refused to cooperate. At times, science was politicised, arguments raged, and social consequences proliferated in ways rarely experienced in modern times. In 2021, the largest-ever vaccine program in Australia began, but the controversies continued, demonstrating that the social aspects of disease control need as much attention as the scientific ones.

EXPLAIN

Europeans brought disease

In 1770, Lieutenant James Cook mapped the eastern coast of Australia. The First Fleet followed, arriving in Sydney in 1788. The most immediate consequence of colonisation by Europeans was a wave of epidemic diseases including smallpox, typhoid, pneumonia, whooping cough, venereal diseases, measles and influenza, to name a few.

Many of the European colonist populations had resistance to diseases like measles, bronchitis and scarlet fever, as they had already been exposed to the responsible pathogens before arriving in Australia. However, these diseases spread rapidly through, and annihilated, many Aboriginal and Torres Strait Islander communities, who had never been exposed before.

Figure 8A–1 Captain James Cook and the monument to his landing, Duncombe Bay, Norfolk Island

Figure 8A–2 Traditional Aboriginal foods

It is estimated that, within 15 months of the First Fleet's arrival, around half of the Aboriginal peoples living in the Sydney area had died from smallpox. Even before Europeans began arriving in the Melbourne area, smallpox had spread southwards from Sydney and had killed around a third of the eastern Aboriginal peoples.

The spread of smallpox was followed by influenza, measles, tuberculosis and venereal diseases, which Aboriginal peoples had no resistance to, and which brought widespread death.

In addition, European invasion had adverse consequences for the health of Aboriginal and Torres Strait Islander communities through alterations to their diet and lifestyle. These factors can have a negative impact on the functioning of the immune system and lead to an increase in disease.

- *Diet*: access to traditional foods was restricted (due to fencing of lands, farming of sheep and cattle, and hunting of native animals), rationing was imposed (low quality and low quantity of foods), and many Aboriginal and Torres Strait Islander peoples were forced to adopt a European style of diet (high sugar and flour based).
- *Lifestyle*: nomadic life morphed into a more stationary lifestyle. Instead of moving in small groups, managing their impact on the land, more Aboriginal and Torres Strait Islander peoples lived in one place, which increased exposure to and spread of pathogens. Some pathogens were fatal, some caused a decline in health, and others affected fertility.

This history of Europeans colonising Australia is an example of emerging disease and the dramatic impact it can have on communities. Note that the course only covers diseases spread by pathogens, so mental health issues and diseases of lifestyle, such as diabetes, are not included. However, ill health from such causes can lead to reduced immune system function and hence susceptibility to pathogens.

Disease
any condition that affects the normal function of either a part of an organism or the complete organism

Infection
when a pathogen has breached the first line of defence and begun to replicate

Getting the terms straight

CHAPTER 6 LINK

CHAPTER 7 LINK

Before this chapter explores how disease emerges or re-emerges, it is important to understand some key terms. Chapter 6 explored what pathogens are, and Chapter 7 covered how the human body and plants deal with pathogens. To recap: pathogens have the capacity to cause disease. A **disease** is not to be confused with an **infection**. An infection occurs when a pathogen has breached the first line of defence and has started to replicate within the host. In contrast, a disease is a consequence of the pathogen's invasion causing damage and having an impact on the normal function of the body's tissues.

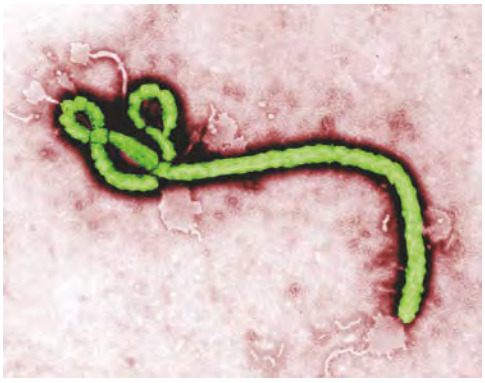


Figure 8A–3 Transmission electron micrograph of an Ebola virus. Ebola causes fever followed by multi-organ system failure.

Two more associated terms are **virulence** and **contagious**. Virulence is the likelihood that a pathogen, once it is in the body, will cause disease and harm to the host. A contagious pathogen is one that is able to spread between individuals. For example, the polio virus has low virulence (most people who catch the disease experience flu-like symptoms; paralysis occurs in fewer than 1% of cases), but it is highly contagious (it is easy to catch the virus from an infected person). Ebola, on the other hand, has a 50–90% **fatality** rate (high virulence) but it is more difficult to catch the virus (less contagious).

Virulence
how likely a pathogen is to cause harm/disease

Contagious
describes a pathogen that is able to spread from an infected person to others

Fatality
the occurrence of death

Professionals who study and track the emergence and re-emergence of diseases are called **epidemiologists**. In 2021, the Australian Chief Medical Officer, Professor Paul Kelly, is an epidemiologist assisting in the battle against COVID-19. The Victorian Chief Medical Officer, Professor Brett Sutton, and the Victorian Deputy Chief Medical Officer, Dr Annaliese van Diemen, are experts in contagious diseases, and their work focuses on preventing and understanding the spread of **infectious** diseases between people.

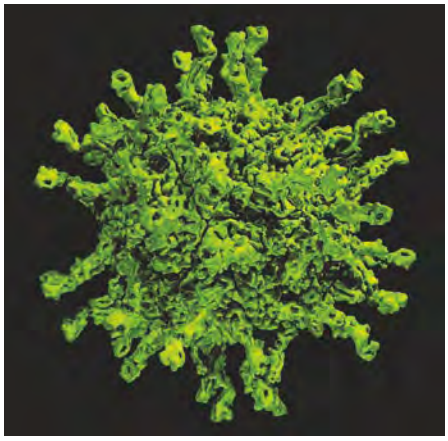


Figure 8A–4 Illustration of the human poliovirus. Individuals with polio can experience flu-like symptoms including sore throat, fever, nausea and stomach pain or, in extreme and rare instances, life-threatening paralysis.

Epidemiologist professional who studies the occurrence of diseases in a population

Infectious able to be transmitted between hosts

Emerging infectious disease a disease not yet seen in people, or a disease that is increasing in incidence or geographical range

Re-emerging infectious disease a disease that appears again after having previously been eliminated

Emergence and re-emergence of pathogens

An **emerging infectious disease** is one that has not occurred in humans before, or has occurred previously but affected only a small number of people, or has occurred throughout time but has only recently been recognised as a disease. In 2007, a report by the World Health Organization (WHO) warned of the threat of emerging infectious diseases. Since the late 1970s, approximately 40 new infectious diseases have been discovered. Examples are SARS, Ebola, avian flu, swine flu, Zika and, more recently, coronavirus disease (COVID-19) caused by the SARS-CoV-2 virus.

Contrast this with **re-emerging infectious diseases**, which are diseases that were once present and had a dramatic decline in case numbers but have returned and are affecting a significant proportion of the population.

Reasons for emerging infectious diseases

A significant contributing factor to the emergence of diseases is human behaviour, and changes in behaviour. Some changes that have occurred over time are:

- population growth – more people are living in closer proximity to each other, providing more opportunities for pathogens to be transmitted between hosts
- international and domestic travel – spreading disease is easier and tracking the origins of disease outbreaks is harder
- poverty – sanitation and water conditions may be lower in quality, causing higher rates of transmission, both direct (for example, lack of water for washing after contact with an infected person) and **indirect transmission** (for example, water-borne pathogens in untreated water)
- ecological damage – the spread of human populations means that more humans live closer to wild animals, making it more likely that pathogens will be passed from animals to humans
- food supply chains – transporting food long distances makes tracing the origins of pathogens associated with the food more difficult
- intensive farming – more animals are kept in smaller spaces that are closer to humans, which increases the chance of pathogen transmission to humans.

Human contribution to climate change is also becoming a contributing factor. As the climate is getting warmer and habitats are changing, diseases are now potentially able to spread into geographical areas they may not previously have survived in. An example is warmer conditions enabling mosquitoes, particularly those capable of transmitting malaria and dengue fever to humans, to move into areas they once would not have inhabited.

VIDEO 8A–1 THE EMERGENCE OF COVID-19

Indirect transmission transmission of a pathogen from a location where it has been away from its host for a long time

LINK **6B** CELLULAR PATHOGENS

Case study in the emergence of a new infectious disease: COVID-19

Pathogen: SARS-CoV-2 (coronavirus)
Mode of transmission: direct transmission through the exchange of droplets

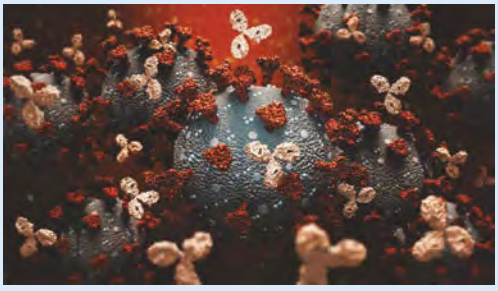


Figure 8A–5 An illustration of monoclonal antibodies (Y-shapes) being used to attack COVID-19 coronaviruses.

COVID-19 is a highly contagious respiratory disease caused by a new RNA virus, a coronavirus similar to the cold virus. The first case of COVID-19 was identified in Wuhan, China in December 2019. The World Health Organization declared a global pandemic in March 2020. By May 2021 there had been more than 164 million cases worldwide with more than 3.4 million deaths, making it one of the deadliest pandemics in history, as well as having an enormous economic and social impact.

The main symptoms of COVID-19 are fever or chills; cough; nasal congestion or runny nose; muscle ache, tiredness and headache; and difficulty breathing. It can progress to serious pneumonia, with the already-sick and elderly being more and more susceptible.

Airborne transmission of the virus is in droplets released from the mouth and nose breathed in by others. This is more likely to happen in close and confined spaces. Transmission by indirect contact, touching a contaminated surface and then touching the lips or nose is a lesser route.

The SARS-CoV-2 coronavirus is believed to have been endemic in a wild animal population, possibly bats, and to have infected the first humans in a so called 'wet market', but this route has not been proven. Other respiratory diseases have jumped from animals to humans in the past and epidemiologists have been warning of this danger for decades.

To fight the virus different strategies were adopted. Prevention of spread has been the priority in Australia with strong measures including widespread testing for the virus, restrictions of movements and gatherings, border closures, quarantining, social distancing and compulsory mask wearing. These have required public health campaigns, legal and public order measures, economic support for those losing income, and a major reorientation of work, education and social life into digital channels. In addition the supply of personal protective equipment for health workers has been vital.

Drugs have not proven effective in stopping the virus and treatment is mainly confined to the relief of symptoms, such as providing oxygen to aid breathing. New technologies such as monoclonal antibodies are also being tried.

Within days of the first outbreaks the largest vaccine development program in history began, using new technologies in some cases. The first vaccines were in use inside 12 months, a dramatic shortening of the usual development. In 2020 Australia brought in a phased program, first vaccinating all quarantine, border and frontline health staff, along with the elderly, immunocompromised and already-sick, and then moving to other groups. Vaccination appears to reduce but not stop transmission, but its main effect is reducing the severity of the disease.

Figure 8A–6 Vaccination programs are the main strategy for combatting COVID-19

During 2021 the main problem that developed was mutation of the virus into new and more transmissible strains, with fears that vaccination would be less effective against them. Genomic sequencing of the strains became a vital tool against them. Vaccination continued to be the main strategy in Australia and most of the world.

See also Video 8A–1 *The emergence of COVID-19* in the Interactive Textbook.

Reasons for re-emerging diseases

There are two key reasons for a disease to re-emerge: resistance or evolution of a pathogen, and a drop in vaccination numbers. An example is penicillin, an antibiotic. Penicillin was introduced in the 1940s for clinical use against bacteria. Alexander Fleming, who discovered penicillin, noticed that by 1943 bacteria were showing signs of resistance to penicillin. Three years later, medical staff in a London hospital estimated that 14% of patients with bacterial infections were showing resistance to penicillin treatment. Today it is estimated that 90% of staphylococcal bacteria are completely resistant to penicillin.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB) are two species of bacteria that are resistant to any antibiotic. MRSA causes skin infections and, in some cases, pneumonia. MDR-TB mostly affects the lungs but is not limited to that area. Symptoms include persistent cough, fever, night sweats and loss of appetite.

Figure 8A–7 MRSA colonies on an agar plate

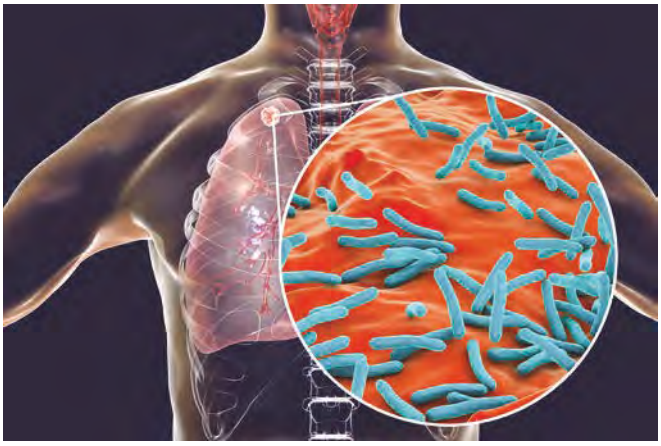


Figure 8A–8 Tuberculosis infection affects the lungs.

Bacteria can either mutate or exchange genetic material with other bacteria of the same or different species. Mutations of bacterial genes could result in the bacteria being able to:

- prevent drugs from adhering to their surface
- decrease the permeability of their plasma membrane to drugs
- actively pump out the drug
- use enzymes that destroy the action of the drug.

Selection pressure the conditions or factors that influence allele frequencies in a population by contributing to the selection of which phenotypes survive in a given environment, e.g. availability of resources, environmental conditions, predators and disease

The likelihood of a mutation being beneficial is low. The exchange of genetic material where a mutation has occurred is more likely. One way in which genetic material is exchanged between bacteria is through a process called conjugation, in which the pilus from one bacterium extends and joins that of another bacterium, forming a bridge enabling the plasmid DNA to be exchanged between the two bacteria. The plasmid could then for example, contain a gene that increases the bacterium's ability to survive medications.

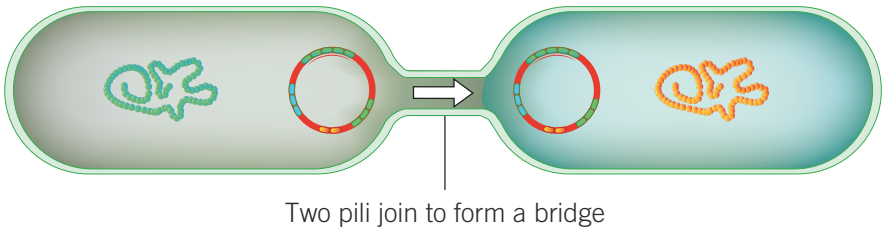


Figure 8A–9 Bacterial conjugation, whereby a plasmid migrates from one bacterial cell to another via a bridge formed by pili

Mutation and exchange of genetic material between bacteria are rare, but the widespread use of antibiotics is acting as a **selection pressure** driving the evolution of stronger and more resistant pathogens. For example, the antibiotics being used will kill most of the bacteria, but any that survive are now resistant and pass on the resistance when they divide Furthermore, with higher population densities,

more people are living closer to each other, allowing easier exchange and providing a rich habitat for pathogenic bacteria to live alongside non-pathogenic bacteria. This provides a further opportunity for bacteria to experience mutations and to exchange genetic material between pathogenic and non-pathogenic bacteria.

Figure 8A–10 A baby getting a measles vaccination

The second reason for the re-emergence of diseases is a decline in vaccination programs. When a well-established vaccination program is successful, some individuals may choose not to vaccinate. An example of this occurring is with measles in the United States. Measles was eliminated from the United States in 2000, but returned in 2019, due to an increase in the number of people opting to not vaccinate their children. This was somewhat fuelled by an anti-vaccination movement spreading invalid claims from a discredited study that linked the measles vaccine with autism. By October 2019, a total of 1249 cases of measles had been reported.

Check-in questions – Set 1

WORKSHEET 8A–1 EMERGING AND REMERGING DISEASES

1 What is a disease?
2 What does it mean if a disease is highly contagious?
3 What is an emerging disease?
4 List three reasons why a disease may emerge.
5 Describe how European colonisation would be a source of emerging diseases.

Figure 8A–11 Helping prevent the spread of pathogens: wearing a mask to reduce aerosol spread, social distancing to prevent people coming into close contact with each other, and increased awareness of good personal hygiene by washing hands.

Preventing the spread of pathogens

During the COVID-19 outbreak, a range of measures were implemented by the Australian and Victorian medical officers, to ensure the safety of the community. These included:

- promoting good hygiene – reminding people to wash their hands regularly and thoroughly
- requiring social distancing – at least 1.5 metres between people
- reducing the movement of people – via lockdown or restrictions on travel
- making the wearing of face masks compulsory
- encouraging people to get tested if they felt unwell.

Other public messages included how to sneeze and cough in a way that reduces the spread of aerosol droplets, cleaning high-touch surfaces, keeping in contact with family members or work colleagues at a distance through means such as phone calls or video call sessions, and keeping healthy by eating a balanced diet and exercising regularly.

A key measure was the reduction of movement and social distancing. During Victoria's lockdown, people were only allowed to leave home for four reasons:

- to shop for food and supplies
- for care and caregiving
- for exercise and recreation
- for study and work if these could not be done at home.

Putting such restrictions in place to help battle COVID-19 was also seen as a reason for the decline in the number of common influenza cases and deaths in Australia. From January to June of 2020, there were 21 000 confirmed cases and 36 deaths from influenza, compared to 132 000 confirmed cases and 430 deaths for the same period in 2019.

Understanding the infectious cycle

In order for epidemiologists to recommend particular control strategies to prevent the spread of a pathogen, they need to understand the infectious cycle of a disease (Figure 8A–12). This involves understanding the site where the infection originates or comes from (**reservoir**), **transmission** between hosts (Table 8A–1), and involvement of any vectors.

Reservoir original or usual site of a disease in relation to its spread

Transmission how a pathogen is passed between hosts

Figure 8A–12 shows that understanding how a pathogen is spread from its origin, to a host and between hosts, allows appropriate control measures to be put into place.

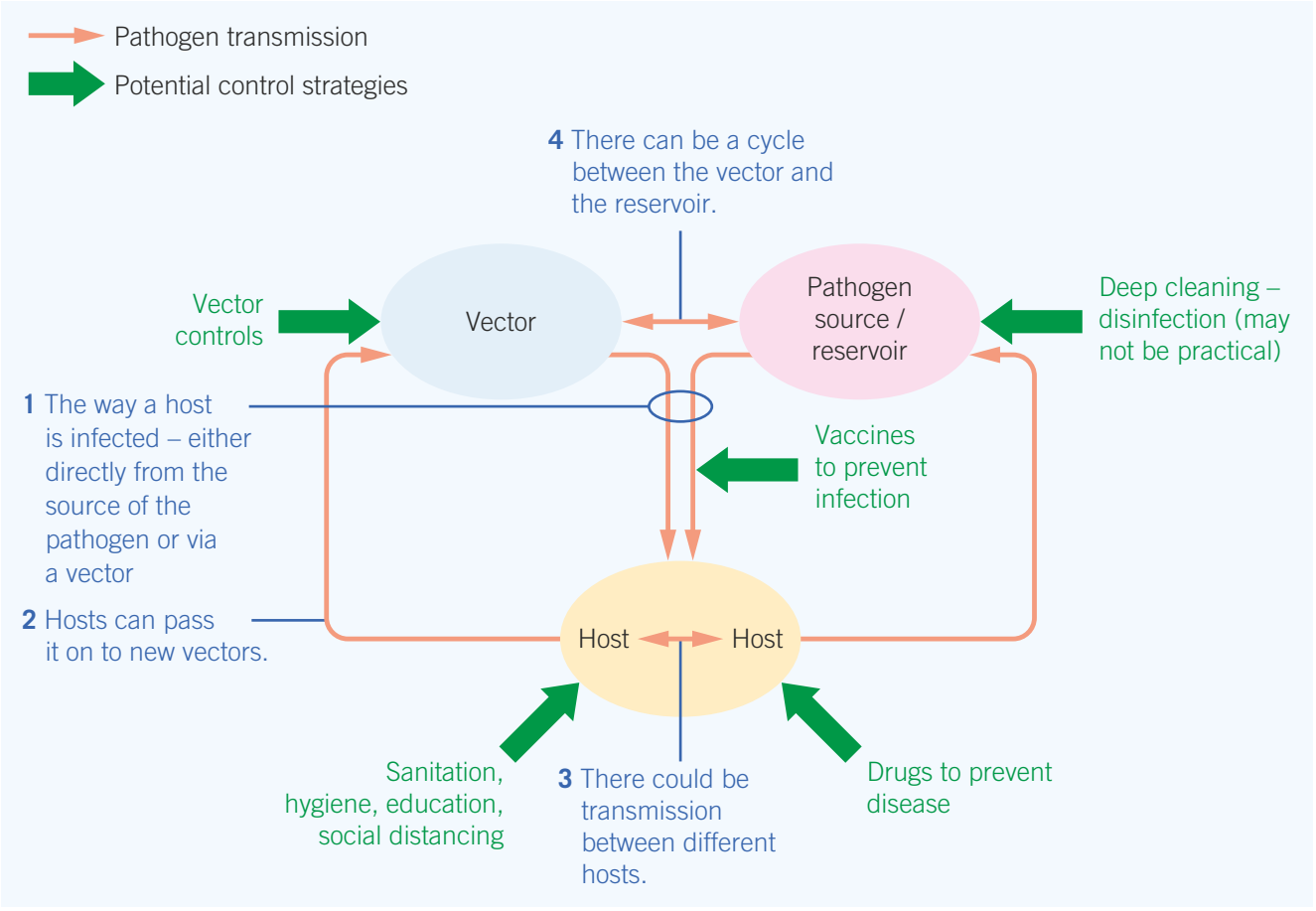


Figure 8A–12 Infectious cycle of a disease. Orange arrows indicate pathogen transmission, green arrows indicate potential control strategies once the infectious cycle is known.

Table 8A–1 Forms of transmission between hosts

Direct transmission	Indirect transmission
• Inhaling droplets from a cough or sneeze • Physical contact, such as rubbing eyes • Eating the pathogen • Being bitten by a vector • Sexual contact	• Touching an object that has come into contact with the pathogen • Picking up pathogen off surfaces • Consuming food or beverage that came into contact with pathogen • Through contamination such as washing food or equipment with dirty water

Public health measures

Apart from responding to specific emerging or re-emerging diseases, ensuring the safety of the community requires fundamental health measures to be maintained at all times. These measures are not always present in all communities – low-income countries, for example, can struggle to implement such procedures. Consequently, there can be outbreaks and health warnings for travellers to such countries.

Health measures aimed at preventing the spread of pathogens include:

- ensuring a safe supply of water for drinking and cleaning
- sewage treatment and disposal
- food safety standards and regulations
- food processing and farming
- border control of entry of exotic species
- pest and animal controls
- vaccination programs
- quarantine procedures (animals, people, plant material).

WORKSHEET 8A–2 PREVENTING THE SPREAD OF PATHOGENS

Check-in questions – Set 2

1 List three control strategies to prevent the spread of a pathogen.
2 What are two direct types of transmission?

VIDEO 8A–2 SKILLS: CHOOSING OPTIONS AND GENERATING LISTS ANSWERS

8A SKILLS

Be mindful when choosing options and generating list answers

A lot of your energy can be consumed with working on memorising all the different terms and concepts in Biology. This textbook has suggested a range of strategies to adopt or modify, to help build your memory. A large part of that memory work is in associating particular terms with their correct context. Not only that, but when answering a question that has follow-up parts, you need to be mindful when choosing the term or concept you are going to use in your answer, as your choice will have flow-on effects.

The parts of a question generally build in complexity. A question may start by asking you to recall or identify, then require you to practise your application skills, and finish by asking you to explain or interpret. Some questions also have a consequential flow. It is important that you look at the question as a whole (during your initial reading time) so that you are aware that what you contribute in the first part of a question will flow on into the subsequent parts. For example:

Question

a Identify a control measure in preventing the spread of a pathogen. (1 mark)

b Explain why this control measure works. (2 marks)

In order to gain the first mark, you have a wide range of answers to choose from. What you need to be mindful of is how strong you are in your explanation of what you identified. You have been given a choice of how you want to respond, so pick the measure you are most comfortable with in order to explain.

All-or-nothing response

At times you might think it is safer to provide two responses in order to 'hedge your bets' instead of committing to one response. This can also happen when you need to generate a list of responses. For example:

Question

Provide three forms of indirect transmission.

If the question is in a content area that you are not comfortable with, it can be tempting to provide more than three options in your response, if you feel that one of the options you've provided is not very strong. What you need to be mindful of is that it is the first three options that will be marked. Stick with your strongest options and know that it is okay to cross out a response and not hedge your bets with an extra response.

Section 8A questions

1 Explain the role of a pathogen in disease.
2 What is the difference between an infection and disease?
3 What are the two key reasons for diseases re-emerging?
4 Outline why high-density living is a contributing factor to emerging diseases.
5 Outline a strategy that would help decrease the chance of a disease re-emerging.
6 Explain how European settlement in Australia affected Aboriginal and Torres Strait Islander peoples' lifestyle in a way that affected disease transmission.
7 Explain why proper sewage disposal and treatment is important in relation to the transmission of disease.
8 Part of the infectious cycle of a pathogen involves being airborne. Describe a control measure you would advise if you were an epidemiologist.
9 Explain why it is important to understand the infectious cycle of a disease.

Figure 8A–13 Vaccination programs are a key health measure in preventing the spread of pathogens.

Treatment of disease

Study Design:
The development of immunotherapy strategies, including the use of monoclonal antibodies for the treatment of autoimmune diseases and cancer

Glossary:
Bispecific monoclonal antibody
Conjugated monoclonal antibody
Hybridoma
Immunotherapy
Monoclonal antibody (mAb)
Myeloma cell

ENGAGE

Multiple sclerosis has a potential treatment

7D THIRD LINE OF DEFENCE LINK

In Section 7D, you read about multiple sclerosis (MS) as an autoimmune disease. In MS, the person's own immune cells attack the central nervous system, resulting in damage to the insulating myelin sheath of nerve cells. Often there are relapsing–remitting stages, where the disease does not become worse for a while, but about 10–15% of MS sufferers have a type of MS called primary progressive multiple sclerosis (PPMS), characterised by gradual worsening of the disease with no periods of relapse or remission. Immunotherapy holds the prospect of a potential treatment

Immunotherapy a treatment that uses activation or suppression of the immune system

Monoclonal antibody (mAb) antibody made by cloning a unique parent immune cell, produced in large quantities in the laboratory as a drug targeting specific cells or substances

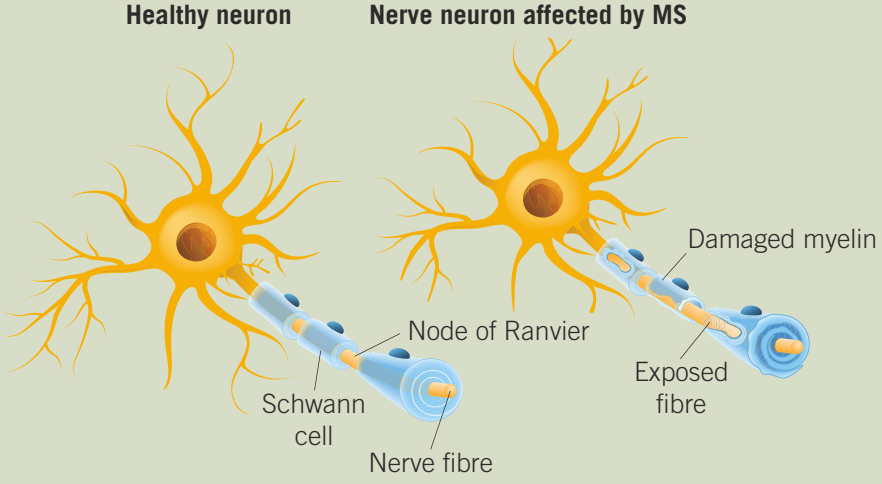


Figure 8B–1 Demyelination as a result of MS, as shown on the right.

EXPLAIN

Immunotherapy

Immunotherapy is a treatment for a disease that causes the activation or suppression of the immune system. The main types of immunotherapy include **monoclonal antibodies (mAbs)**, non-specific immunotherapies and cancer vaccines. The focus of this section is mAbs.

As discussed in Section 7D, antibodies are produced naturally during the adaptive immune response, to target the specific non-self antigens of pathogens. Monoclonal antibodies are made in the laboratory to work in the same way. 'Monoclonal' here means an antibody made in quantity by cloning one parent immune cell. There are numerous types and they can be made to target a variety of cells and substances. Here we will discuss these main types of monoclonal antibodies that target cancer cells and the causes of autoimmune diseases:

7D THIRD LINE OF DEFENCE LINK

- Bispecific mAbs attach to target cells to signal for an immune response.
- Conjugated mAbs deliver a cytotoxic or radioactive 'payload' directly to the target cell.

Bispecific monoclonal antibodies

In Section 7D you learned about the shape and specificity with which antibodies bind with specific antigens, and that the antigen-binding sites are the same on each arm of an antibody. A **bispecific monoclonal antibody** is laboratory-made with two different antigen-binding sites, so it can attach to two different antigens at one time.

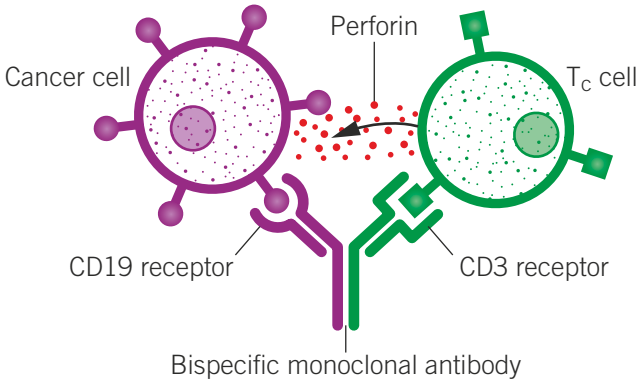


Figure 8B–2 The bispecific monoclonal antibody has different antigen-binding sites on its two arms, so it is able to join a cancer cell with a T cell, which enables the T cell to perform its role and kill the cancer cell.

An example of a bispecific mAb is blinatumomab (Blincyto®), which is used to treat different types of the blood cancer leukaemia. One arm of the mAb binds to a CD19 receptor of the leukaemia cell, while the other arm attaches to the CD3 receptor of a T_c cell (Figure 8B–2). The action of this mAb ensures that the T cell from the adaptive immune system comes into direct contact with a cancer cell, where it can perform its role in destroying the cancerous cell.

Bispecific monoclonal antibody
a monoclonal antibody that has two different antigen-binding sites

Conjugated monoclonal antibody
an antibody that has been combined with a radioactive substance or chemotherapy agent (a cytotoxin)

Conjugated monoclonal antibodies

A **conjugated monoclonal antibody** is one that has been combined with a chemotherapy drug (a cytotoxin) or radioactive substance (Figure 8B–3). The conjugated mAb can be used to deliver the drug or radioactive substance directly to the cancerous cells, preventing their growth and lessening the damage to healthy cells in the area.

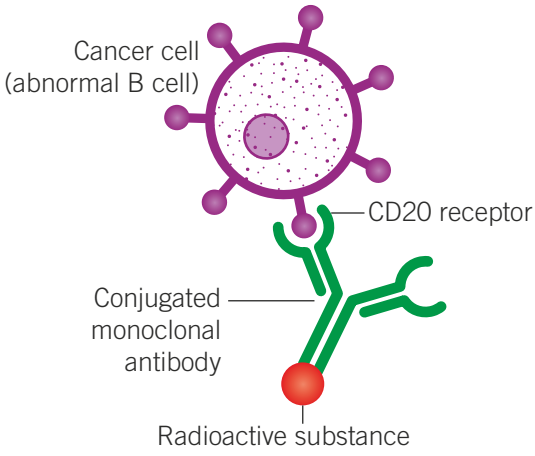


Figure 8B–3 Conjugated monoclonal antibody with radioactive substance associated with it. The antigen-binding sites are the same on both arms of the antibody.

VIDEO 8B–1
IMMUNOTHERAPY: MONOCLONAL ANTIBODIES

An example of a conjugated mAb is rituximab, which is used to deliver a radioactive substance to fight diseases such as non-Hodgkin lymphoma. Rituximab targets the CD20 receptor of a B cell. As non-Hodgkin lymphoma is a cancer of the immune system, there are abnormal B cells; this treatment targets those cells and delivers the radioactive substance to stop their growth.

Monoclonal antibodies against autoimmune diseases

Autoimmune diseases are a malfunction of the immune system. Using drugs against the malfunctioning cells is difficult due to the risk of weakening the immune system so much that it allows pathogens to take hold. Ideally, treatments should specifically target only the malfunctioning cells or molecules of the immune system, not the whole system.

In 2017 the Australian Therapeutic Goods Administration approved a monoclonal antibody drug called ocrelizumab as the first approved treatment for primary progressive multiple sclerosis. Ocrelizumab is a monoclonal antibody that targets the CD20 receptor of B cells. It reduces the attacks by the immune cells on the central nervous system, and has been shown to be a successful treatment for MS.

Anti-inflammatory monoclonal antibodies are also used to inhibit cytokines that are involved in abnormal inflammatory responses, such as in rheumatoid arthritis, inflammation and ulcers of the colon. Another example is the use of mAbs to inhibit IgE involved in moderate to severe allergic asthma.

Possible side effects of mAbs

Taking mAbs can cause side effects, such as an allergic reaction, rash, fever, headaches, vomiting and low blood pressure. For example, cetuximab – a mAb that targets a cell protein called EGFR, which is found on some cancer cells but also on normal skin cells – can cause serious rashes in some people.

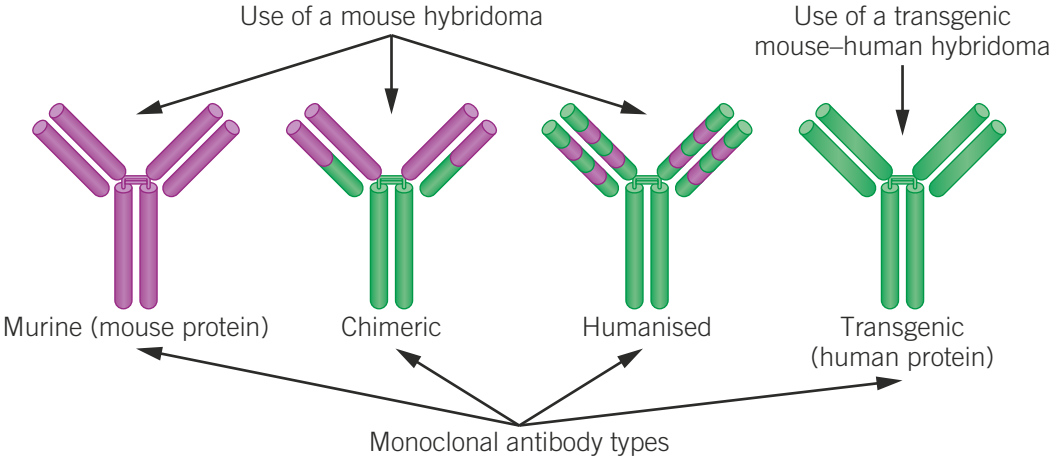
Check-in questions – Set 1

1 Define monoclonal antibody.
2 What can a bispecific monoclonal antibody do?
3 Outline how a conjugated monoclonal antibody compares to a bispecific one.

Making monoclonal antibodies

Four types of mAbs can be distinguished according to the source of the protein that makes them up (Figure 8B–4):

- murine mAbs – made of mouse proteins
- chimeric mAbs – a mixture of mouse and human proteins
- humanised mAbs – mouse protein attached to predominately human proteins
- transgenic mAbs – fully human proteins produced with the use of transgenic mice.



6A RECOGNISING SELF FROM NON-SELF LINK
3A COMMON DNA TOOLS AND TECHNIQUES LINK

Figure 8B–4 The four types of monoclonal antibodies

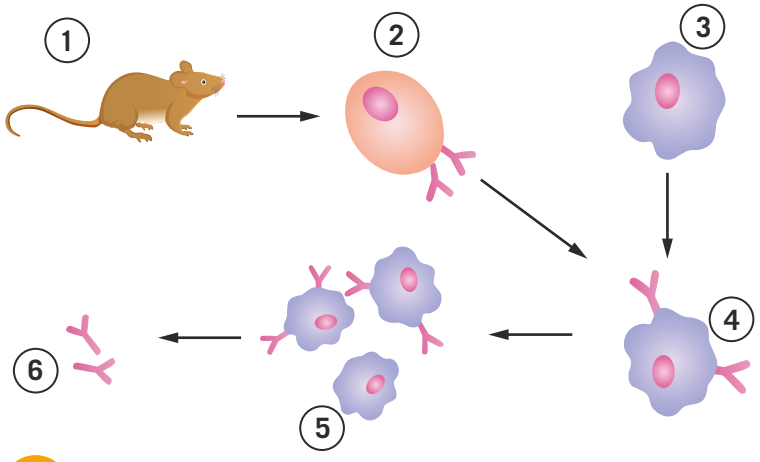


Figure 8B–5 Producing monoclonal antibodies from mice. The numbers refer to the steps explained in the text.

The first mAbs were mouse mAbs – the process for producing these is outlined in Figure 8B–5. In order for the mAbs not to be rejected by humans (as mouse mAbs would be considered non-self), researchers used recombinant DNA techniques to produce chimeric, humanised and transgenic mAbs. Transgenic mAbs are made up of all human proteins, in comparison to the other types. The process is:

1 A mouse is injected with antigen from cancer cells, stimulating adaptive immune response to produce B lymphocytes that secrete specific antibody.

2 B lymphocytes are extracted from the mouse's spleen.
3 A tumour cell, called a myeloma cell, is prepared.
4 B lymphocytes are combined with myeloma cells to form a hybridoma, which divides quickly while still being able to produce antibodies.
5 The hybridoma grows by cloning itself, producing many cells. Those capable of still secreting the antibody are selected.
6 The monoclonal antibodies are collected and purified.

The B lymphocyte that produces the specific antibody has a limited life span. To help provide multiple copies and therefore large quantities of antibodies, the B lymphocytes are combined with a **myeloma cell** to form a **hybridoma**. The myeloma is a tumour plasma cell that is highly capable of replicating many times.

Myeloma cell an abnormal plasma cell used in the production of monoclonal antibodies

Hybridoma a cell that is a result of combining a B lymphocyte and a cancer cell

Check-in questions – Set 2

1 In producing mAbs from mice, what does the mouse need to be injected with first?
2 What cell does the B lymphocyte join with to form a hybridoma?
3 What is the benefit of using the cell you identified in Question **2**?
4 What could happen to an individual if a monoclonal antibody from a mouse was used repeatedly on them?

WORKSHEET 8B–1 IMMUNOTHERAPY

8B SKILLS

VIDEO 8B–2 SKILLS: USING DUAL CODING IMAGERY TO CREATE EXPLANATIONS

Using dual coding imagery to create explanations

Understanding the functions of different processes is an important skill in Biology. Even more important is the ability to convey your understanding when writing explanations. This can be achieved by using a diagram as a stimulus and building your response. This consolidates and extends on the work of dual coding using image association as discussed in the 7E Skills section. The skill of knowing how to break down the information and then build it back up again in an explanation is vital, and it is essential to have a clear sequence in your response.

LINK **7E** ACTIVE AND PASSIVE IMMUNITY

In this Skills section, the use of conjugated monoclonal antibodies will be used as an example. You can then practise by using the same approach for bispecific monoclonal antibodies. Here is an example:

Question

Explain how a conjugated monoclonal antibody assists with the fight against cancer.

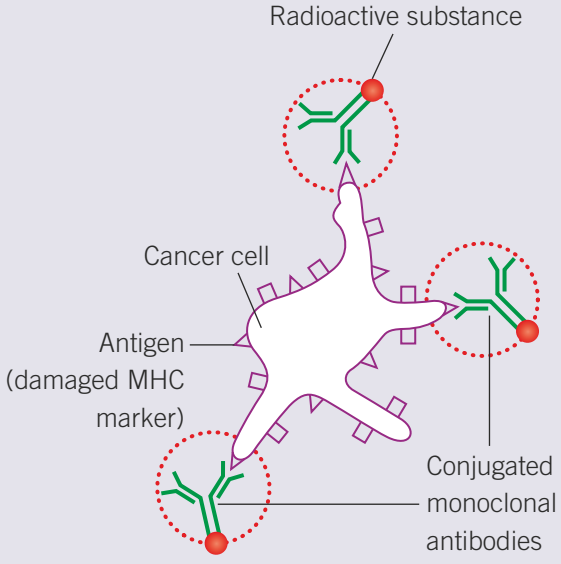
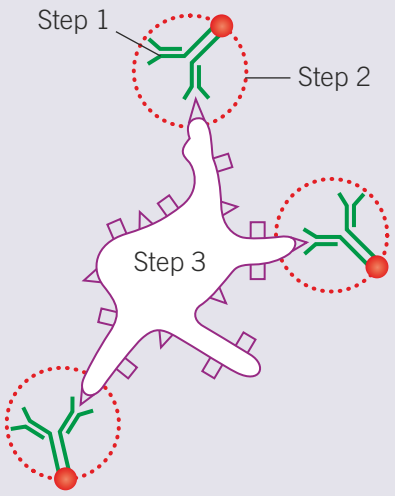


Figure 8B–6 Diagram of dual coding to help remember conjugated monoclonal antibody

The thinking behind your answer can be shown in diagrammatic form (Figure 8B–7).

The sequential steps of the answer:

Diagram	Steps	Answer
 **Figure 8B–7** The steps needed for answering the question	1 Identify the key element from the diagram	A conjugated monoclonal antibody has a radioactive substance associated with it.
	2 Connect and extend to what happens next	The radioactive substance is released towards the target cancer cell due to the binding of the mAbs to the cancer cell antigens.
	3 Link back to the context of the question	As a result of the radioactive substance, the cancer cell is killed, helping to combat the cancer.

When explaining your answer, think of the three steps like this:
Step 1 = What is it that you need to *identify?*
Step 2 = *Elaborate* with the connection or the point of what you identified.
Step 3 = *Link* back to the context.

Through each of the steps, use the context of the question. For example, if the name of the drug or monoclonal antibody was provided in the question, you would use it in your answer. If the question context stated what the type of cancer was, you would use the name of the cancer, not the generic phrase 'cancer cell'.

The more of these thinking routines you use, the better your explanations will be, and you will be able to mentally do them without the assistance of a template. If using a diagram at the start of your explanations helps, you can do that in your response in an assessment, as long as you elaborate on your response by following the three steps outlined above.

Section 8B questions

1 Describe what immunotherapy attempts to achieve.
2 Monoclonal antibodies are used to target cancer or autoimmune diseases. What is the promising benefit of using monoclonal antibodies for treatment?
3 If a monoclonal antibody has a radioactive substance attached to it, what kind of monoclonal antibody is it?
4 Describe what a chimeric monoclonal antibody is.
5 If a cancer cell had an antigen with a sphere shape, and a T cell had a triangle receptor shape, draw the monoclonal antibody that you would use.
6 What type of monoclonal antibody did you draw for Question **5**?
7 Explain why a mouse would be injected with an antigen to make a monoclonal antibody.
8 Explain why it is important to have transgenic monoclonal antibodies.

Chapter 8 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
8A.1	Recall and define the terms associated with emerging and re-emerging diseases, and preventing the spread of pathogens	5☐, 11☐
8A.2	Compare emerging and re-emerging infectious diseases with examples of the impact of European arrival on Aboriginal and Torres Strait Islander peoples	12☐, 13☐, 14☐
8A.3	Explain why diseases emerge or re-emerge	4☐, 15☐, 16☐, 18☐
8A.4	Identify different scientific and social measures to control and prevent the spread of a pathogen	2☐, 17☐
8A.5	Understand the infectious disease cycle as a relationship between pathogen, host and forms of transmission	3☐, 19☐, 20☐, 21c☐
8A.6	Understand, compare and explain the different types of transmission	1☐, 21a☐, b☐
8B.1	Recall and define the terms associated with immunotherapy and monoclonal antibodies	6☐, 10☐
8B.2	Describe and explain the function of monoclonal antibodies	9☐, 26☐
8B.3	Compare the different types of monnoclonal antibodies	24☐, 25☐
8B.4	Draw the different types of monoclonal antibodies	22☐
8B.5	Outline and explain the benefits of monoclonal antibodies	7☐, 8☐, 21d☐, 23☐

Multiple-choice questions

1 Which is *not* an example of a direct form of transmission?

- **A** rubbing your eyes after sneezing into your hands
- **B** eating food from a chopping board that has not been washed properly
- **C** inhaling droplets from a cough or sneeze
- **D** having sexual contact with another person

2 Which is the best reason for implementing social distancing during a COVID-19 outbreak?

- **A** Making social distancing mandatory prevents people from being offended that other people are keeping a distance from them.
- **B** It reduces the likelihood that an uninfected person will touch an infected person or touch a surface that the infected person has touched.
- **C** The chance of direct transmission from an infected person is reduced, especially when coughing or sneezing.
- **D** It reduces the number of people exposed when the air is infected in a particular location.

3 What does an infectious cycle include?

A how a pathogen is transmitted from host to host
B the reason why a vector transmits the pathogen to the reservoir
C only how a vector transmits a pathogen to a host
D the life cycle of the pathogen

4 Which is *not* a contributing factor to an emerging disease?

A the condensing of populations and people living closer to other people
B intensive farming practices
C people travelling between different locations
D the nomadic lifestyle of Indigenous populations before settlement by other cultures

5 A new variant of coronavirus has a mutation that has changed the spike proteins on the surface of the virus, making it better at invading a host's cells. Which of the following options best identifies the virulence of this variant coronavirus?

A The new variant has a lower virulence than the original coronavirus.
B The new variant has a higher virulence than the original coronavirus.
C The new variant has the same virulence as the original coronavirus.
D The new variant does not have a virulence associated with it.

6 Immunotherapies

A cause a pathogen to become more infectious.
B are yet to be created and are currently a purely hypothetical cancer treatment.
C activate or suppress an individual's immune system.
D involve injecting people with vaccinations that contain active antigens from a pathogen.

7 The benefit of a conjugated monoclonal antibody is that it

A is able to join a T cell and the cancer cell close together.
B is able to deliver the radioactive treatment more closely and specifically against the cancer cell.
C can cause more harm to the healthy cells so that they have a smaller chance of becoming cancerous.
D will have two different antigen-binding sites in order to deliver the radioactive substance to kill the cancerous cells.

8 Monoclonal antibodies are used to fight autoimmune diseases because they can

A suppress malfunctioning immune cells and release cytokines.
B turn off the immune system.
C increase the responses of the immune system.
D suppress malfunctioning immune cells and inhibit inflammatory response molecules.

9 What feature of a target cell, such as a cancer cell, does a monoclonal antibody use?

A the nucleus
B the antigen on the plasma membrane
C a thick plasma membrane that prevents the entry of pathogens
D irregular shape, so it can become easily identified

10 What other cell is a mouse B lymphocyte combined with to make monoclonal antibodies?

A hybridoma
B myeloma cell
C T lymphocyte
D spleen cell

Short-answer questions

11 Define disease. (1 mark)

12 Compare an emerging and a re-emerging disease. (2 marks)

13 European colonisation of Australia led to the emergence of pathogens new to Aboriginal and Torres Strait Islander peoples as well as to the re-emergence of pathogens they had previously been exposed to. Explain how these two kinds of impacts happened. (2 marks)

14 Many of the European colonists had resistance to the diseases they brought with them to Australia. Describe how these colonists were acting like a vector, just like a mosquito would. (1 mark)

15 Doctors advise you to complete your course of medication when you have a disease. Explain the importance of doing this. (2 marks)

16 How does over-subscribing of antibiotics contribute to a pathogen being able to re-emerge? (1 mark)

17 During the COVID-19 pandemic, in Australia there were border restrictions not only for international travellers, but between states. Outline a reason why such restrictions were put in place, in relation to how the spread of pathogens is controlled. (2 marks)

18 Is COVID-19 an example of an emerging or re-emerging disease? Justify your answer. (2 marks)

19 Explain the importance of knowing the infectious cycle of a pathogen. (2 marks)

20 Describe a control measure against a vector, such as a mosquito. (2 marks)

21 Between 1994 and 2016 there were 70 cases of Hendra virus infection in horses in Australia. The virus originated from fruit bats. Seven people were infected and four of those died.

- **a** Horses are thought to become infected by eating food that has been contaminated by fruit bats. What form of transmission is this? (1 mark)
- **b** Of the seven people who contracted Hendra virus, five were veterinarians. What type of transmission was this and how did the veterinarians become infected? (2 marks)
- **c** Describe a strategy to prevent a horse from getting the virus and a human from getting the virus. (2 marks)
- **d** Monoclonal antibodies are a potential treatment for humans. Explain how a monoclonal antibody could assist in treating people infected with Hendra virus. (2 marks)

22 Draw a fully labelled bispecific monoclonal antibody. (1 mark)

23 Explain what the benefit of a bispecific monoclonal antibody is. (2 marks)

24 What are two differences between a monoclonal antibody and a natural antibody? (2 marks)

25 Explain why mouse monoclonal antibodies cause an immune response. (2 marks)

26 Explain the importance of forming a hybridoma in the production of monoclonal antibodies. (2 marks)

27 Use your knowledge of the adaptive immune response to explain the following.

- **a** Explain why the Europeans who first colonised Australia would have been resistant to diseases they brought with them, such as smallpox, while Aboriginal and Torres Strait Islanders had a higher death rate from such pathogenic diseases. (2 marks)
- **b** What present-day medical technology, had it been available, would have been most effective in preventing Aboriginal and Torres Strait Islander peoples from dying from smallpox, measles and influenza brought by the first European arrivals? (1 mark)
- **c** Outline how vaccinations work, and explain what is the ideal kind of vaccine to produce long-term protection. (2 marks)

UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES?

CHAPTER 9

EVOLUTION: GENETIC CHANGES IN POPULATIONS OVER TIME

Introduction

Thus far in Biology, you have spent a lot of time studying living organisms, but there is much more to Biology. Evolutionary biology, for example, is the study of the evolutionary processes that have resulted in the diversity of living organisms we now see on Earth. This chapter explores these processes and how they impact the alleles in a population. The biological consequences of changing the allele frequencies of a population, in terms of genetic diversity, are also covered. The role humans play in the manipulation of allele frequencies and evolutionary processes is also investigated, to gain a full understanding of the changes seen over time.

Curriculum

Area of Study 2 Outcome 2
Genetic changes in a population over time
Changes in species over time

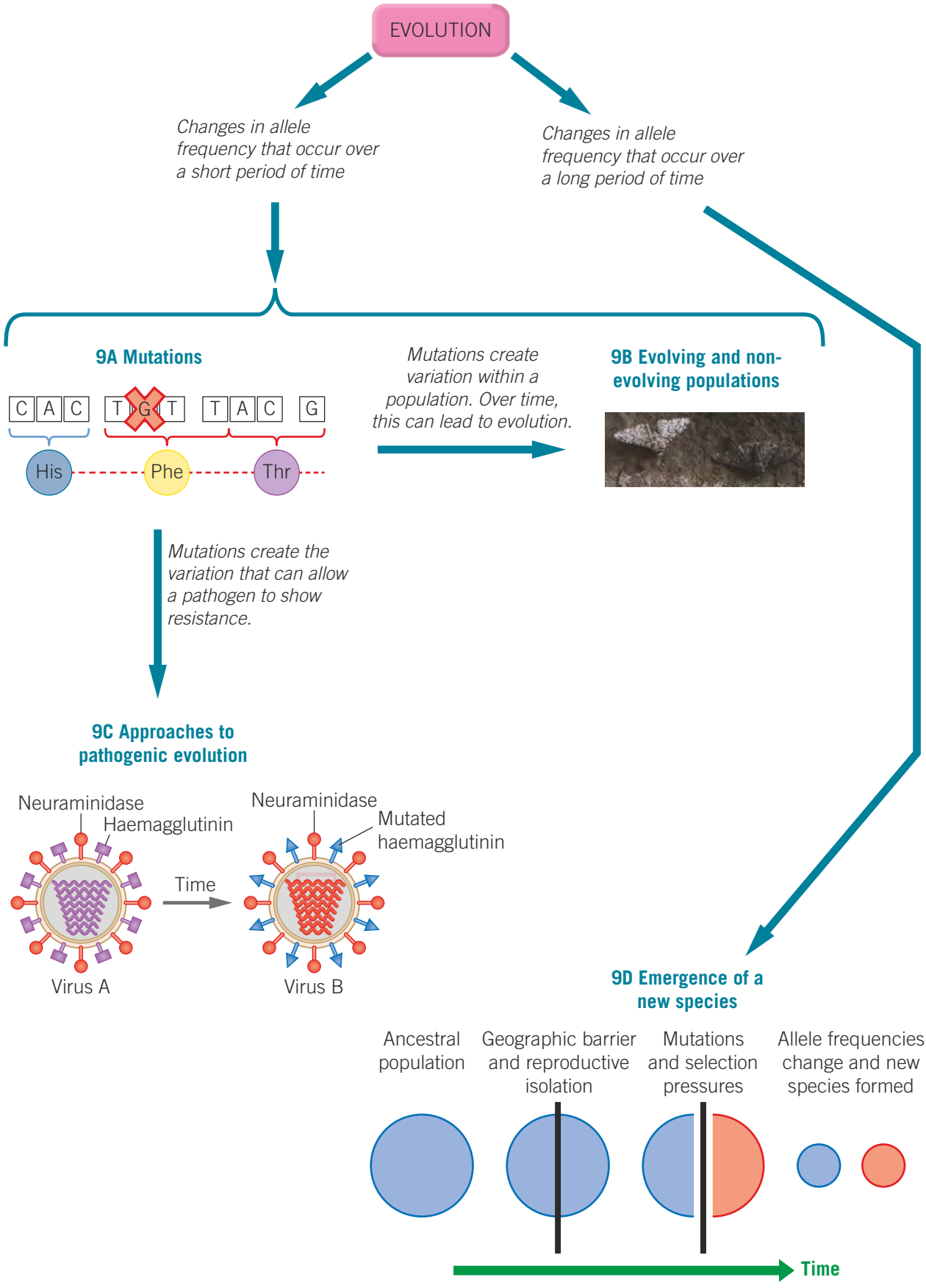
Study Design	Learning intentions – at the end of this chapter I will be able to:
Genetic changes in a population over time • Causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow; and mutations as the source of new alleles • Biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity	**9A Mutations** **9A.1** Define population, species, gene pool, allele frequency and evolution **9A.2** Outline how mutations can be the source of new alleles and the consequences of this for genetic diversity **9A.3** Define and summarise how point mutations, including the different types of frameshift and substitution mutations, cause a change in allele frequencies in a population's gene pool **9A.4** Define and summarise how block mutations, including translocation, deletion, inversion and duplication, cause a change in allele frequencies in a population's gene pool **9A.5** Define and summarise how chromosome abnormalities, including aneuploidy and polyploidy, cause a change in allele frequencies in a population's gene pool

Study Design	Learning intentions – at the end of this chapter I will be able to:
Genetic changes in a population over time • Causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow; and mutations as the source of new alleles • Biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity • Manipulation of gene pools through selective breeding programs	**9B Evolving and non-evolving populations** **9B.1** Define and summarise how gene flow causes a change in allele frequencies in a population's gene pool **9B.2** Define and summarise how genetic drift, including the bottleneck effect and the founder effect, cause a change in allele frequencies in a population's gene pool **9B.3** Define and summarise how environmental selection pressures cause a change in allele frequencies in a population's gene pool through the process of natural selection **9B.4** Outline how artificial selection, including selective breeding programs, can cause a change in allele frequencies in a population's gene pool **9B.5** Distinguish between those evolutionary processes that have the potential to increase the genetic diversity of a population and those that have the potential to decrease the genetic diversity of a population
Genetic changes in a population over time • Consequences of bacterial resistance and viral antigenic drift and shift in terms of ongoing challenges for treatment strategies and vaccination against pathogens	**9C Approaches to pathogenic evolution** **9C.1** Define bacterial resistance, antigenic drift and antigenic shift **9C.2** Give reasons why bacterial resistance causes ongoing challenges for treatment strategies against bacteria **9C.3** Give reasons why antigenic drift and shift cause ongoing challenges for treatment strategies and vaccination against viruses
Changes in species over time • Evidence of speciation as a consequence of isolation and genetic divergence, including Galapagos finches as an example of allopatric speciation and Howea palms on Lord Howe Island as an example of sympatric speciation	**9D Emergence of a new species** **9D.1** Define viable, fertile, reproductively isolated and speciation **9D.2** Distinguish between allopatric and sympatric speciation **9D.3** Outline the steps involved in the allopatric speciation of the Galapagos finches **9D.4** Outline the steps involved in the sympatric speciation of the *Howea* palms on Lord Howe Island

Glossary

Adaptations
Adaptive radiation
Allele frequency
Allopatric speciation
Aneuploidy
Antigenic drift
Antigenic shift
Artificial selection
Bacterial resistance
Block mutation
Bottleneck effect
Chromosome abnormality
Conjugation
Divergent evolution
Epidemic
Evolution
Fertile
Founder effect
Frameshift mutation
Gene flow
Gene pool
Genetic diversity
Genetic drift
Genotype
Karyotype
Missense mutation
Mutagenic agent
Mutation
Natural selection
Nonsense mutation
Pandemic
Phenotype
Point mutation
Polyploidy
Population
Reproductive isolation
Selection pressures
Selective breeding
Silent mutation
Speciation
Species
Substitution mutation
Sympatric speciation
Viable

Concept map



See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

Mutations

Study Design:

- Causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow; and mutations as the source of new alleles
- Biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity

Glossary:

Adaptations
Allele frequency
Aneuploidy
Block mutation
Chromosome abnormality
Evolution
Frameshift mutation
Gene pool
Genetic diversity
Genotype
Karyotype
Missense mutation
Mutagenic agent
Mutation
Nonsense mutation
Phenotype
Point mutation
Polyploidy
Population
Silent mutation
Species
Substitution mutation

ENGAGE

The unbreakable mutation

In 1994, a young man in Connecticut, USA, was involved in a serious car accident but, incredibly, walked away uninjured. X-rays were unable to reveal a single fracture in his body! In fact, they showed that this young man had a bone density eight times higher than other men his age. A team of scientists investigated further and found that he and 21 other members of his family have a mutation that disrupts the functioning of a gene called LRP5. In the past, researchers had recorded that a mutation in the LRP5 gene caused osteoporosis, a condition that makes bones brittle and weak. However, it now appears that a different type of mutation in the same gene could have the opposite effect, giving some people extremely dense bones that are practically unbreakable.

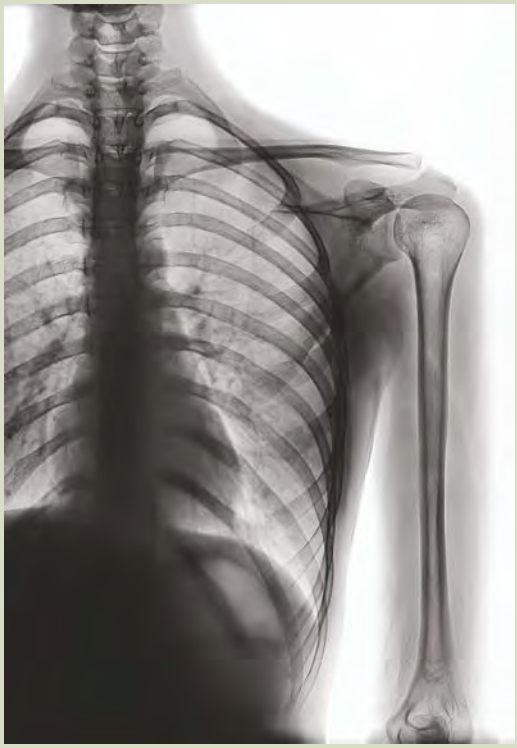


Figure 9A–1 A mutation in the gene LRP5 can cause weak and brittle bones, but another type of mutation in the same gene can cause the opposite – unbreakable bones.

This section looks at different types of mutations, including beneficial mutations like the one on LRP5, all from the perspective of evolution. The focus is on how mutations can change the genetic make-up of individuals and, over time, influence the genetic information in a population, causing evolution to occur.

EXPLAIN

Introducing evolution

LINK 2A NUCLEIC ACIDS

LINK UNIT 2

VIDEO 9A–1 INTRODUCING EVOLUTION

Revisiting key ideas

Working through Units 1–3 of VCE Biology, you've learnt many of the key ideas that are the foundations for understanding evolution. To recap:

- DNA is a type of nucleic acid that contains genes.
- Genes are sections of DNA containing the nucleotide sequence that codes for the production of a polypeptide or protein.
- There are alternative forms of genes, called alleles, and these code for variations of a specific trait or characteristic.
- For each gene locus, one allele is inherited from the mother and one from the father, and together they form the **genotype** of the individual.
- The genetic make-up or genotype of an individual and the influence of the environment both contribute to the **phenotype**, or expression of the physical characteristic the gene codes for.
- Sexual reproduction and meiosis can increase the variation, or diversity, of the genetic material in an individual through crossing over, independent assortment, random mating and non-disjunction.
- Changing the genetic material of an individual may alter the range of phenotypes expressed in a population.
- Increasing the **genetic diversity** of a population (having more genetic variation between individuals in a population) increases the chance of survival of a species.
- **Adaptations** are the behavioural, physiological and structural features of an organism that help them survive and flourish in an environment.

Genotype
the genetic make-up or the combination of alleles for a particular gene of an organism

Phenotype
a physical characteristic determined by genotype and environment

Genetic diversity
the genetic variability within a species

Adaptations
the behavioural, physiological and structural features of an organism that help them survive in an environment

Variation

There is always variation between the phenotypes of individuals in a population. This variation results from the different combinations of alleles (genotypes) that have arisen due to sexual reproduction and meiosis, and the influence of the environment. For example, a plant may have a genotype that allows it to grow tall, but it will only be able to do so if it has access to all the requirements for growth. If these requirements are lacking, then the plant will not grow to the size determined by its genotype. The environment has influenced the phenotype.

There are other types of variation that may be present in a population. For example:

- *morphological*: differences in the structure of an organism; for example, body shape, tail length in dogs
- *behavioural*: differences in patterns of activity; for example dog behaviour (herding, retrieval, guarding)
- *biochemical*: differences in the composition of cells; for example, blood groups and the different pigments in cat hair
- *physiological*: differences in the functioning of an organism; for example, differences in people's ability to detect tastes, odours and colours
- *developmental*: differences that occur as an organism ages; for example, a snake changing colour over time
- *geographic*: differences in location; for example, different possum body mass in different regions of Australia.

Figure 9A–2 Top: Juvenile green tree pythons (*Morelia viridis*) are either bright yellow or red. Bottom: As they move into adulthood, they turn green. This is an example of developmental variation.

9D EMERGENCE OF A NEW SPECIES LINK

It is important to note that when we talk about evolution, we are not focusing on one individual but on all the individuals of a species living in the same region at the same time. This is the definition of a **population**. A **species** is a group of organisms that can interbreed and produce fertile and viable offspring in their natural environment (explored further in Section 9D).

Population a group of individuals of the same species living in the same region at a given time

Species a group of organisms that can interbreed, producing fertile and viable offspring

Gene pool the sum total of alleles present in a population of organisms

Allele frequency the proportion of a particular allele within a population

Evolution a change in the allele frequencies of a population over time

The gene pool and allele frequency

A **gene pool** is the sum total of alleles present in a population of organisms. For example, consider Figure 9A–3. In this simplified gene pool, there are 6 individuals and 12 alleles, as each individual has two copies of a gene (one from their mother and one from their father). We can use gene pools to determine **allele frequency**, which is the proportion of a particular allele within a population. In this case, D has a frequency of 8/12 (67%) in the population and d has a frequency of 4/12 (33%).

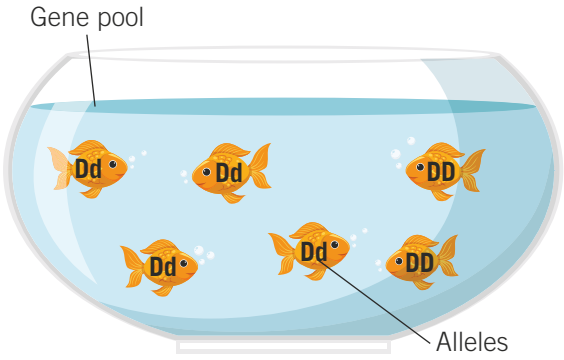


Figure 9A–3 A gene pool is the sum total of alleles in a particular population. This population consists of 6 goldfish. There are 12 alleles in the gene pool.

The range of variations possible in a population is limited by the alleles that are available in the gene pool. In a large gene pool, there is likely to be a greater number of different alleles, and therefore more genetic diversity, while in a small gene pool, there is likely to be less genetic diversity.

Defining evolution

When the allele frequencies in a population changes over many generations, the population is said to be evolving. Therefore, **evolution** can be defined as a change in the allele frequencies of a population over time. These changes result in diversification within a species. In Section 9D you will explore changes that occur over a long period of geological time (such as eras or epochs) and result in the emergence of new species.

9D EMERGENCE OF A NEW SPECIES LINK

What causes allele frequencies in a population to change?

Table 9A–1 Factors that result in changing allele frequences over time

Factor	Explanation
Gene flow	The movement of alleles into (or out of) a population as a result of migration
Genetic drift	Random changes in allele frequency; occur naturally in every population, due to chance events
Selection pressures	Differentially selective environmental pressures; these can occur naturally (random mating) or artificially (non-random mating)
Mutations	A random change in the genetic composition of an organism due to alterations in the DNA base sequence. Mutations do not reshuffle alleles that are already present, but can produce completely new alleles

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

This section focuses on how mutations are a source of new alleles, and Section 9B examines how allele frequencies can change in a population through gene flow, genetic drift and selection pressures.

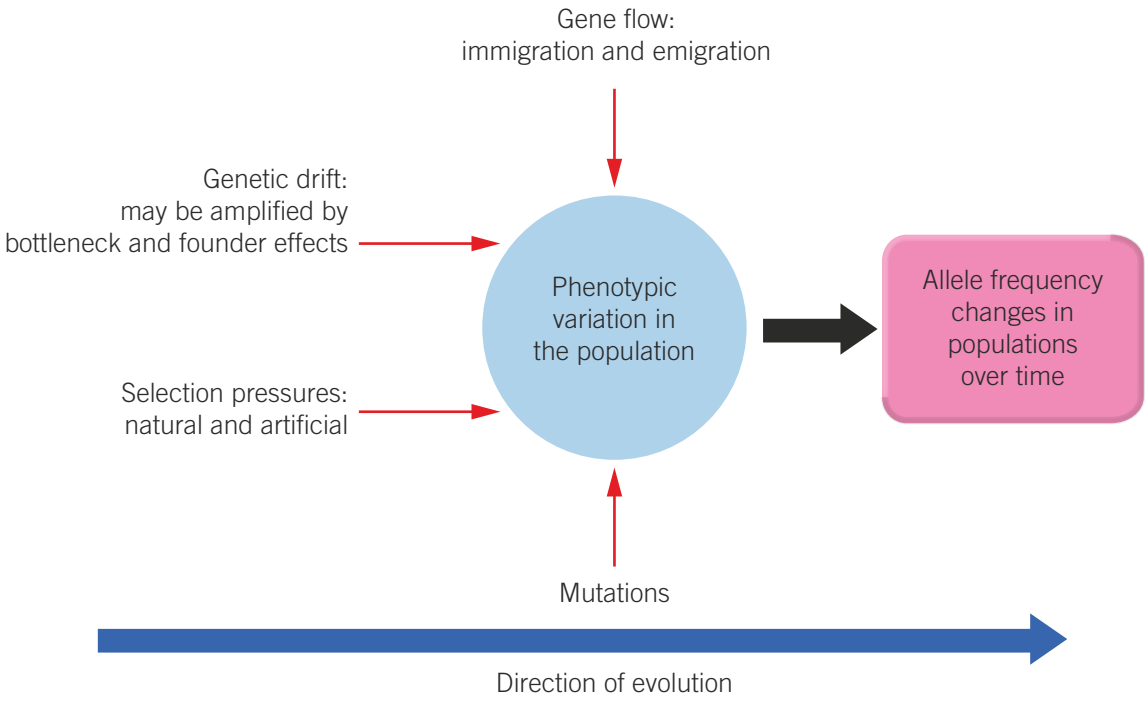


Figure 9A–4 Evolution occurs as a consequence of allele frequencies changing in a population over time. There are many reasons for this change: gene flow, genetic drift, selection pressures and mutations.

Check-in questions – Set 1

1 Define population, species, gene pool, allele frequency and evolution.
2 A geneticist is calculating the allele frequency of a population of 100 individuals. How many alleles for a specific trait are in the gene pool of this population?
3 State the causes of variation in a population – that is, what causes allele frequencies to change in a population over time.

Introducing mutations

Defining mutations

DNA is usually stable; however, genetic material can change, especially when errors occur during DNA replication. When this change is permanent, it is called a **mutation**. An organism's DNA influences its structure, how it behaves, and its physiological processes. So changes to the nucleotide sequence of a section of DNA coding for a specific trait, a gene, can affect the structure and function of the protein it encodes. Therefore, mutations can create new versions of old alleles, which can cause significant variation between individuals. New alleles mean there will be changes in the allele frequencies of a population over time and this, you will recall, is the definition of evolution.

Mutation
a permanent change in the nucleotide sequence of a section of DNA

VIDEO 9A–2
MUTATIONS

Mutations can be classified as somatic or germline.

- A somatic mutation occurs in a body cell of an organism and will only affect that individual cell, so it is not passed on.
- A germline mutation occurs in a gamete and so is passed on to offspring. It is these mutations that have a role in the evolution of a population.

Causes of mutations

Mutations can occur naturally or be induced.

- Spontaneous mutations are naturally occurring changes caused by errors in DNA replication. They create new alleles instantly.
- Induced mutations are caused by exposure to external elements. Agents known to cause mutations, like radiation sources (UV radiation, X-rays, nuclear radiation) and chemicals (benzene, pesticides, drugs), are called **mutagenic agents**. Induced mutations alter DNA at a slower rate than naturally occurring mutations.

Mutagenic agents agents known to cause mutations; for example, some radiation sources and chemicals; also called mutagens

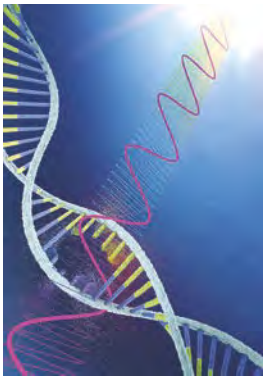


Figure 9A–5 Ultraviolet radiation can damage the DNA in a gene.

Effects of mutations

The effects of gene mutations can be beneficial, detrimental or neutral.

Table 9A–2 Effects of gene mutations

Effect	Explanation
Beneficial	The mutation increases the organism's chance of survival and reproduction.
Detrimental	The mutation decreases the organism's chance of survival and reproduction.
Neutral	The mutation has no discernible effect on the organism's chance of survival and reproduction.

Point mutation a change to one base in the nucleotide sequence of a section of DNA; includes base substitutions and frameshift mutations

Point mutations

Defining point mutations

A **point mutation** is a small, localised change to one base in the nucleotide sequence of a gene. Base substitutions may create silent, missense or nonsense mutations, while insertions and deletions cause frameshift mutations.

2B THE GENETIC CODE AND GENE EXPRESSION LINK

Recall that genetic sequences are read in sets of three nucleotides. In DNA this is called a triplet, and in messenger RNA a codon. These triplets code for specific amino acids, of which there are 20 different types. The genetic code is degenerate – that is, multiple codons code for the same amino acid. When the nucleotide sequence of a gene is altered, the triplets are altered, gene expression is altered and potentially so too is the production of a functional protein.

Substitution mutation a type of point mutation that occurs when one nitrogenous base in a gene is replaced with another base; includes silent, missense and nonsense mutations

Substitution mutations

A **substitution mutation** occurs when a single nitrogenous base is replaced by a different base in the nucleotide sequence of a gene. There are three types of substitution mutations: silent, missense and nonsense (Table 9A–3).

Table 9A–3 Types of substitution mutations

	No mutation	Substitution mutations		
		Silent	Missense	Nonsense
DNA	TTT	TTC	TGT	ATT
mRNA	AAA	AAG	ACA	UAA
Amino acid	Lys	Lys	Thr	STOP

Silent mutations

A **silent mutation** occurs when the base change in the nucleotide sequence of the DNA doesn't change the amino acid that is coded for. Consequently, the protein structure and function remain the same. This is possible because the genetic code is degenerate. You can see in Table 9A–3 how, despite there being a substitution point mutation (TTT → TTC), the amino acid for that triplet remains lysine.

LINK 2B FROM DNA TO PROTEIN

Silent mutation
a mutation where the change in the nucleotide sequence doesn't change the amino acid that is coded for

Missense mutations

A **missense mutation** occurs when the base change in the nucleotide sequence of the DNA codes for a different amino acid. Consequently, the protein is still produced; however, the effect that this new amino acid has on the structure and function of the protein is uncertain. The effects can be minor or severe, depending on which amino acid is altered in the amino acid chain. As you can see in Table 9A–3, when there is a missense substitution point mutation (TTT → TGT), the amino acid for that triplet is changed from lysine to threonine.

LINK 2C PROTEINS

Missense mutation
a mutation in which the base change in the nucleotide sequence of the DNA changes the amino acid that is coded for; a type of substitution point mutation

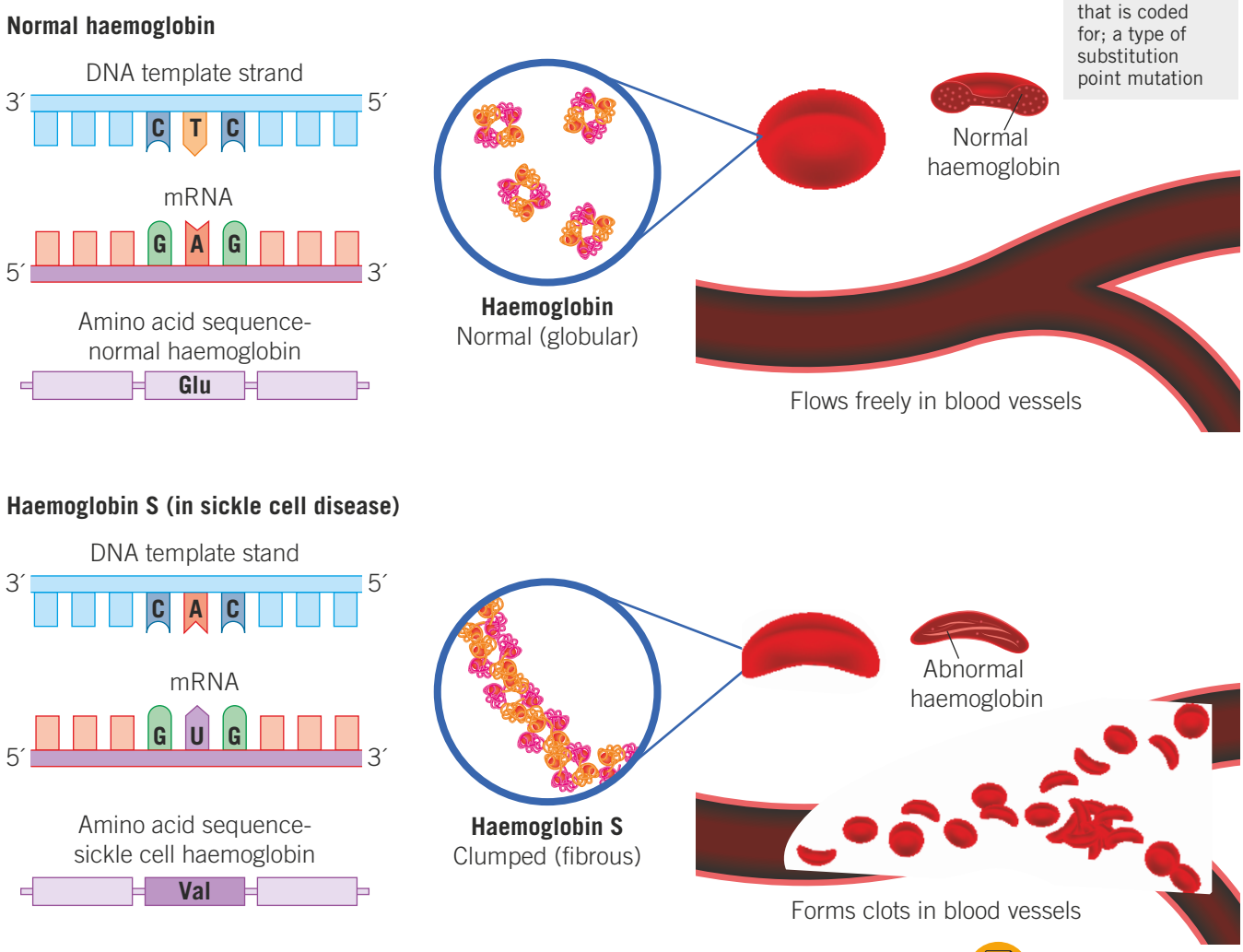


Figure 9A–6 Sickle cell anaemia is a disorder caused by a substitution missense mutation.

Nonsense mutation a mutation that occurs when the base change in the nucleotide sequence of the DNA codes for a STOP codon, prematurely halting the production of the polypeptide; a type of substitution point mutation

Frameshift mutation a type of point mutation that occurs when one base is inserted into or deleted from a gene, causing an incorrect reading of the codons due to a shift in the reading frame

Nonsense mutations

A **nonsense mutation** occurs when the base change in the nucleotide sequence of the DNA codes for a STOP codon. Consequently, the production of the protein is prematurely halted or truncated. The impact of this change depends on the location of the mutation – if it is early in a gene, the effect will be greater than if the change is late in the gene. As you can see in Table 9A–3, when there is a nonsense substitution point mutation (TTT → ATT), the triplet codes for STOP.

NOTE

Specific examples of mutations are not examinable. Remember, however, that assessors set questions in context, so examples may be used in that way.

Frameshift mutations

A **frameshift mutation** occurs when there is either insertion or deletion of a single base in a gene. This alters the codon reading frame and therefore affects every codon, and therefore every amino acid, beyond the point (downstream) of the mutation. This has a major effect on the resulting protein structure and function. Typically, frameshift mutations have more severe consequences than substitution mutations. Examples of diseases caused by, or in part by, frameshift mutations include Crohn's disease and some cancers.

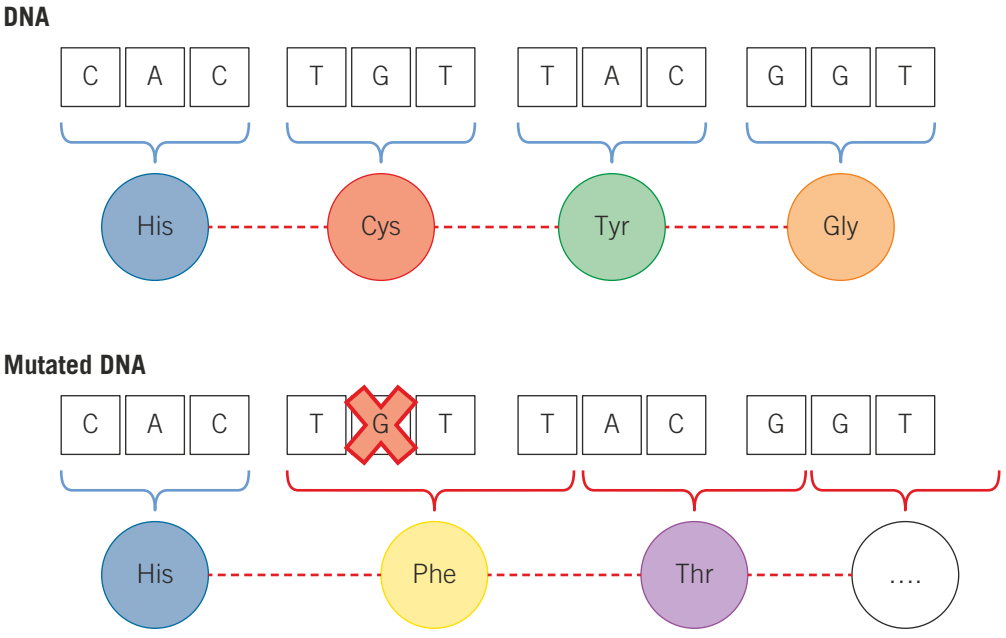


Figure 9A–7 An example of a frameshift mutation: when the base 'G' is deleted from the nucleotide sequence, the reading frame (or triplet structure) changes, resulting in a different amino acid from the point of the mutation onwards.

Check-in questions – Set 2

WORKSHEET 9A–1 POINT MUTATIONS

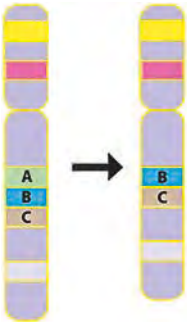
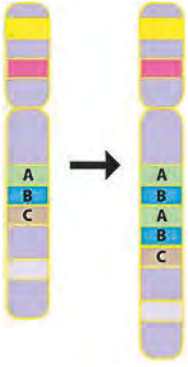
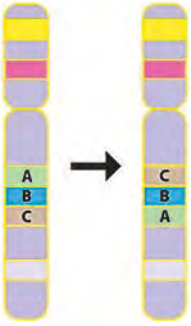
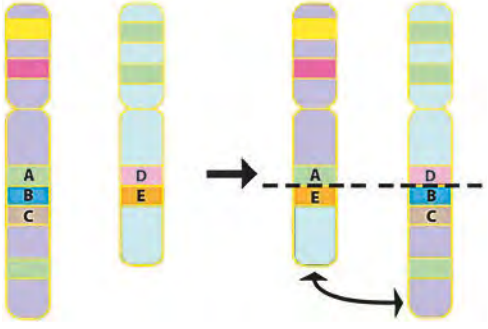
1 Outline how mutations can be the source of new alleles and how this affects genetic diversity.
2 Distinguish between a substitution mutation and a frameshift mutation.
3 Distinguish between silent, missense and nonsense mutations.

Block mutations

Block mutations are also called chromosome mutations, as they alter large sections of DNA containing multiple genes. Such mutations are usually a consequence of spontaneous errors during meiosis. There are several types of block mutations and each can have significant effects on an organism's phenotype, depending on the location of the change. For example, genes may be disrupted, removed or disabled.

Block mutation a type of mutation that affects large sections of DNA, typically containing multiple genes; also called chromosome mutation

Table 9A–4 Summary of the types of block mutation

Diagram	Description
	**Deletion** • A section of DNA is removed from the chromosome. • Leads to disrupted and/or missing genes. • Large impact on growth and development, sometimes fatal.
	**Duplication** • A section of chromosome is repeated multiple times (sometimes thousands of times) on the same chromosome. • Can lead to an increase in gene expression.
	**Inversion** • A section of chromosome is removed, rotated 180° and reattached in reverse order. • Can be as small as two bases, or as large as several genes. • Usually does not cause abnormalities, but can cause lowered fertility.
	**Translocation** • A section of one chromosome attaches to a non-homologous chromosome or sections are exchanged between the two. • Leads to disruptions in gene sequences or interruptions in gene regulation. • Can cause some cancers.

Chromosome abnormalities

UNIT 2 LINK

Chromosomal abnormalities are mutations that involve whole chromosomes, or a change in the number of chromosomes. To identify whether a cell contains a chromosomal abnormality, a **karyotype** can be used prenatally.

Chromosome abnormality mutation that involves a whole chromosome, or a change in the number of chromosomes, which can be identified using a karyotype; examples are aneuploidy and polyploidy

Karyotype a pictorial representation of chromosomes that allows a geneticist to determine size, banding pattern, shape and number of chromosomes in an individual's somatic cell; allows the determination of diploid number, gender and chromosomal abnormalities

Aneuploidy when a cell has one more or one less chromosome than expected, usually due to non-disjunction

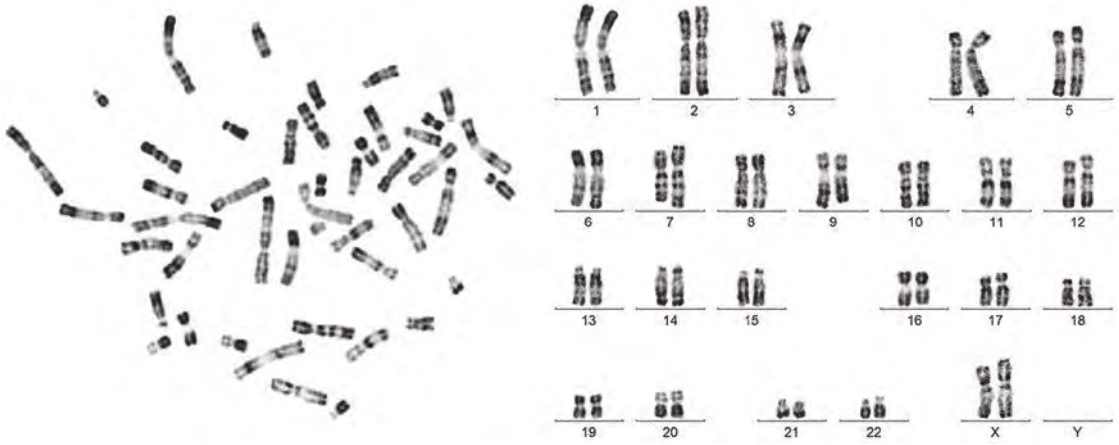


Figure 9A–8 A karyotype can be used to identify chromosomal abnormalities. A photograph is taken when the chromosomes have condensed (left), and they are then stained and arranged according to their structure (right). Example above shows a normal female karyotype.

Aneuploidy

Aneuploidy is when there is the addition of or loss of *one* chromosome from a cell. This can be caused by non-disjunction during meiosis, when the chromosomes fail to separate during anaphase I or anaphase II. The result is daughter cells or gametes forming with an incorrect number of chromosomes, as shown in Figure 9A–9.

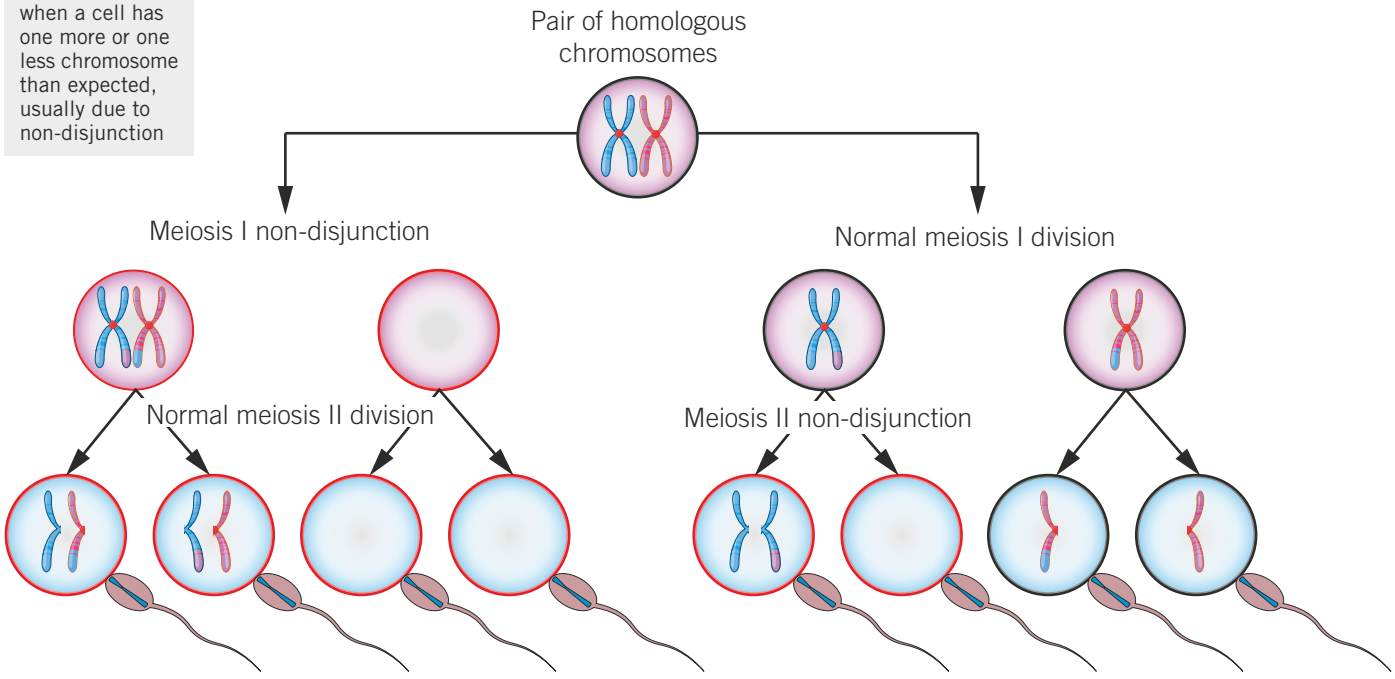


Figure 9A–9 Aneuploidy is a consequence of non-disjunction, where chromosomes do not separate as they should during meiosis I or meiosis II. The affected cells, outlined in red, have one more or one less chromosome than expected.

Polyploidy

Polyploidy is a condition in which an organism has more than two full sets of chromosomes in its cells. Recall how a cell normally has two copies of every chromosome (diploid, $2n$), as the gametes are haploid (n) and one is received from each parent. However, if errors occur during meiosis, when gametes are made, a gamete may be diploid, so at fertilisation the zygote ends up being polyploid. If one gamete is diploid, triploidy results ($3n$), and if both gametes are diploid, tetraploidy results ($4n$).

Polyploidy
a condition in which an organism has more than two full sets of chromosomes in its cells; more common in plants than animals

The polyploid organism will not be able to reproduce to create fertile and viable offspring unless it is able to mate with an individual that has the same chromosomal error, which is unlikely. Remember, to form offspring, pairs of chromosomes are needed, not odd numbers. However, in the case of plants, self-fertilisation and asexual reproduction are common, and so in this way a polyploid plant can create offspring. This is why polyploidy is more common in plants than in animals. Figure 9A–10 shows examples of naturally occurring and artificially created polyploids.

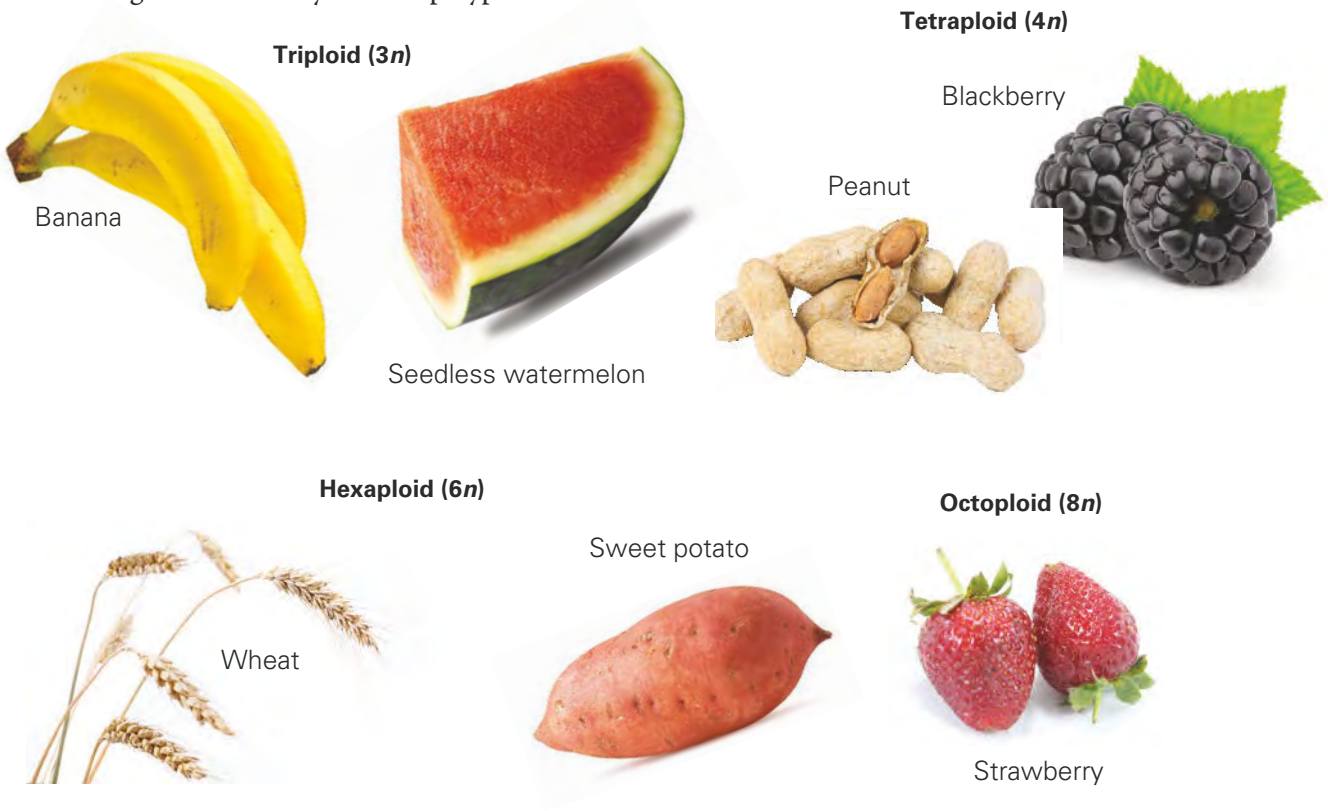


Figure 9A–10 Polyploidy is more common in plants than in animals. Scientists believe this is a survival advantage that has developed accidentally in plants. Plant breeders can also intentionally develop polyploids with desirable traits, such as seedless watermelons.

Check-in questions – Set 3

1 Distinguish between a block mutation and a chromosome abnormality.
2 List the different types of block mutation and write three words to describe each.
3 Distinguish between aneuploidy and polyploidy.

WORKSHEET 9A–2 BLOCK MUTATIONS AND CHANGES IN CHROMOSOME NUMBER

VIDEO 9A–3 SKILLS: UNDERSTANDING THE LINK BETWEEN NUCLEIC ACIDS

9A SKILLS

Understanding the links between nucleic acids

You have now covered the structure of nucleic acids, transcription and translation, proteins and mutations. Many assessment questions link all these ideas together. Earlier chapters have covered the value of mind maps, linking different concepts and completing as many practice questions as possible. Now our focus is on identifying the nucleic acids from the information provided in a question. For example, some questions may give you an amino acid sequence, some will give you the code on the DNA template strand, and others the mRNA codons. For these types of questions, a codon table will be provided. Here is one from the 2017 VCAA Biology examination, followed by two questions that use it:

Codon table to determine amino acids

1st position (5′ end) ↓	2nd position				3rd position (3′ end) ↓
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Figure 9A–11 Codon table from the 2017 VCAA Biology examination © VCAA 2017

The following nucleotide sequence occurs on the template strand of a DNA molecule.

AAA GCT ACC TAT CGG TTA

The codon table provided can be used to determine the sequence of amino acids coded for by a nucleotide sequence.

Question 27

In a mutation, the eighth nucleotide in this sequence was changed from C to T.

What would be the result of this mutation?

A The peptide chain would be shortened.

B The third amino acid would change from Thr to Ile.

C The fourth amino acid would change from Ile to Tyr.

D There would be no change in the amino acid sequence.

Question 28

In a different mutation, a T was inserted after the fourth nucleotide on the given template strand.

The result of this mutation would be that

A all amino acids in this entire sequence would change.

B only the first amino acid in the sequence would change.

C only the second amino acid in the sequence would change.

D all amino acids after the first in the sequence would change.

Points to keep in mind when approaching this type of question:

- The table provided is called a 'codon table'. The term 'codon' implies that the table is for mRNA. Indeed, among the nitrogenous bases listed around the outside, uracil (U) is present, so it *is* RNA. Take care to check this, as a table of triplets that is DNA could just as easily have been provided.
- The information above the questions says that the nucleotide sequence given is on the template strand of a DNA molecule. The sequence confirms that this is DNA, as thymine is present. Again, assessors could provide the coding strand of DNA, or an mRNA strand, so take care to check.
- Also remember that the template strand can be referred to as the non-coding strand.

Now you are ready to attempt the questions.

Question 27

Count along to the eighth base in the sequence provided, and change it to a 'T'. You are not ready to use the codon table yet, as you are working with DNA. The complementary mRNA codon to this DNA triplet in the template strand would be ATC → UAG (previously UGG). Now you can use the codon table, and you can see that a STOP is now coded for (previously Trp). Therefore, the answer is A.

Question 28

For this question you don't need to refer to the codon table. Inserting a nucleotide in the second triplet of the DNA template strand means the second amino acid will be affected. However, insertions and deletions of this sort (point mutation) cause a frameshift, and therefore the answer is D.

To prepare for questions that combine all these elements, ensure you can move forwards and backwards through the process of transcription and translation. For example, if provided with the amino acids, you should be able to work backwards to the template DNA to identify the mutation, and vice versa. Mutations are permanent changes to the nucleotide sequence of a section of DNA, so a question could ask about the change itself or about the consequences of the change.

VIDEO 9A–4 SKILLS: MUTATIONS, EVOLUTION AND GENETIC DIVERSITY

2B THE GENETIC CODE AND GENE EXPRESSION LINK

Mutations, evolution and genetic diversity

Although it is easy to focus on DNA, proteins and the different types of mutations, remember that this chapter is about evolution – that is, how allele frequencies can change in a population over time.

You know that some mutations have *no* impact on the survival of an organism, so these mutations are not likely to contribute to a population evolving. This may be because the:

- mutation is in a non-coding DNA region (between genes or in a gene intron).
- mutation doesn't change the amino acid that is coded for
- change to the function of the gene or protein is not helpful or harmful.

However, mutations can be a source of new alleles if they occur within or alter an exon of a gene, or affect the structure and function of a protein by altering the amino acid chain, or an entire gene. Therefore, mutations do have the potential to change allele frequencies in the gene pool of a population. However, they will only contribute to evolutionary change if the mutations occur in the genetic information of cells that can be passed onto future generations – that is, in germline cells. Additionally, the organism must survive to maturity and reproduce, so the new allele can enter the next generation.

What about the impact of mutations on genetic diversity? Recall the following dot point in the Study Design:

- Biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity

Do mutations cause an increase or decrease in genetic biodiversity? Genetic variation, or genetic diversity, is a measure of the range of genetic differences that exist within a population or within an entire species. This means a population with many different alleles for a single gene locus has a greater amount of genetic variation than a population in which there is only one possible allele at a single gene locus. Therefore, if a mutation creates a new allele, and this allele is inheritable, this will increase genetic diversity.

Figure 9A–12 The great diversity of *Harmonia axyridis*, commonly called the harlequin or Asian ladybeetle

Section 9A questions

1 A population of koalas has three genotypes: EE, Ee and ee. There are 20 individuals with the EE genotype, 20 with the Ee genotype and 10 with the ee genotype.

- **a** Define gene pool.
- **b** How many individuals are in this koala population?
- **c** How many alleles are there in this population of koalas?
- **d** How many alleles for the dominant trait (E) are there? How many alleles for the recessive trait (e) are there?

2 A germline mutation occurred in a person and resulted in a change in a DNA triplet from CTA to GTA. CTA codes for the amino acid aspartic acid. GTA codes for the amino acid histidine.

- **a** Name the type of mutation that resulted in the change from CTA to GTA.
- **b** Explain why this mutation could be harmful.
- **c** Suggest why this mutation is likely to have ongoing effects.
- **d** This type of mutation does not always result in a change in the amino acid coded for by the affected triplet. Explain why.
- **e** Two other types of mutation are the insertion and deletion of bases. Suggest why the addition or deletion of a single nucleotide is often more harmful to an organism than the mutation that occurred here.

3 Edwards syndrome is characterised by three copies of chromosome 18. This is an example of aneuploidy.

- **a** Define aneuploidy.
- **b** How is this type of chromosome abnormality detected prenatally?

4 There are several types of chromosome or block mutations.

- **a** Name two of these.
- **b** Summarise the similarities and differences between the two types of mutations you listed in part **a**.
- **c** If a chromosome still contains all its genes following inversion, suggest why the genes on that section may not be expressed.

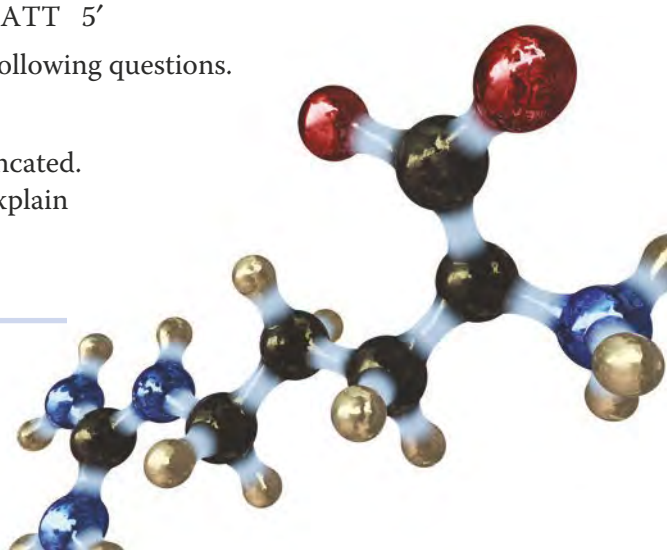
5 Give reasons why mutations are said to increase the genetic diversity of a population.

6 Consider the hypothetical gene below. The DNA template strand is shown.

3′ TAC AAA CCG GCC TTT GCC ACC AAC CCC CTA ATT 5′

Using the codon table in the 9A Skills section, answer the following questions.

- **a** Write the sequence of amino acids this gene codes for.
- **b** A mutation causes the polypeptide being built to be truncated. Identify which of the nucleotides must have changed. Explain your choice.
- **c** Name this type of mutation.



9B Evolving and non-evolving populations

Study Design:
- Causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow; and mutations as the source of new alleles
- Biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity
- Manipulation of gene pools through selective breeding programs

Glossary:
Artificial selection
Bottleneck effect
Founder effect
Gene flow
Genetic drift
Natural selection
Selection pressures
Selective advantage
Selective breeding

ENGAGE

The fastest land animal

The world's fastest land animal, the cheetah, can sprint at speeds of around 120 km/h! They can accelerate faster than most sports cars but, sadly, they can't outrun the possibility of extinction.

Figure 9B–1 The fastest land animal could be facing extinction. Despite surviving two unpredictable events that dramatically decreased the size of the population, the cheetah is once again fighting for survival.

Pressure from climate change, hunting by humans, and habitat destruction are currently reducing their populations. But cheetahs also face problems as a consequence of their own genetic information – they have a low rate of reproductive success, meaning that as a species they are not always able to reproduce. However, this is not the first time in history that cheetahs have faced extinction. Genetic analysis of wild cheetahs shows that they have already survived at least two major catastrophic events, called bottleneck events. A bottleneck event occurs when, for example, a natural disaster dramatically reduces the size of a population. When this happens, as it did in the case of the cheetah, the few surviving individuals end up mating with relatives (inbreeding) and this reduces the genetic diversity of the population. Even though the size of the cheetah population in Africa had recovered to some degree by the 19th century, the cheetahs are almost all genetically identical and heavily inbred. When there is such low genetic diversity in a population, it is very difficult for the population to adapt to changes in their environment. If one individual is affected, all individuals are affected. As the population of wild cheetahs declines, only time will tell if they can survive the current genetic bottleneck.

This section explores how allele frequencies can change due to gene flow, genetic drift and environmental selection pressures (natural and artificial). The consequences for the genetic diversity of a population are also explored.

EXPLAIN

Changing allele frequencies

Gene flow

Gene flow is the exchange of genetic information, specifically alleles, between populations of the same species. In animals, gene flow results from their movement into (immigration) or out of (emigration) a population. In plants, it results from the movement of seeds and pollen.

Gene flow
the exchange of genetic information, specifically alleles, between populations

VIDEO 9B–1
CHANGES TO ALLELE FREQUENCY

When genes 'flow' from one population to another, that flow may increase genetic diversity of the gene pool of the receiving population, affecting the allele frequency. This may also decrease the variability between the two populations involved, making them more similar or homogenous.

NOTE

Interbreeding between the organism carrying an allele and a member of the population the organism is immigrating into, must also occur in order for this allele to become part of the gene pool.

Figure 9B–2 When genes flow, the genetic diversity of the populations involved may change. With the movement of the 'a' allele from the left population into the right population, the allele frequencies of the receiving population will change and increase in diversity.

PPS

Genetic drift

You have seen how allele frequencies can change as organisms move between populations, but they also change randomly. This is called **genetic drift**. In genetic drift, no allele is favoured; they are all equally subject to being affected.

Genetic drift
a random change in allele frequency, occurring naturally in every population, due to chance events

In small populations, genetic drift can have a much more dramatic effect than in large populations, as it can lead to a reduction in genetic diversity. As time progresses, alleles may be lost from the gene pool (frequency is 0%) or fixed as the only allele present for the gene (frequency is 100%). Larger populations are proportionately less affected by these unpredictable events and retain more stable allele frequencies.

9A MUTATIONS LINK

Events in the environment may 'magnify' genetic drift, by randomly killing a part of the population. Such events may be volcanic eruptions, landslides, floods and bushfires – but they also include the impact of disease, predators and so on.

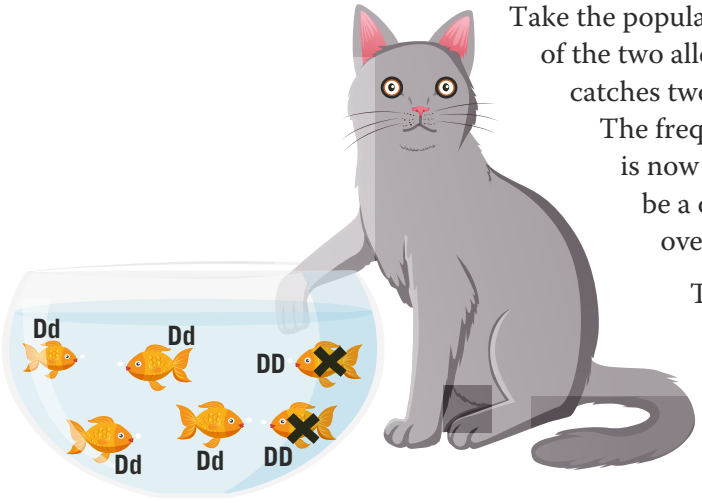


Figure 9B–3 Genetic drift is a change in the allele frequencies of a population that occurs because of a random or chance event.

Take the population of goldfish in Figure 9B–3, where the frequency of the two alleles present is very different. Suppose a cat randomly catches two fish, which happen to both have the genotype DD. The frequency of each allele left in the population, D and d, is now equal. As the fish reproduce from now on, there will be a change in the allele frequencies of the population over time, meaning that evolution is occurring.

There are two effects in genetic drift that may be severe: the bottleneck effect and the founder effect.

Bottleneck effect

If a population is reduced to low numbers by an unpredictable and catastrophic event (natural or human-induced), there may be a severe reduction in the population's genetic diversity, which causes changes in the allele frequency of the gene pool, and thus the population is evolving. The population may recover and increase its numbers again, having squeezed through a 'bottleneck' of low numbers. However, as the surviving members of the species begin to repopulate, the allele frequencies of the new population are no longer representative of the original gene pool. This is called the **bottleneck effect**.

Bottleneck effect when a population is drastically reduced to low numbers by a random or chance event and the allele frequencies of the surviving population do not reflect the genetic diversity of the original population

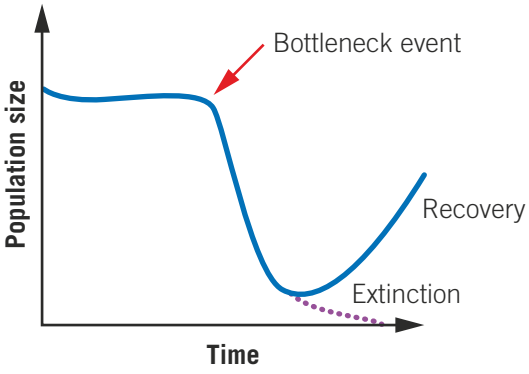


Figure 9B–4 The impact of a bottleneck event on population size is clear when represented in a graph.

The smaller the population, the larger the impact that the bottleneck effect has. Alleles may be lost entirely after one natural disaster or may disappear after several generations of reproducing. Remember the cheetah, which has been through two bottleneck events. The first occurred around 100 000 years ago and the second about 10 000 to 12 000 years ago, at the end of the last ice age. As a consequence, many cheetahs are homozygous at a very high percentage of their gene loci; that is, there is less than 5% variation between members of the species. This puts the cheetah at high risk of extinction. When a population has low genetic diversity or variation, like the cheetah, it is less likely that there will be an allele that offers a survival advantage if selection pressures or the environment change.

Founder effect when a small sample of a large population moves away to colonise a new area and becomes isolated; the allele frequencies of the founder population do not represent the genetic diversity of the larger original population

Founder effect

The **founder effect** in genetic drift occurs when a small sample of the original population moves away to colonise a new area and becomes isolated. The individuals in that small colony are unlikely to have the same allele frequencies that were in the original population's gene pool and so, as time passes and the population increases through interbreeding, the gene pool of the founder population will no longer represent the gene pool of the original population. Like the bottleneck effect, alleles in the founder population may be lost entirely, or with inbreeding occurring, may even disappear after several generations, thus reducing its genetic diversity.

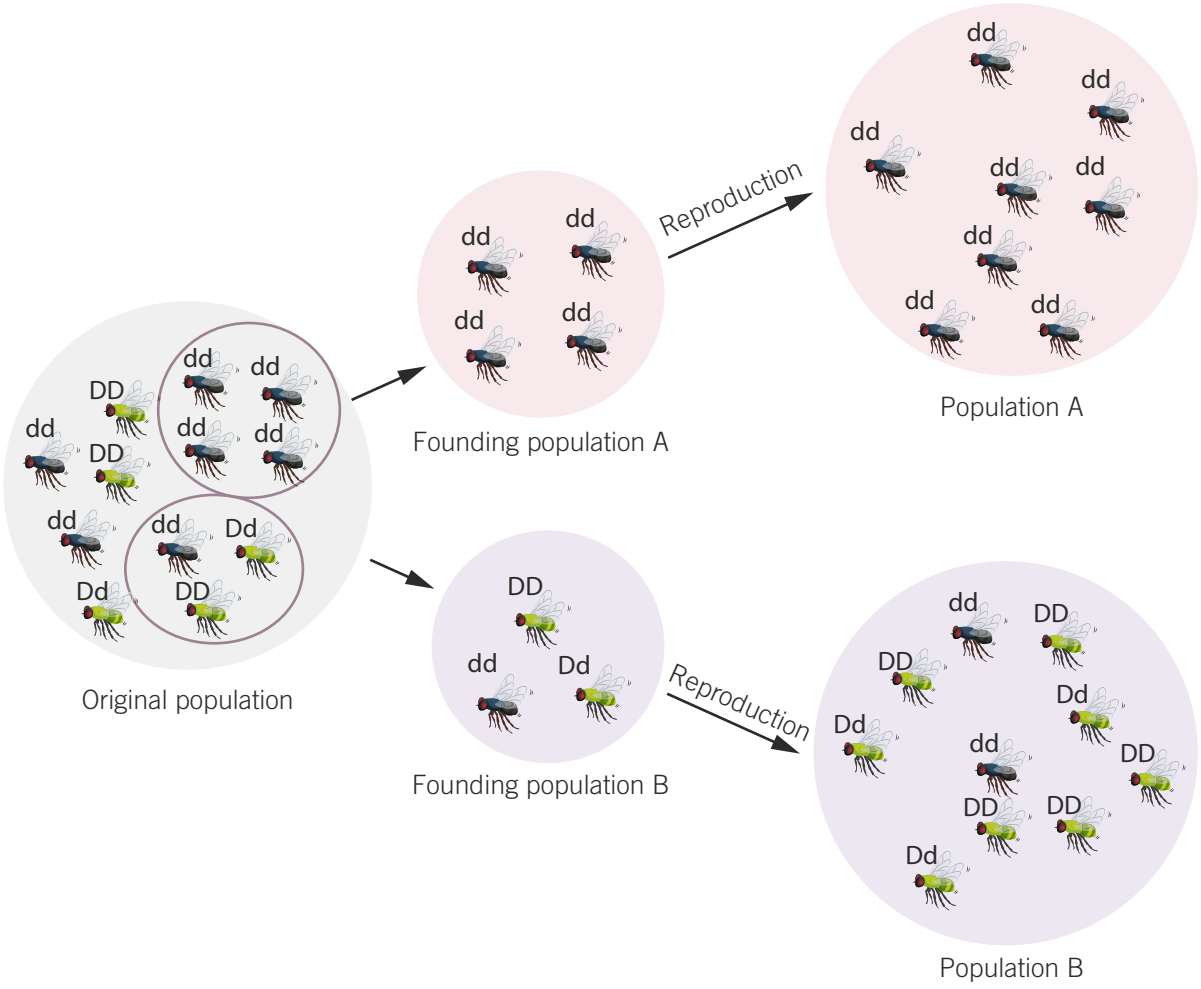


Figure 9B–5 When a small sample of a large population moves away to colonise a new area and becomes isolated, the allele frequencies of the founder population change over time and eventually do not represent the genetic diversity of the larger original population. Two examples are shown here.

The environmental pressures experienced by the founding population in the new area are likely to be different than those experienced by the original population. Therefore, the founding population and the original population will evolve differently. The founding population with lower genetic diversity is less likely to have alleles that may provide a survival advantage and so is more vulnerable in changing conditions.

Figure 9B–6 Macaroni penguins live on sub-antarctic islands and the Antarctic Peninsula. Most have black faces (left) and a small proportion have white faces. A population of penguins on Macquarie Island is identical to the macaroni penguin except they all have white faces (right). It may be that, by chance, the small founder population that first occupied Macquarie Island consisted only of white-faced macaroni penguins, now called royal penguins.

Check-in questions – Set 1

1 Define gene flow.
2 a Explain what is meant by 'genetic drift'.
  b Outline why genetic drift can have a greater effect on the genetic variation of a small population than on a large population.
3 List the similarities between the bottleneck and founder effects.

WORKSHEET 9B–1 THE GENE POOL AND RANDOM CHANGES TO THE ALLELE FREQUENCY

Selection pressures the conditions or factors that influence allele frequencies in a population by contributing to the selection of which phenotypes survive in a given environment, e.g. availability of resources, environmental conditions, predators and disease

Selection pressures

If a particular phenotype in a population gives an individual a survival advantage, that individual is more likely to survive, reproduce and pass on its alleles to the next generation. The conditions or factors that influence allele frequencies in a population in this way are known as **selection pressures**. They are pressures that contribute to the selection of phenotypes that will be advantageous in a given environment. Examples of selection pressures are given in Table 9B–1.

Table 9B–1 Examples of selection pressures

Factor	Examples
Abiotic	• Availability of resources (e.g. shelter) • Environmental conditions (e.g. temperature, natural disasters and geographic features)
Biotic	• Predators • Disease

9A MUTATIONS LINK

Selection pressures only influence the allele frequencies in a population when there is variation in the population, with some members of the population being better suited to the environmental conditions than others to the particular environmental conditions. This variation is usually the consequence of a mutation. For example, a snails' shell colour does not matter until a new predatory bird moves into the area. The bird then becomes a selection pressure and the snails with shells that provide camouflage with their environment are at an advantage. These snails survive, as they are better suited to the environment, while other snails without well-camouflaged shells are less likely to survive. As you can see in this example, selection pressures can decrease the occurrence of a trait or increase the occurrence of a trait, but in both cases, there is the potential to change the allele frequencies of a population over time, thus causing evolution.

Selection pressures can be natural environmental pressures (*natural selection*) or artificial pressures brought about by humans through selective breeding or *artificial selection*.

Figure 9B–7 A predatory bird is considered an example of a biotic selection pressure as it can influence the allele frequencies in a population of snails, by determining which shell phenotypes confer survival in a given environment.

Natural selection

The theory of **natural selection** was conceived by Charles Darwin (and independently by Alfred Russel Wallace), who popularised it as 'survival of the fittest' (a term already in use). However, this does not literally mean survival of the fittest organism – otherwise the cheetah would not be in so much trouble! Natural selection is the influence of selection pressures on the allele frequency of a population. It is the selection pressures that affect the survival of an individual in the population, but remember, this can only occur if there is already phenotypic variation in the population. That is, those individuals that are best suited to the environment or have a phenotype that offers a survival advantage (called a **selective advantage**) are more likely to survive, reproduce and pass on their favourable alleles to the next generation.

Natural selection an evolutionary process whereby those individuals in a population that have a particular set of alleles are best suited to the environment and will survive, reproduce and pass on their genetic information to the next generation

Selective advantage a trait or phenotype that provides a survival advantage

Therefore, due to natural selection, the frequency of alleles will change over generations, causing evolution to occur. Beneficial phenotypes result in more offspring and therefore the responsible allele(s) are more likely to increase in frequency, while detrimental or less suitable phenotypes result in fewer offspring and hence the responsible allele(s) become less common in the gene pool. If there is a change in environmental conditions or selection pressures, those phenotypes that were once beneficial may no longer be, and thus the allele frequencies in a population are constantly evolving.

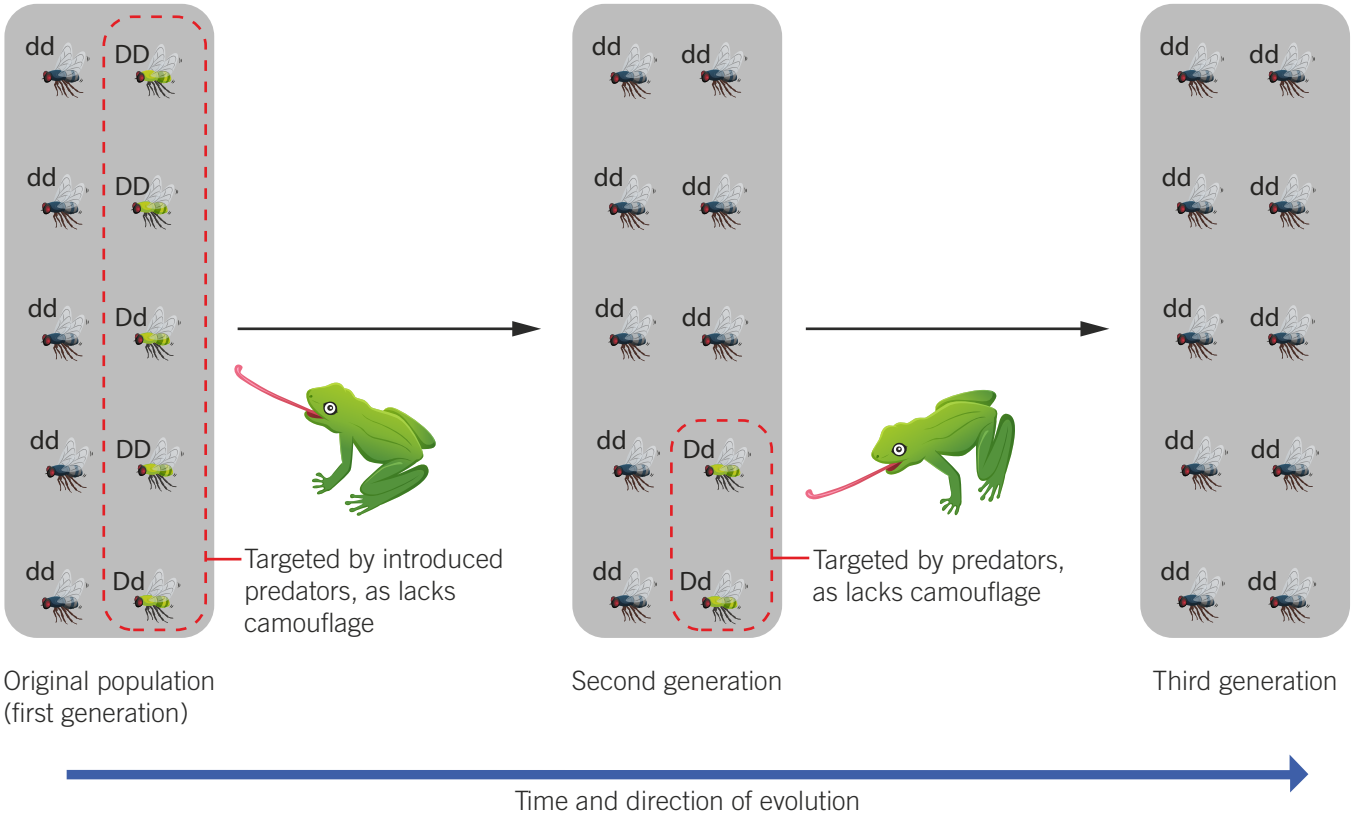


Figure 9B–8 Variation exists in the original population of these flies. A selection pressure, a predatory frog, eats individuals with a selective disadvantage (green colour standing out) and, over time, individuals with a selective advantage (grey colour against a grey background) survive and reproduce, passing on their alleles to the next generation. Allele frequencies in the population change – evolution is occurring.

Adaptations, which are the phenotypes of an organism that make it better suited to its environment, are favoured by natural selection. How this leads to the emergence of new species is covered in Section 9D.

LINK UNIT 2
LINK 9D EMERGENCE OF A NEW SPECIES

Check-in questions – Set 2

1 Define selection pressures, selective advantage and natural selection.
2 The peppered moth has two phenotypes: a light grey form and a melanic dark form. By day the moths live on tree trunks and are a food source for local birds. In the early 1800s, the moth population consisted mainly of the light grey form, with only a few of the dark form. By the late 1800s, as a result of the Industrial Revolution, woodland trees became stained with soot and the frequency of dark moths increased.

a In the early 1800s, which phenotype had a selective advantage? Which was at a selective disadvantage?
b In the late 1800s, which phenotype had the selective advantage? Which was at a selective disadvantage?
c What is the selection pressure on the moths?

Artificial selection

Selective breeding or artificial selection occurs when humans deliberately intervene in the reproductive process. Organisms that have the desired phenotypic characteristics are selected by humans to reproduce and pass on their alleles, while those with undesired characteristics are prevented from doing so. In this way, breeders manipulate the gene pool and keep the desired features (and their alleles) in a population that are economically beneficial or aesthetically pleasing. However, this process significantly reduces the genetic diversity of the breeding population and consequently increases their vulnerability to selection pressures.

Selective breeding or artificial selection a process whereby humans intervene in the breeding of a species to keep desired features in a population that are economically beneficial or aesthetically pleasing, by selecting which organisms are to reproduce

There is no guarantee that all offspring will show the desired characteristics, and so offspring with the desired characteristics are selected and bred together. This process is repeated for many generations before all offspring will reliably show the selected characteristics.

A comparison of natural and artificial selection is shown in Table 9B–2.

Table 9B–2 Comparison of natural and artificial selection

Natural selection	Artificial selection
Occurs naturally	Occurs when humans intervene
Results in populations with characteristics that best suit the environment	Results in populations with characteristics desired by humans (e.g. aesthetically pleasing). Not always best suited to the environment
Usually takes a long time to occur	Takes less time than natural selection

Plants

Plants have been selectively bred by humans for thousands of years, for many reasons including disease resistance, increased crop yield, drought tolerance, sweeter-tasting fruit and larger or more colourful flowers.

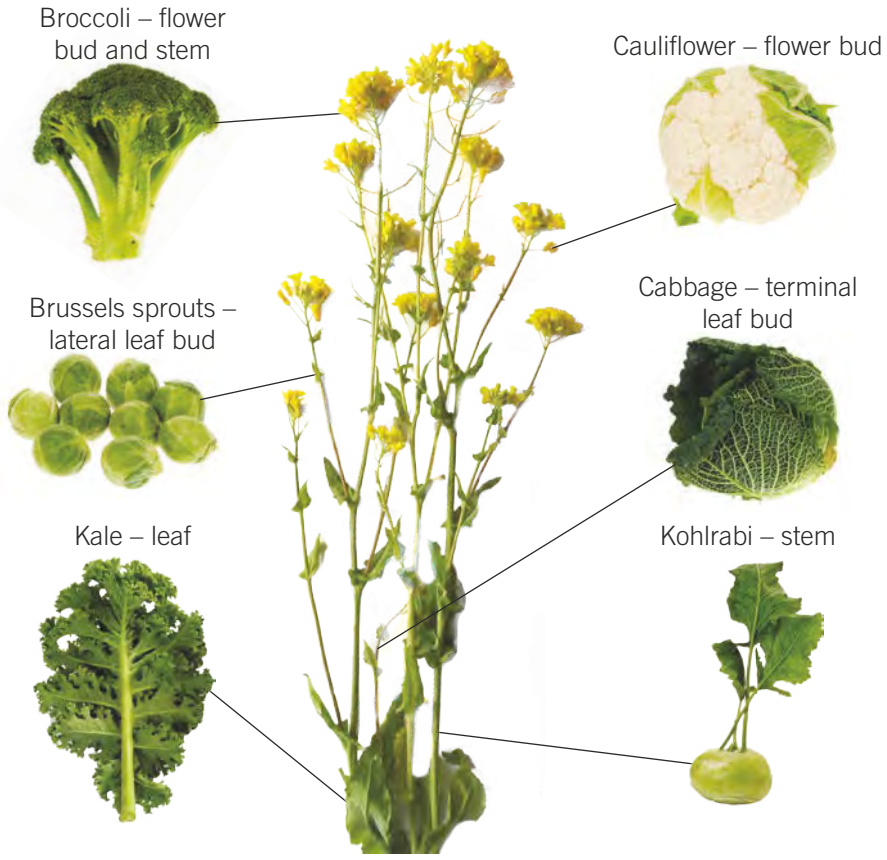


Figure 9B–9 An example of a plant that has been selectively bred is wild brassica, which has given rise to cauliflowers, cabbages, broccoli, Brussels sprouts, kale and kohlrabi.

Animals

Humans have been selectively breeding animals for at least 8000 years. Animals are selectively bred for various characteristics, including: sheep that produce high-quality wool; chickens that lay large eggs; domestic dogs that are good at herding; horses that are strong for pulling ploughs; cows that produce lots of milk.

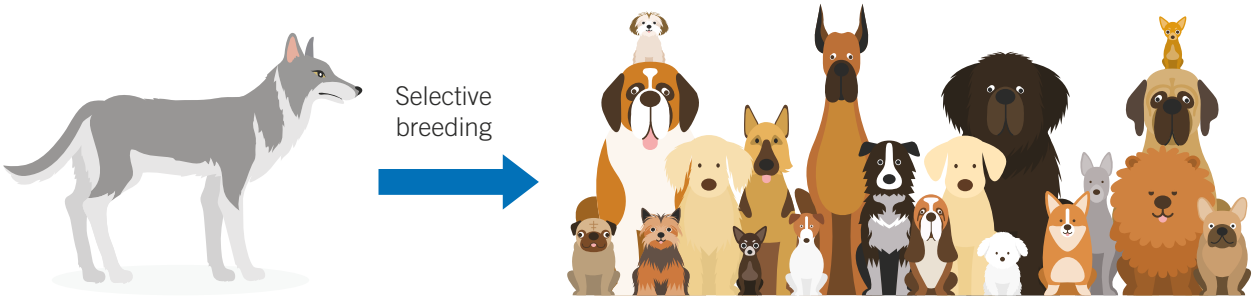


Figure 9B–10 An example of an animal that has been selectively bred is the domestic dog. All breeds are descended from a single species, *Canis lupus*, the grey wolf.

CHAPTER 3 LINK

Today, modern selective breeding techniques often employ reproductive technologies, like artificial insemination, and more complex molecular technologies like genetic engineering and gene editing. You were introduced to these methods in Chapter 3, and how they can be used to alter the characteristics of an organism in a targeted and specific way.

WORKSHEET 9B–2 CHANGES TO THE ALLELE FREQUENCY BASED ON SELECTION: NATURAL AND ARTIFICIAL

Check-in questions – Set 3

1 Outline how artificial selection can cause a change in allele frequencies in a population's gene pool.
2 Identify the key differences between natural and artificial selection.

VIDEO 9B–2 SKILLS: SCENARIO BASED QUESTIONS

9B SKILLS

Scenario-based questions

There are thousands of examples of gene flow, genetic drift and natural selection in the world, and your assessors will not expect you to be familiar with all of them! However, they will expect that you can apply your knowledge to whichever example you are given. Remember to convey your understanding of the concept, not the specific scenario.

Let's use natural selection as an example. Information about natural selection may be presented as a description, a table of data or a graph. Consider the following scenario and the possible questions that follow. Keep in mind that you cannot just state the key steps in natural selection. You need to demonstrate to your assessor you can make the link between the concept and the stimulus material provided, so make sure you refer to the example regularly as you write your answer.

Question:

In 1950, *Myxoma virus* was introduced into Australia to try to control the impact of the European rabbit. Initially the virus was highly successful, killing over 98% of the rabbits it infected, in just days. However, as time passed, the virus became less effective and the rabbit population slowly began to increase again.

Give reasons for the increase in the rabbits' population.

OR: Using the information, explain how natural selection is 'survival of the fittest'.

OR: Outline the steps that resulted in the rabbit population surviving *Myxoma virus*.

Possible answers:

Natural selection has occurred.

- *Variation* existed in the population: rabbits that are susceptible to the virus and rabbits that are resistant to the virus (beneficial phenotype)
- This variation is *inherited.*
- There is a *selection pressure*: the virus.
- Those individuals with alleles for the beneficial phenotype are at a selective advantage/are *best suited to the environment*, so they are selected for. Those individuals with alleles for the unfavourable phenotype are selected against.
- Those with the beneficial phenotype (resistance to the virus) are more likely to *survive, reproduce and pass on their alleles* to the next generation.
- Over *generations,* these *alleles will increase in frequency* in the gene pool of the population.
- Therefore, the rabbit population increasingly becomes composed of individuals that carry the beneficial allele that confers resistance to the virus.

OR: Therefore the rabbits that have the resistant phenotype are at a selective advantage in this environment.

The use of acronyms to remember processes is discussed in the 9D Skills section. Natural selection can be summarised using the acronym VISEG:

V: Variation *I: Inherited* *S: Selection pressure* *E: Environment* *G: Generations*

Note that the terms that these letters stand for have been included in italics in the answers above.

Genetic diversity

VIDEO 9B–3
SKILLS: GENETIC DIVERSITY

Remember this dot point from the Study Design?

- Biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity

How does this apply to what you have learnt in this section, about gene flow, genetic drift (bottleneck and founder effects) and selection pressures (natural and artificial)? Read back through this section, focusing on information related to genetic diversity. Then summarise your findings in your notes, under sub-headings. Alternatively, if you are a visual learner, make a mind map or a poster and annotate it with definitions, examples, diagrams and an explanation of whether genetic diversity is increased or decreased.

Why have we not just given you the answer here? If you have to work something out for yourself, you will need to think and problem solve, and by interacting with the content, you are more likely to make connections in your long-term memory. If you are still unsure of your answer, ask a classmate and try to work it out together.

Section 9B questions

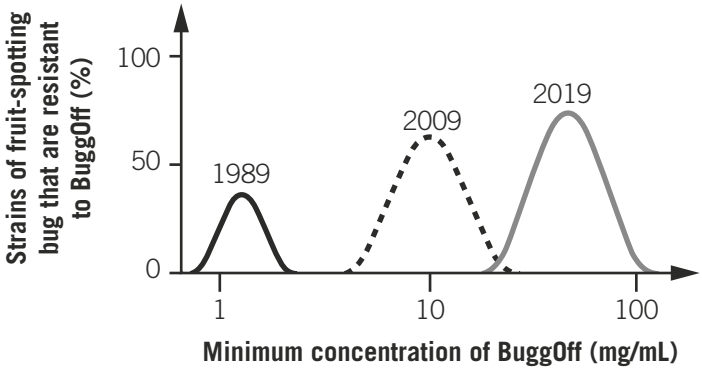
1 Some dogs are selectively bred so that they do not cause allergic reactions in their owners.
   - **a** Give two more reasons why people might selectively breed dogs.
   - **b** Explain how the 'allergy-free' dog has been produced by selective breeding.
   - **c** Explain the effect of selective breeding on the genetic diversity of this dog population.

2 The mountain pygmy possum (*Burramys parvus*) is found in only two locations in Victoria: Mt Hotham and Mt Buller. In 1996, there were around 300 possums in the Mt Buller population but by 2010 this number had dropped to around 40. This is due to the loss of habitat as a result of bushfires, encroaching ski resort developments and hunting by feral cats.

   - **a** Name and define the evolutionary process that has occurred.
   - **b** Explain why the genetic diversity of the possum population also decreased between 1996 and 2010.

   In 2010, scientists began an intensive recovery program to help the possums. They began by restoring the possums' habitat and controlling predators. Then they relocated several males from the Mt Hotham population into female territory of the Mt Buller population. Since 2014, the numbers of mountain pygmy possum have increased.
   - **c** Normally, if a population has low genetic diversity and the population is able to increase in size over time, the diversity still remains low. Outline why, in the case of the possums, the genetic diversity of the population has increased as the population size has increased.

3 Australia exports macadamias to the rest of the world. Farmers who grow macadamia tress often need to use a pesticide, called BuggOff, to try and reduce the impact of fruit-spotting bugs on the crop yield. Three studies were conducted, tracing the resistance of the fruit-spotting bugs to BuggOff. The results of the studies are shown in the graph below.



- **a** Describe the trend shown in the graph.
- **b** Why didn't all the fruit-spotting bugs die after the first exposure to BuggOff?
- **c** Define mutation and explain how understanding mutations related to this scenario.
- **d** Define selection pressure and state the selection pressure in this case.
- **e** Which fruit-spotting bugs are selected for and which are selected against?
- **f** Predict what might happen if the fruit-spotting bugs were sprayed with a different pesticide 10 years after the last study.

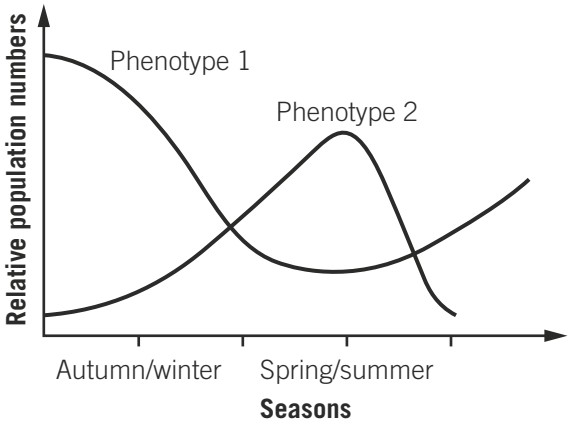
4 The snail *Cepaea nemoralis* has a yellow, pink or brown shell. Each colour shell may have up to five dark bands or no bands. Both shell colour and number of bands are genetically controlled and are not affected by the environment. The snails are eaten by birds such as thrushes, which hunt by sight.

The following observations were made:

- Most snails living on a uniform background, such as short grass, have no bands.
- Most snails living on a green background, such as grass, are yellow.
- Most snails living on a non-uniform background, such as rough vegetation, have bands.

- **a** Suggest an explanation for these observations.
- **b** Predict the phenotype of snails living on a dark background of dead leaves.

The graph shown here illustrates the changes in the snail population for two different phenotypes over a year. These snails live in a deciduous woodland (where the trees drop their leaves), where in autumn and winter the snails will be living among dead leaves. In spring and summer, the vegetation the snails live among is green.



- **c** Describe the trends shown in the graph, including mention of what you would expect phenotype 1 and phenotype 2 to look like.

5 Explain why, in terms of genetic diversity, genetic drift has a greater impact on a smaller population than on a larger population.

Approaches to pathogenic evolution

Study Design:
Consequences of bacterial resistance and viral antigenic drift and shift in terms of ongoing challenges for treatment strategies and vaccination against pathogens

Glossary:
Antigenic drift
Antigenic shift
Bacterial resistance
Conjugation
Epidemic
Pandemic

ENGAGE

Using mathematical modelling

Scientists often use complex mathematical models to predict the spread of disease through populations. They first collect information on how the pathogen spreads and then use computers to determine the speed and distance of spread of the pathogen. However, when a pathogen mutates or evolves, the model has to be changed, as these variations are not taken into consideration by mathematical models.

In early 2020, researchers in Pennsylvania developed a mathematical theory that takes evolutionary changes into consideration. The researchers are hoping that, by including evolution in their modelling, they can better anticipate how a virulent pathogen strain may emerge. This would allow better decision making by the health officials and governments to prevent dangerous mutant pathogen strains from evolving further and spreading worldwide. Interestingly, their testing included using real-word networks like Twitter, where the spread of information was likened to the spread of disease.

An understanding of how pathogens evolve in the first place is therefore crucial. This section builds on Sections 9A and 9B, and looks at how pathogens, specifically bacteria and viruses, can evolve. It also examines the challenges faced by the medical and pharmaceutical industries in the prevention and treatment of diseases caused by these evolving pathogens.

LINK **9A** MUTATIONS
LINK **9B** EVOLVING AND NON-EVOLVING POPULATIONS

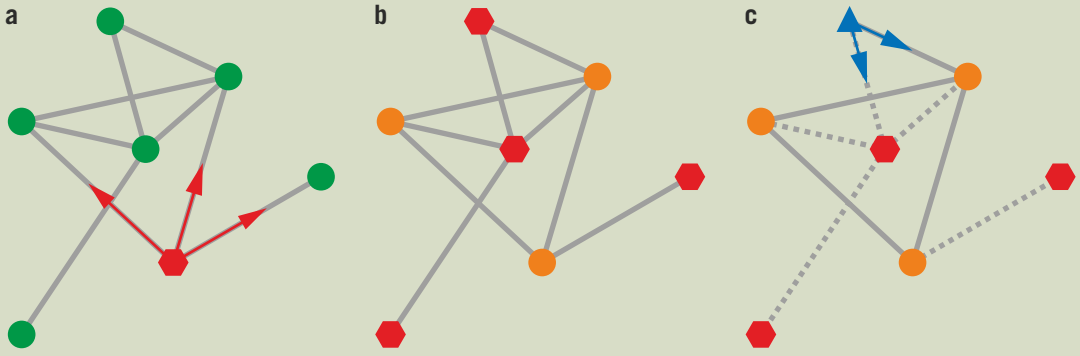


Figure 9C–1 Mathematical modelling is used to predict the spread of a disease, and can now take evolution into account. **a** Model showing a population of susceptible individuals (green circles) and a single infected individual (red hexagon). **b** The infection spreads and there is balance between infected individuals and those that have recovered (orange circles). **c** The pathogen in one individual mutates, creating a new strain (blue triangle).

EXPLAIN

Bacterial evolution

The development of bacterial resistance

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

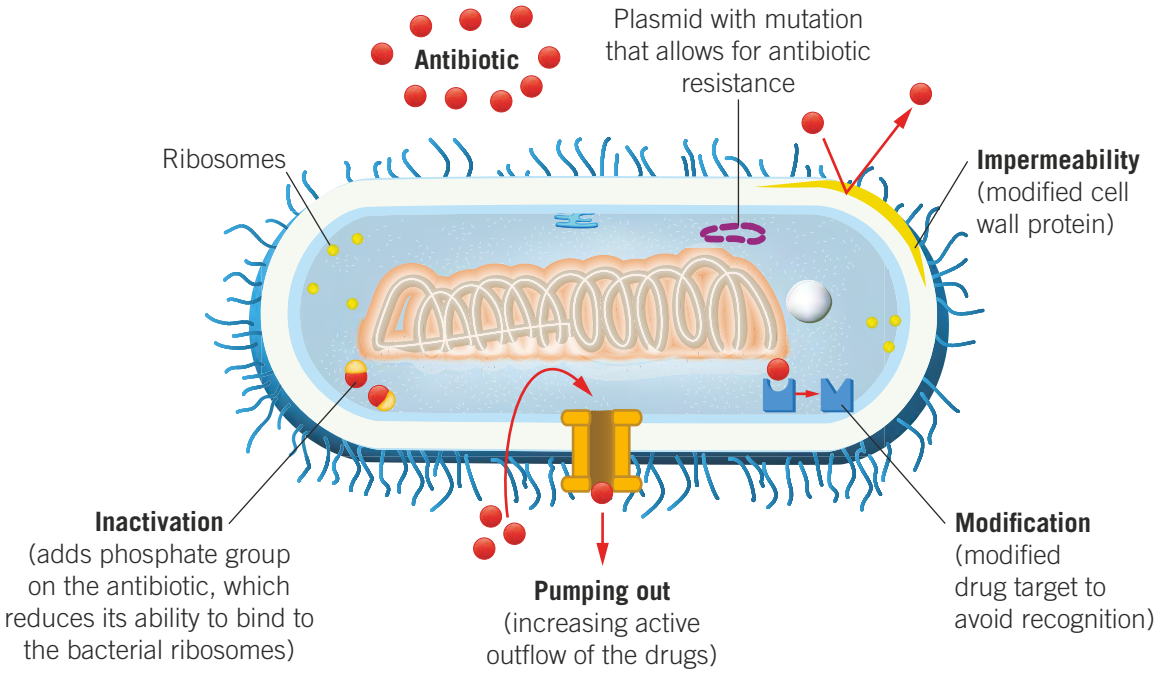
Remember that natural selection is based on 'survival of the fittest'. That is, the alleles for phenotypes that are best suited to the environment increase in frequency within the gene pool. What does this have to do with the development of **bacterial resistance**?

9A MUTATIONS LINK

Recall from Section 9A that mutations are the source of new alleles. A random mutation might cause some bacteria to become resistant to an antibiotic. Figure 9C–2 illustrates some of the ways that bacteria can demonstrate resistance to antibiotics. Bacteria only have one chromosome, and they have plasmids, but they only have one copy of each gene, so this means the impact of a mutation is immediate.

6B CELLULAR PATHOGENS LINK

Bacterial resistance the ability of bacteria to survive and reproduce in the presence of an antibiotic that has been designed to slow their growth or kill them; arises by mutation and becomes widespread when antibiotics are over-used



VIDEO 9C–1 PATHOGENIC EVOLUTION: BACTERIA

Figure 9C–2 Some of the mechanisms by which bacteria can demonstrate resistance to antibiotics as a consequence of a random mutation

When treated with antibiotics, the resistant bacteria are selected for, as they have the survival advantage. They will survive, reproduce and pass on their alleles for resistance to the next generation. Those bacteria that are sensitive to the antibiotic will die, as they are selected against. This means resistant bacteria will flourish as there is no longer competition from the sensitive strains of bacteria. In this situation the antibiotic acts as the selection pressure. The allele responsible for the beneficial resistance phenotype will increase in frequency within the population. This is an example of natural selection.

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

Over time, the whole population of bacteria becomes antibiotic resistant because the resistant strains are best suited to their environment. This happens quickly as bacteria reproduce, on average, every 20 minutes. Consequently, their evolution can occur at a rapid pace.

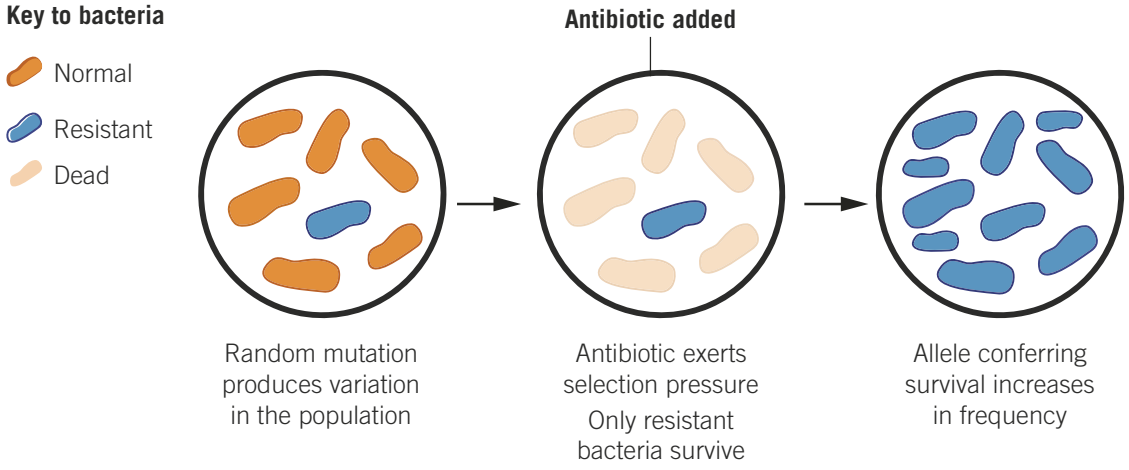


Figure 9C–3 Bacterial resistance to antibiotics develops via natural selection. Bacteria with the selective advantage survive, reproduce and pass on beneficial alleles to the next generation.

Genes for antibiotic resistance often occur on plasmids, which you will recall are small loops of DNA. Plasmids can be transferred from one bacterium to another, even between different species, through a process called **conjugation**, also known as horizontal gene transfer. This is when a tube forms between two bacteria to allow the movement of a plasmid from a donor bacterium to a recipient, passing on the allele for resistance.

Conjugation the process by which genetic material is exchanged between a donor bacterium and a recipient bacterium; occurs through a tube via direct cell–cell contact; also known as horizontal gene transfer

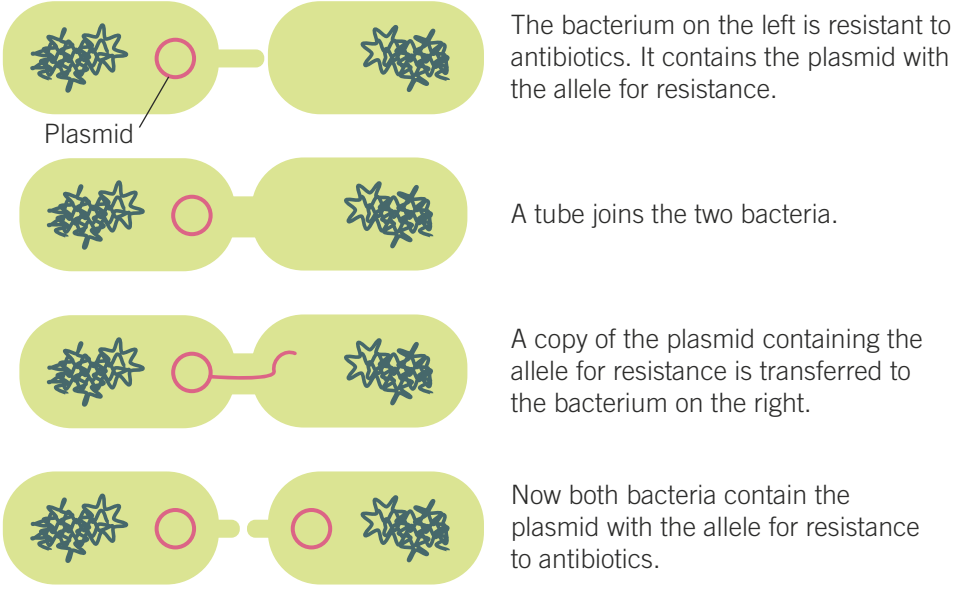


Figure 9C–4 In conjugation, genetic material is exchanged between bacteria through direct cell–cell contact. In this way, a plasmid carrying an allele for antibiotic resistance can be passed on.

LINK **3B** APPLICATION AND IMPLICATIONS OF DNA MANIPULATION TECHNIQUES

Keep in mind what you learnt in Section 8A about the ease and frequency with which people now travel and how this contributes to the emergence and re-emergence of pathogens. Antibiotic resistance is a global problem that requires the combined effort of all nations and sectors to control.

LINK **8A** EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT

Check-in questions – Set 1

1 Explain what is meant by bacterial resistance.
2 Outline how bacterial resistance develops in a bacterial population.

Challenges for treatment strategies

Increasing numbers of antibiotic-resistant bacteria in the population means that infections and diseases caused by such bacteria are becoming harder to control. It is increasingly difficult to find antibiotics that these strains of bacteria are not resistant to. Consequently, the effects of bacterial infections are becoming more severe, longer lasting and more expensive, and often result in death.

Remember, where there is widespread use of antibiotics, such as in hospitals or on farms, resistance quickly spreads among different species of bacteria, by conjugation.

MRSA, super bug

Staphylococcus aureus, or golden staph, is a usually harmless bacterium that lives on the skin or in the nose. However, if it gets past one of the body's first lines of defence, it can cause a range of mild to severe infections. In the past, the antibiotic methicillin was used to treat these infections. However, at some point, bacterial strains arose that were resistant to this antibiotic. Methicillin-resistant *Staphylococcus aureus* (MRSA) is now resistant to most antibiotics, because of conjugation. It is for this reason that MRSA is called a superbug – it is almost impossible to destroy.

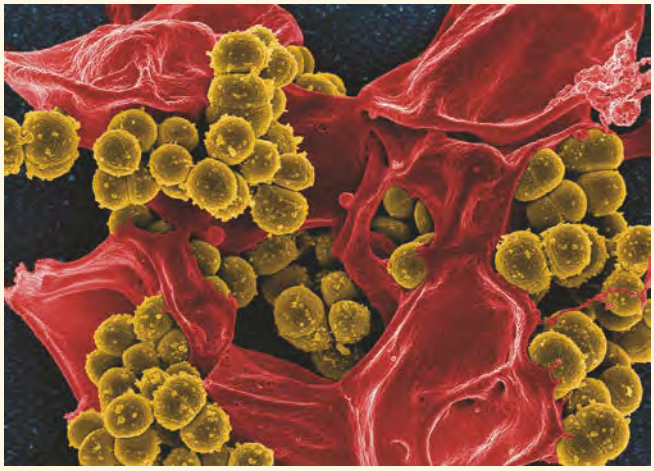


Figure 9C–5 Coloured scanning electron micrograph of MRSA in the process of being phagocytised by a human neutrophil

MRSA infections are especially prevalent in hospitals, where methicillin has been most commonly used. Doctors now prescribe alternative antibiotics, like vancomycin, to treat infections caused by *Staphylococcus aureus*.

The search for new antibiotics

There is a constant race to find novel, or new, antibiotics as the number of resistant bacterial strains continues to rise. So why, then, has the discovery of new antibiotics slowed significantly over the past 20 years?

There are several reasons why finding novel antibiotics is so challenging. Researching, testing, developing and running clinical trials of new antibiotics is expensive, and this reduces their appeal to the pharmaceutical industry. Even if a new, effective antibiotic is developed, the need to minimise exposure of bacteria to antibiotics in order to limit the development of resistance means the antibiotic will only be prescribed sparingly. This is, again, not an appealing prospect for the pharmaceutical industry. Additionally, the clinical life of any new antibiotic will be limited, as resistance to it inevitably develops.

To develop a novel antibiotic, pharmacologists need to:

- find a way to modify the chemical structure of an existing antibiotic so that a resistant bacterium will not be resistant to the altered form
- find new molecules that target bacterial biochemical pathways or cell structures that are different between bacteria and host, and work differently from antibiotics currently in use.

The search for alternatives to antibiotics

Scientists have also been looking at alternatives to antibiotics as a way of treating antibiotic-resistant bacteria. A tool that is emerging as particularly valuable in combating bacterial resistance by altering the allele for resistance, is CRISPR. The CRISPR-Cas9 system, as you will recall from Section 3D, is believed to be the cheapest, fastest and easiest-to-use form of genetic engineering. It has applications in gene editing, as described in Sections 3D and 5E, but researchers are discovering that it also has applications in detecting and altering bacterial resistance. The following box covers some of the current research into use of the CRISPR-Cas9 system to treat infections caused by antibiotic-resistant bacteria.

LINK **3D** THE FUTURE OF GENOME EDITING

LINK **5E** BIOTECHNOLOGICAL APPLICATIONS OF BIOCHEMICAL PATHWAYS

Using the CRISPR-Cas9 system

One new approach to using CRISPR-Cas9 is the 'pro-active' genetic system, or Pro-AG. This is a new CRISPR-based system that disables the gene that makes bacteria antibiotic resistant. It works by editing its targets rather than destroying them. This means the edited plasmid is still able to replicate and spread to other bacteria but is no longer able to produce antibiotic resistance.

Another novel approach is to program Cas9 to view itself as an enemy and therefore to make cuts in its own genome. This is done by encoding a plasmid with instructions for CRISPR enzymes designed to disable *Salmonella* DNA. The plasmid is then placed inside *E. coli* bacteria and, through conjugation, the engineered plasmid is transferred from the *E. coli* to the *Salmonella*. The CRISPR system is then activated and destroys the *Salmonella* bacteria, leaving the *E. coli* undamaged. This highlights an advantage of CRISPR: it can be programmed to destroy only specific pathogenic bacteria and leave the other, healthy microbes untouched.

These new approaches are in the pre-clinical stages, but you can see how eventually there may be a place for CRISPR-Cas9 in treating bacterial infections that do not respond to antibiotics.

Minimising the development of resistance

There are many ways to try to minimise the development of bacterial resistance, such as:

- using antibiotics only when necessary (last resort) and appropriate (not for viral infections)
- reducing the over-prescription of antibiotics by doctors
- using antibiotics that are specific to the infection (known as narrow spectrum) — this requires the antibiotic to be tested against the strain of the bacterium isolated from the infected person, so the most effective medication can be used
- ensuring that patients complete their course of antibiotics, so the bacteria are entirely destroyed
- ensuring that unused antibiotics are not kept for self-medication in the future or for someone else to use as they may not be appropriate for a future infection.

Check-in questions – Set 2

1 Suggest two reasons why bacterial resistance causes ongoing challenges for treatment strategies.

DOC **WORKSHEET 9C–1** BACTERIAL EVOLUTION AND THE CHALLENGES FOR TREATMENT

6C NON-CELLULAR PATHOGENS LINK
8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT LINK
VIDEO 9C–2 PATHOGENIC EVOLUTION: VIRUSES

Viral evolution

Example: influenza A virus

In Section 6C, the structure of non-cellular pathogens, including viruses, was covered. To illustrate the evolution of viruses and the challenges this poses for treatment and vaccination, this section focuses on the influenza A virus. Keep in mind that the ideas and concepts covered here can be applied to other viruses. Human influenza A viruses cause seasonal **epidemics** (known as the flu season) almost every winter, and are also known to cause flu **pandemics** (global epidemics).

Epidemic the rapid spread of an infectious disease to a large number of people within a population

Influenza A viruses are divided into subtypes based on two glycoproteins on the surface of the virus: haemagglutinin (H) and neuraminidase (N). There are many subtypes of haemagglutinin and many subtypes of neuraminidase. Each subtype is allocated a number (H1 to H18 and N1 to N11).

- Haemagglutinin is an attachment protein that mediates entry of the virus into a host cell.
- Neuraminidase is an enzyme that facilitates the release of the virus from the host cell after replication.

6A RECOGNISING SELF FROM NON-SELF LINK
7D THIRD LINE OF DEFENCE LINK

Recall from Section 6A that the H and N surface proteins of influenza viruses are antigens, which means they are recognised by our immune system as non-self, and are capable of triggering an adaptive immune response. Remember from Section 7D that an adaptive immune response means antibodies are produced, as well as B memory cells, which provide long-term immunity in case of subsequent exposure to the same pathogen (and antigen).

Pandemic an outbreak of infectious disease that occurs over a wide geographical area, affecting a large number of people

Antigenic drift mutations altering viral surface antigens, making the virus unrecognisable to the host's immune system; can result in an epidemic

Antigenic shift reassortment of genes on genomes from different viruses infecting the same host cell, altering viral surface antigens, resulting in novel strains that can cause pandemics

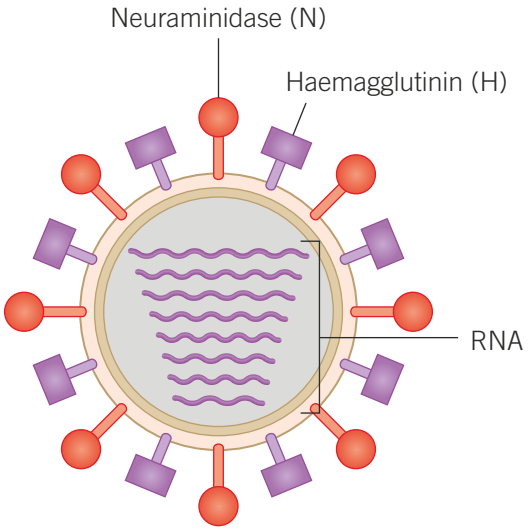


Figure 9C–6 Generalised structure of an influenza A virus. Note the antigens or surface glycoproteins H and N, and the eight strands of RNA in the centre.

Normally, due to an organism's natural immunity, influenza A would eventually cease to exist. However, the virus undergoes constant genetic variation, and this is why it can persist and spread so well, evading the adaptive immunity of its host. Viruses can evolve in two ways:

- mutation (gene change), called **antigenic drift**
- reassortment (gene swapping), called **antigenic shift**.

Antigenic drift

LINK 9A MUTATIONS

The RNA in the influenza virus is more prone to mutations than genes made of DNA. Recall that a mutation is a permanent change in the nucleotide sequence of a gene. This is due to the virus's replication machinery not having a 'proofreading' mechanism – that is, errors that occur are not corrected by enzymes, as they would be in DNA. Over time, mutations accumulate as they continually occur when the genetic material in the virus replicates. Many of the mutations that occur are random changes in a single nucleotide, or point mutations. When a mutation alters a gene coding for either or both of the surface proteins, the shape of these molecules changes. The specific antibodies created in response to a person having the flu in the past are now unable to recognise the shape of the viral antigens and bind to them. This process is known as antigenic drift.

LINK 8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT

LINK 7D THIRD LINE OF DEFENCE

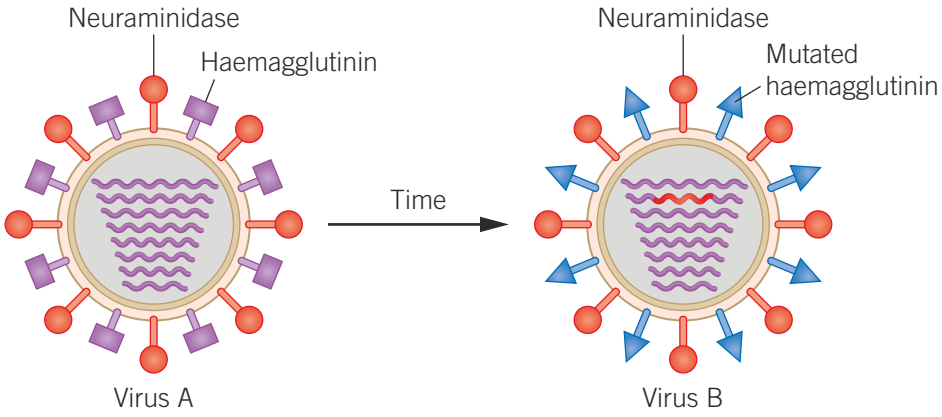


Figure 9C–7 Antigenic drift: when an accumulation of mutations alters the gene coding for the viral antigens (H and N), the shape of these molecules changes, making the virus less recognisable by the host cell's immune system. Note the altered shape of the haemagglutinin glycoprotein in this situation.

Antigenic drift can result in an epidemic occurring – that is, more than the expected number of cases of disease, in this case the flu, occurring in a particular community or region, during a given period of time.

Antigenic shift

LINK 8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT

Influenza viruses are constantly changing. You have seen how influenza viruses change all the time due to mutations or antigenic drift. Antigenic shift happens abruptly, less frequently than antigenic drift and at unpredictable intervals.

Antigenic shift (gene reassortment) refers to the mixing of RNA in the virus genome, which occurs when several different viruses infect the same host cell at the same time. This shift typically occurs when a human flu virus crosses with a flu virus that usually affects animals (such as birds or pigs). In effect, influenza viruses have an enormous global pool of genetic material that can come together in different combinations, creating a major change in the virus. In this case, segments of their genetic material may recombine to produce viruses that have entirely new H or N surface proteins. Such animal-origin viruses can contain an H or H/N combination that is so different from the same subtype in humans that the antibodies made by the host's immune system in response to the previous subtypes don't recognise the new subtype, so a large portion of the human population is vulnerable to infection by the new strain. If this new virus causes illness in infected people, and can spread easily from person to person, an influenza pandemic can occur.

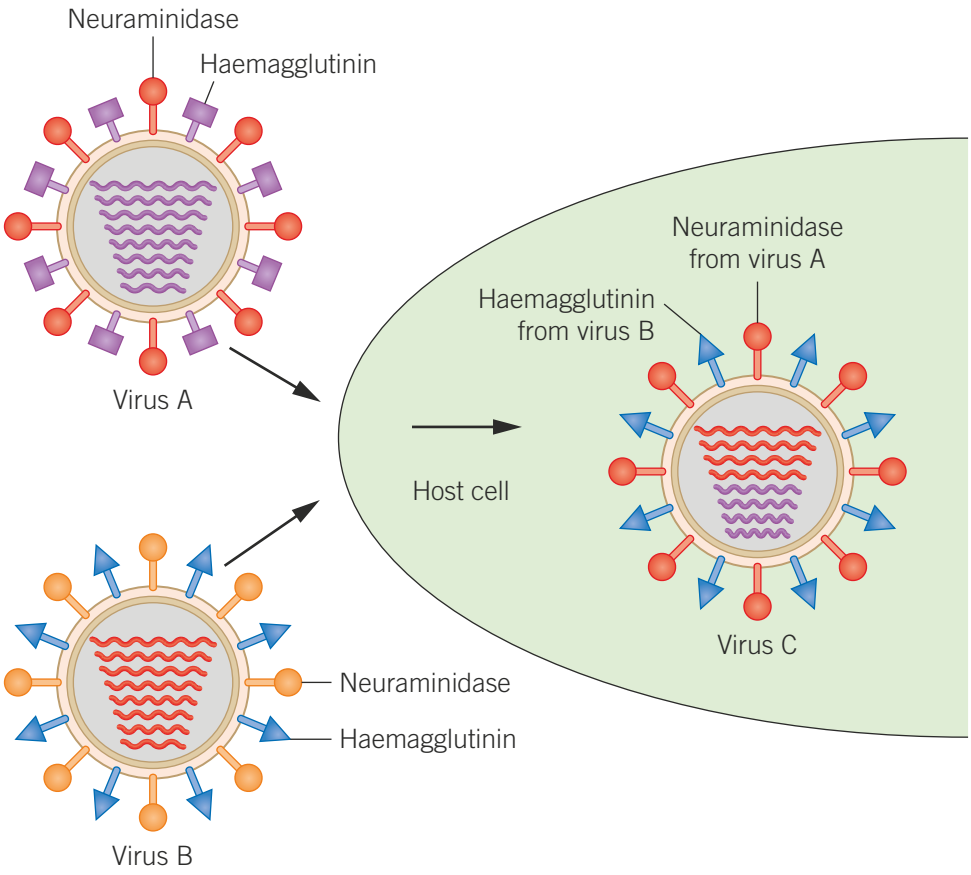


Figure 9C–8 Antigenic shift: when several different viruses infect the same host cell at the same time, segments of their genetic material may recombine to produce viruses that have entirely new H or N surface proteins. This makes the virus unrecognisable by the host cell's immune system. Note that the RNA and the surface proteins of virus C are a combination of the invading viruses.

Summarising antigenic drift and antigenic shift

Table 9C–1 Summarising antigenic drift and shift

Antigenic drift	Antigenic shift
Influenza A (and B)	Influenza A
Point mutations	Reassortment of genes
Gradual change	Sudden change
Continually occurring	Less frequent
Minor changes in the virus	Major changes in the virus
Same subtype	New subtype created
Causes epidemic	Causes pandemic

The consequences of antigenic drift and antigenic shift for treatment of infections and vaccination is discussed in the rest of this section.

Check-in questions – Set 3

1 Influenza (flu) viruses change constantly. State the two ways they can alter their genome.
2 Which type of change can result in:
  a an epidemic?
  b a pandemic?
3 Antigenic drift refers to changes in the influenza virus that happen slowly over time. Summarise how this occurs.
4 Antigenic shift refers to changes in the influenza virus that happen quickly. Summarise how this occurs.

Challenges for treatment strategies and vaccination

LINK 6C NON-CELLULAR PATHOGENS

Whether a virus evolves due to antigenic drift or antigenic shift, there are two key approaches to preventing and treating influenza viruses: medication and vaccination. The evolution of viruses creates challenges for both approaches.

Medication

Successful antiviral medication will interfere with the life cycle of a virus (Figure 9C–9).

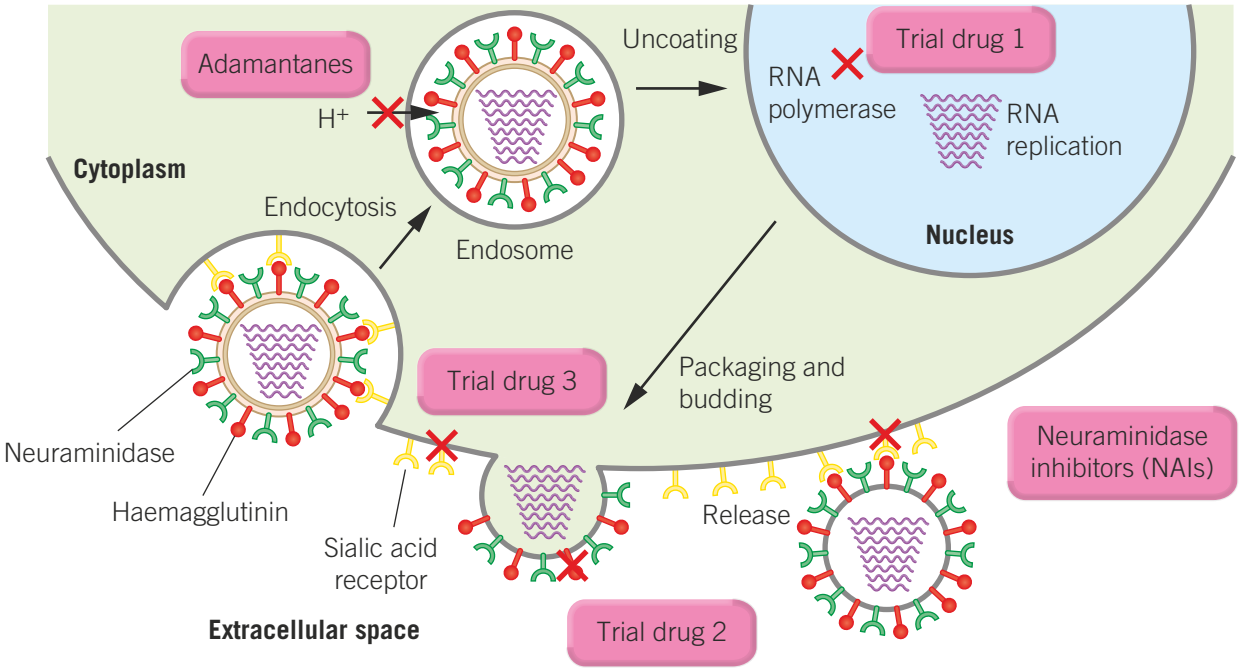


Figure 9C–9 The life cycle of an influenza virus and where antiviral medication can interfere, preventing the continued spread of the virus through the host

Antiviral drugs are recommended only for people who are very sick, immunocompromised, pregnant or at high risk of serious complications. A key reason why their use is limited in this way is to decrease the chance that a virus will exist that has resistance to the drug. Returning to the example of influenza A, as a virus that is continually evolving through antigenic drift and shift, there are challenges as a consequence of this in terms of prevention and treatment strategies.

Neuraminidase inhibitors (NAIs) have become the most commonly used antiviral medication since the influenza virus developed resistance to the adamantane family of drugs, several years ago. Their mechanism of action is by preventing the liberation of the virions from the host cell, shown in Figure 9C–9, is only effective if they are administered in the first 48 hours of the onset of symptoms. During the 2007–2009 influenza seasons worldwide, a virus strain emerged that was naturally resistant to NAI treatment. A mutation had changed the genetic code in the virus, resulting in an amino acid substitution in the neuraminidase enzyme. Consequently, the shape of the NAI was no longer complementary to the enzyme active site, allowing the influenza viruses to evade the action of NAIs. This is an example of antigenic drift. Given the frequency at which antigenic drift occurs in the influenza A virus, it is best to have as many options as possible for treating influenza infections.

To combat future influenza virus epidemics (evolution due to antigenic drift) and even pandemics (evolution due to antigenic shift) we need medication that can replace or work in combination with the NAIs. A number of drugs that act on different targets than those of the NAIs are being researched and tested, and these are summarised in Table 9C–2.

Table 9C–2 Antiviral medications currently being developed.

Acts on virus or host cell?	Mechanism of action	Name	Figure 9C–9 reference
Virus	Targets viral RNA polymerase, which is involved in RNA replication within the host cell	Favipiravir	Trial drug 1
	Blocks maturation of the viral H surface protein at the post-translational stage	Nitazoxanide	Trial drug 2
Host cell (epithelium of the respiratory tract)	Cleaves sialic acid receptors from the host cells to inhibit virus entry	Fludase	Trial drug 3

NOTE

You do not need to know the names of these novel medications. However, it is important to understand how and why successful antivirals interfere with the virus life cycle.

Compared to the NAIs, these novel drugs are expected to have a longer clinical life, making them more effective in the long term. Those that act on the host cell have the added benefit of being even less likely to encourage drug resistance.

One ongoing challenge in the design of novel antiviral medication is to ensure that the drug targets a process or structure that is unique to the virus. Recall that viruses use the host cells to multiply, so it is hard to destroy viruses without killing the host cells as well. This is increasingly difficult because of the ever-changing nature of the virus's structure, due to their evolution. In addition to this, there is great diversity among viruses – some have DNA, others have RNA – and it is therefore unlikely that a broad-spectrum antiviral drug can be created.

6C NON-CELLULAR PATHOGENS LINK

To manage the evolution of viruses and reduce the likely development of resistant virus strains, scientists are also researching and testing:

- treatments that involve the delivery of a combination of antivirals, each acting on a different aspect of the influenza virus's life cycle
- a possible role for CRISPR in the treatment of viral infections (see the 'Using CRISPR' box).

Using CRISPR

The CRISPR-Cas9 system, you will recall, has potential applications in detecting and altering bacterial resistance. The specificity of CRISPR is also appealing to researchers who are looking to target pathogenic viruses. It is a logical progression to take a tool that cuts up viruses from a bacterium (CRISPR), where it exists naturally, and use it to treat viral infections in humans. Scientists have shown that Cas13, another CRISPR enzyme, can be programmed to damage the viral genome of the influenza A virus, so much so that it can no longer infect new cells.

The advantage of CRISPR is that, if a virus evolves, it is comparatively easy to change the CRISPR system to keep up with whatever the virus is doing. CRISPR antiviral medications are still in development, but they have shown early signs of success in the laboratory and also promise to be cost effective.

Vaccination

LINK **7D** THIRD LINE OF DEFENCE
LINK **7E** ACTIVE AND PASSIVE IMMUNITY

In Section 7D you learnt about the third line of defence and the important roles that antibodies play in defending against pathogens once they have entered our body. In Section 7E you saw how antibodies can be artificially induced through vaccinations to prevent viral infections. This means that people who have been vaccinated against the flu will be less likely to display symptoms if exposed to the same strain of influenza they were vaccinated against. But we now know that viruses evolve, so what does this mean for the success of vaccinations as a preventative measure for viral infections?

As mentioned earlier, you can get sick from the seasonal flu every year even though you've had it before – this is because of antigenic drift. Every year, a slightly different version of the virus emerges. This means that every time the virus evolves, the vaccine for influenza A must be updated so that the antibodies it induces are specific to the antigens of the new strains.

Currently, researchers predict which flu viruses are likely to cause a problem in the upcoming flu season and then make seasonal influenza vaccines. In Australia, the flu vaccines released to the public are usually quadrivalent; that is, they contain four viral strains. Scientists know that influenza viruses evolve and possibly will do so before the flu season begins, so by including four strains they hope that at least one strain will be similar enough to the evolving virus to give some protection.

Finding a universal vaccine, one that will be successful no matter what virus is being targeted, is the challenge of defending against evolving viruses. One way of finding a universal vaccine is to target a conserved area of the virus – this is an area of its structure that doesn't change despite antigenic drift and shift. Haemagglutinins, or H surface proteins, have two regions: a head domain and a stem (or stalk) domain (Figure 9C–10). Traditionally, vaccines are based on producing antibodies in the host organism that can target and neutralise the head domain, which is the part required for cell attachment. However, the head domain is highly variable between strains of influenza, and is the domain that experiences more mutations.

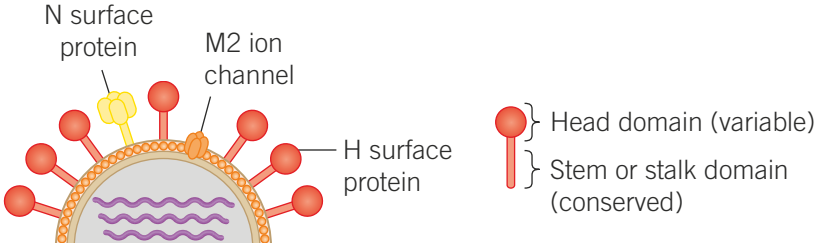


Figure 9C–10 To make a universal influenza virus vaccine, the regions of the virus that are targeted need to be those that remain the same, or do not evolve often, such as the stem or stalk of the H surface protein and the M2 protein.

And what of a vaccine program for viruses that have evolved because of antigenic shift? Until the virus appears, a vaccine cannot be designed and manufactured. As we have seen in the case of COVID-19, a new subtype of virus has evolved all of a sudden and people have little or no protection.

The new vaccines target the stem or stalk domain, which is highly conserved (even between different subtypes of virus) and experience fewer mutations. Consequently, these vaccines should confer broad-spectrum and long-lasting protection. The M2 protein is another feature of interest due to its highly conserved regions.

WORKSHEET 9C–2 VIRAL EVOLUTION AND THE CHALLENGES FOR TREATMENT AND VACCINATION

Check-in questions – Set 4

1 Suggest why antigenic drift and shift cause ongoing challenges for treatment strategies and vaccination against viruses.

9C SKILLS

VIDEO 9C-3 SKILLS: READING THE NEWS

Reading the news

You may think that reading the news is not something you have time for during Units 3 and 4 Biology. However, consider your assessors' point of view. You already know that questions are often based on scenarios and relevant contemporary examples. Where does the information for these questions come from? The news. We are not recommending that you spend hours reading online journals, but when your teacher mentions a new discovery, or you hear someone talking about a new species of mammal, or perhaps something relevant from a trusted news source pops up on your Facebook feed, take note. Perhaps signing up to a science news alert email could be handy, so you can scroll through recent work that scientists around the world have published, because if there is something related to evolution, or any other Unit 3/4 topic, your assessors may think it is exciting enough to share with you in your assessments.

For example, below is a summary of what some questions in recent VCAA examinations were based on:

- VCAA 2019:
  - Zika virus – between 2015 and 2018 there was a high incidence of Zika virus-related infections around the world.
  - Thunderstorm-related asthma – in Victoria in 2017 there was a higher than usual mortality rate due to 'thunderstorm asthma'.
  - Genetic screening – in 2018 the Genomics Health Futures Mission announced that it would begin screening over 10 000 couples for 500 severe or deadly recessive gene mutations.
  - Human evolution – in 2018 evidence was published that the Denisovans were a separate group from Neanderthals.
- VCAA 2018:
  - Measles – outbreaks in 2018 killed more than 140 000 people worldwide.
  - Golden Rice – in 2017 Food Standards Australia, and in 2018 the Food and Drug Administration of the United States, approved Golden Rice for consumption.
- VCAA 2017:
  - Tammar wallaby – in 2017 scientists found that the milk of the wallaby had antimicrobial properties.

- Human evolution – in 2017 a new species of hominin was identified, based on 1500 fossil bones found in South Africa in 2013.
- Evolution – in 2016 research was published that Australia's First Nations peoples had coexisted with megafauna for at least 17 000 years.

Remember, assessors are not expecting you to know everything about any one of these findings specifically. They will use findings like these as a context in which to determine your understanding of the Study Design. So your job is to read relevant articles and practice making links between what you read and what you have learnt in class. In this way, if new information appears in an assessment situation, you will feel comfortable with it.

An example of current research into the treatment of pathogens is in the area of phage therapy. This was referred to in the VCAA 2019 examination when describing how bacterial resistance causes ongoing challenges in the treatment of bacteria (discussed in Section 9C). The question related specifically to the treatment of bacterial infections. Read the following information summarised from news reports, and see how links can be made from the published research and the content you have covered in Biology.

VIDEO 9C–4 SKILLS: PHAGE THERAPY

Phage therapy

Bacteriophages, or phages, are viruses that infect and kill bacterial cells. The therapeutic use of bacteriophages in treating bacterial infections, called phage therapy, was first trialled close to a century ago. Phage therapy showed some success clinically, but as soon as antibiotics entered the picture, phage therapy research was put aside. Now, with the rise of antibiotic resistance, interest in phage therapy has been reignited in the search for alternatives to antibiotics. Despite the appeal of phage therapy, the gaps in scientists' understanding must be filled before phage therapy can be tested clinically and used in mainstream medicine.

Advantages of phage therapy compared to antibiotics:

- *Phages have a higher level of host specificity.*
- *The cost of development is lower.*
- *There are fewer known side effects (so far).*
- *Phages have been more successful at treating multi-drug resistant bacteria.*
- *There are many more types of phage than there are bacteria, so there are a wider range of possibilities.*

In addition to this, DNA technologies, such as genetic engineering, CRISPR and genome sequencing, will allow phages to be matched for their specific bacteria-killing properties.

An important consideration for phage therapy is whether there is any possibility of bacterial resistance developing.

Figure 9C–11 Generalised structure of a bacteriophage, a virus that infects and kills bacteria

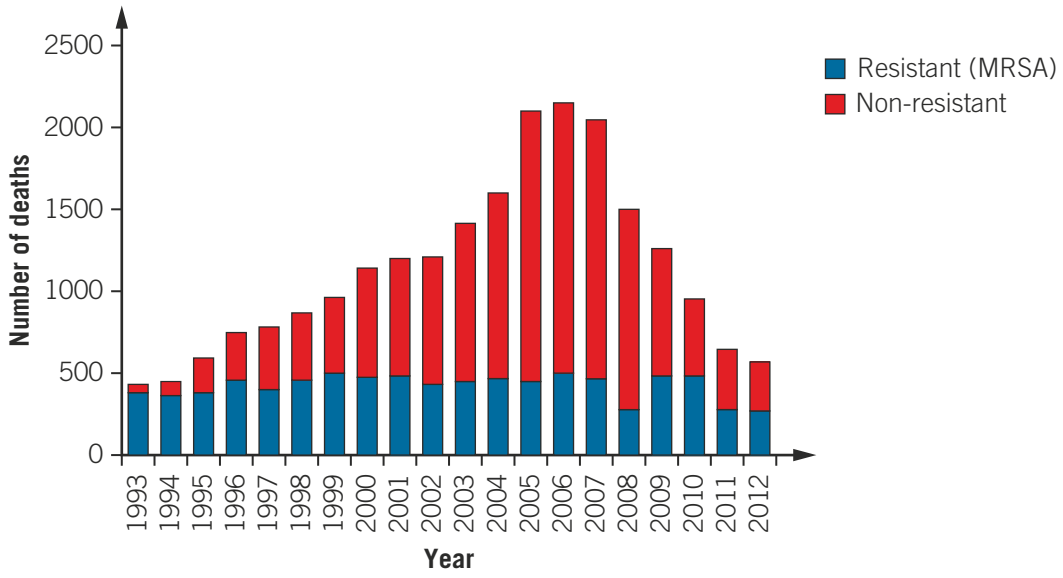
Hopefully you can see from the above information that there are links to many aspects of this course: viruses, bacteria, bacterial infections, treatment using antibiotics, antibiotic resistance, specificity, genetic engineering and CRISPR.

Section 9C questions

1 Even if you have been vaccinated for this year's seasonal flu, you must be vaccinated again next year. Which type of antigenic change does this describe and how does this occur?

2 Write a paragraph using the following terms to summarise the evolution of bacterial resistance:
selective advantage, pre-existing variation, random mutation, resistant bacteria, sensitive bacteria, selection pressure, antibiotic, increase in frequency, selected against, survive and reproduce, gene pool, allele.

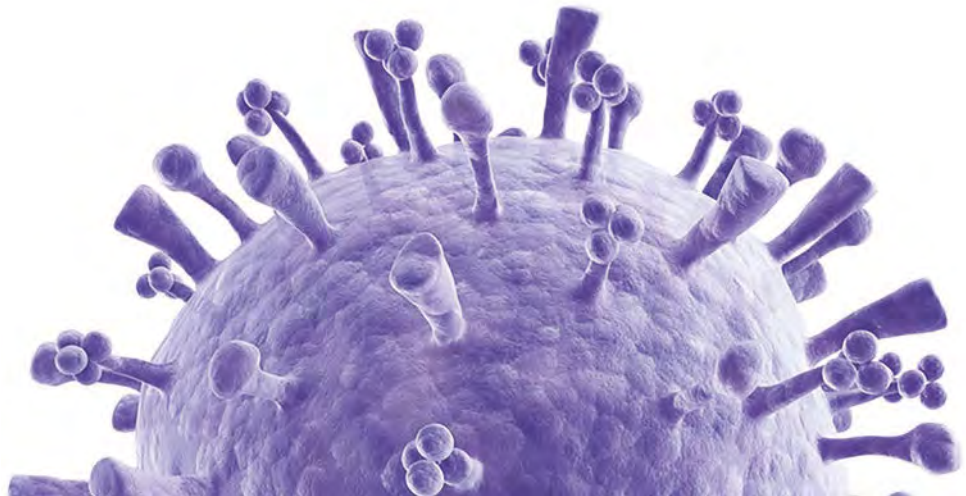
3 Methicillin is an antibiotic used to treat infections caused by *Staphylococcus aureus*. However, many populations of this bacterium have become resistant to this antibiotic and are known as methicillin-resistant *Staphlyococcus aureus*, MRSA. The graph shows changes in number of cases and deaths from MRSA in a European country between 1993 and 2012. The questions that follow relate to the graph.



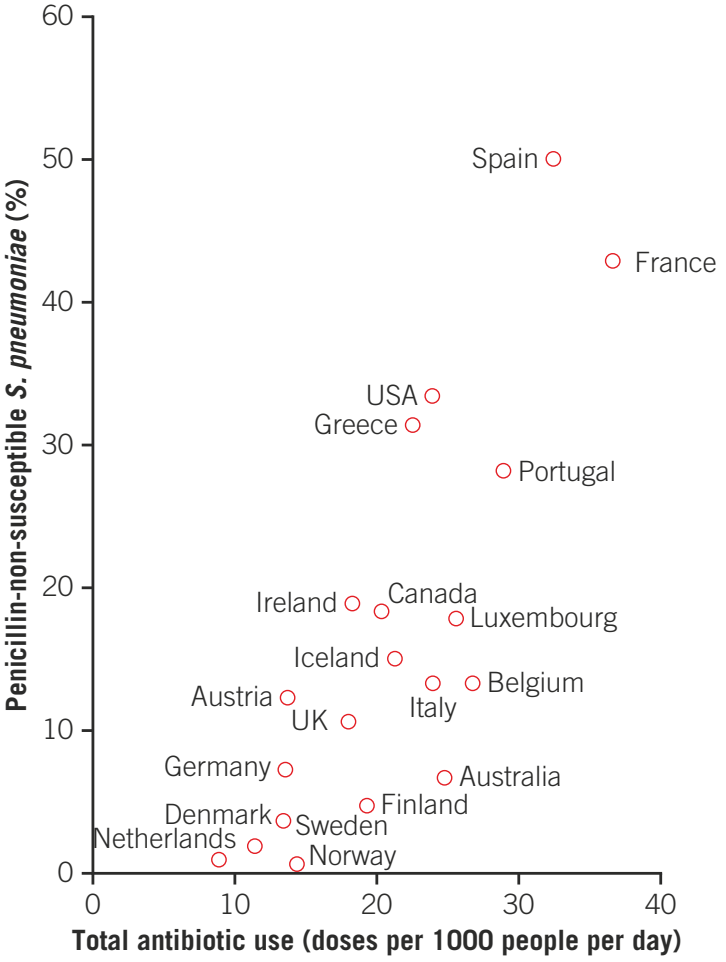
a Describe the differences between the trends in deaths from methicillin-resistant *S. aureus* (MRSA) and non-resistant *S. aureus*.

b Many cases of MRSA develop in hospitals. Suggest why this is so.

c In the mid-2000s, healthcare professionals were asked not to prescribe antibiotics unless strictly necessary. Suggest how this could explain the pattern shown by the graph between 2007 and 2012.



4 In 2004, scientists in Switzerland published the results of a study to see if there was a correlation between the use of antibiotics and the number of cases of antibiotic resistance. They recorded the use of penicillin in outpatient departments of hospitals and penicillin resistance in bacteria recorded in the patients attending those departments. The scientists collected data from hospitals in the United States, Canada and 18 European countries. The results of the study are shown in the scatter graph.



a The scientists concluded that bacterial resistance is directly associated with antibiotic use.

i State the evidence in the scatter graph that supports the scientists' conclusion.

ii State one piece of evidence from the scatter graph that does *not* support their conclusion.

b Summarise the implications of antibiotic resistance for the health of the global population, in terms of the impact on possible treatment strategies.

5 Swine influenza (H1N1), known as swine flu, is the influenza A virus with H1 haemagglutinin and N1 neuraminidase proteins. In 2009, over 37 000 Australians were infected with the virus, and 191 people died as a result of infection. The outbreak was caused by a new virus that had a mix of antigens from human, avian and swine (pig) influenza viruses.

a Name the antigenic change that occurred.

b Draw a diagram to show how swine flu would have come about by the process you identified in part **a**.

c It is difficult to produce effective vaccines against new types of influenza virus, such as swine flu. Explain why this is the case.

d Medication is another approach to managing the treatment of influenza viruses. Besides the virus evolving, what challenges must be overcome for medications to be successful?

9D Emergence of a new species

Study Design:
Evidence of speciation as a consequence of isolation and genetic divergence, including Galapagos finches as an example of allopatric speciation and *Howea* palms on Lord Howe Island as an example of sympatric speciation

Glossary:
Adaptive radiation
Allopatric speciation
Divergent evolution
Fertile
Reproductive isolation
Speciation
Sympatric speciation
Viable

ENGAGE

The natural perfume of insects promotes speciation

9A MUTATIONS LINK

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

Evolution is the change in allele frequencies of a population over time, through mutations, gene flow, genetic drift or natural selection, as you saw in Sections 9A and 9B. Sometimes, these changes can result in the development of a new species.

It is well accepted that the development of new species involves natural selection, but scientists have found that, in the case of stick insects, the right scent is just as crucial. Research has shown that stick insects in different populations and species were unwilling to interbreed because of the scent of the chemicals on the insects' exterior. These chemicals prevent the stick insects from drying out but also play an important role in 'signalling' to attract suitable mates. The populations that were not mating differed most in their chemical profiles and their genetic make-up. This led the researchers to conclude that the insects' perfume acted as a barrier that prevented the different populations from interbreeding and mixing their genetic information. Over time this led to the development of a new species.

In this section we will explore the evolutionary process of forming a new species, and consider the examples specified in the Study Design.

Figure 9D–1 Over two decades, researchers in three countries investigated 100 populations of stick insects, including 11 species. They concluded that the development of a new stick insect species involves more than just natural selection.

EXPLAIN
Speciation

VIDEO 9D–1 SPECIATION

LINK 9A MUTATIONS

Recall from Section 9A that members of a species have similar structural features, and are able to interbreed and produce **fertile** and **viable** offspring in their natural environment. **Speciation** is the creation of new species from the previously existing, or ancestral, species. This process occurs when a population is divided in some way and, over time, because the sub-populations are isolated from each other, genetic differences accumulate.

For speciation to occur, the division in the population must prevent the sub-populations from successfully interbreeding – that is, the sub-populations must be **reproductively isolated**. This also means the sub-populations are genetically isolated, so there is no gene flow between the groups. There are different reasons why groups of organisms may be unable to interbreed and produce offspring. For example, it may be that two sub-populations of the same species reproduce at different times of the year, or have developed different mating rituals.

Two basic mechanisms through which speciation can occur are:

- **allopatric speciation**, where a geographical barrier divides the ancestral population
- **sympatric speciation**, where no geographical barrier is involved.

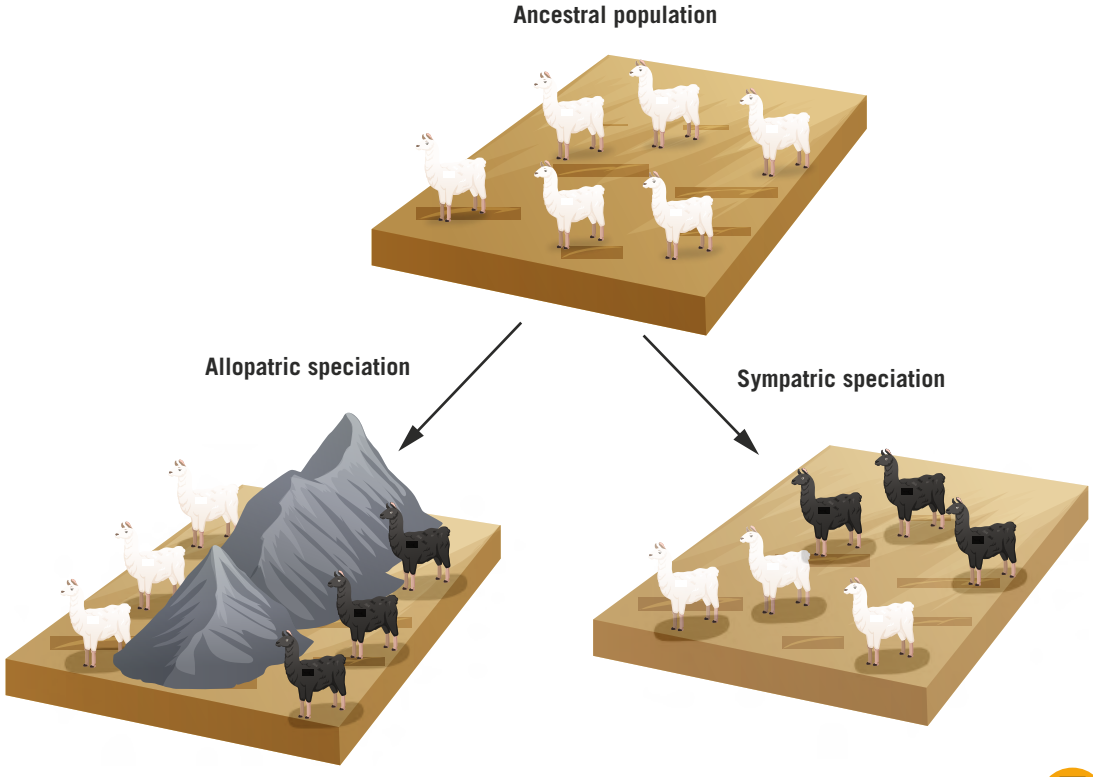


Figure 9D–2 Speciation, or the formation of a new species from an existing species, can occur by allopatric speciation or sympatric speciation.

Fertile
able to reproduce

Viable
able to survive

Speciation
the evolutionary process of forming a new species from a pre-existing ancestral species

Reproductive isolation
the inability of two groups of organisms to interbreed successfully; genetic isolation also ensures there is no gene flow between the two populations

Allopatric speciation
a form of speciation that occurs when a geographical barrier physically divides a population; the two sub-populations accumulate so many genetic differences that they become reproductively isolated and are considered different species

Sympatric speciation
a form of speciation that occurs without the involvement of a physical barrier; often occurs in plants as a consequence of polyploidy

Check-in questions – Set 1

1 Define fertile, viable and speciation.

Allopatric speciation

Allopatric speciation occurs when a geographical barrier physically separates a population and speciation results. The term comes from *allo-* meaning 'other', and *-patric* meaning 'place'. The steps in the process of allopatric speciation (Figure 9D–3) are as follows:

1 Begins with an interbreeding ancestral population of one species that naturally contains variation.
2 A geographical or physical barrier separates a population into two. For example, the barrier may be a mountain, a desert forming, a river changing course or a freeway going through a forest.
3 The two populations are reproductively isolated, so there is no gene flow between them.
4 Different mutations may arise randomly in the two populations or be pre-existing.
5 Selection pressures on either side of the barrier may be different. Therefore, different phenotypes will be at a selective advantage as natural selection occurs.
6 Mutations also arise that affect the ability of individuals to reproduce. The allele frequencies of each population change as the two populations evolve in different ways.
7 Eventually, the accumulated genetic changes in the two gene pools prevent the formation of fertile and viable offspring if the two populations were brought back together. They therefore remain reproductively isolated and are now two distinct species.

9A MUTATIONS LINK

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

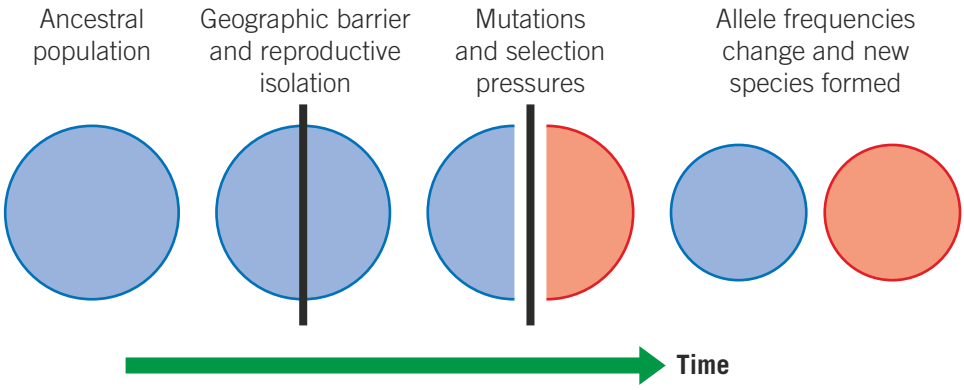


Figure 9D–3 The evolutionary process of allopatric speciation

The effects of natural selection and genetic drift can lead to more rapid speciation if the population is smaller.

Example: the Galapagos finches

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

The Galapagos finches are so named because they are different species of finches living on different islands in the Galapagos archipelago, in the Pacific Ocean off South America. Originally there was just one ancestral species of finch that colonised the islands from mainland South America: a ground-dwelling, seed-eating finch. However, as the finches on different islands were geographically isolated from each other by the ocean, over millions of years, the ancestral finch species evolved into many different species. At least 15 species developed: some species are ground-dwelling seed-eaters; some live on cactuses, eating seeds; some live in trees, eating seeds; and some species are tree-dwelling insect-eaters. Each species of finch has a uniquely shaped beak, because individuals with that beak shape have a selective advantage in that particular environment. Therefore, they survive and are able to reproduce, resulting in viable and fertile offspring. For example, finches that have large blunt beaks can crack open the hard exterior of nuts and seeds that grow on trees, while finches with long thin beaks can get their beaks all the way into cactus flowers to get the nectar.

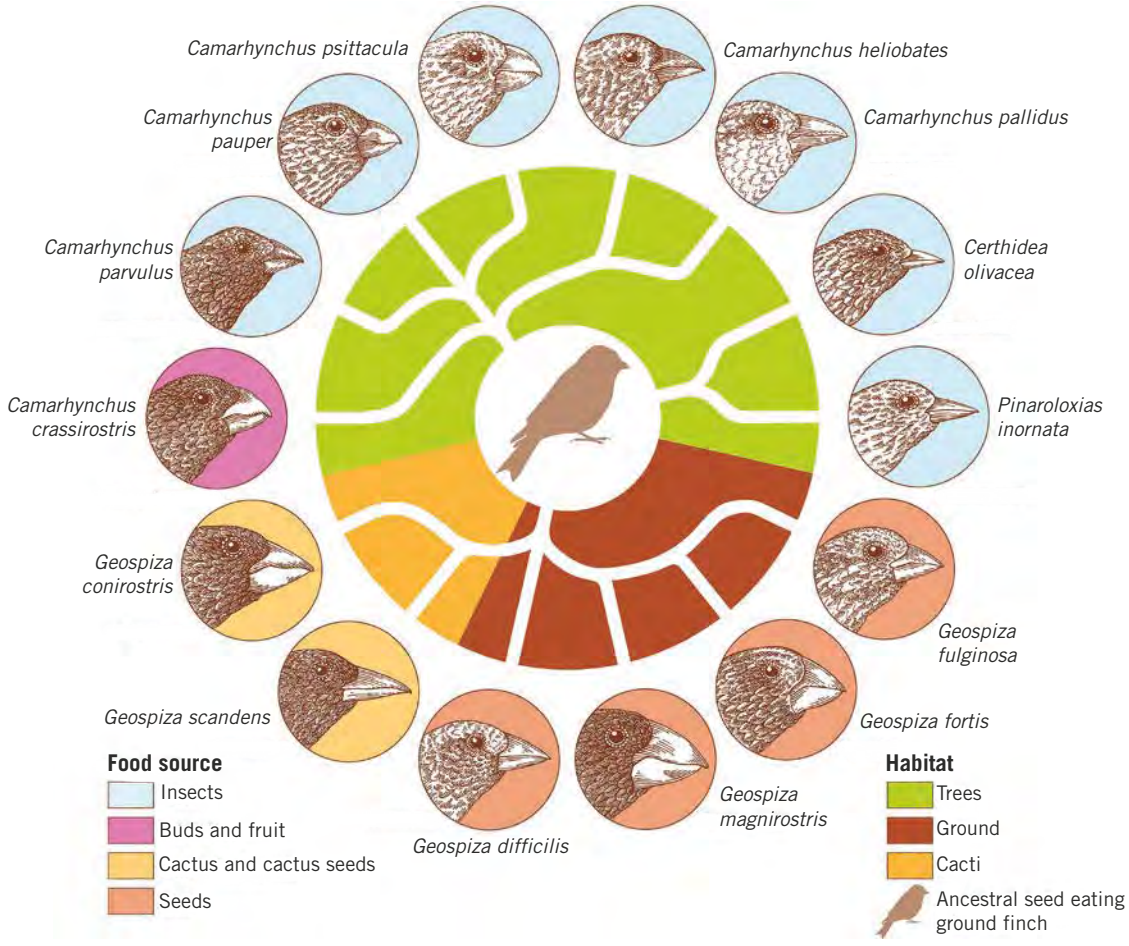


Figure 9D–4 The Galapagos finches developed from one ancestral species by allopatric speciation. The ancestral finch population was first geographically isolated and as a result of different selection pressures and mutations, the sub-populations accumulated so many genetic differences that they formed different species.

Remember, the Study Design specifically mentions the Galapagos finches as an example of allopatric speciation. Therefore, the next step is to put the information about the Galapagos finches together with the steps of the evolutionary process of speciation. Work through each of the steps of allopatric speciation outlined previously and identify how you could reword each numbered step to fit the case of the Galapagos finches. (The Check-in questions that follow will assess your understanding.)

The Galapagos finches not only provide a clear example of allopatric speciation, but they are also an example of:

- **divergent evolution**, where two or more species develop from a single ancestral species over time. As the two new species continue to accumulate differences and evolve, they become less and less similar
- **adaptive radiation**, where divergent evolution occurs rapidly, and a large number of species develop from a single ancestral species. It occurs when the ancestral species occupies various niches within an environment, and unique adaptations exist, each favoured by particular selection pressures in their environment.

These concepts are explored further in Section 10C, when you will learn how structural morphology provides evidence of evolution.

Divergent evolution where two or more species form from a single ancestral species over time

Adaptive radiation the rapid diversification of a large number of related species from a single ancestral species

LINK **10C** EVIDENCE OF EVOLUTIONARY RELATIONSHIPS

Check-in questions – Set 2

1 Using Figure 9D–3 as a guide, summarise the steps involved in the allopatric speciation of the Galapagos finches.
2 Define divergent evolution and adaptive radiation.

DOC **WORKSHEET 9D–1** ALLOPATRIC SPECIATION AND THE GALAPAGOS FINCHES

Sympatric speciation

Sympatric speciation occurs when new species are formed from an ancestral species within the same location – that is, without the involvement of a geographical or physical barrier. For this reason, it is sometimes thought of as being the opposite of allopatric speciation. The term comes from *sym-* meaning 'same', and *-patric* meaning 'place'. Sympatric speciation is rare, and it is difficult to tell whether it has occurred. However, it has been seen in a range of organisms, including plants, bacteria, cichlid fish and the apple maggot fly.

How could a population become reproductively isolated and reduce gene flow without the involvement of a physical barrier? Why do the members of the population stop interbreeding when they still live in the same environment? How can the population possibly speciate? There are several ways that sympatric speciation can occur, but the most common occurs in plants, when there is reproductive isolation because of genetic abnormalities. For example, if a non-disjunction mutation results in a polyploid individual, as you saw is possible (in Section 9A), the affected individual will not be able to reproduce and create fertile and viable offspring with individuals that do not have the same chromosomal error. This is because reproduction with an individual from the normal, non-mutated original parent population results in offspring with an uneven number of chromosomes.

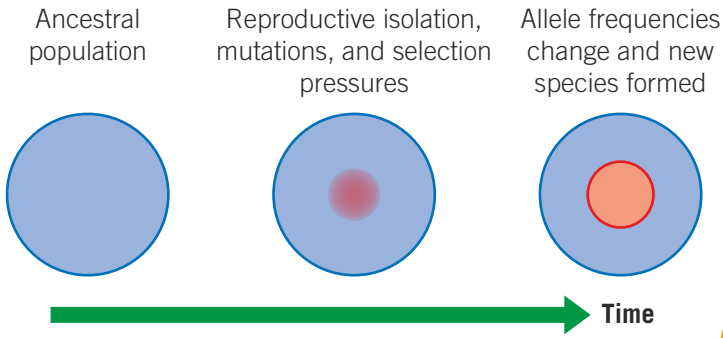


Figure 9D–5 The evolutionary process of sympatric speciation

9A MUTATIONS LINK

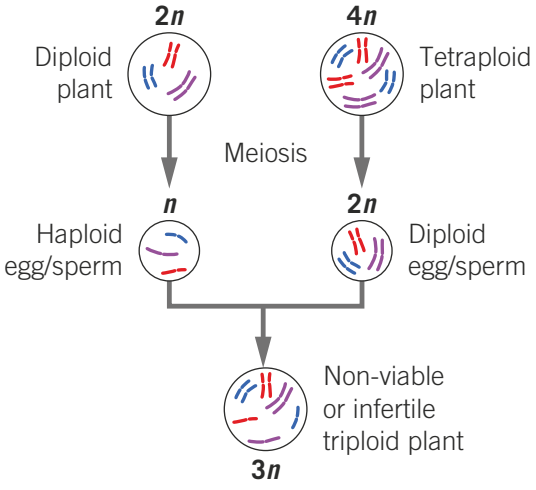


Figure 9D–6 A tetraploid plant (4*n*) cannot successfully produce offspring with a diploid plant (2*n*), as the offspring would have an uneven number of chromosomes.

This is why sympatric speciation is more common in plants than in animals, because it is easier for plants to self-fertilise (asexual reproduction) than it is for animals. A tetraploid (4*n*) plant cannot successfully produce offspring with a diploid (2*n*) plant (Figure 9D–6), but a tetraploid plant can self-fertilise to create offspring.

Sympatric speciation can also occur in other ways, although, as mentioned earlier (e.g. stick insects and scent), these are uncommon. For example, it may occur if members of a population use different resources or niches within the same geographical location or have mismatched reproductive organs or timings, or different mating dances. The following example, looking at *Howea* palms, will help you understand how sympatric speciation occurs in this situation.

Example: *Howea* palms

Lord Howe Island is a small volcanic island in the Tasman Sea, approximately 700 km north-east of Sydney. It was formed some 6.9 million years ago, and it has a subtropical climate, rocky peaks, a volcano crater and densely vegetated lower ground.

It is home to four species of palm trees, two of which, *Howea forsteriana* and *Howea belmoreana*, provide an example of sympatric speciation. These palms are found nowhere else in the world; they are endemic to Lord Howe island. Table 9D–1 compares them.

Table 9D–1 Comparing the features of the two species of *Howea*

	Flower spikes	Leaf shape	Soil type	Flowering
H. forsteriana	Many flower spikes	Straight leaves	Grows in alkaline or calcareous soil	Flowers roughly six weeks before *H. belmoreana*
H. belmoreana	One flower spike	Curved leaves	Grows in more acidic volcanic soils	Flowers roughly six weeks after *H. forsteriana*

LINK **9B** EVOLVING AND NON-EVOLVING POPULATIONS

About 5 million years ago, an ancestor of the two palm species colonised Lord Howe Island from Australia. This species grew in neutral and acidic soils on the island.

Eventually, some seeds of the ancestral palm must have germinated in the more calcareous soils. Growing in calcarenite soils is stressful for the plant, as these soils have a high pH (alkaline) and contain few nutrients. This physiological stress has been shown to affect flowering time. Therefore, these trees were unable to pollinate, or be pollinated by, the trees growing in volcanic soils as their reproductive cycles were different. They became reproductively isolated and gene flow could not occur between the two palms. Over time, the different selection pressures acting on them resulted in phenotypic differences and each became better adapted to growing in the alkaline or calcareous soil. Eventually, the accumulated genetic changes in the two gene pools prevented the formation of fertile and viable offspring when the two populations were brought back together. They therefore remain reproductively isolated and are now two distinct species (Figure 9D–7).

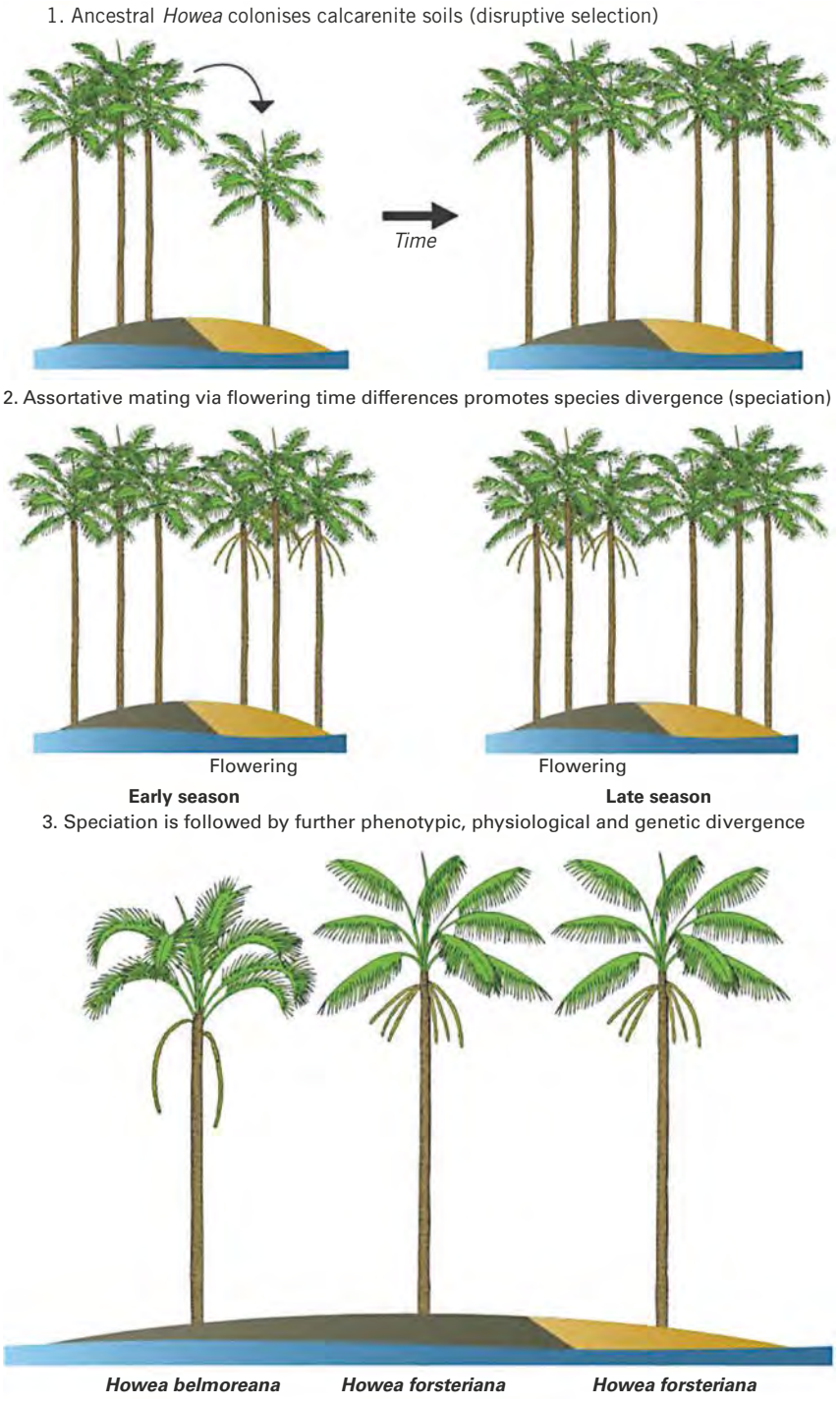


Figure 9D–7 *Howea* palms are an example of sympatric speciation, where speciation occurs without the involvement of a physical barrier.

Check-in questions – Set 3

WORKSHEET 9D–2 ALLOPATRIC AND SYMPATRIC SPECIATION

1 Using the steps in Figure 9D–5 as a guide, summarise the process of sympatric speciation of *Howea* palms.
2 Complete the following table, which compares allopatric and sympatric speciation.

Allopatric speciation	Sympatric speciation
Sub-populations occupy different geographical areas	
Physical separation of the populations	
Natural selection causes differentiation	
Common in nature	
Example: Galapagos finches	

9D SKILLS

VIDEO 9D–2 SKILLS: ANSWERING QUESTIONS ON SPECIATION

Answering questions on speciation

Answering questions about any biological process involves knowing what the examiners are looking for. For example, in the case of speciation:

- Know the definition of 'species' accurately, as there is no room for error: a species is a group of organisms that can interbreed, producing fertile *and* viable offspring in their natural environment.
- Ensure you can identify that the question is about speciation. The definition of speciation is 'the evolutionary process of forming a new species from a pre-existing ancestral species', so look for these terms (new species, pre-existing species, ancestral species, species from) as clues in the scenario and question.
- Know the basic steps in speciation, both allopatric and sympatric. Keep your steps simple and clear so they can be applied to any scenario, as you saw is also the case with natural selection (in 9B Skills). Using acronyms can be one way to do this.

VIDEO 9D–3 SKILLS: USING ACRONYMS

Using acronyms

Consider the steps in the process of allopatric speciation. How could you use an acronym to remember all the key details that you would need to include in an answer to an assessment question? Begin by pulling out the terms that summarise each point:

- V*ariation*: this begins with an interbreeding ancestral population of one species that naturally contains variation (pre-existing variation).
- I*solation*:
  - A geographical or physical barrier separates a population into two.
  - The populations are reproductively isolated, so there is *no gene flow* between the two isolated populations.
- M*utations*: Different mutations may arise in the two populations.
- *Selection pressures*:
  - Selection pressures on either side of the barrier may be different. Therefore, different phenotypes will be selected for as natural selection occurs over generations.
  - After many generations, phenotypic differences, or different adaptations, may appear between the two populations.
- A*llele frequencies change*: The allele frequencies of each population change as the two populations evolve in different ways.

- N*ew species*: Eventually, the accumulated genetic changes in the two gene pools prevents the formation of fertile and viable offspring if the two populations were brought back together. They therefore remain reproductively isolated and are now two distinct species.

Now take the letters from the start of each word: V, I, M, S, A, N. How will you remember this sequence of letters? You could just remember VIMSAN, or you might find it easier to remember a sentence: 'Vegetables In Marshmallows Sounds Awfully Nutritious'.

Now you can look at other processes you need to know, and make acronyms for them too. Perhaps you could start with natural selection.

Section 9D questions

1 Define speciation.
2 Distinguish between allopatric and sympatric speciation.
3 Sympatric speciation is most common in plants, when there is reproductive isolation because of genetic abnormalities – for example, if a mutation or non-disjunction results in a polyploid individual.
   a Explain why it is more difficult for a polyploid animal to reproduce and create offspring than it is for a plant to do so.
   b In Biology class, a student mentions that when sympatric speciation involves the production of polyploid offspring, the formation of a new species is occurring in just one generation. Is this true? Explain your answer.
4 *Howea forsteriana* and *Howea belmoreana* are two species of palm tree growing on Lord Howe Island, off the coast of Australia. The table shown here lists some key differences between the two species. Referring to the information provided, summarise how these two different species developed in the same geographic location.

	H. forsteriana	*H. belmoreana*
Optimum soil pH	8	6
Habitat altitude (above sea level)	30–60 m	above 120 m
Month of flowering	October	December

Figure 9D–8 *Howea forsteriana* (left) and *Howea belmoreana* (centre) growing side by side

5 The heliconid butterflies of South America have brightly coloured patterns on their wings. A hybrid of two species, *Heliconius cydno* and *H. melpomene*, has wing patterns that are different from both parental species. An investigation was carried out to see whether the hybrid was a new species. Separate groups of four butterflies, each consisting of a male and a female of one of the parental species, and a male and a female of the hybrid, were placed together and their choices of mates recorded. The results are shown in the table.

Figure 9D–9 *Heliconius* hybrid

	Number of matings	
	***H. melpomene* male**	**Hybrid male**
***H. melpomene* female**	15	0
Hybrid female	0	15
	***H. cydno* male**	**Hybrid male**
***H. cydno* female**	5	3
Hybrid female	0	5

a Define species.
b With reference to the information in the table, explain whether or not the results of the investigation suggest that the hybrid butterfly is a separate species.
c From the information and your answer to part **b**, how sure are you that the offspring from the mating between the *H. cydno* female and the hybrid male would produce viable and fertile offspring?
d What does it mean for two species to be reproductively isolated from each other?
e How likely is it that the butterflies would be isolated by a geographical barrier?
f Outline how allopatric speciation occurred in this case.

6 Examine the role of variation in the adaptation and speciation of the Galapagos Island finches, using an example of two different beak shapes from Figure 9D–4.

Chapter 9 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
9A.1	Define population, species, gene pool, allele frequency and evolution	8☐, 11☐
9A.2	Outline how mutations can be the source of new alleles and the consequences of this for genetic diversity	2☐, 11☐, 17c☐
9A.3	Define and summarise how point mutations, including the different types of frameshift and substitution mutations, cause a change in allele frequencies in a population's gene pool	6☐, 12a☐, b☐, d☐
9A.4	Define and summarise how block mutations, including translocation, deletion, inversion and duplication, cause a change in allele frequencies in a population's gene pool	3☐, 17a☐, b☐, c☐
9A.5	Define and summarise how chromosome abnormalities, including aneuploidy and polyploidy, cause a change in allele frequencies in a population's gene pool	4☐
9B.1	Define and summarise how gene flow causes a change in allele frequencies in a population's gene pool	1☐, 8☐
9B.2	Define and summarise how genetic drift, including the bottleneck effect and the founder effect, cause a change in allele frequencies in a population's gene pool	7☐, 13e☐
9B.3	Define and summarise how environmental selection pressures cause a change in allele frequencies in a population's gene pool through the process of natural selection	13a☐, b☐, c☐, 16d☐
9B.4	Outline how artificial selection, including selective breeding programs, can cause a change in allele frequencies in a population's gene pool	18a☐, b☐, c☐, d☐, e☐
9B.5	Distinguish between those evolutionary processes that have the potential to increase the genetic diversity of a population and those that have the potential to decrease the genetic diversity of a population	5☐, 13f☐, 18e☐
9C.1	Define bacterial resistance, antigenic drift and antigenic shift	15☐, 16a☐, c☐, d☐
9C.2	Give reasons why bacterial resistance causes ongoing challenges for treatment strategies against bacteria	16b☐, e☐

Success criteria – I am now able to:		Linked question
9C.3	Give reasons why antigenic drift and shift cause ongoing challenges for treatment strategies and vaccination against viruses	9☐, 10☐, 19a☐, b☐, c☐
9D.1	Define viable, fertile, reproductively isolated and speciation	14a☐, b☐
9D.2	Distinguish between allopatric and sympatric speciation	13g☐, 14c☐
9D.3	Outline the steps involved in the allopatric speciation of the Galapagos finches	13g☐
9D.4	Outline the steps involved in the sympatric speciation of the *Howea* palms on Lord Howe Island	1☐, 14a☐, c☐

Multiple-choice questions

1 Lord Howe Island has two species of palm trees: *Howea forsteriana* and *Howea belmoreana*. The two species do not breed with each other, despite existing in close proximity to each other. What evolutionary process does *not* occur?

A genetic drift of both species of palm

B gene flow between the two species

C natural selection in both species

D mutations in *Howea belmoreana*

2 Which of the following statements about mutations are true?

Statement 1: Mutations can result in new alleles.

Statement 2: Mutations can create genetic variation in a gene pool.

Statement 3: Only mutations that occur in gametes have an impact on evolution.

Statement 4: Mutations are unable to influence allele frequencies.

A Only 1, 2 and 3 are true.

B Only 1 and 2 are true.

C Only 2 and 4 are true.

D 1, 2, 3 and 4 are all true.

3 In humans, Charcot-Marie-Tooth (CMT) disease is caused by mutations in the gene encoding for the peripheral myelin protein, on chromosome 17. It occurs because of a duplication in two or more genes in the chromosome. If the number of nucleotides in the duplicated section is divisible by three, then

A the transcribed RNA will contain many STOP codons.

B the translated protein will be longer than the peripheral myelin protein found in a person without CMT.

C the length of each of the genes duplicated will increase by three nucleotides.

D the peripheral myelin of these patients will show one amino acid change in the sequence compared to normal peripheral myelin.

4 Polyploidy refers to

A extra copies of a gene on a chromosome.

B complete extra sets of chromosomes.

C a chromosome that has replicated but not divided.

D aneuploidy – they are the same thing.

5 Which of the following would lead to increased genetic variation?

A an increase in mutagens in the environment

B a random catastrophic change in the environment

C a selective breeding program

D ensuring that fewer than normal offspring are produced in each generation

6 Quentin has type I osteogenesis imperfecta, a brittle bone disorder. His DNA sequence for a section of the collagen type I gene is shown below. The corresponding 'normal' sequence is also shown.

Quentin:
5′-AAACTCCACTTCTTCCAGTAC-3′

Normal:
5′-AAACTCACTTCTTCCAGTAC-3′

What type of mutation does Quentin carry?

A substitution point mutation

B frameshift as a result of deletion

C frameshift as a result of insertion

D deletion as a result of a block mutation

7 The western swamp tortoise was believed to be extinct, until it was rediscovered in 1953. As the tortoise was one of the most endangered reptiles in Australia, the Perth Zoo began a captive breeding program in 1989 and has now bred more than 900 western swamp tortoises. Many of the tortoises bred in this way have been released into the wild. However, a significant problem can result – low genetic diversity. This is due to

A gene flow.

B natural selection.

C founder effect.

D bottleneck effect.

8 Two populations of sugar gliders live in the canopies of two neighbouring *Eucalyptus* forests.
In population 1, the B allele has a frequency of 0.7, while in population 2, the frequency is 0.1.
If 30% of population 1 migrates to population 2:

- The allele frequency of the B allele in population 2 will _____ .
- The genetic diversity of population 2 will _____ .
- Evolution _____ occur.

Select the option below that has the correct words for the blanks in the statements above. The words are in order of the statements.

A increase; increase, will not
B decrease; not change; will not
C increase; not change; will
D decrease; decrease; will

Use the following information to answer Questions 9 and 10.

Nitazoxanide is a broad-spectrum antiparasitic medication that has been repurposed as a broad-spectrum antiviral medication. Nitazoxanide blocks maturation and intracellular transport of the viral haemagglutinin protein at the post-translational stage.

9 With prevention of the production of the H protein, the newly produced viruses will not be able to

A release themselves from the host cells.
B attach to new host cells.
C undergo antigenic shift.
D undergo antigenic drift.

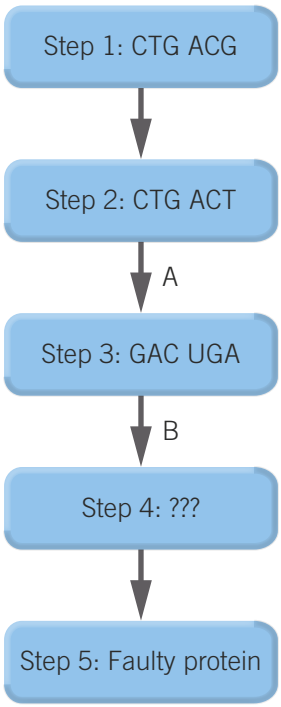
10 As nitazoxanide is in clinical trials for influenza treatment, there are no influenza viruses currently resistant to it. If and when resistance occurs, it is most likely to be because of a mutation in the viral DNA. For nitazoxanide to no longer be effective, this mutation most likely would have caused a change in the shape of

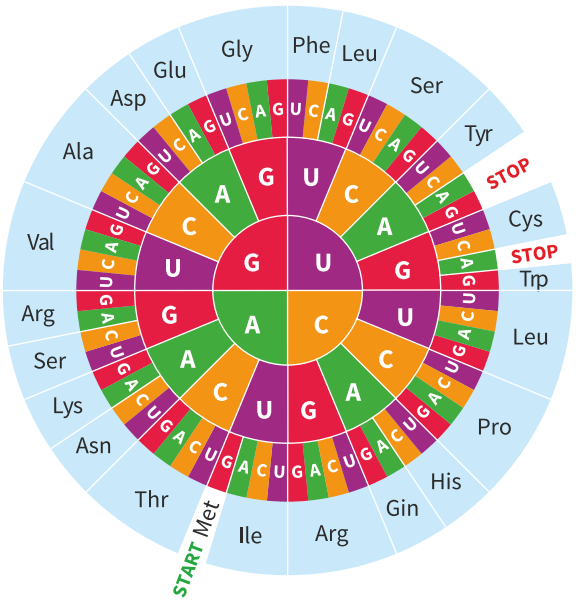
A a binding site on the endoplasmic reticulum.
B the ribosomes of the influenza virus.
C the N protein.
D nitazoxanide.

Short-answer questions

11 Compare the effects of somatic and germline mutations on the genetic diversity and evolution of a species. Include definitions of the following key terms: mutation, somatic mutation, germline mutation, evolution, allele frequency. (5 marks)

12 Use the information in the left-hand diagram to answer parts **a** to **c**.





a Between which two steps has a mutagen affected the genetic code? Explain how you know. (2 marks)

b What type of mutation is this? (1 mark)

c State what occurred during processes A and B that resulted in a faulty protein. (2 marks)

d Describe the impact of the mutation on Step 4 and whether there are consequences for the organism. (2 marks)

13 Daphne Major is an island in the Galapagos archipelago where scientists Peter and Rosemary Grant studied variation in a species of ground finch *Geospiza fortis*. These finches have been identified as preferring to eat tiny seeds because, with their small beaks, they are unable to crack open larger seeds. The graphs on the next page show the Grant's findings in 1976 and 1978.

a What characteristics of the graphs show that there was variation in the population? Include reference to the differences in the range of variation between 1976 and 1978. (2 marks)

b What happened to the population size between 1976 and 1978? What other change occurred in the population? (2 marks)

c The lack of rain in 1977 affected the seeds that the finches ate. The table below shows how the seeds were affected.
Name and outline the process by which beak sizes changed between 1976 and 1978. (5 marks)

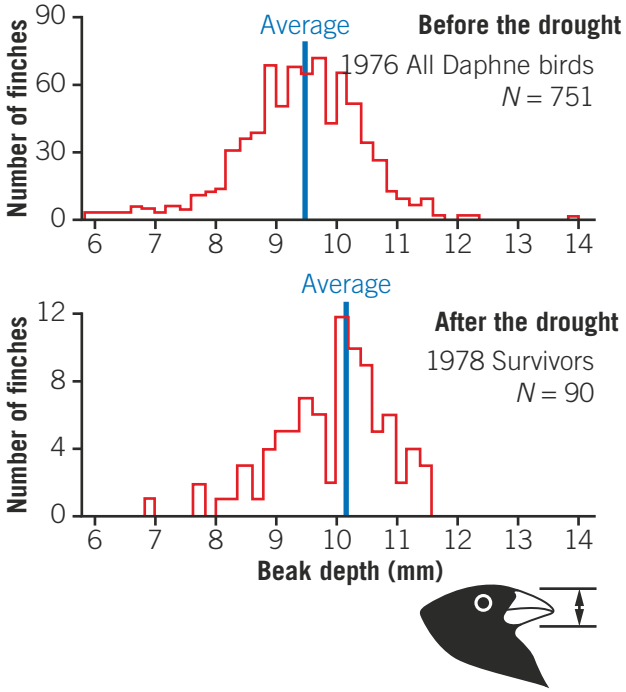
Year	Mean no. seeds per m^2	Mean mass of each seed (mg)
1976	8.5	3.5
1978	2.8	4.2

d Would you predict an increase or decrease in beak depth after 10 years of no drought? (1 mark)

e If the changes in beak size are not due to the process you outlined in part **c**, but to genetic drift, what would you expect to see in future generations? Explain your answer and include a definition of genetic drift. (3 marks)

f Suggest whether the two evolutionary processes covered in this question (that is, genetic drift and the process you named in part **c**) have the potential to increase or decrease the genetic diversity of a population. (3 marks)

g List two similarities between allopatric speciation and the process you named in part **c**. (2 marks)



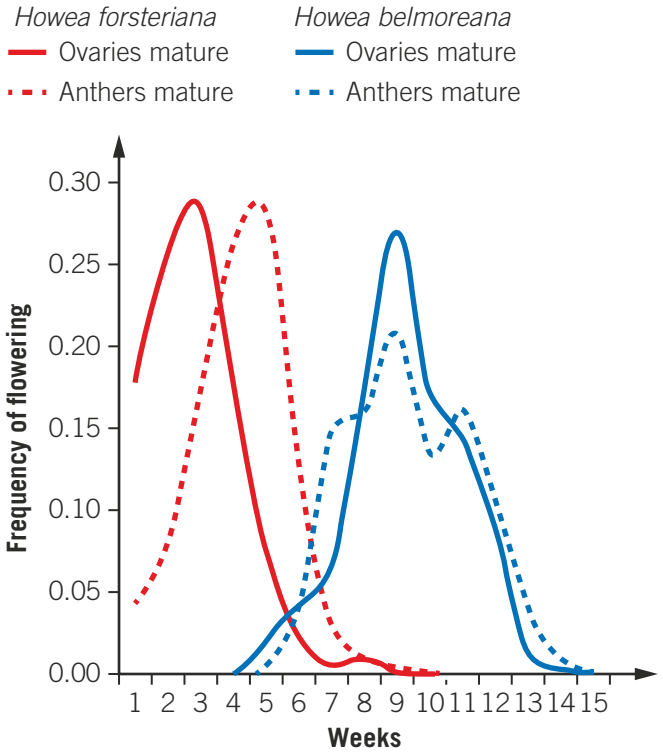
14 The flowering times of two species of *Howea* palms, *H. forsteriana* and *H. belmoreana*, are given in the graph below.

Note: The anthers are the part of a plant that contain the pollen or male gametes; the ovaries are the part of a plant that contain the female gametes.

a Does the data provide evidence that the two types of palms are reproductively isolated? Explain your answer. (2 marks)

b When two populations of organisms become reproductively isolated, speciation can result. Define speciation and include reference to the terms viable and fertile. (2 marks)

c Scientists have evidence that the *Howea* palms *H. forsteriana* and *H.belmoreana* underwent sympatric speciation. State the key difference between allopatric speciation and sympatric speciation. (1 mark)

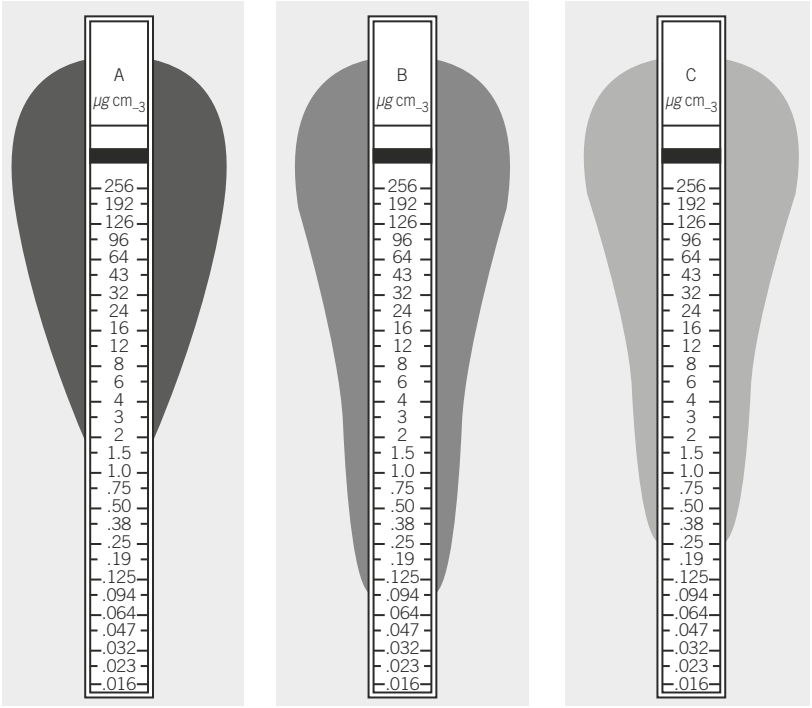


15 Distinguish between antigenic drift and antigenic shift. (2 marks)

16 The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antibiotic required to prevent the growth of bacteria. It is used to determine whether a pathogen is susceptible or resistant to an antibiotic. E-test strips are used to determine the MIC for specific antibiotics.

- Each E-test strip is a plastic strip that is placed on an agar plate that has been inoculated with the bacteria to be tested.
- As soon as an E-test strip is put on an inoculated agar plate, the antibiotic is released immediately to establish a concentration gradient in the agar along either side of the strip.
- The agar plates are left overnight to allow time for the bacteria to grow.
- After incubation, a symmetrical inhibition ellipse is produced as shown in the drawing below.
- The MIC is determined by reading the scale on the E-test strip at the lowest point where bacterial growth is prevented.

Bacteria of a strain of *Staphylococcus aureus* were tested with E-test strips for three antibiotics, **A**, **B** and **C**. The results are shown in the diagram.



a State the MIC for each of the antibiotics. (1 mark)

b With reference to the results in the diagram, suggest the advantages of using E-test strips. (2 marks)

c Make a drawing to show the result you would expect if the MIC for an antibiotic was 8 μg cm^{-3}. (1 mark)

d List the numbers of the following statements in the correct order, to explain the evolution of resistance to the antibiotic streptomycin by the bacterium *Escherichia coli.* (1 mark)

1 Most of the population of *E. coli* is resistant to streptomycin.

2 A random mutation in a DNA triplet of a plasmid, changing TTT to TTG, gives an *E. coli* bacterium resistance to streptomycin.

3 The resistant bacterium divides and passes copies of the R plasmid (plasmid with gene for resistance to antibiotic) to its offspring.

4 Sensitive bacteria die in the presence of streptomycin as a selective pressure.

5 The frequency of the mutated allele in the population increases.

6 The resistant bacterium has a selective advantage and survives.

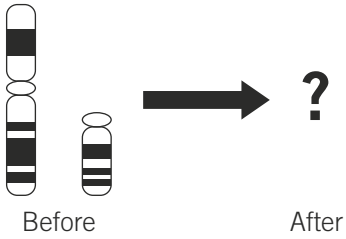
e Give two reasons why bacterial resistance causes ongoing challenges for treatment against bacterial infections. (2 marks)

17 'Accidents' can occur to chromosomes. Such accidents are usually a consequence of spontaneous errors that occur during meiosis. One type of accident is when a section of one chromosome becomes permanently attached to another.

a Name the type of chromosome mutation that is being referred to. (1 mark)

b Complete the image provided on the right, by drawing what this mutation would look like. (1 mark)

c How can this type of chromosome mutation cause evolution when it is only occurring in one cell? (3 marks)



18 The table shows the changes in milk yield and nutrient content in a herd of Jersey cattle in which artificial selection for high yields of high-quality milk was carried out. The figures in the table are the mean results per cow for one year.

a Describe the trend in the mean milk yield over the 10-year period of the breeding experiment. (1 mark)

b Describe the trends in the nutrient content of the milk over the 10-year period. (2 marks)

c It was found that the herd of cows in 1999 had more health problems than those in 1989. Suggest why this happened. (2 marks)

d Outline the four basic steps that apply to all forms of selective breeding. (2 marks)

e Identify the relationship between artificial selection, allele frequency and genetic diversity. (2 marks)

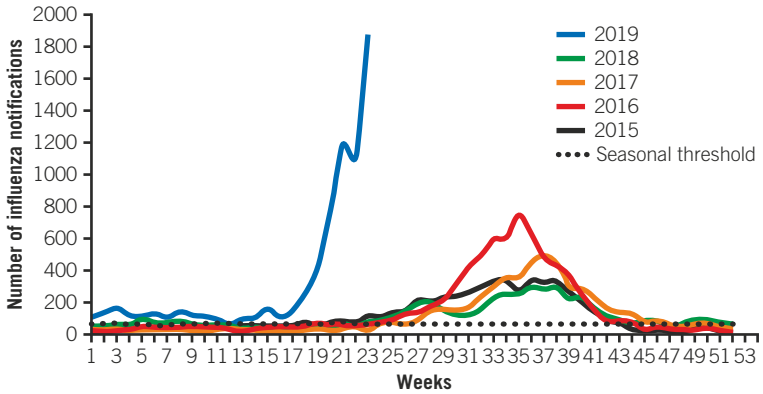
Year	Mean milk yield per cow (kg)	Percentage protein content	Percentage fat content
1989	4104	3.83	5.40
1990	4104	3.84	5.40
1991	4123	3.84	5.42
1992	4151	3.84	5.41
1993	4182	3.83	5.40
1994	4245	3.82	5.37
1995	4281	3.81	5.35
1996	4311	3.81	5.35
1997	4370	3.80	5.35
1998	4412	3.79	5.33
1999	4470	3.79	5.32

19 Influenza is an example of a disease caused by a virus. In some years there are influenza epidemics. The graph below shows the number of influenza notifications in Western Australia in 2015–2019.

a Give three reasons why it is difficult to treat and vaccinate against diseases caused by viruses. (3 marks)

b In most years, the number of influenza notifications is relatively low. Give possible reasons, in terms of the influenza virus and the body's immune system, why there were large numbers of influenza notifications in 2019. (2 marks)

c Explain how vaccination makes a person immune to a viral disease. (3 marks)

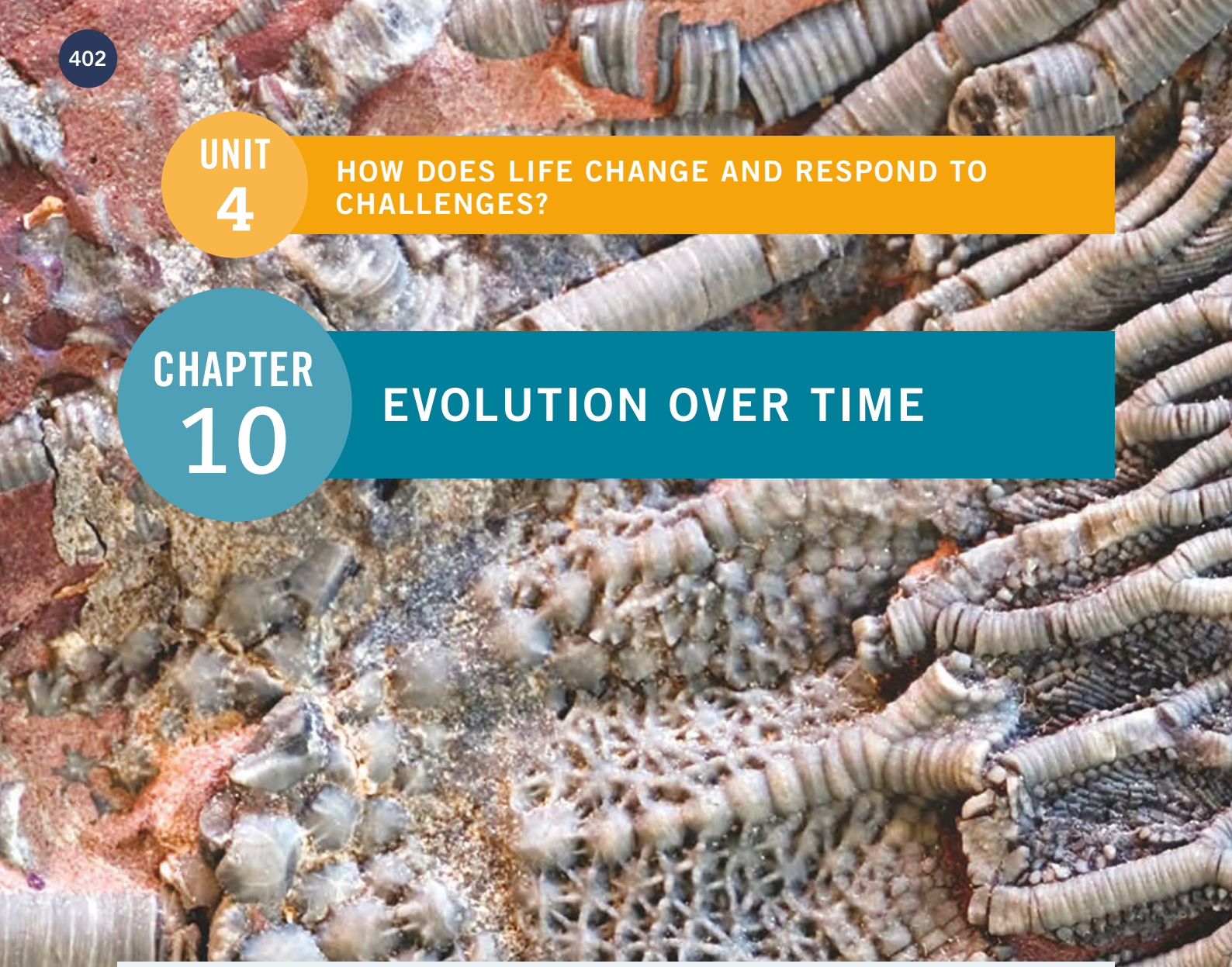


UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES?

CHAPTER 10

EVOLUTION OVER TIME



Introduction

In this chapter you will take a trip back in time through the 4.5 billion year history of Earth. We examine the development of life through the eras and periods of geological time, from the emergence of the first primitive life forms in deep ocean vents, to the wide biodiversity on our planet today.

Fossil evidence has played an essential role in developing scientific understanding of life that has come before us. New discoveries help us to build a more complete image of how life was shaped, in some cases adding to what we already know but often challenging existing ideas about how all the pieces of life fit together. In this chapter you will explore many different types of fossils, including 'transitional fossils', and how they have helped scientists make connections from one species to the next. The process of fossilisation is explained, along with the various techniques used to date fossils and the rocks they are found in.

The types of evidence that are collected and analysed by scientists to determine the evolutionary relationships between species are investigated. Recent advances in technology have enabled scientists to analyse evidence at a molecular level, in addition to the structural and anatomical evidence (often from fossils) that they have relied on in the past. Finally, you will develop an understanding of how this evidence is used to create diagrams known as phylogenetic trees, which can be interpreted to trace the ancestry of not only all current life forms, but those that have come and gone in the millions of years before us.

Curriculum

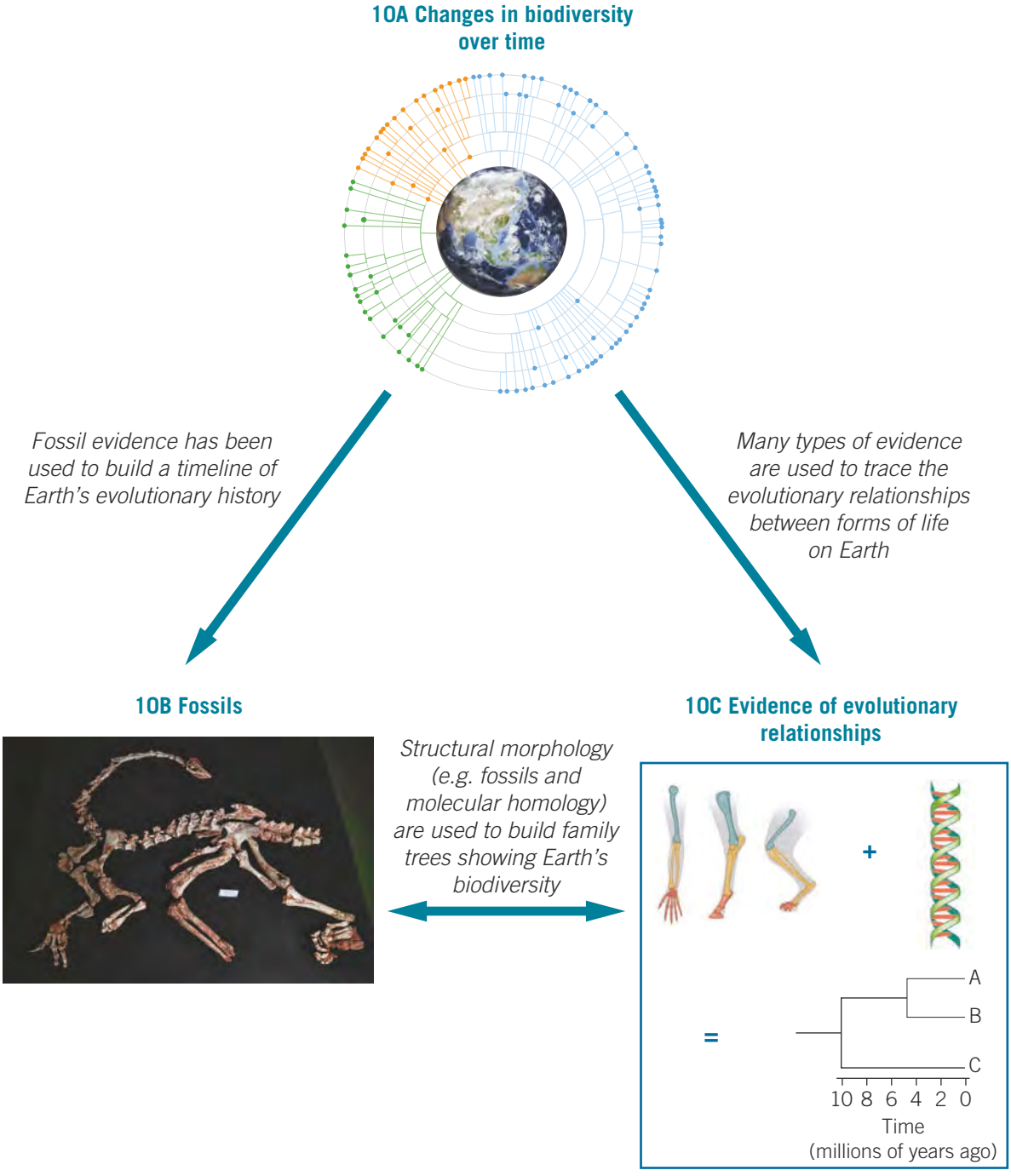
Area of Study 2 Outcome 2
Changes in species over time

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transitional fossils, relative and absolute dating of fossils	**10A Changes in biodiversity over time** **10A.1** Explain the geological time scale as a dating system that shows the history of life on Earth **10A.2** Identify that the geological time scale is divided into eras and periods according to major changes in biodiversity/ complexity as inferred from fossil evidence **10A.3** Describe the consequences that changing conditions on Earth (e.g. temperature, natural disasters, atmospheric composition) had on biodiversity at the time **10A.4** Describe the role of extinction in evolution.
• Changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transitional fossils, relative and absolute dating of fossils	**10B Fossils** **10B.1** Define fossil, and identify the different forms in which fossils can be preserved (petrified, cast, mould, carbon impression, trace) **10B.2** Explain the process that results in fossilisation **10B.3** Describe the environmental conditions that must be present for fossilisation to occur **10B.4** Use an example to explain the significance of transitional fossils in demonstrating the evolution of related species **10B.5** Distinguish between relative and absolute dating with reference to index fossils and radiometric dating **10B.6** Analyse half-life graphs of various isotopes to determine the absolute age of a specimen
Determining the relatedness of species • Evidence of relatedness between species: structural morphology – homologous and vestigial structures; and molecular homology – DNA and amino acid sequences • The use and interpretation of phylogenetic trees as evidence for the relatedness between species	**10C Evidence of evolutionary relationships** **10C.1** Distinguish between the different types of structural morphology and explain how they can be used to determine relatedness between species **10C.2** With the use of examples, describe the significance of vestigial structures as evidence of evolution **10C.3** Explain how molecular homology (DNA and amino acid sequences) can be used as an indication of relatedness between species **10C.4** Analyse phylogenetic trees for evidence of relatedness between species

Glossary

Absolute dating
Amphibians
Arthropod
Background extinction rate
Biodiversity
Brachiopod
Cambrian explosion
Cast fossil
Chordate
Cyanobacteria
Divergent evolution
Eon
Era
Fossil
Fossil record
Geological timescale
Half-life
Hominin
Hominoid
Homologous structure
Index fossil
Invertebrate
Isotope
Mass extinction
Megafauna
Metamorphic rock
Molecular homology
Mould fossil
Myriapod
Niche
Palaeontologist
Period
Petrification
Phylogenetic tree
Phylogeny
Plate tectonics
Primate
Radiometric dating
Relative dating
Reptiles
Sauropod
Sediment
Sedimentary rock
Stratigraphy
Stromatolite
Structural morphology
Supereon
Tetrapod
Theropod
Trace fossil
Transitional fossil
Vertebrate
Vestigial structure

Concept map



See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

Changes in biodiversity over time

Study Design:
Changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transitional fossils, relative and absolute dating of fossils

Glossary:
Amphibians
Arthropod
Background extinction rate
Biodiversity
Brachiopod
Cambrian explosion
Chordate
Cyanobacteria
Eon
Era
Geological time scale
Hominin
Hominoid
Invertebrate
Mass extinction
Megafauna
Myriapod
Niche
Period
Plate tectonics
Reptiles
Sauropod
Stromatolite
Supereon
Tetrapod
Theropod
Vertebrate

ENGAGE

Plate tectonics

As you sit here and read about the geological timescale, you may be unaware that the ground beneath your feet is slowly moving north (and slightly east) at a rate of approximately 7 cm per year. While this doesn't seem like much, the accumulation of this movement over the past few years now means that Australia is 'out of sync' with GPS and has resulted in the government formally requesting that our country's longitude and latitude be updated to reflect our new position. This movement of our country is due to the action of tectonic plates, pieces of Earth's crust, on which all the planet's continents rest. While all continents are showing signs of movement or 'drift', the plate that Australia rests on is moving considerably faster than any other plate.

Plate tectonics a scientific theory that focuses on the separation of Earth's crust into plates that move across the underlying mantle

Planet Earth consists of four layers: inner core, outer core, mantle and crust (Figure 10A–1). The crust is broken into seven large pieces (and a few smaller ones) that glide over the mantle below it. The movement of these plates over the mantle is referred to as **plate tectonics** and it is this process that explains the movement of planet Earth's continents throughout geological time. Plate tectonics is also responsible for the formation of the landscapes that we see today. From mountain ranges to valleys, volcanoes to deep ocean trenches, all these natural wonders are the result of tectonic plate interactions.

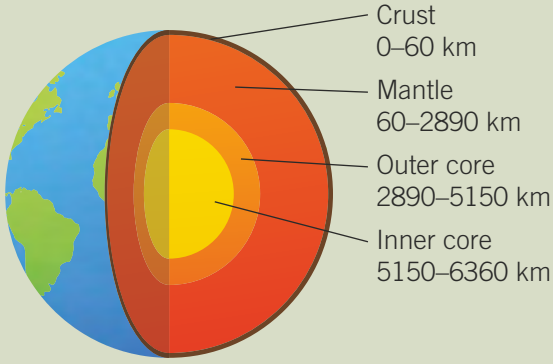
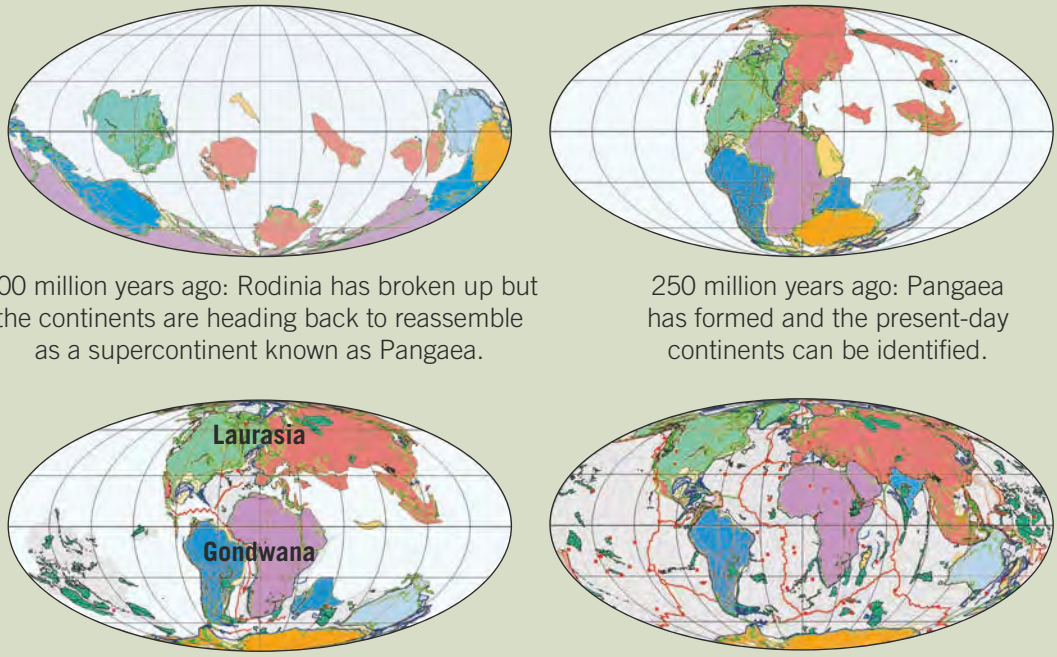


Figure 10A–1 The four layers of Earth. The outer layer (crust) is made up of pieces known as tectonic plates, and these are all moving.

It is now widely accepted that approximately 1 billion years ago, all the modern-day continents were joined, in a supercontinent known as Rodinia. Since then the planet's land masses have assembled into different arrangements (Figure 10A–2).



500 million years ago: Rodinia has broken up but the continents are heading back to reassemble as a supercontinent known as Pangaea.

250 million years ago: Pangaea has formed and the present-day continents can be identified.

100 million years ago: Pangaea has split up into the minor supercontinents Laurasia and Gondwana.

Present day

Figure 10A–2 Four maps showing how Earth's continents have moved over time.

As the continents move, new environments help to increase the planet's biodiversity and, in some instances, may also lead to mass extinctions. With plate tectonics continuing to drive the movement of today's continents towards each other, scientists believe another supercontinent is only a matter of time away. If this prediction turns out to be accurate, future generations may be able to walk from Australia all the way to North America!

EXPLAIN

The geological time scale

The history of life on planet Earth is represented on a geological time scale. This time scale was developed in the 19th century, when geologists observed that particular rocks were characterised by similar types of fossils. The **geological time scale** acts like a calendar depicting the key changes in Earth's **biodiversity** over time. It is used by scientists to describe the timing and relationships between the key events that have led life to where it stands today. A complete geological time scale is shown in Figure 10A–3.

For convenience, the geological time scale is divided into categories of time intervals named after some aspect of the rocks or fossils laid down at that time. This chapter uses four of the categories as follows, in ascending order of size:

- **Period**: an interval of about 50 million years
- **Era**: an interval of two or more periods, about 50 million to 300 million years
- **Eon**: an interval of two or more eras, about 550 million to 2000 million years
- **Supereon**: an interval of more than one eon.

Geological time scale
a scale dividing Earth's history into intervals according to the geological and biological events and conditions present at that time

Biodiversity
the variety of plant and animal life in an ecosystem at any given time

Period
a time interval characterised by specific rock layers; periods are subdivisions of eras

Era
a subdivision of an eon

Eon
a long period of time that consists of at least two eras

Supereon
a period of geological time that consists of more than one eon

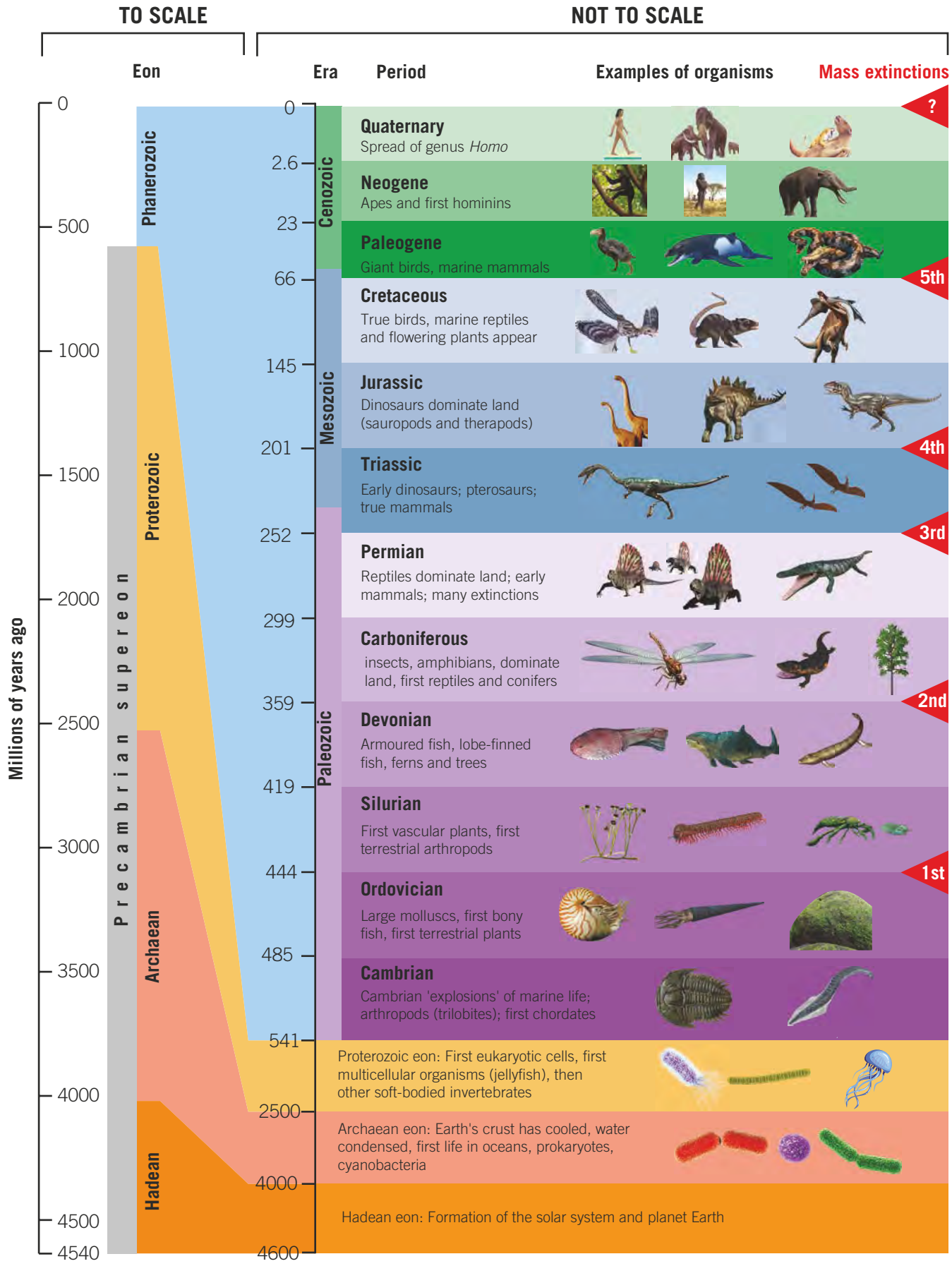


Figure 10A–3 The geological time scale, showing time in millions of years ago, to scale on the left. Moving to the right, the Phanerozoic eon is greatly enlarged to fit in its very much greater variety of organisms. This part is therefore not to scale. On the right, the five biggest mass extinction events are marked when they occurred.

The Precambrian supereon

The Precambrian supereon is the only supereon currently recognised in geological time. Although the Precambrian division of time accounts for approximately seven-eighths of Earth's geological history, it has contributed very little to the current fossil record.

NOTE

The Precambrian supereon is about nine times longer than the time from its end to the present day.

Hadean eon

Occurrence: 4.6–4 billion years ago

Key events:

- The solar system formed from a cloud of dust and gas.
- Planet Earth formed as a result of a collision between two solar bodies.
- Regular asteroid collisions bombarded the newly formed Earth and Moon for the remainder of Hadean time.

Archaean eon

Occurrence: 4–2.5 billion years ago

Key events:

- Approximately 70% of continental land mass was formed.
- Water formed in the atmosphere and cooled Earth as it fell, filling the oceans.
- Life began in the oceans (3.5–2.8 billion years ago), most probably around hot volcanic vents on the ocean floor.
- With the assistance of radiation or heat from volcanic lava, organic molecules arose from inorganic substances such as ammonia, methane and hydrogen.
- These organic molecules assembled into **cyanobacteria**, an aerobic prokaryote.
- Cyanobacteria formed large microbial mats known as **stromatolites** on the ocean floors. By the end of Archaean time, the ocean was teeming with bacterial life.

Cyanobacteria a group of prokaryotic microorganisms that are capable of photosynthesis; recognised as the earliest form of life on Earth

Stromatolite a structure that consists of layered deposits made by cyanobacterial colonies; fossilised stromatolites are among the earliest fossils known

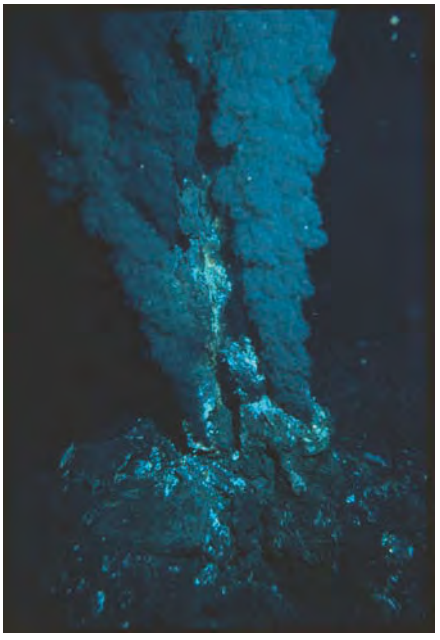


Figure 10A–4 Archaean life. Left: Stromatolites like those seen in Shark Bay, Western Australia, are layered mounds of rock formed by the growth of cyanobacteria. Fossilised stromatolites are the earliest record of life on Earth, with the earliest evidence of stromatolites found in Western Australia and dated at approximately 3.48 billion years old. Right: Deep ocean hydrothermal vents are thought to be the site where life originated billions of years ago.

Proterozoic eon

Occurrence: 2.5 billion – 541 million years ago

Key events:

- Oxygen accumulated in the atmosphere from photosynthetic cyanobacteria.
- Different types of prokaryotes formed symbiotic relationships, resulting in the formation of Earth's first eukaryotic cells. This is known as the endosymbiotic theory (see Figure 10A–5).
- Plate tectonics forces caused the collision of continental rock that resulted in the formation of Earth's first supercontinent, Rodinia.
- By the end of the eon, the first multicellular organism, a soft-bodied type of jellyfish known as a cnidarian had appeared.
- By the end of the eon, an abundance of soft-bodied **invertebrates** existed.

Invertebrate
an organism that does not have a backbone

VIDEO 10A–1
FROM SINGLE CELLS TO MULTICELLULAR ORGANISMS

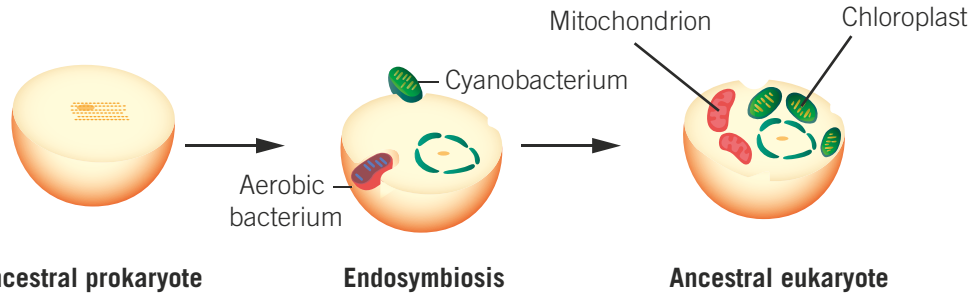


Figure 10A–5 The endosymbiotic theory explains the origin of eukaryotic cells through the engulfment of more efficient energy-converting prokaryotes into another prokaryotic cell.

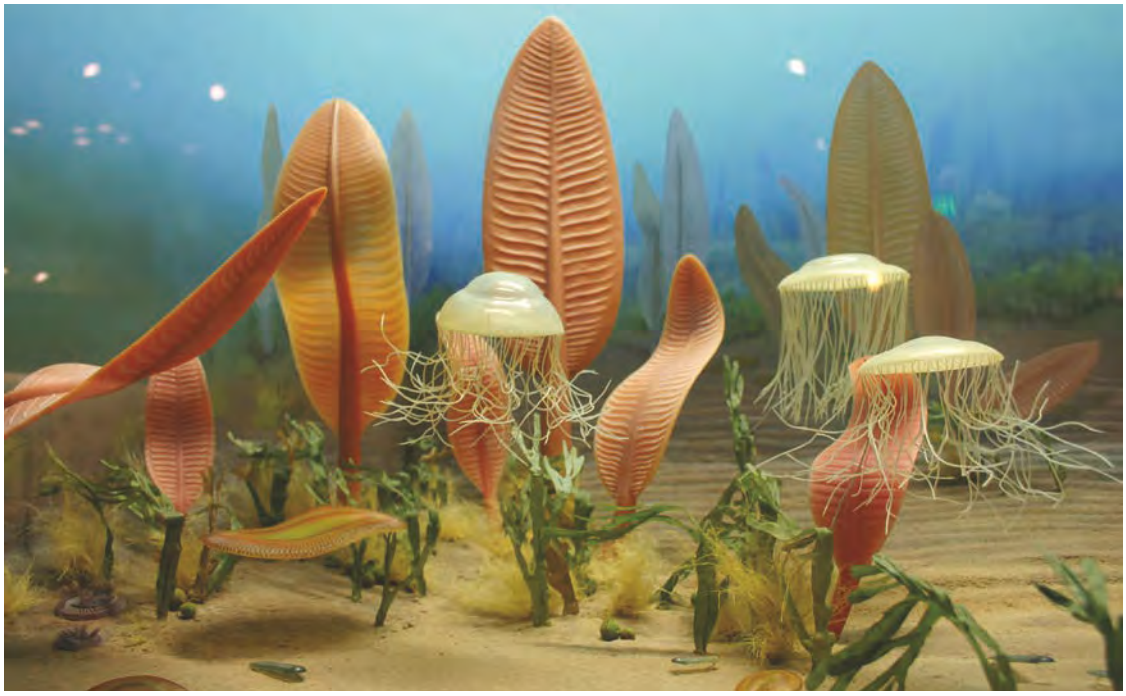


Figure 10A–6 An artist's depiction of how life on the ocean floor would have appeared in the late stages of the Proterozoic eon

Check-in questions – Set 1

1 Name the three eons that make up the Precambrian supereon.
2 Explain the significance of photosynthetic cyanobacteria development in the Archaean eon.
3 Describe what the first multicellular organisms are thought to have looked like.

Phanerozoic eon

Following the Precambrian supereon is the Phanerozoic eon, the current geological eon. It is characterised by an explosion of life that was not present in earlier Precambrian times.

The Phanerozoic eon is divided into three eras (Paleozoic, Mesozoic and Cenozoic), which are further divided into 12 periods. Each of these three eras represents a major stage in the development of life according to the fossil record. The appearance of the first vertebrates, fish, and their evolution into tetrapods that walked on land provides a good example of change in biodiversity over time.

LINK 10B FOSSILS

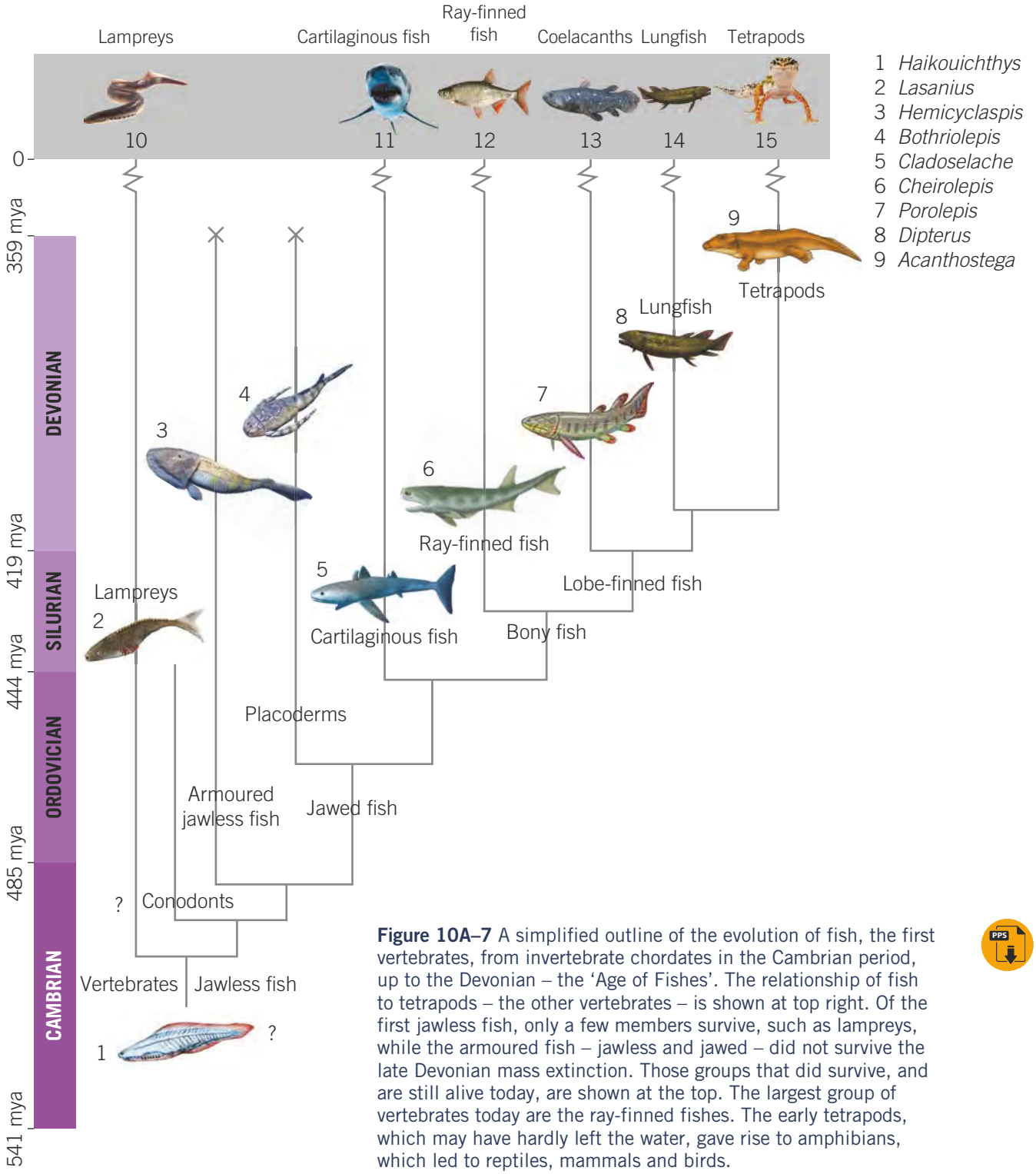


Figure 10A–7 A simplified outline of the evolution of fish, the first vertebrates, from invertebrate chordates in the Cambrian period, up to the Devonian – the 'Age of Fishes'. The relationship of fish to tetrapods – the other vertebrates – is shown at top right. Of the first jawless fish, only a few members survive, such as lampreys, while the armoured fish – jawless and jawed – did not survive the late Devonian mass extinction. Those groups that did survive, and are still alive today, are shown at the top. The largest group of vertebrates today are the ray-finned fishes. The early tetrapods, which may have hardly left the water, gave rise to amphibians, which led to reptiles, mammals and birds.

Paleozoic era – old life

Occurrence: 541–252 million years ago

The Paleozoic era is the longest of the three eras in the Phanerozoic eon, spanning 289 million years. It consists of six periods starting with the rapid increase in biodiversity in the Cambrian period. Prior to this, the fossil record remains relatively empty.

10B FOSSILS LINK

Cambrian period

Occurrence: (541–485 million years ago)

Key events:

- The **Cambrian explosion**: evolution of the greatest number of organisms in a single geological period. All major animal species existing today arose from life that began here.
- This resulted in the development of **arthropods** (ancestors of today's insects and crustaceans), **brachiopods** and early **chordates** (Figure 10A–8).
- Ocean life flourished and almost all current marine orders evolved throughout this time. Most notable was the appearance of the trilobite, a well-recognised form of prehistoric life (Figure 10A–9).

Cambrian explosion
a rapid increase in complex biodiversity within the fossil record that occurred at the beginning of the Cambrian period

Arthropod
a type of animal that has no internal backbone, a segmented body and a hard, external covering known as an exoskeleton

Brachiopod
a marine animal with no internal backbone and a hinged upper and lower shell

Chordate
any animal that contains evidence of a spinal cord at some point in its development

Figure 10A–8 *Pikaia gracilens*, the earliest chordate, making it the earliest known ancestor of all modern vertebrates

Figure 10A–9 A fossilised trilobite. These early organisms belong to the arthropod family and contained a thick exoskeleton. They dominated the ocean floor for 300 million years.

Ordovician period

Occurrence: 484–444 million years ago

Key events:

- Trilobites continued to dominate the ocean environment.
- Bony fish, the planet's first true **vertebrates**, continued to evolve and it is believed that by the end of this period they had developed jaws.
- The first permanent form of terrestrial life appeared, in the form of non-vascular plants most likely resembling modern-day mosses.
- Earth's surface became almost entirely frozen, resulting in an ice age that caused the Ordovician–Silurian extinction, the first of five mass extinctions that occurred on Earth.

Figure 10A–10 The chambered nautilus is classified as a mollusc and is often referred to as a 'living fossil' as it has changed very little over millions of years.

Figure 10A–11 *Cameroceras* ('chambered horn') is a type of giant mollusc that lived mainly during the Ordovician period.

Silurian period

Occurrence: 444–419 million years ago

Key events:

- Warming of Earth removed the glaciation effects seen in the late Ordovician period.
- Jawless fish became more abundant and jawed fish continued to develop.
- Species of mollusc and coral thrived in the warm waters, and arthropods continued to dominate the ocean.
- On land, vascular plants began to emerge (Figure 10A–12), paving the way for diversification of all future terrestrial plant life.
- The oldest creature currently believed to have lived permanently on land arose: a species of **myriapod** known as *Pneumodesmus newmani* (Figure 10A–13).

Vertebrate
having a backbone

LINK UNIT 1

Myriapod
a subphylum of mostly terrestrial arthropods including millipedes, centipedes and other 'many-legged' invertebrates

Figure 10A–12 *Cooksonia*, an extinct primitive plant, is the oldest known plant to have a stem, making it the first vascular plant on Earth.

Figure 10A–13 Myriapods. Left: Reconstruction of *Pneumodesmus newmani*, a myriapod thought to be the first animal that lived solely on land. Right: Fossil of *P. newmani* that was found in 2004 by Mike Newman, a bus driver from Scotland.

Figure 10A–14 Jawless, armoured fish continued to thrive in Devonian waters.

Figure 10A–15 The giant prehistoric fish *Dunkleosteus* grew to 10 metres in length and was the top predator of its time.

Devonian period

Occurrence: 419–359 million years ago

Key events:

- Jawless fish (Figure 10A–14) remained dominant in the oceans.
- The diversification of fish gave rise to lobe-finned fish, which eventually evolved into the first terrestrial vertebrates.
- Oxygen levels reached present-day levels of approximately 21%.
- On land, plant life exploded and, by the end of the period, complex root and shoot systems had developed, giving rise to large ferns and trees.
- Arthropods diversified to include wingless insects and early spiders.
- The first known land vertebrate, a **tetrapod** known as *Tiktaalik* (Figure 10A–16), appeared. It is often referred to as the 'fish out of water' as it had gills and primitive limbs that enabled it to prop itself up on land. This hybrid appearance makes *Tiktaalik* the current evolutionary link between fish and all land vertebrates.
- The period ended with the second of Earth's mass extinctions, when it is believed that 70–80% of all marine invertebrates became extinct.

Tetrapod
any vertebrate animal that has four legs or limb-like attachments, e.g. amphibians, reptiles, birds and mammals

10B FOSSILS LINK

Figure 10A–16 *Tiktaalik roseae* is an extinct lobe-finned fish from the late Devonian period (375 mya).

Carboniferous period

Occurrence: 359–299 million years ago

Key events:

- Tropical swamps were prevalent and the development of seed-producing plants, like conifers, allowed p ant life to move further inland.
- Lush forests produced large amounts of oxygen and other organic matter, which allowed insect life to flourish.
- In the oceans, sharks and bony fish continued to thrive, while the once-dominant trilobite, hit hard by the previous Devonian extinction, continued to fade away.
- **Amphibians** thrived and, in a significant evolutionary moment, **reptiles** evolved to become the first animal based solely on land.

Amphibians
a class of tetrapods that are semi-terrestrial, with young living in water before moving to land as adults; examples are frogs, toads and salamanders

Reptiles
a class of air-breathing tetrapods with skin covered in scales, that lay eggs on land

Figure 10A–17 An artist's impression of a typical Carboniferous landscape

Figure 10A–18 *Meganeura*, one of the largest ever flying insects

Permian period

Occurrence: 299–252 million years ago

Key events:

- Reptiles flourished in the dry inland environment (due to the supercontinent Pangaea being surrounded by a single ocean).
- The end of the period is marked by the disappearance of 95% of all life, in Earth's third and largest extinction event, known as The Great Dying.

Figure 10A–19 The supercontinent Pangaea (pictured here with modern continental borders) was formed in the Permian period.

Figure 10A–20 A group of dimetrodons. These large tetrapods dominated the Permian landscape. Despite their appearance, dimetrodons were not true reptiles. In fact, they were a type of primitive mammal, making them a distant relative of all modern-day mammals.

Check-in questions – Set 2

1 How many periods make up the Paleozoic era and what are their names?
2 Explain the significance of the Cambrian explosion.
3 What is a tetrapod and why did its appearance on land in the Devonian period mark a significant milestone in evolutionary history?
4 Insect life flourished in the Carboniferous period. What conditions existed at the time that supported the exponential growth of such organisms?

Mesozoic era – middle life

Occurrence: 252–66 million years ago

Theropod
a type of carnivorous dinosaur with short forelimbs that ran on powerful, hind legs

The Mesozoic era is the second era of the Phanerozoic eon. It lasted 186 million years and is commonly referred to as the Age of Dinosaurs. The catastrophic Permian extinction event that occurred at the end of the previous era saw 75% of all reptiles wiped out. However, in the three periods of the Mesozoic era, reptiles recovered and went on to dominate land, air and sea for the next 150 million years.

Triassic period

Occurrence: 252–201 million years ago

Figure 10A–21 *Coelophysis*, one of the earliest dinosaurs on record, emerged in the late Triassic period. It was a slender, bipedal carnivore that grew to approximately 3 metres.

Figure 10A–22 Pterosaurs take to the sky. These were the planet's first flying reptiles.

Key events:

- With 95% of all life wiped out in The Great Dying, a mammal-like reptile known as *Lystrosaurus* was one of the few surviving vertebrates.
- By the middle Triassic period, Pangaea had begun to break up and mountain ranges had continued to develop.
- Reptiles had increased in size and moved into the sea, with turtles and crocodiles appearing.
- Reptiles continued to evolve on land, with dinosaurs appearing in the form of small, carnivorous **theropods** such as *Coelophysis* (Figure 10A–21).
- For the first time, reptiles took to the sky in the form of pterosaurs (Figure 10A–22), the first flying vertebrates.
- Mammal-like reptiles continued to evolve and, by the end of the period, some were true mammals.
- The period ended with the Earth's fourth mass extinction, which saw the destruction of most marine invertebrate life.

Jurassic period

Occurrence: 201–145 million years ago

Key events:

- Pangaea drifted apart, leading to the positioning of the present-day continents.
- Modern-day sharks dominated the oceans.
- An abundance of vegetation on land supported the herbivorous dinosaurs that were now flourishing and reached the gigantic sizes associated with **sauropods** such as *Brachiosaurus* (Figure 10A–23) and *Diplodocus*.
- Mammals continued to develop.

Sauropod
a large herbivorous dinosaur characterised by its long neck and tail, and four-legged stance

Figure 10A–23 *Brachiosaurus*, one of the most famous sauropods, had a long neck that allowed it to feed on leaves higher up in the trees, avoiding the need to compete for food with smaller dinosaurs such as *Stegasaurus*.

Figure 10A–24 *Stegosaurus*, a large herbivorous dinosaur famous for its two rows of plated armour running the length of the spine.

Cretaceous period

Occurrence: 145–66 million years ago

Key events:

- Pangaea continued to drift apart, creating many distinct geographical environments. This led to the eventual extinction of all giant sauropods.
- New environments led to the diversification of dinosaurs into many new species.
- *Tyrannosaurus rex* emerged as the apex carnivore.
- The first true birds took to the sky, as several species of pterosaur perished.
- Flowering plants appeared, along with pollinating insects such as bees and butterflies, and became the dominant terrestrial plants.
- The end of the Cretaceous period was marked by an asteroid impact that caused the fifth and final mass extinction event. The impact of the asteroid, 10–14 kilometres in diameter, created the present-day Chicxulub crater in Mexico 66 million years ago, and is widely believed to have caused the extinction of 75% of all life (including the dinosaurs and anything else with a body mass greater than about 10–20 kg). The crater measures some 150 kilometres in diameter.

Figure 10A–25 The Chicxulub crater, shortly after its formation 66 million years ago off the coast of present-day Mexico. It has become buried in silt and covered by the sea.

Check-in questions – Set 3

1 How many periods make up the Mesozoic era? Name them in order.
2 Explain why the Mesozoic era is known as the age of the dinosaurs.
3 As true birds took to the sky for the first time in this era, the pterosaurs that dominated the skies disappeared. What may have been the cause of this?
4 What is believed to have caused the mass extinction at the end of the Cretaceous period? Give at least one piece of evidence for this cause.

Cenozoic era – recent life

Occurrence: 66 million years ago – present

The Cenozoic era is the current one, and part of the Phanerozoic eon. The mass extinction of the dinosaurs in the previous Cretaceous period allowed for the rapid diversification and spread of mammals across the globe. The proliferation of mammals in the absence of dinosaurs has ultimately brought us to where we are today – the age of human domination of life on Earth.

Niche
the role that a species plays within its ecosystem

Hominoid
a superfamily consisting of all current and extinct humans and apes

Paleogene period

Occurrence: 66–23 million years ago

Key events:

- The climate warmed and jungles spread from pole to pole.
- Sharks dominated the ocean as a result of the extinction of most large reptiles.
- The king of the Paleogene jungle was an anaconda-like snake, Titanoboa, over 12 metres long and weighing more than a tonne. It was able to eat any animal, including the enormous crocodiles that roamed the shorelines.
- Mammals thrived in a dinosaur-free world, and diversified to fill the **niches** left vacant.
- Flightless, carnivorous birds were the top predators until they died out in the Quaternary period.
- Seasonal climates saw the rapid expansion of grassland savannahs, and evergreen trees appeared.
- The period ended with the evolution of the ancestors of many current-day species including early whales, horses, primates (e.g. lemurs), elephants, rodents, bats, dogs and cats.
- Fossil evidence has shown that early **hominoids** were also present at this time.

Figure 10A–26 *Titanoboa cerrejonensis* with prey.

Figure 10A–27 Left: *Gastornis*, one of the huge carnivorous 'terror birds' that dominated the food chain on land. Right: An early precursor of modern whales, *Janjucetus hunderi* inhabited coastal waters of Australia around 25 million years ago.

Neogene period

Occurrence: 23–2.58 million years ago

Key events:

- Grasses spread further around the world, reducing the prevalence of the planet's forests.
- Continental plate collisions occurred, resulting in the formation of mountain ranges such as the Andes and the Himalayas.
- Hoofed animals flourished, as did the apes, with the evolution of 30 new species.
- *Danuvius guggenmosi*, a recently discovered extinct ape from the early Neogene period, could walk on two legs. The last common ancestor of the great apes and humans could have looked like *Danuvius*.
- **Hominins** appear for the first time, with the development of *Australopithecus* in Africa marking the beginning of the human lineage.
- Earth's continents and oceans moved into the positions we see today.

Hominin
the subfamily consisting of all current and extinct bipedal primates

LINK **11A** CLASSIFICATION AND CHARACTERISTICS OF MODERN HUMANS

Figure 10A–28 An early hominin species, *Australopithecus* is one of the earliest bipedal ancestors of modern humans.

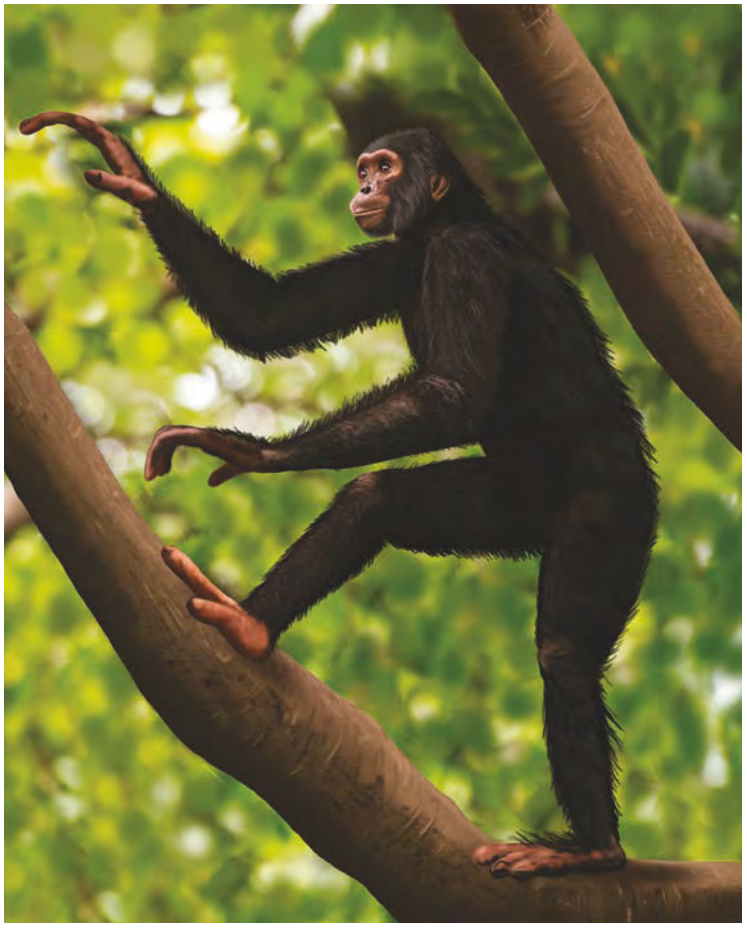


Figure 10A–29 *Danuvius guggenmosi* is an extinct ancestor of humans and great apes that lived in the Neogene period 11.6 million years ago in what is now Germany. It had adaptations for both hanging from tree branches and standing on two legs (bipedalism). It may have been very similar in climbing and walking to the last common ancestor between humans and other apes.

Quaternary period

Occurrence: 2.58 million years – present

WORKSHEET 10A–1 THE GEOLOGICAL TIMESCALE

Megafauna large or giant mammals that were still living in the Quaternary period

Key events:

- This period began with an ice age, during which sheets of ice and alpine glaciers covered large parts of Earth.
- Woolly mammoths (Figure 10A–30), giant ground sloths, sabre-toothed tigers (Figure 10A–31) and wolves were all prevalent.
- An extinction event around 11 000 years ago wiped out most of this **megafauna**.
- Early humans of the *Homo* genus had by now migrated throughout Africa, the Middle East and Asia.
- *Homo sapiens*, today's modern humans, were by now the dominant species, and civilisation began to resemble life as we now know it.

Figure 10A–30 Woolly mammoths were a species of megafauna that thrived in the icy conditions of the Quartenary period, until they were hunted to extinction.

Figure 10A–31 A sabre-toothed tiger attacks a large ground sloth, a common occurrence during the Quaternary period.

Human impacts on other species

11A CLASSIFICATION AND CHARACTERISTICS OF MODERN HUMANS **LINK**

With the Quaternary period still in progress, we cannot yet reflect on the full effect of humans on the natural world. However, one thing is clear: in the entire history of planet Earth, no other species has had the impact that humans have had on the environment and on biodiversity in the little time that we have existed for.

You can read the full details of the evolutionary patterns that have resulted in the formation of *Homo sapiens* from our early ancestors in Section 11A.

Background extinction rate the normal extinction rate expected to occur over a period of time due to natural environmental factors

There is little question that we are currently in the grip of a sixth major extinction event. **Background extinction rate** calculations predict the extinction of 0.1–1 species per 10 000 over 100 years. The current rate of extinction is thought to be between 10 and 10 000 times higher than the background extinction rate, and accelerating. With these numbers, it is hard to argue that humans are not having a devastating impact on the natural world. The exploitation of our planet's resources by humans either directly (e.g. hunting) or indirectly (e.g. climate change) in the past 100 years has resulted in species loss that would have taken up to 10 000 years at the background extinction rate. According to the International Union for the Conservation of Nature, this equates to a loss of 1.2 species every two years, and it is estimated that 60% of all vertebrates on Earth have already been lost in the past 50 years.

While recovery will take time, the situation is not without hope. Recent scientific advances in fields like gene technology may provide the resources to undo some of the damage and bring back species from the brink of extinction. Organisations such as the San Diego Zoo are collecting and storing the genetic material of endangered animals as part of their Frozen Zoo program. The Frozen Zoo contains over 10 000 living cell cultures as well as the gametes and embryos of many vulnerable species, essentially acting as a wildlife 'biobank'. The program is currently sequencing the genome of 10 000 species, which will provide valuable information to help their conservation. The Frozen Zoo has recently provided reproductive scientists with samples of the critically endangered northern white rhino. With the last male of the species dying in 2018, and only two females left, it is hoped that new stem cell technology will bring this species back from the brink of extinction. It is programs like those of the Frozen Zoo that could help ensure that our planet and all the remaining biodiversity is around for the enjoyment of many future generations.

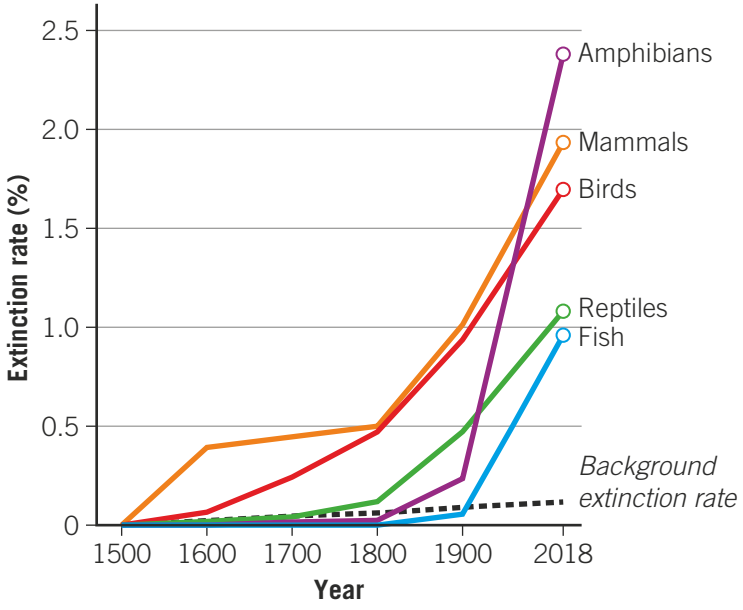


Figure 10A–32 Cumulative percentage of vertebrate species driven to extinction by human activity

LINK **3C** GENETICALLY MODIFIED AND TRANSGENIC ORGANISMS
LINK **3D** THE FUTURE OF GENOME EDITING
LINK **9B** EVOLVING AND NON-EVOLVING POPULATIONS

Figure 10A–33 Preserving species. Left: A scientist at the Frozen Zoo removes frozen embryos and eggs from a tank filled with liquid nitrogen, which preserves the gene pool of endangered species such as the northern white rhinoceros. Right: A northern white rhinoceros.

Check-in questions – Set 4

1 How many periods does the Cenozoic era consist of? Name them in order.
2 What is the most significant feature of the Cenozoic era?

VIDEO 10A–2
SKILLS:
INTERPRETING
THE GEOLOGICAL
TIME SCALE

10A SKILLS

Interpreting the geological time scale

As new discoveries are made and evidence is added to the fossil record, scientists are constantly updating and evaluating their understanding of Earth's biological timeline. While the emergence of a new fossil discovery may sometimes provide the missing piece of the puzzle, it can also add another layer of complexity that causes us to redefine what we already know, making the geological time scale a 'moving target'.

For this reason, it is important to understand that the end-of-year examination will assess your ability to *apply* the geological time scale, rather than memorise it. The key points below provide a summary of the information you will need to know in order to successfully answer questions on this topic.

1 *How the geological time scale is organised*

It is important that you know that the time scale is divided into eras and periods. While you *do not* need to memorise dates, you should be able to recognise the names of the eras that the time scale has been divided into, and know what types of organisms appeared before others in the evolution of life on Earth.

2 *What the geological time scale represents*

The geological time scale is a calendar that gives a visual summary of key chronological events throughout Earth's history. The fossil record shows that significant changes in biodiversity have occurred, and the order in which new species appeared (and disappeared), and is highly organised rather than random. Each era of the time scale represents a significant change in the Earth's biodiversity at the time, with the appearance of new dominant groups.

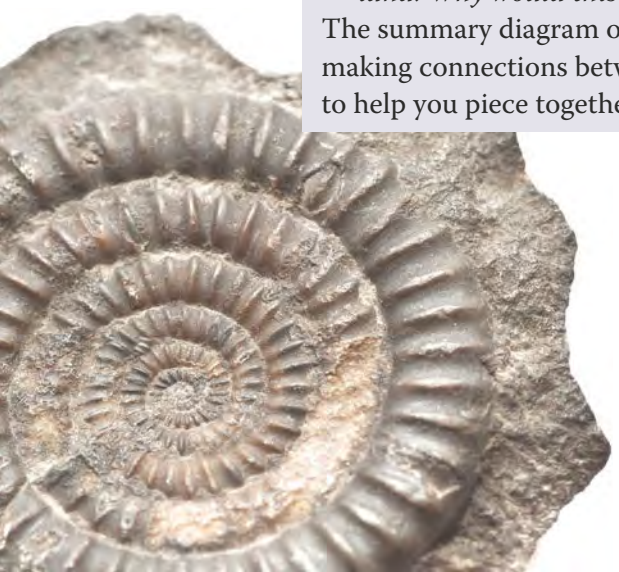
For example, the change from the Mesozoic era to the Cenozoic era saw the loss of the dinosaurs (Mesozoic) and the emergence of mammals (Cenozoic).

3 *The consequences of change*

Finally, and perhaps most importantly, you need to be able to apply your knowledge to explain the significance of change. This change might be a change in the environment or a change in the behaviour or structure of an organism. Here are two examples of the types of questions you could be asked:

- *Explain the significance of the build-up of oxygen in the Earth's atmosphere in terms of biodiversity.*
- *Early invertebrates are believed to have temporarily left the water to lay their eggs on land. Why would this behaviour have been advantageous for the invertebrates?*

The summary diagram of the geological time scale (Figure 10A–34) is a useful way of making connections between all the key events that have occurred. Use this as a resource to help you piece together all the significant events of Earth's 4.5 billion year history.



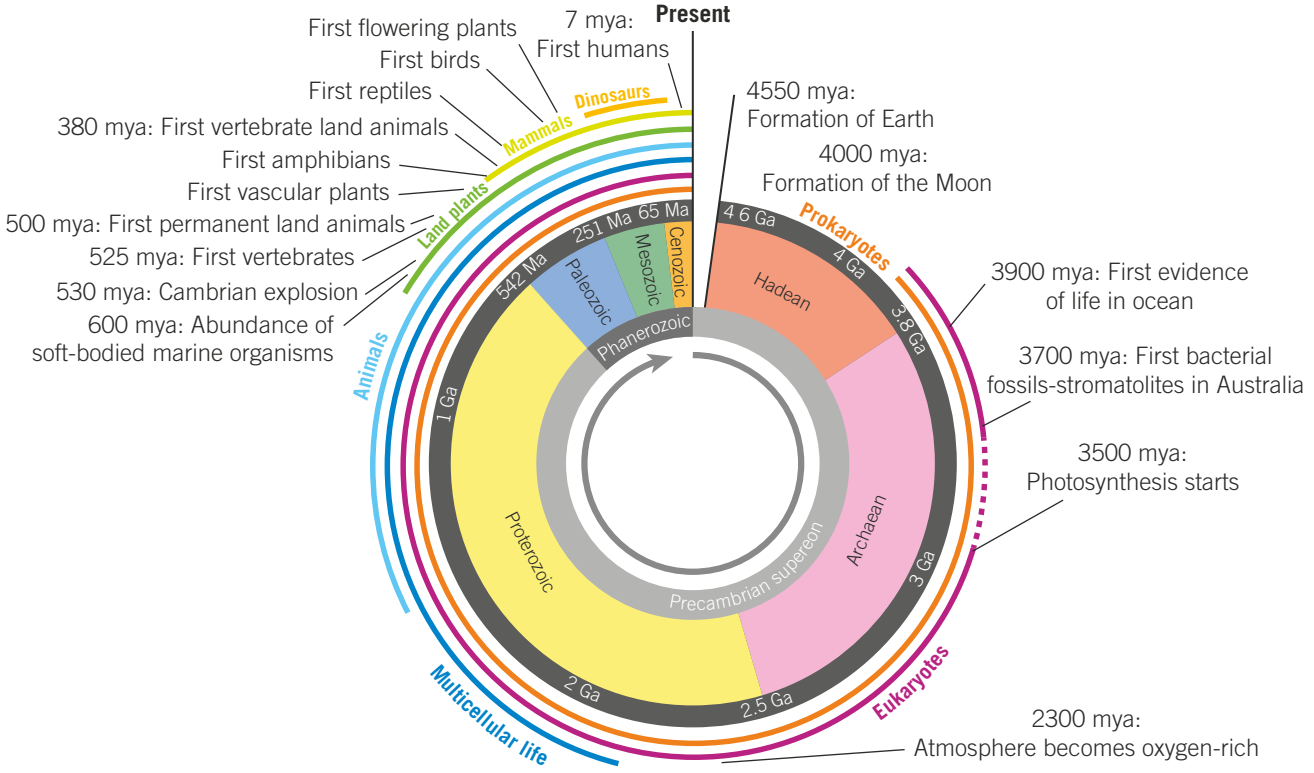


Figure 10A–34 A summary of key events in Earth's geological history

Section 10A questions

1 Put the following events into the correct order (1 = least recent, 6 = most recent):
- Emergence of grassland savannahs
- Evolution of reptiles
- Cambrian explosion
- Build-up of oxygen in the atmosphere
- The Great Dying extinction event
- Permanent life on land

2 Identify these statements as true or false. Rewrite any false statements to make them true.
- **a** Mammals did not appear for the first time until the extinction of the dinosaurs.
- **b** A period is a larger portion of time than an eon.
- **c** Soft-bodied organisms were the first multicellular life forms in the ocean.
- **d** The Mesozoic era occurred prior to the Cenozoic era
- **e** Life began on land before moving to the ocean.

3 Why was the build-up of oxygen in the atmosphere so significant in the evolution of life?

4 Explain, with a diagram, how multicellular life is believed to have originated.

5 What events are believed to be the likely cause of life emerging from the sea onto land?

6 Briefly outline the emergence of mammals and explain why the extinction of the dinosaurs was so significant in the development of mammals.

7 Flowering plants are the most successful of all terrestrial (land) plants. Explain why the development of flowers has given these types of plants a distinct advantage.

8 The idea of the Cambrian explosion of life developed from fossil discoveries of the mid 1800s. To characterise this sudden development as an explosion is now thought to be exaggerated. Outline the kinds of discoveries made since that would support this view.

9 What evidence is there to suggest that we are currently in the midst of Earth's sixth mass extinction? What is the major difference between this extinction and previous ones? Use examples in your answer.

Fossils

Study Design:
Changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transitional fossils, relative and absolute dating of fossils

Glossary:

Absolute dating	Palaeontologist
Cast fossil	Petrification
Fossil	Radiometric dating
Fossil record	Relative dating
Half-life	Sediment
Index fossil	Sedimentary rock
Isotope	Stratigraphy
Metamorphic rock	Trace fossil
Mould fossil	Transitional fossil

ENGAGE

A remarkable discovery

In March 2011, a Canadian miner named Shawn Funk was at work. Using heavy-duty equipment, he was digging as he had done a thousand times before, unaware that he was about to make one of the most remarkable discoveries that science had ever seen.

As he excavated the layers of dirt beneath him, Shawn stumbled across an unusual assortment of rocks. Closer inspection of these rocks showed an intricate pattern of detail. What Shawn didn't realise at the time was that he had just found a 110 million year old nodosaur preserved in the best condition of any dinosaur fossil on record. While most animal fossil discoveries consist of bones and the occasional tooth, this nodosaur fossil was so complete that it was more like a life-like statue than the fossilised remains of an ancient dinosaur.

Figure 10B–1 A front view of the nodosaur fossil. The fossil is so life-like that the dinosaur appears to be sleeping. Scientists believe that the nodosaur was fossilised whole, but only its front half was stable enough to be recovered.

To remove the nodosaur from its rocky tomb, museum experts spent days chipping away at the rock, until it was ready to be hoisted out of the ground. However, removing the 7000 kg slab rock that contained the dinosaur did not go to plan. As the rock emerged from the ground, it shattered, splitting the nodosaur into pieces. Eventually, the team were able to safely transfer the front half (snout to hips) of the nodosaur to the museum's workstation, where recovery continued over the next five years.

Figure 10B–2 Artist's impression of a nodosaur. A Cretaceous herbivore, the nodosaur grew to over 5 metres long and weighed well over 1000 kg.

The reward for this painstakingly delicate project is a true-to-life dinosaur that looks as if it had walked Earth just weeks ago. From the scales on the skin that covered its bony armour, to the tendons that helped support the nodosaur's tail, the detail captured in its stone-like state is extraordinary. Finds like this not only help us learn more about Earth's prehistoric times, they also motivate us to continue searching for clues, as we wonder what else is out there, just waiting to be stumbled upon.

Fossil
the remains or traces of a pre-existing life form

Fossil record
a record of organisms that once lived, through geological time, as documented by fossils

EXPLAIN

Types of fossils

Fossils are the remains or traces of a pre-existing life form. They come in a range of sizes and types and are generally formed from the hard parts (shells, bones, teeth) of an organism. As new fossil evidence is found, it is added to the fossil record. The **fossil record** is a system that tracks all the changes in Earth's biodiversity over time. The geological time scale that you read about in Section 10A has been built according to fossil record evidence.

LINK 10A CHANGES IN BIODIVERSITY OVER TIME

Currently the oldest known fossil on record is of bacteria dated at over 4 billion years old. Despite the current fossil record being extensive, it still has many gaps, which **palaeontologists** hope to fill with new finds in the future.

Palaeontologist
a scientist who studies fossils

Figure 10B–3 Left: Stromatolites are currently the oldest known fossil on record. Right: A palaeontologist recovers the vertebrae of fossilised dinosaur remains.

Fossils can be divided into two broad types:

- body fossils – parts of the organism itself (e.g. teeth, bones, shells)
- **trace fossils** – evidence of the organism's presence (e.g. footprints and coprolites (faeces)).

Trace fossil
fossilised signs or remains of an organism's activity, e.g. tracks

Body fossils

A body fossil can be formed in different ways. Each of the processes that results in this type of fossil is outlined in Table 10B–1

Petrification the replacement of an organism's organic matter with minerals, turning it into a stony material

Mould fossil an impression that forms from the decay of the organism within a rock

Cast fossil fossil formed when an organism decays, leaving an impression, which fills with minerals, resulting in a 3D object of the organism's external surface

Table 10B–1 Processes that result in the formation of body fossils

Process	Petrification	Mould/cast formation	Carbon impressions	Mummification (unaltered preservation)
Description	The replacement of parts of the organism's organic material with minerals, converting them into a stone-like fossil. Harder organic parts such as bone and shell petrify better than softer parts.	The formation of a **mould fossil** occurs when an organism decays, leaving behind an impression in the rock. When the mould fills with minerals (or other materials), it forms a 3D cast of the organism's external form, known as a **cast fossil**.	The carbon contained within the organism is deposited onto rock over time, leaving behind a thin, dark film in place of the organism.	A rare type of fossilisation where the organism is left in its original state. Unlike other fossilisation processes, the soft parts do not deteriorate away, and a complete, unaltered fossil is formed.
Common examples	Bones, petrified wood, the exoskeletons of trilobites, shells of ammonites	Shells or exoskeletons of marine life, e.g. bivalves, trilobites	Plant material, e.g. leaves	Fossilisation of insects in amber, or woolly mammoths and other organisms in permafrost

Petrified dinosaur skeleton

Trilobyte mould and cast

Carbon impression of an insect

Yuka the mummified baby woolly mammoth

Trace fossils

Unlike body fossils, trace fossils do not contain parts of the organism itself. Instead, they are the signs or remains of an organism's activities within its environment. Common examples are footprints, bite marks, burrows, eggs and coprolites (faeces).

Figure 10B–4 Trace fossils: coprolite (left), dinosaur tracks (middle) and dinosaur eggs (right)

Trace fossils are useful for determining the behavioural patterns of animals. In many cases, trace fossil evidence pre-dates evidence of the organism itself, giving scientists knowledge of life that may never be found directly. For example, one of the earliest body fossil finds that shows our human ancestors walked on two legs has been dated at 3.2 million years old. However, trace fossils of footprints making their way across volcanic ash have been dated at 3.75 million years old, which shows that our ancestors walked on two legs much earlier than suggested by the body fossil that was found.

LINK 11A CLASSIFICATION AND CHARACTERISTICS OF MODERN HUMANS

Sedimentary rock a type of rock that is formed from the accumulation of sediment into layers

Metamorphic rock a type of rock that arises from the transformation of existing rocks

Fossil formation

Fossilisation is the process that results in the preservation of the hardened remains or traces of organisms. The process usually occurs in **sedimentary rock**, due to the layering effect that forms this type of rock, although fossils have occasionally been found in **metamorphic rocks**.

Figure 10B–5 shows the typical fossilisation process.

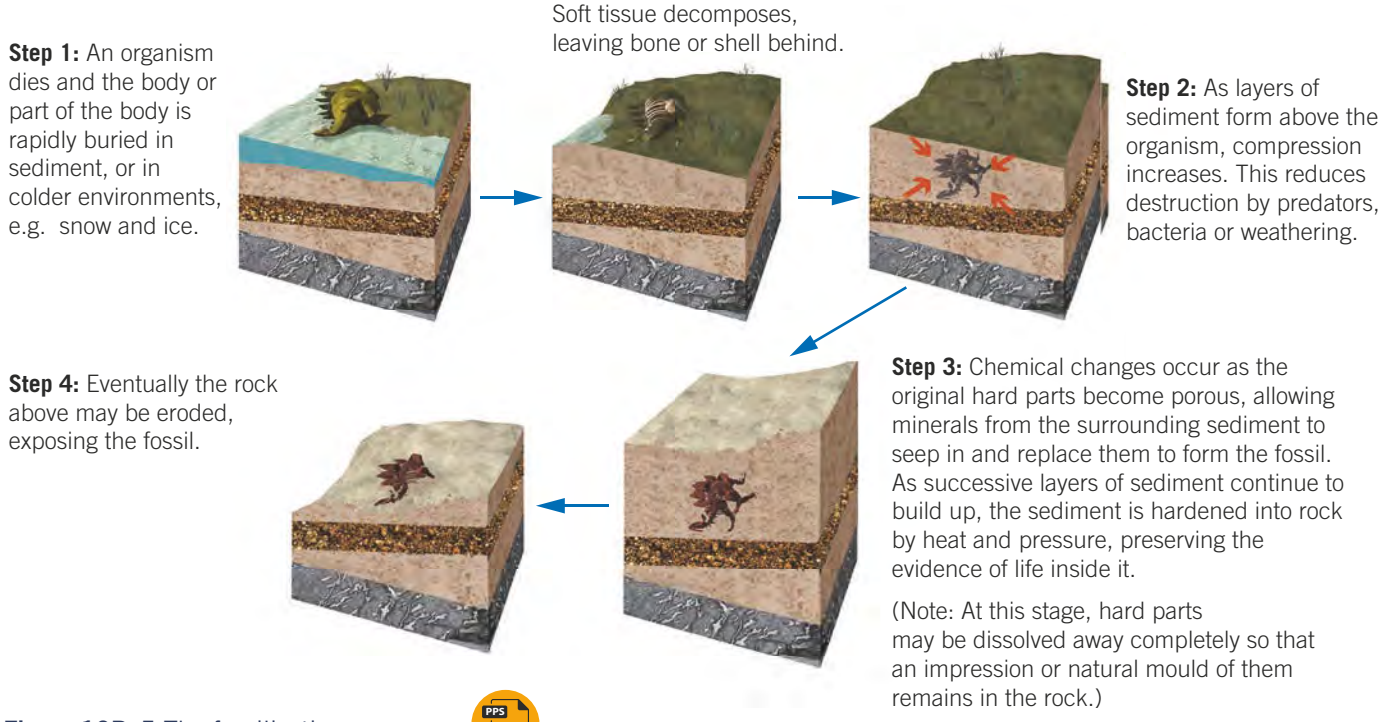


Figure 10B–5 The fossilisation process

For fossilisation to occur, a very specific set of circumstances must exist. Specific details of these conditions are summarised in Table 10B–2.

Table 10B–2 Conditions required for fossilisation

Condition	Explanation
Rapid burial	Provides protection and therefore preservation against erosion and other environmental damage
Lack of scavengers	Ensures remains are not eaten and specimen is preserved
Lack of decomposers	Ensures organic material of the specimen is not broken down or decomposed
High pressure	Produced as a result of the **sediment** layers that form on top of the specimen, which promotes mineralisation of the organism's structures
Lack of oxygen	Necessary to protect against decay by decomposers
Presence of hard structures	These structures are replaced by minerals in the fossilisation process (bone, shell etc.)

Sediment naturally occurring material that is formed through the effects of weathering and erosion

Transitional fossil a hybrid fossil that shows traits of both an ancestral group and a descendant group

The conditions required for fossilisation do not commonly occur in the natural world. This is one of the reasons why the fossil record still contains significant gaps. Can you think of any other reasons?

Transitional fossils

Transitional fossils are hybrid organisms that bridge the evolutionary gap between two different species. This type of fossil contains features in common with the ancestral group it has arisen from, and with its descendants, making it an intermediate between the two.

10A CHANGES IN BIODIVERSITY OVER TIME LINK

Two well-known examples of transitional fossils are explained below.

Figure 10B–6 *Archaeopteryx* fossil. The feathered impression in the rock surface indicates the presence of wings and a feathered tail.

Archaeopteryx: reptiles to birds

Archaeopteryx, discovered in 1861, is a transitional fossil believed to be the evolutionary link between reptiles and birds. Fossil evidence has shown that this species had traits in common with both dinosaurs and birds, which led scientists to place it as the intermediate form between these two very different groups.

In common with the theropod dinosaurs were *Archaeopteryx*'s long bony tail, jaws lined with sharp teeth, sharp claws for grasping prey and, most importantly, a furcula or fused clavicle (wishbone), which is only found in birds and some theropod dinosaurs, providing evidence of the relationship between the two.

Unlike dinosaurs, *Archaeopteryx* also had feathers that were structured for flight, as well as wings that allowed it to fly for short periods. These features resemble those of modern birds.

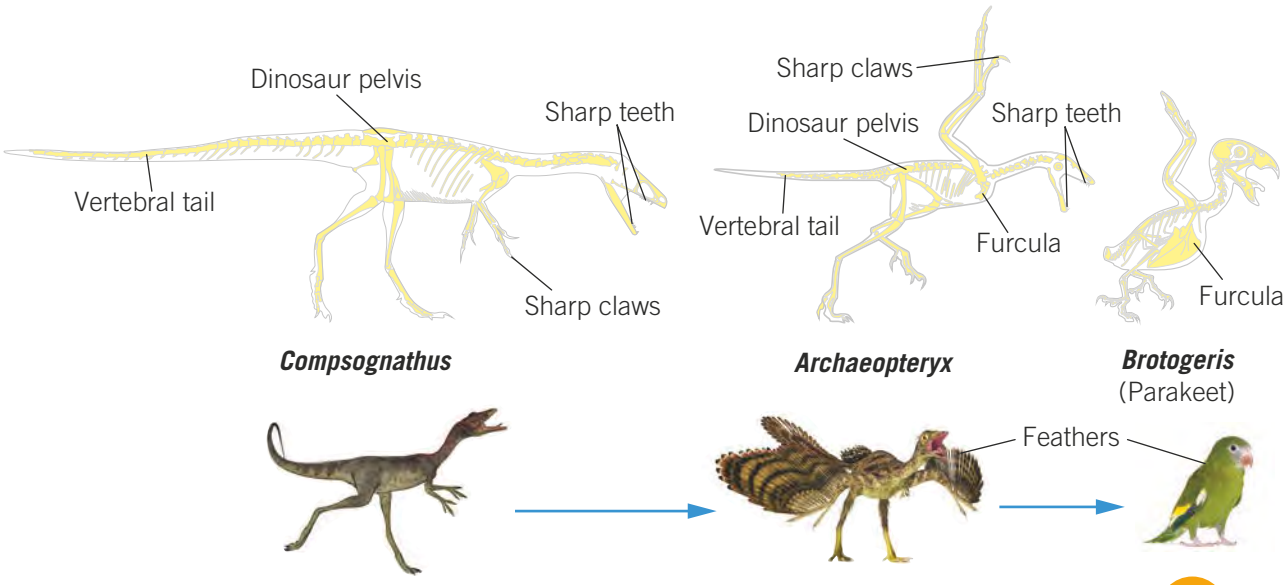


Figure 10B–7 The skeletal structure of *Archaeopteryx* contains features of both the *Compsognathus* dinosaur and modern birds, making it an intermediate form between the two.

From the ocean to the land: fish to amphibians

LINK **10A** CHANGES IN BIODIVERSITY OVER TIME

As you read in Section 10A, life began in the oceans of Earth, before eventually making its way onto land through ancestral amphibians like *Ichthyostega* (Figure 10B–8).

These early amphibians are believed to be descendants of a transitional species known as *Tiktaalik*. Fossil evidence of *Tiktaalik* has shown the presence of early wrist and finger bones that would have assisted it to prop itself up in shallow waters. These skeletal features show an intermediate form between the stronger limbs of amphibians like *Ichthyostega* and the fins of marine fish that *Tiktaalik* arose from, leading scientists to believe that *Tiktaalik* is the bridge between ocean life and terrestrial life.

Tiktaalik also shows the presence of both gills and primitive lungs, reinforcing its classification as a transitional fossil. *Tiktaalik*'s predecessors were fish such as *Eusthenopteron*, which breathed through gills typical of modern fish, while early amphibians such *Ichthyostega* breathed through lung-like structures that are associated with modern amphibians and other terrestrial life. The presence of ribs indicates the presence of lungs, and *Tiktaalik* had ribs, smaller than those of *Ichthyostega*.

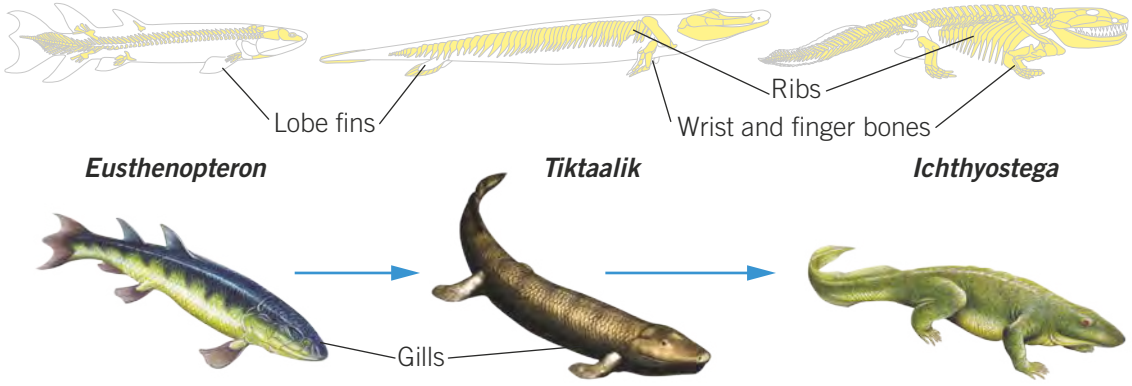


Figure 10B–8 The evolution of life from ocean to land can be seen through the transitional fossil *Tiktaalik*, a lobe-finned fish that has features of other lobe-finned fish like *Eusthenopteron*, as well as features of the tetrapod amphibian *Ichthyostega*

Check-in questions – Set 1

1 Define fossil.
2 Distinguish between a body fossil and a trace fossil.
3 Draw a flow chart summarising the fossilisation process.
4 Why is rapid burial such an important feature of fossilisation? Name two other conditions that must occur for a fossil to form.
5 Explain the significance of transitional fossils in the study of evolution.

Dating of fossils

Absolute dating determines the actual age of the specimen being analysed

Relative dating determines the age of a specimen by comparing its placement with that of other fossils or the rock layers it is found in

Stratigraphy a branch of geology that uses the 'principle of rock succession' to examine the order and position of strata in connection with fossilised remains

Knowing when an organism lived plays an important part in the reliable construction of our evolutionary family tree. Accurate dating techniques allow scientists to place evidence into the fossil record with precision, which is necessary to create accurate sequences of evolutionary change over time.

There are two methods scientists can use to date fossils: they can date the fossil directly (**absolute dating**), or they can analyse the rock samples that the specimen is found in, to determine its age (**relative dating**).

Relative dating

Relative dating of fossils and/or their surrounding rocks occurs when the specimen is compared to another sample of known age. For example, if you were to find an ammonite on the rock shores of an area that was already known to be around 150 million years old, you could use this information to estimate that your ammonite fossil is also approximately 150 million years old. The basis for this method of dating comes from a branch of science known as **stratigraphy**.

As you read earlier, fossils are almost exclusively found in a type of rock known as sedimentary rock. Sedimentary rock is formed through a layering effect in which multiple layers of sediment (strata) are laid down on top each other. As the layers accumulate, increasing pressure and compression over time lead to the production of sedimentary rock.

Figure 10B–9 The strata in this sedimentary rock wall are clearly indicated through the layers of colour throughout.

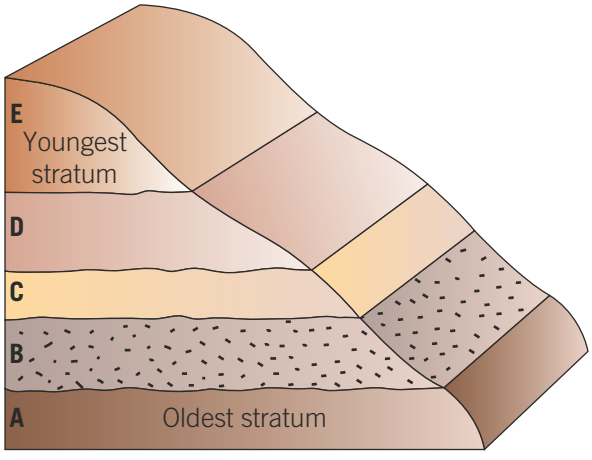


Figure 10B–10 The principle of rock succession states that the layers towards the bottom are older than the layers towards the top.

The principle of rock succession states that, in an undisturbed environment, the oldest strata lie at the bottom, with the layers becoming progressively younger towards the top. From this, we can conclude that each layer is younger than the one below it, and older than the one on top of it. This rule also applies to any fossils that are contained within the layers of rock.

Index fossil
a fossil that is used to date and correlate the strata within which it is found

Index fossils

Like rock layers, fossils do not appear randomly. Rather, they follow each other through time in a regular and predictable pattern. For this reason, some types of fossils can be used as a tool for determining the age of samples. We call these **index fossils**.

Index fossils are known to have occurred during a very specific time frame. They also have a wide geographical distribution, which means they are commonly found in many parts of the world.

VIDEO 10B–1
INDEX FOSSILS

Figure 10B–11 Typical index fossils: trilobites (top left), ammonites (top right) and brachiopods (bottom)

To use an index fossil, you need to be able to correlate the rock layer that your fossil has been found in with layers of rock that index fossils are typically found in. Once you are able to make this correlation (Figure 10B–12), you will be able to obtain a relative age for the fossil in question (or the rock it was found in). The presence of index fossils in rock strata, even those at different sites, separated by large geographical distances, can be used to infer that the strata are of the same approximate age.

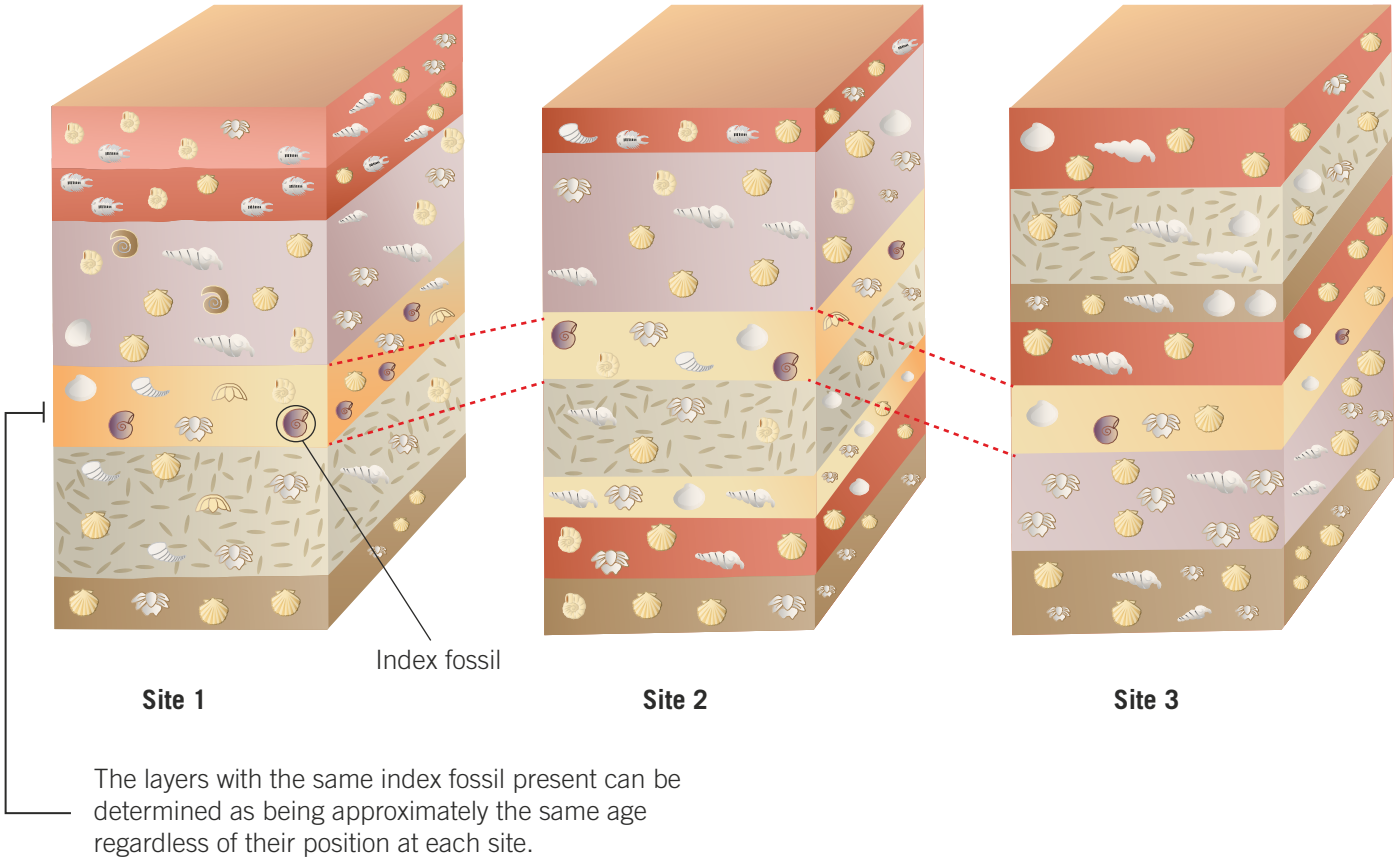


Figure 10B–12 Using index fossils to correlate rock layers when determining the relative age of samples

While relative age is a common and useful tool for dating, it does not give an exact age for the specimen. To get this information, absolute dating techniques must be used.

Check-in questions – Set 2

1 Explain the relationship between sediment, strata and sedimentary rock (you may wish to use a diagram in your answer).
2 What does the principle of rock succession state, and how is this relevant to fossil dating?
3 Identify some common examples of index fossils.
4 Use a diagram to explain how index fossils are used to date a specimen.

Absolute dating

When the approximate age that relative dating provides is not specific enough, another technique, absolute dating, is used. Where relative dating simply compares rock layer ages with reference to the layers above and below, absolute dating determines the actual ages of rocks and fossils. This is done using **radiometric dating**, which draws upon naturally occurring radioactive **isotopes** in the minerals that make up fossils and rock samples. Isotopes which are radioactive decay at a known rate that is unaffected by pressure or temperature (Figure 10B–13). This makes them useful as a natural geological clock.

Radiometric dating
a method of absolute dating that uses the concept of isotope decay to determine the age of a geological sample

Isotope
variant of an element that differs in the number of neutrons in the nucleus

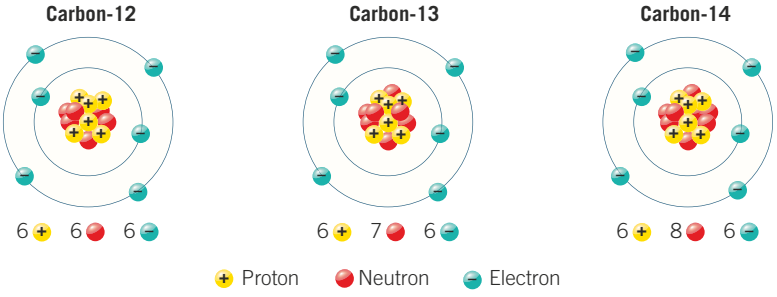


Figure 10B–13 Isotopes of carbon. Note the difference in neutron numbers between each isotope. Carbon-12 and Carbon-13 are stable, but Carbon 14 is not and over time it will lose a neutron and gain a proton, which results in the formation of a new, more stable element. This is referred to as 'decay'.

When using radiometric dating, it is important to understand the concept of a **half-life**. All radioactive isotopes consist of an unstable parent isotope that decays into a stable daughter isotope. The time it takes for half of the parent isotope to decay into the daughter isotope is referred to as a half-life (Figure 10B-14).

Half-life
the time taken for 50% of a an unstable parent isotope to decay into its corresponding stable daughter isotope

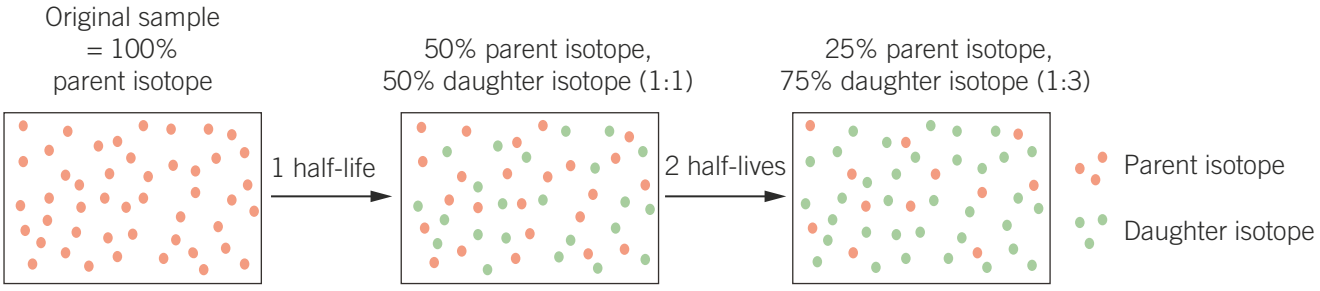


Figure 10B–14 Absolute age is determined through the ratio of parent to daughter isotope in a geological sample.

By measuring the ratio of the amount of parent isotope to the amount of the daughter isotope in the sample, and analysing this against the isotope's known half-life, the absolute age of the specimen can be determined. The concept of half-lives and their use in radiometric dating is explored further in the 10B Skills section.

Scientists use a range of isotopes when calculating the absolute age of a sample. A list of those commonly used and their half-lives is given in Table 10B–3.

Table 10B–3 Isotopes commonly used in absolute dating

Parent isotope (unstable)	Daughter isotope (stable)	Half-life (years)	Dating range (years)
Carbon-14	Nitrogen-14	5730	< 50 000 years
Potassium-40	Argon-40	1.3 billion	100 000 – 4.5 billion
Uranium-238	Lead-206	4.5 billion	10 million – 4.6 billion

WORKSHEET 10B–1 RELATIVE VS ABSOLUTE DATING

Check-in questions – Set 3

1 How does absolute age differ from relative age?
2 Explain the concept of 'half-life' and how it is used to date fossils or the rock strata they are found in.
3 If a fossil was estimated to be approximately 48 000 years old, which isotope would be used to determine its absolute age? Explain.

10B SKILLS

VIDEO 10B–2
SKILLS: USING HALF-LIFE GRAPHS TO DETERMINE AGE

Using half-life graphs to determine age

A typical way of presenting data that relates to absolute dating and half-life is as a graph. Figure 10B–15 shows a general representation of the radioactive decay of an unstable parent isotope into its stable daughter isotope.

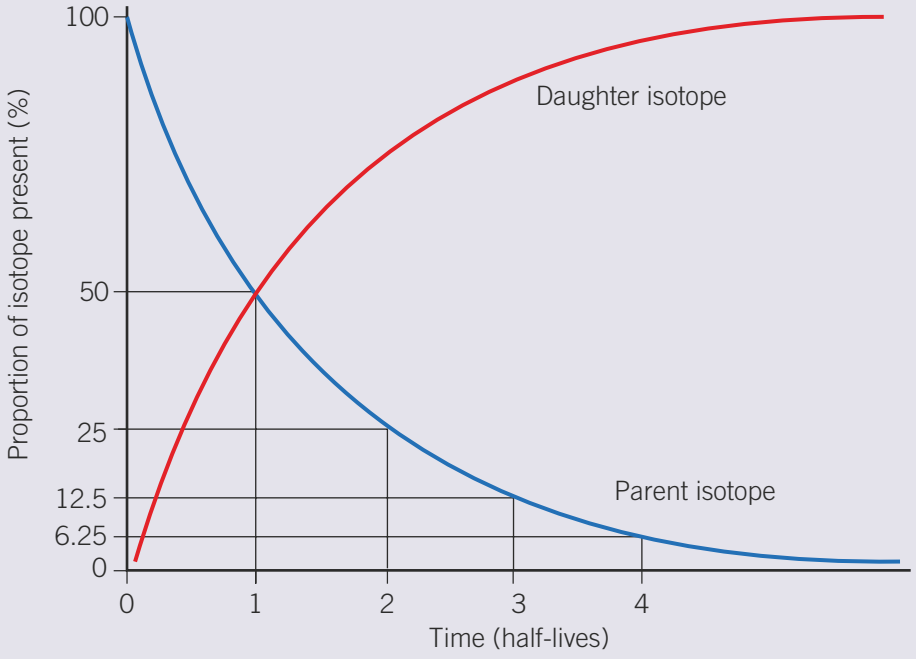


Figure 10B–15 A typical half-life graph of radioactive isotope decay

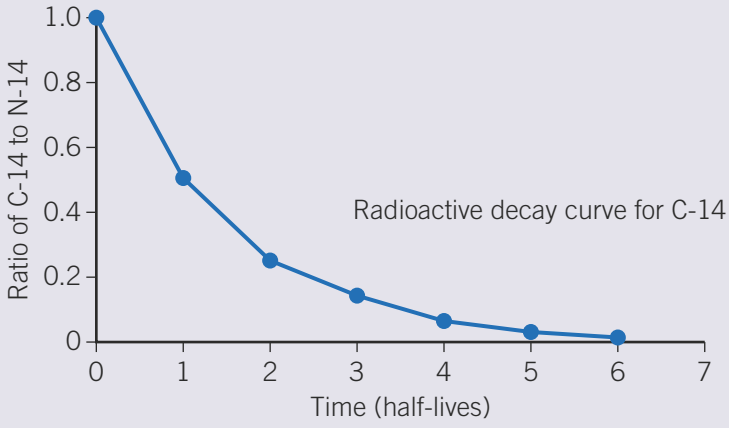
As you can see from the graph, after one half-life has passed, 50% of the parent isotope has been replaced by the daughter isotope and there is now a 50:50 ratio of each isotope present within the sample.

It is important to note that, after a second half-life, the parent isotope *does not* disappear completely to be replaced entirely by the daughter isotope. Rather, the 50% that remained after the first half-life is reduced by half again, leaving 25% of the parent isotope (and 75% of the daughter isotope) in the sample.

After a third half-life, the remaining 25% of parent isotope is halved again, leaving 12.5% of the parent isotope, with the rest made up of the daughter isotope.

This process continues as the years progress, with the passing of each half-life resulting in the loss of another half of the remaining parent isotope. By comparing the ratios of parent to daughter isotopes in the sample, scientists are able to apply this information to the isotope's known half-life, which allows them to determine absolute age.

Let's take a closer look, with an example based on a commonly used isotope: carbon-14 (C-14). The graph below shows the rate of decay of C-14 into nitrogen-14 (N-14).



The half-life of C-14 is 5730 years.

Question

In 2017, a palaeontologist unearthed a strange rock that turned out to be the molar of a sabre-toothed tiger. Scientists carried out carbon-14 dating analysis and determined that the tooth had approximately 6% of its original carbon-14 left.

a How many half-lives have passed?

Half-lives passed	0	1	2	3	4	5	6
% of original C-14 that remains	100	50	25	12.5	6.25	3.13	1.57

Answer

If 6% of carbon-14 remains in the tooth, this means that 4 half-lives have passed.

Question

b How much nitrogen-14 would be contained in the tooth?

Answer

100% – 6.25% (which is the amount of parental C-14 that is left) = 93.75%
There would be 93.75% of N-14 in the sabre-toothed tiger's molar.

Question

c How old is the sabre-toothed tiger's molar?

Answer

1 half-life of C-14 = 5730 years

4 half-lives have passed, so 4 × 5730 = 22 920

Answer

The sabre-toothed tiger's molar is 22 920 years old.

Now consider this question:

Question

In 1998, a 7 kg *Tyrannosaurus rex* coprolite was found in a cave in Canada. Explain why scientists were unable to use carbon dating analysis to date this fossil.

Answer

Dinosaurs are known to have become extinct 65 million years ago, which means the coprolite is *at least* 65 million years old. As carbon-14 has a relatively short half-life, it only has a dating range up to about 50 000 years old. Beyond this point, too much of the C-14 has decayed for scientists to be able to determine an exact age without compromising the reliability and validity of the experiment.

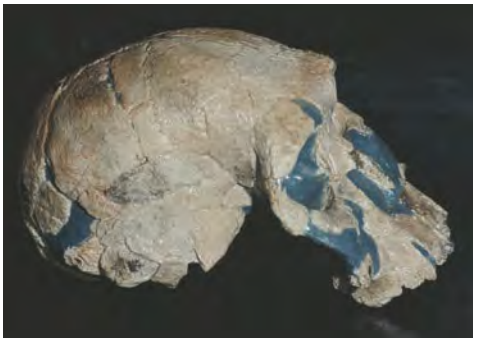
Finally, it is important to understand that the isotopes used in absolute dating have limitations. As you have seen in the question above, age ranges associated with the various isotopes can be one barrier. The table below lists some other considerations for scientists to remember when applying absolute dating techniques.

Isotope system	Key considerations
Carbon–nitrogen	The sample must be 'relatively' young and be organic in origin for it to contain carbon (e.g. bone, wood, teeth, shell).
Potassium–argon	Used to date rock layers only. Widely used because most rock samples contain potassium and it has a larger age range than carbon, making it useful in dating older fossils from earlier eras.
Uranium–lead	Also used to date rock layers only. The rock sample for dating must contain uranium-bearing minerals, which is not always the case, so it is not as widely used as the isotopes above.

Section 10B questions

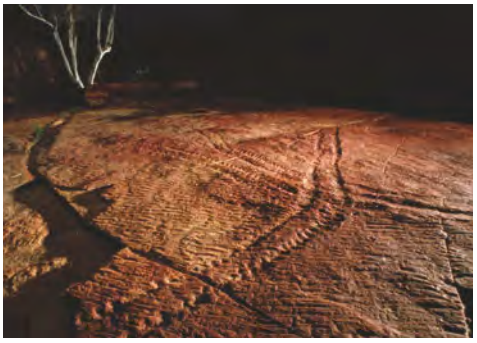
1 Use the images below to identify the type of fossil shown in each case. For those that are body fossils, identify the process that resulted in their preservation.

a



b

c



d

2 How do petrification, cast/mould and carbon fossilisation processes differ from mummification in their preservation of organisms?

3 In 2011, this almost complete 110 million year old nodosaur fossil (at right) was found. It is the best preserved of any dinosaur on record.
   - **a** What made this fossil so different from most fossils that are found?
   - **b** Explain the conditions that would have needed to be present for the successful preservation of this animal.

4 The image below shows the fossilised remains of a whale. Explain the process that would have resulted in the fossilisation of this specimen.

5 Will the fossil record ever be complete? Discuss.

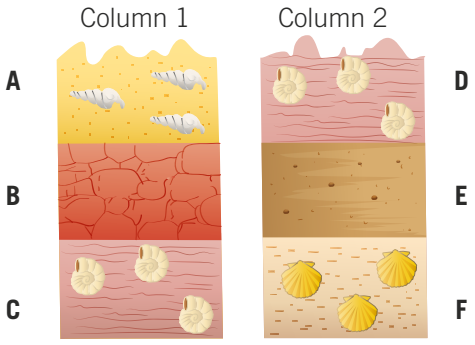
6 Construct a Venn diagram that shows the relationship between relative and absolute dating.

7 The table shown here provides information about several fossil species.
   - **a** Based on this information, explain which fossil would be most useful for determining the most precise age of the rock strata in which it is found.
   - **b** Which of these species would be the least useful in determining the age of the rock strata that it was found in? Explain.

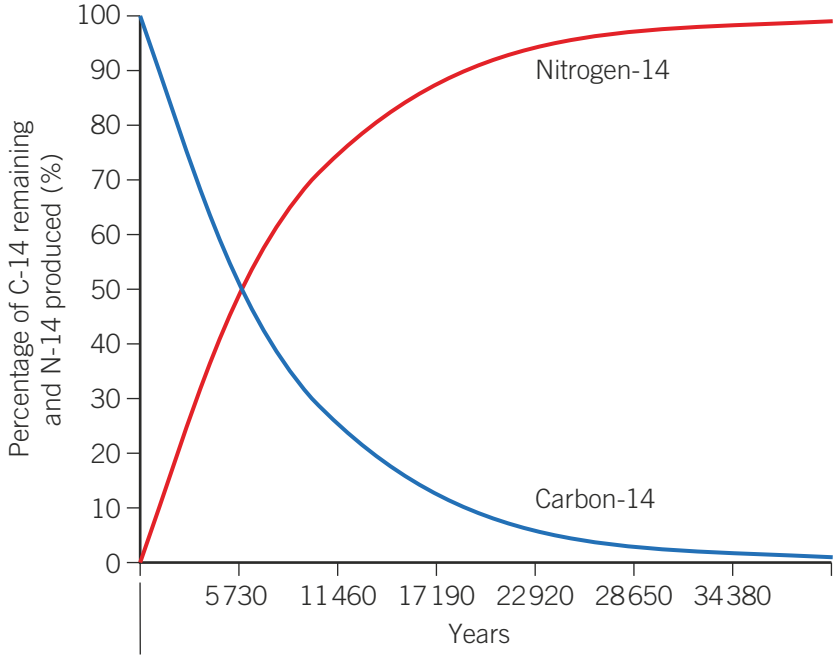
Fossil	Name	Appearance in the fossil record
	Brachiopod	540 million years ago – present
	Ammonite	250–150 million years ago
	Trilobite	550–250 million years ago
	Crinoid	485 million years ago – present

8 Use the diagram on the right to answer the following questions.

- **a** Which two layers are approximately the same age? Explain.
- **b** Which layer is the oldest? Explain.
- **c** Which layer is the youngest? Explain.



9 The following graph shows the decay of C-14 into N-14.



- **a** Complete the following table.

Decay of carbon-14									
Percentage of C-14 remaining (%)	100	50	25	12.5	6.25	3.125	1.56	0.78	0.39
Years ago	0	5730							

- **b** How old would a coprolite be if it contained about 24% of its original C-14?
- **c** Scientists in Antarctica removed an ice core from a glacier. At the bottom of this core they found some plant matter that contained about 5% of its C-14. How old was the bottom of the glacier?
- **d** According to the graph, do scientists need to wait until a half-life has been completed in order to use absolute dating? Explain.

Evidence of evolutionary relationships

Study Design:

- Evidence of relatedness between species: structural morphology – homologous and vestigial structures; and molecular homology – DNA and amino acid sequences
- The use and interpretation of phylogenetic trees as evidence for the relatedness between species

Glossary:

Divergent evolution
Homologous structure
Molecular homology
Phylogenetic tree
Phylogeny
Primate
Structural morphology
Vestigial structure

ENGAGE

99.9% identical

Did you know that your DNA is 99.9% identical to that of the person sitting next to you? Human DNA is 99% identical to that of our closest living relative, the chimpanzee.

The completion of the Human Genome Project in 2003 has not only provided scientists with the precise sequence of all 3.2 billion base pairs within our genome, but also provided the opportunity for us to compare our DNA with just about any other organism. The results of some of these comparisons are shown in Figure 10C–1.

As you will learn in this section, comparisons of DNA are used to determine evolutionary relationships between species. This is not the only technique, however, with scientists using a range of structural and molecular methods in their analysis of relatedness between all species, both past and present.

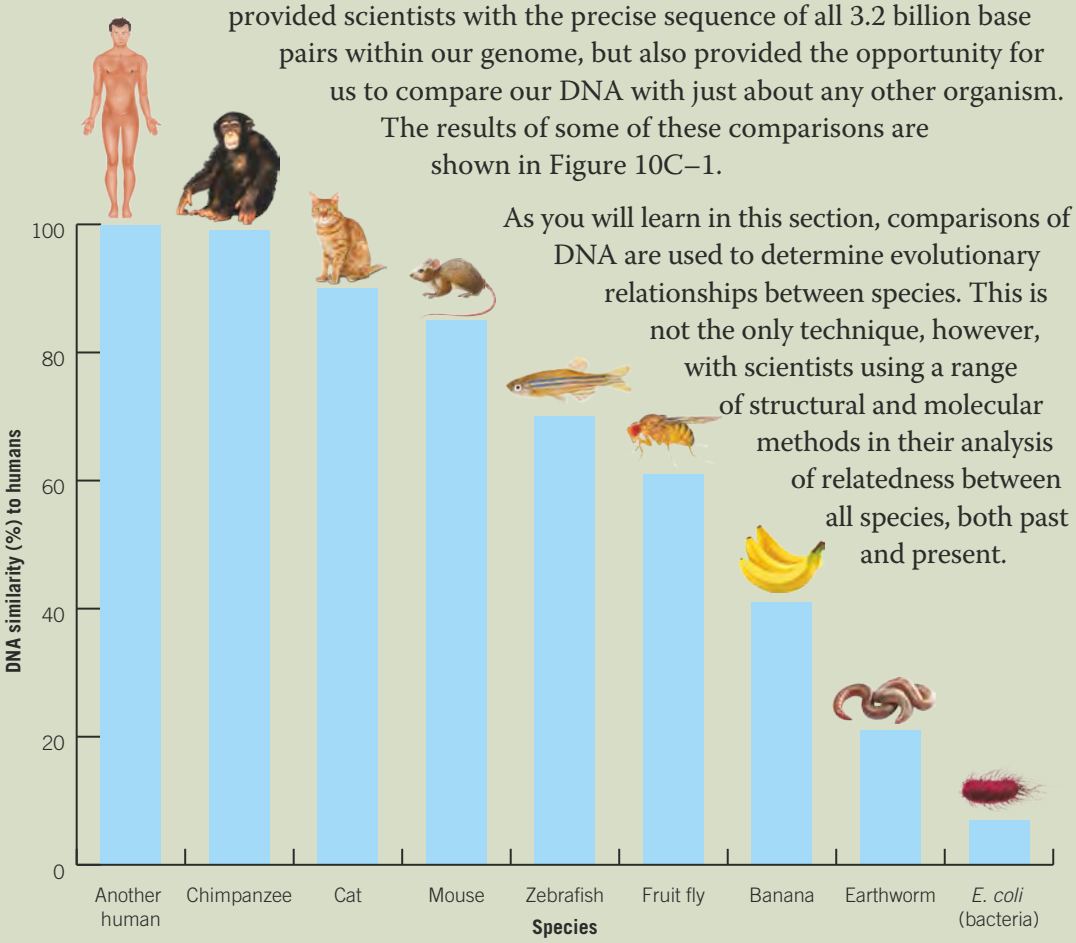


Figure 10C–1 The percentage of the DNA of various organisms that is shared with humans

EXPLAIN

Structural morphology

Structural morphology plays an important role in determining the relationships between species. While the analysis of structural similarities and differences is useful in comparing modern species, more importantly, it has provided a means for scientists to make connections between today's biodiversity and extinct ancestral species.

10B FOSSILS LINK

10A CHANGES IN BIODIVERSITY OVER TIME LINK

Structural evidence in the fossil record has assisted scientists to assemble an evolutionary timeline of how life on Earth has progressed over billions of years. Remember *Archaeopteryx*, the transitional fossil discussed in Section 10B? Or from Section 10A, *Tiktaalik*, the ancestral fish that moved onto land? It was the structural features preserved in the fossilised remains of these animals that allowed scientists to make the link between dinosaurs and birds, and the progression of life from water to land.

Structural morphology the study of an organism's features and form to determine the evolutionary relationship of species

Homologous structure a structure within a group of species that performs a different function yet has the same underlying structure

Homologous structures

Take a look at the animals in Figure 10C–2. At first glance, they do not appear to have much in common. They look different and so do the environments they occupy.

While outwardly these animals do not appear to have any similarities, if you take a closer look at their internal environment, you will see similarities in their bone structure (Figure 10C–3). We call these similar structures **homologous structures** because, while they may perform different functions according to the needs of the organism, they are similar in structure. The pentadactyl limb is a good example. Homologous structures can be seen in a range of organisms that share a *recent common ancestor*. The more similar the homologous structure between species, the more closely related these species are, and the more recently they shared a common ancestor.

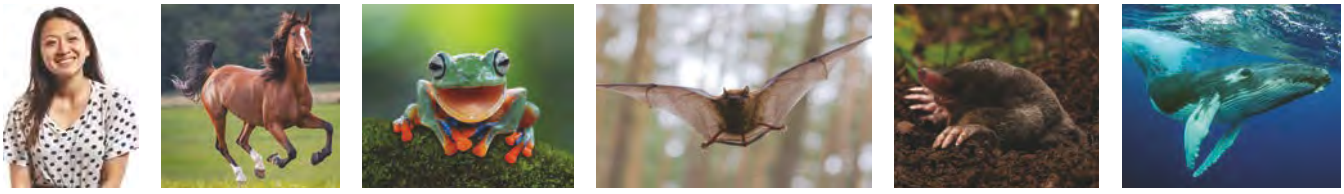


Figure 10C–2 What do all these animals have in common?

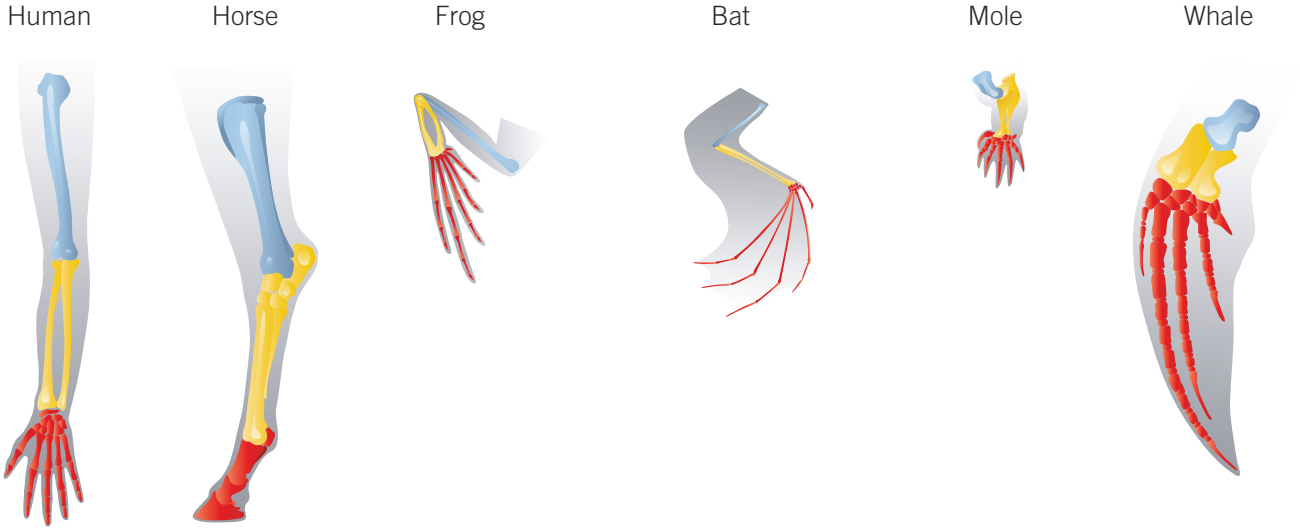


Figure 10C–3 The pentadactyl limb. The colour coding in each image highlights the similarities in structure between each animal.

As you can see, mammals, birds, amphibians (and even reptiles) all have the same basic underlying bone arrangement. The overall shape differs, however, according to the function that the limb performs for the animal – for example, flying (birds and bats) versus swimming (whales).

LINK 9D EMERGENCE OF A NEW SPECIES

Structural evidence such as that of the pentadactyl limb can be used to infer that all these organisms have emerged from one recent common ancestral species of tetrapod vertebrate. In other words, **divergent evolution** has occurred, which has seen the separation of an ancestral species in different environments. The different selection pressures within each environment have resulted in the original five-digit appendage of the ancestral species evolving into the various forms seen in its descendant groups today.

Divergent evolution where two or more species form from a single ancestral species over time

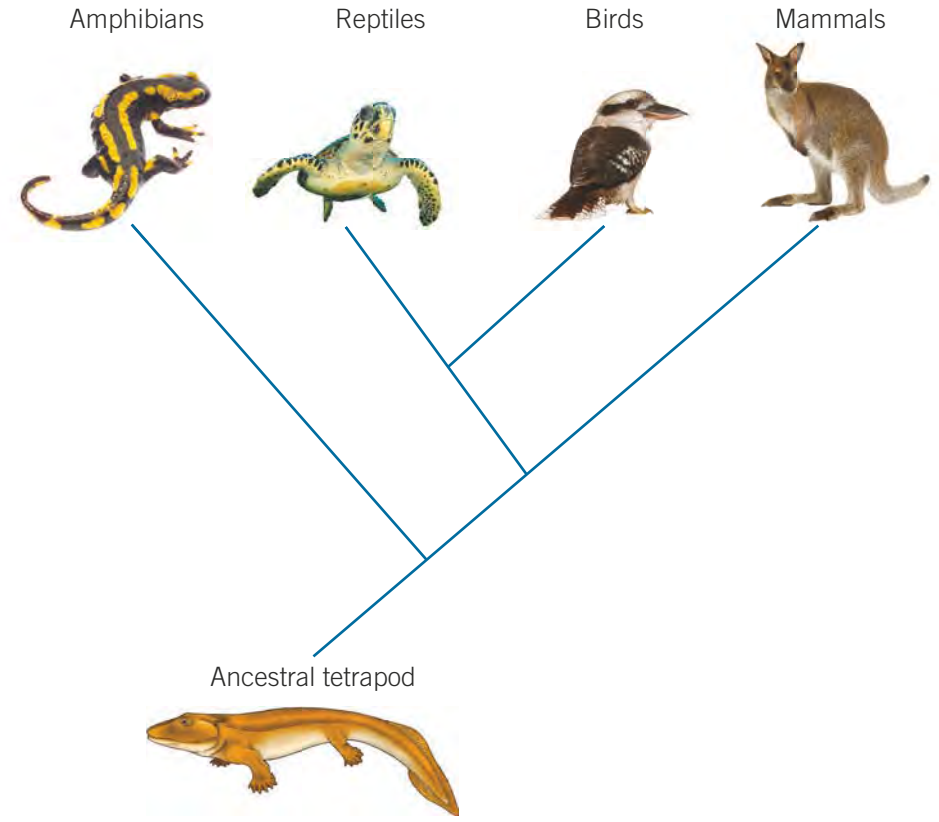


Figure 10C–4 Structural evidence such as that of the homologous pentadactyl limb supports the pattern of divergent evolution.

Other examples of homologous structures providing evidence of divergent evolution include the following.

- Insect wings: the two pairs of wings of ancestral insects are represented by homologous structures in modern insects — the wings themselves, hardened wing cases (seen in beetles), and balancing organs called halteres in flies.
- Arthropod limbs and segments
- Vertebrate eyes
- Plant stems, leaves and roots, forming storage and protective structures
- Bones of the inner ear in mammals
- carpels, stamens, petals, and sepals in flowering plants

Vestigial structures

The grotto salamander is a cave-dwelling amphibian that spends its adult life underground, in the deep network of caves that make up its habitat. This species of salamander is completely blind as a result of its eyelids fusing shut early in adulthood. Blindness is no disadvantage to the salamander, because the caves it lives in are pitch black, so eyesight would be of no use.

Vestigial structure a structure within an organism that is no longer functional but served a purpose in a common ancestor

Given the blindness of the salamander (Figure 10C–5), the presence of rudimentary eye bulbs seems unnecessary. Why go to the effort of developing these structures if they are useless? Any structure that exists within an organism that is no longer functional is referred to as a **vestigial structure**. While the eye bulbs of the grotto salamander serve no purpose for it now, they once had a purpose in a common ancestor.

Figure 10C–5 The grotto salamander is blind despite having two small beady eye bulbs.

Another example of a vestigial structure is found in whales. A close look at the bone structure of a whale reveals evidence of a small pelvis and hind limbs. Because whales are marine mammals, the pelvis and hind legs have no purpose, but their presence is evidence that whales have descended from terrestrial mammals that walked on legs.

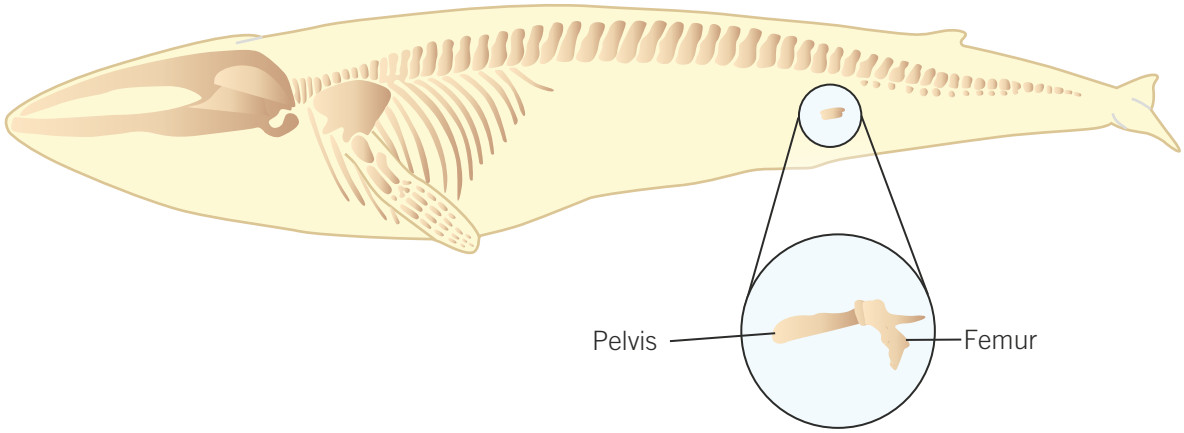
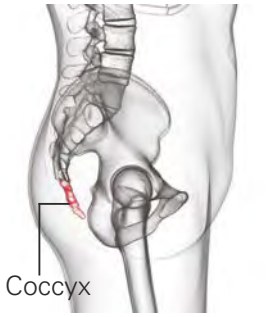
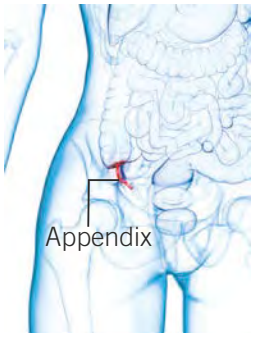
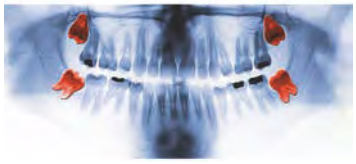
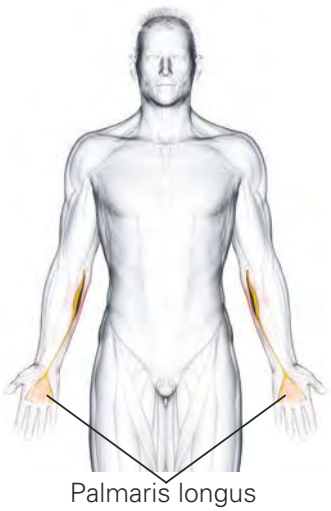


Figure 10C–6 The reduced pelvic and femur bones are vestigial structures that are evidence of the emergence of whales from an ancestor that walked on land.

There are many other examples of vestigial structures throughout the fossil record, but you don't have to look to fossils to find them all. Your own body contains examples of vestigial structures – you just need to know where to look!

Table 10C–1 Vestigial structures of the human body

Vestigial structure	Image	Ancestral purpose
Coccyx (tailbone)		Early human embryos have a tail, but the vertebrae fuse before birth, forming the coccyx. This suggests that our ancestors at one stage had a tail, which supports the theory that we share a common ancestor with other **primates**.
Appendix		Plant-eating vertebrates have a much larger appendix than our human version. For this reason, it is believed that the appendix is a vestigial organ from a plant-eating ancestor.
Wisdom teeth		Wisdom teeth are believed to be remnants of our large-jawed ancestors. A change in diet, along with better hygiene practices, means that they are no longer needed.
Plica semilunaris		The inner membrane fold of the corner of the eye is evidence of a third eyelid, which was used for protection or visibility under water. Some primates (e.g. gorillas) still have such a structure, which supports the theory that we share a common ancestor with them.
Palmaris longus muscle		This muscle is absent in 10% of the human population. It is believed to have played a role in grip or even hanging from trees, which supports the theory that we share a common ancestor with primates such as the great apes.

LINK 11A CLASSIFICATION AND CHARACTERISTICS OF MODERN HUMANS

Primate the order consisting of all current and extinct humans, apes and monkeys, characterised by having dextrous hands with opposable thumbs and a relatively large and developed brain

Check-in questions – Set 1

1 Identify the different types of structural morphology that scientists use to determine evolutionary relationships.
2 Explain how homologous structures provide evidence of species relatedness.
3 What evidence do vestigial structures provide in support of evolution?

Molecular homology

Advances in technology have given scientists the capacity to make more sophisticated assessments of relationships between both modern-day and extinct species. With the development of techniques that can sequence DNA and proteins, we can now compare species at a molecular level. This is particularly useful when the species of interest have no structural similarities (for example, a kangaroo and a eucalyptus tree). Structural morphology techniques would be of little use in determining the relationship between these two species, but because DNA is universal, DNA and the proteins of an organism can be analysed to determine their degree of relatedness

CHAPTER 2 LINK

The use of **molecular homology** in determining species relatedness can be described as a 'molecular clock'. This 'clock' is based on two fundamental ideas:

Molecular homology the analysis of DNA and amino acid sequences as evidence of evolutionary relationships

1 The greater the similarily in the DNA or amino acid sequences between the species, the less time that has passed, and therefore the more recently these species shared a common ancestor.
2 The greater the differences in these sequences between species, the more time that has elapsed for these changes to accumulate and, hence, the further back in time a common ancestor was shared.

Comparing DNA sequences

All organisms contain DNA. This means that all organisms, both extinct and present-day, are descended from a common ancestor. By comparing the DNA of different species, we can determine how recently (or long ago) the common ancestor was shared.

2A NUCLEIC ACIDS LINK

Recall that DNA is made up of the repetitive linkage of nucleotides, with each nucleotide consisting of a nitrogenous base. When conducting DNA analysis to determine relatedness, the sequence of bases between the two (or more) species is compared, to identify similarities and differences. The greater the degree of similarity, the more recently the species shared a common ancestor.

Figure 10C–7 compares a DNA sequence for part of a gene found in humans, rats and mice.

Human	CCAATGGGGCGGGGGCGCTGGGGCTCACCATATAAGGAGCGGCCTCGCCATAAAAGGAAACATTGTATCTCTTTATA
Rat	CCAATGGGGCGGGGGCGCTGGGGCTCGCCATATAAGGAGCGGCCTCGCCATAAAAGGAAACATTGTATCTCTTTATA
Mouse	CCAATGGGGCGGGGGCGCTGGGGCTCGCCATATAAGGAGCGGCCTCGCCATAAAAGGAAACATTGTATCTCTTTATA

Figure 10C–7 A comparison of human, rat and mouse DNA for a common gene

From this information, it can be seen that there is no difference in bases between the rat and the mouse for this part of the gene. The human gene segment contains one base that is different (highlighted in purple) from the rat and the mouse. From this we can infer that the:

- rat and the mouse have a very recent common ancestor (being identical in sequence for this part of the gene) and are therefore very closely related
- human had a common ancestor with both the rat and mouse longer ago (given the single base difference) and is therefore more distantly related to both these species.

Mitochondrial DNA

Most of the DNA within a eukaryotic cell is packaged within the nucleus. There is, however, a small amount of DNA inside the mitochondria, referred to as mitochondrial DNA (mtDNA).

As mitochondria were once free-living prokaryotes, it makes sense for them to carry their own genome. mtDNA is a small circular chromosome (similar to a plasmid) that is passed almost exclusively from mother to offspring. Upon fertilisation, the small amount of mtDNA within the sperm cell is destroyed, leaving only the mtDNA of the female ovum. As a consequence, except in rare cases, the ability to pass on mtDNA from the father to future generations is lost.

VIDEO 10C–1 USING MTDNA AS EVIDENCE OF EVOLUTION

LINK 3B APPLICATION AND IMPLICATIONS OF DNA MANIPULATION TECHNIQUES

LINK 10A CHANGES IN BIODIVERSITY OVER TIME

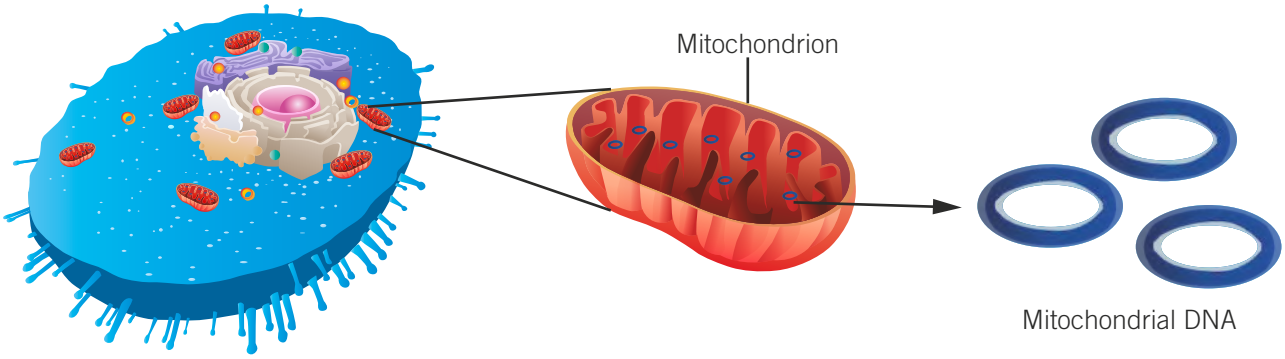


Figure 10C–8 Each mitochondrion contains several mtDNA molecules and each cell has several hundred mitochondria, resulting in the high number of copies that is associated with mtDNA.

As you can see in Figure 10C–9, nuclear DNA is inherited as a combination of both maternal and paternal DNA. The recombinant nature of this DNA results in changes to DNA sequences at every generation. For example, recombination of your parents' DNA resulted in your DNA, a third version that is distinct from both your parents. This pattern continues throughout generations, making backtracking through a species' ancestry a complicated process.

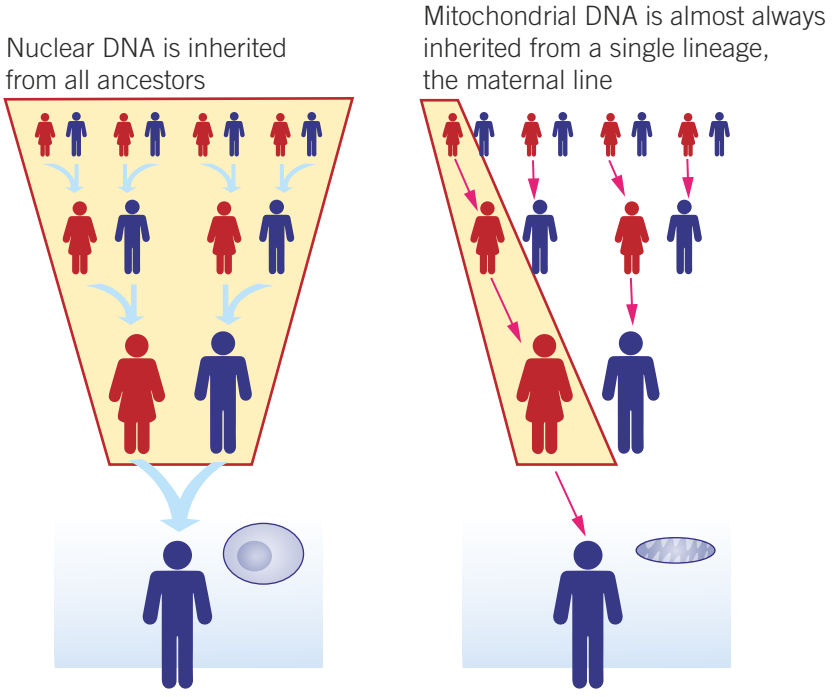


Figure 10C–9 The inheritance of mtDNA is matrilineal, inherited only from the female or 'maternal' side, except in rare cases.

In contrast, mtDNA is derived only from the maternal line, so no recombination occurs. While mtDNA does have a faster mutation rate than nuclear DNA, these mutations occur in a timely and systematic manner, making them easy to account for. The fast mutation rate also allows for comparisons to be made, not just between species, but also between different populations of the same species. These characteristics of mtDNA make it a valuable tool for scientists tracing the ancestry of a species.

LINK UNIT 2

For example, a study in 1980 compared the number of mutations in the mtDNA of two humans and two chimpanzees. It was found that there were half as many mtDNA differences between the two humans as there were between the two chimpanzees. From this, it can be concluded that the two humans (with fewer mtDNA mutations) shared a more recent common ancestor than the two chimpanzees (who had more mtDNA mutations).

11B MIGRATION OF MODERN HUMANS LINK

11C CLASSIFYING NEW DISCOVERIES OF HOMININS AND EVIDENCE OF INTERBREEDING BETWEEN SPECIES LINK

Studies have also found that, of all racial groups, African people have the greatest diversity in their mtDNA, as measured by the number of mutations. From this we can infer that African mtDNA is the oldest, and therefore the origin of humans lies in Africa. In fact, using mtDNA, geneticists have been able to trace every human on Earth back to one single female ancestor who lived approximately 200 000 years ago, known as Mitochondrial Eve. You will learn more about the role of mtDNA in tracing the evolution of humans in Sections 11B and 11C.

Comparing amino acid sequences

Like DNA, proteins can also be analysed to determine species relatedness. This is done by analysing amino acid sequences in proteins that are common between the species of interest.

A common example is the analysis of amino acids that make up a portion of the haemoglobin molecule (Table 10C–2).

From the information in Table 10C–2, you can see that humans and chimpanzees have identical amino acid sequences for the portion of haemoglobin analysed. This means that they are the most closely related of the five species and therefore share the most recent common ancestor.

Table 10C–2 Comparison of the amino acid sequence in the same part of the haemoglobin protein molecule, between species

Species	Sequence of amino acids
Human	Lys-Glu-His-Iso
Horse	Arg-Lys-His-Lys
Gorilla	Lys-Glu-His-Lys
Chimpanzee	Lys-Glu-His-Iso
Zebra	Arg-Lys-His-Arg

Conversely, humans have the least similarity with the horse and the zebra, as in both instances, three of the four amino acids shown are different. This means that humans are least closely related to horses and zebras, and therefore shared a common ancestor longer ago.

It is possible to draw many other conclusions about the relationships between the species in the table. For example, what does a comparison of the amino acid sequences of the horse and zebra tell us? What about the gorilla and the chimpanzee? Even with just a small amount of information, it is possible to learn a lot about the evolutionary relationships between species.

Other types of proteins can also be analysed for amino acid sequences. While haemoglobin is useful when comparing the relatedness of vertebrates, it is not useful for analysing the relationships between a wider variety of species, as not all organisms contain haemoglobin. For situations like this, the cytochrome c protein is useful, as it plays an important role in the electron transport chain of aerobic cellular respiration, which is used by almost every organism in the production of ATP.

5C CELLULAR RESPIRATION LINK

Table 10C–3 Number of amino acid differences in cytochrome c between humans and other species

Organism	Number of amino acid differences to humans
Chimpanzee	0
Rhesus monkey	1
Rabbit	9
Cow	10
Pigeon	12
Rattlesnake	14
Bull frog	20
Fruit fly	24
Garden snail	29
Wheat germ	37
Yeast	44
Mould	48

As you can see, using cytochrome c allows a wider analysis that extends beyond the animal kingdom, to species such as wheat and mould. We can determine from the information in the table that, once again, humans and chimpanzees share the most recent common ancestor, as indicated by the identical nature of their cytochrome c proteins. Humans and mould, on the other hand, are the most distantly related, with the highest number of differences (48) between their respective amino acid sequences.

It is also possible to interpret the amino acid sequence of a protein from the organism's DNA. Recall from Section 2B that the instructions for proteins are stored within the genome of the organisms. Accurate interpretation of the nucleotide sequence within the genome provides information on the amino acid sequence that will be produced through transcription and translation.

WORKSHEET 10C–1 EVIDENCE OF EVOLUTION

LINK 2B THE GENETIC CODE AND GENE EXPRESSION

Check-in questions – Set 2

1 How does the comparison of DNA sequences between species determine relatedness?
2 Explain how the inheritance of mtDNA differs from the inheritance of nuclear DNA.
3 What advantages does mtDNA offer when determining the relatedness of species?
4 Why would using amino acid analysis of cytochrome c be more advantageous than haemoglobin analysis?

Phylogeny a branch of science that studies the evolutionary relationships between a group of species

Phylogenetic tree a branching diagram used to represent the evolutionary relationships between species

Phylogenetic trees

The study of the evolutionary history of a group of organisms is referred to as **phylogeny**. Scientists use branching diagrams known as **phylogenetic trees** to represent the relationships between species over time. Phylogenetic trees can be constructed from both structural and molecular evidence. The more evidence that is considered when constructing the tree, the more accurate and meaningful it will be in showing species relatedness.

LINK 9B EVOLVING AND NON-EVOLVING POPULATIONS

LINK 9D EMERGENCE OF NEW SPECIES

Shown here is an example of a simple phylogenetic tree that highlights the relationship between five species (represented by A to E).

How to read a typical phylogenetic tree

When reading a phylogenetic tree, it is important to note the following:

- The species of interest are at the ends of the tree's branches.
- Each node or branching point represents the most recent common ancestor of the descendant species that have developed as a result of divergent evolution.
- Each branch represents the process of speciation that has resulted in the formation of the final species from the common ancestor. (Several ancestors of the species may have occurred along the branch.)
- The root of the phylogenetic tree represents a series of ancestors that lead up to the most recent common ancestor of all the species of interest represented in the tree.

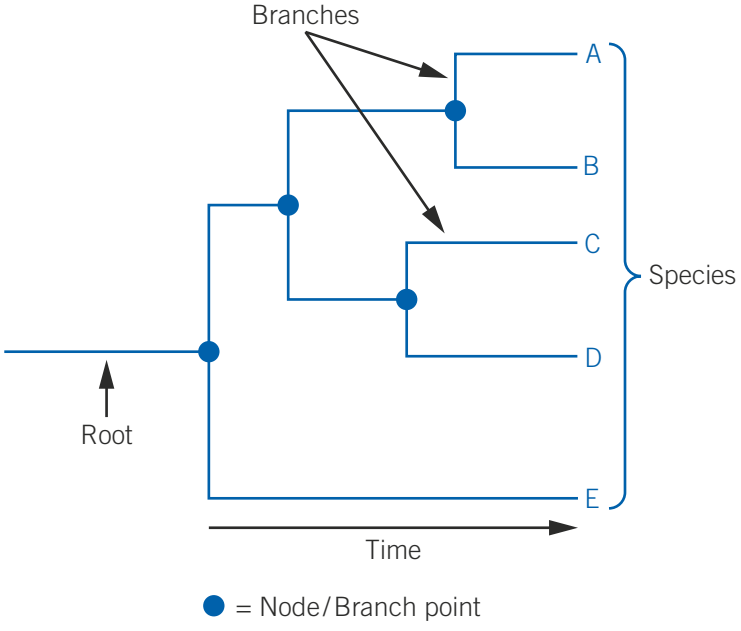


Figure 10C–10 A phylogenetic tree showing the relationship between five species A to E

As you can see in Figure 10C–11, phylogenetic trees focus on showing how species have evolved from a series of common ancestors. Determining how recently species shared a common ancestor can then be used to infer the relatedness between them. In general:

- The more recently species shared a common ancestor, the more closely related the species are.
  - For example, species A and B are the most closely related as they have the most recent common ancestor.
  - They would also have the greatest number of features (structural and molecular) in common, as the least amount of time has passed for mutations to accumulate.
- The less recent the common ancestor, the more distantly related the species are.
  - For example, A and C are less closely related than A and B as they had a common ancestor less recently.
  - A and C are more closely related than A and E, as their common ancestor occurs more recently (8 mya) than the common ancestor of A and E (10 mya).
  - A and C would therefore have more features in common than A and E.
  - The line for species E is shown to end at 2 mya. This shortened line indicates that species E is extinct and also gives the time at which this extinction occurred.

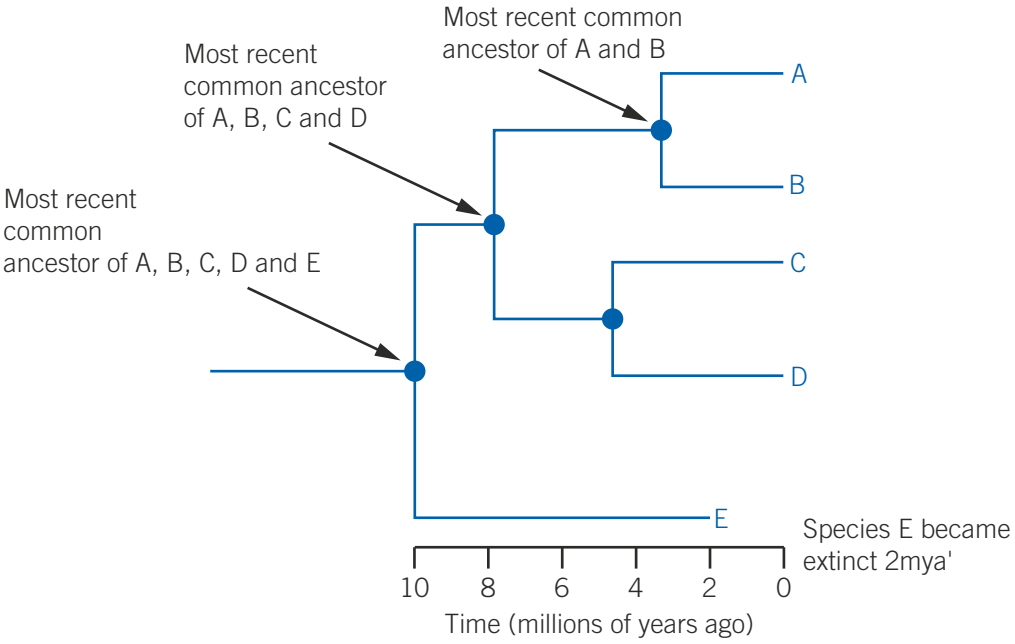


Figure 10C–11 Showing relatedness through a phylogenetic tree

Phylogenetic trees can be presented in different ways (see Figure 10C–12). They may be rotated onto their side, or drawn using diagonal lines. Just remember to focus on the branching patterns that occur in the body of the tree, as this is where the important information lies. Regardless of the tree's form, the information presented in it does not change, with the focus always on representation of evolutionary relatedness between species.

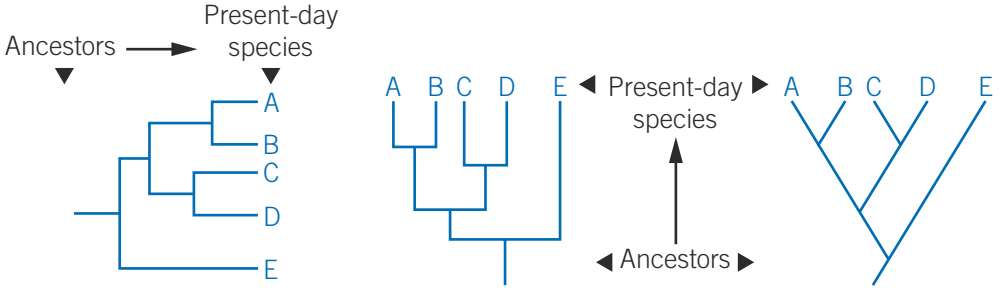


Figure 10C–12 Phylogenetic trees can be presented in a variety of forms.

WORKSHEET 10C–2 PHYLOGENETIC TREES

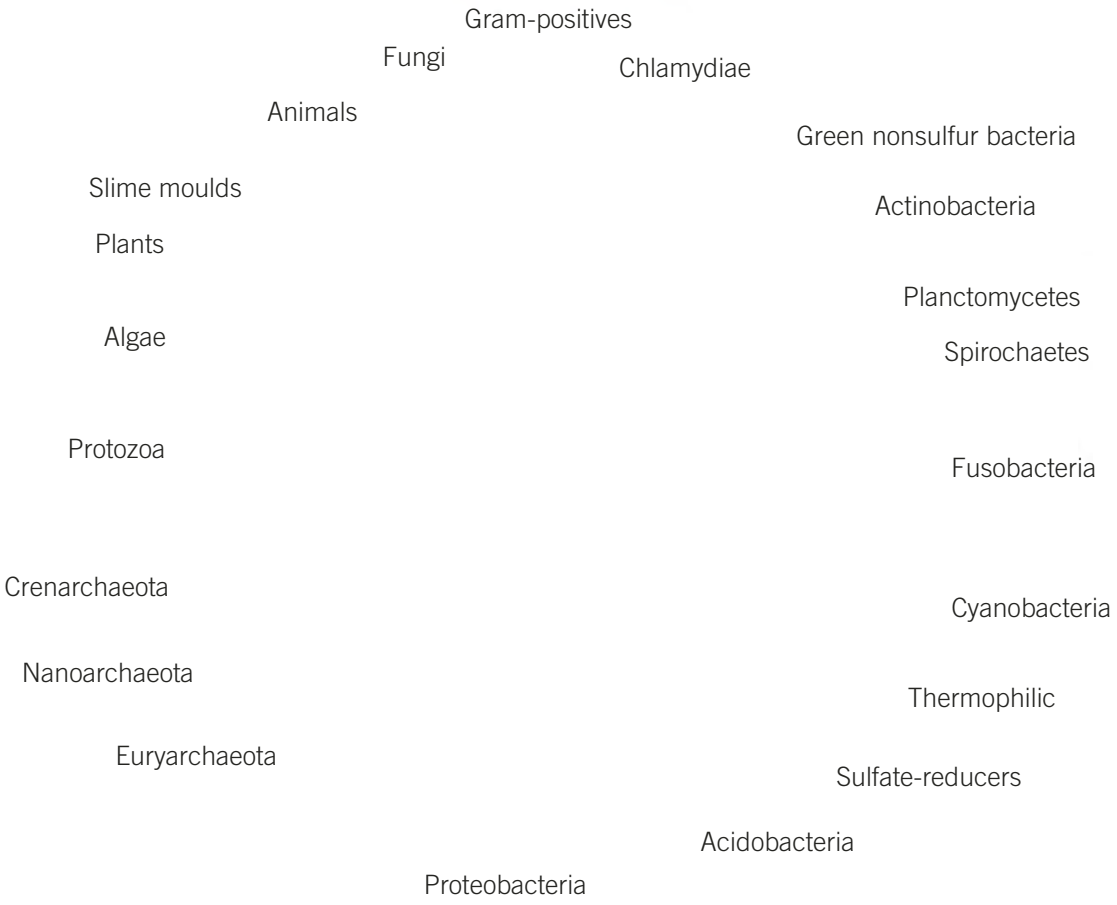
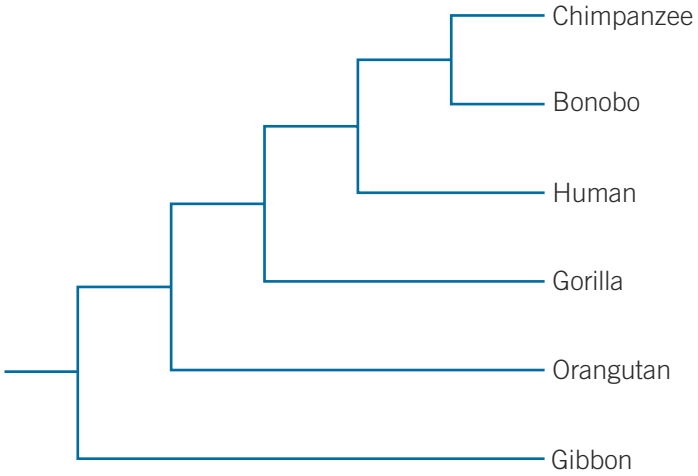


Figure 10C–13 A phylogenetic tree showing evolutionary relationships of a broad range of species, mostly prokaryotes, with eukaryotes shown by the brown branches at top left. As it would be very wide if set out horizontally, it has been curved round into a circle, with the root at the centre, but otherwise it conveys the same information as other phylogenetic trees.

The analysis and interpretation of phylogenetic trees is explored further in the 10C Skills section.

Check-in questions – Set 3

1 What does the term 'common ancestor' mean?
2 What does a branch point represent?
3 Use the phylogenetic tree below to answer the questions that follow.



a Which two species are the most closely related? Explain.
b Which two species would have the least features in common? Explain.

4 Use the information in Figure 10C–7 to create a simple phylogenetic tree that shows the relationship between humans, mice and rats.

VIDEO 10C–2 SKILLS: INTERPRETING PHYLOGENETIC TREES

10C SKILLS

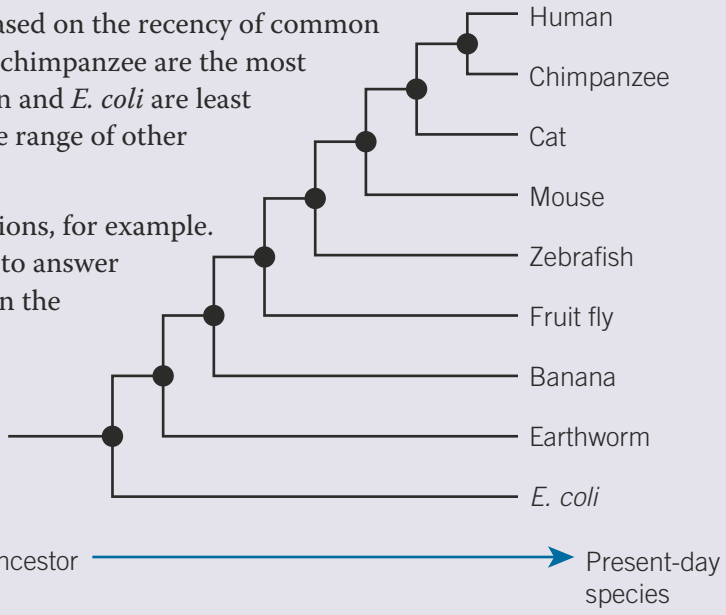
Interpreting phylogenetic trees

The ability to use and interpret phylogenetic trees for species relatedness is a specific dot point in the Study Design. It is therefore important that you feel confident in your ability to draw accurate conclusions from them, as they will certainly feature in your end-of-year examination.

At the beginning of this section, you were presented with a graph that showed the similarity of DNA within different species. The information in that graph has been converted into the phylogenetic tree below.

While it is easy to see that, based on the recency of common ancestry, the human and the chimpanzee are the most closely related and the human and *E. coli* are least closely related, there is a wide range of other information in this diagram.

Consider the following questions, for example. All the information required to answer them accurately is provided in the diagram.



Question

1 Identify the species that would have the most features in common with a banana.

Answer

The earthworm. To answer this correctly, look for which other organism the banana shared its most recent common ancestor with. The diagram shows this to be the earthworm.

Question

2 Consider the zebrafish and the mouse. Which of these would you expect to have more in common with the fruitfly?

Answer

The zebrafish. Once again, it is a matter of using the diagram to determine who has the most recent common ancestor with the fruitfly.

Question

3 The teeth and skeleton of a mouse, a cat and a chimpanzee were examined. More similarities were observed between the mouse and the chimpanzee than between the cat and the mouse. Is this expected, based on the information in the phylogenetic tree?

Answer

No. According to the phylogenetic tree, the mouse and the chimpanzee had a common ancestor longer ago than the mouse and the cat, who shared a common ancestor more recently. This means there has been greater opportunity for mutations to accumulate between the mouse and the chimpanzee, which would result in their skeletons looking less similar than the skeletons of the mouse and the cat, where there has been less time for mutations to occur.

Molecular homology: are DNA or amino acid comparisons better?

DNA and amino acid sequence comparisons are both valuable tools for scientists investigating species relatedness. But is one more useful than the other?

In general, comparing DNA sequences is more useful than comparing amino acid sequences. This is a good opportunity for you to reflect on Section 9A, where you investigated mutations. Recall specifically the work you did on silent substitution point mutations. These types of mutations have no impact on the formation of amino acid sequences that make up a protein (see Figure 10C–14). Therefore, if you were analysing amino acid sequences alone, this mutation would not be detected, and any conclusions that were drawn on species relatedness from this analysis would be misleading.

LINK 9A MUTATIONS

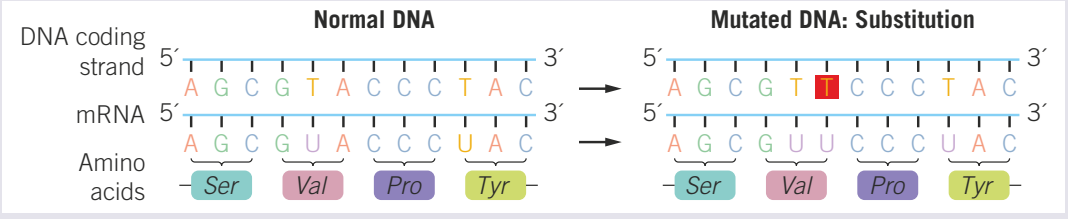


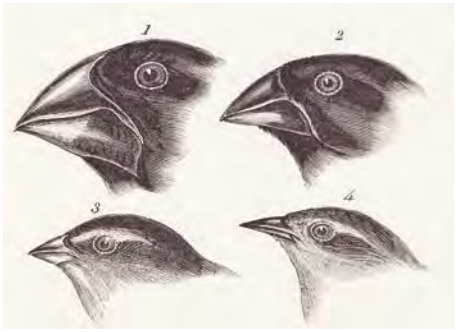
Figure 10C–14 Note the substitution of a thymine nucleotide in place of an adenine nucleotide. This mutation still results in the addition of the amino acid valine (Val) to the growing polypeptide chain, so if amino acid sequences alone were analysed, this mutation would not be identified.

Analysing DNA sequences, on the other hand, would pick up all mutations, including the silent substitution point mutations. It is for this reason that molecular homology using DNA sequences is deemed by scientists to be the most accurate and preferred option.

Section 10C questions

1 Use the information in Figure 10C–3 to copy and complete the table.

Pentadactyl limb	Modification	Function
Human		
Horse		
Bat		
Whale		
Mole		
Cat		

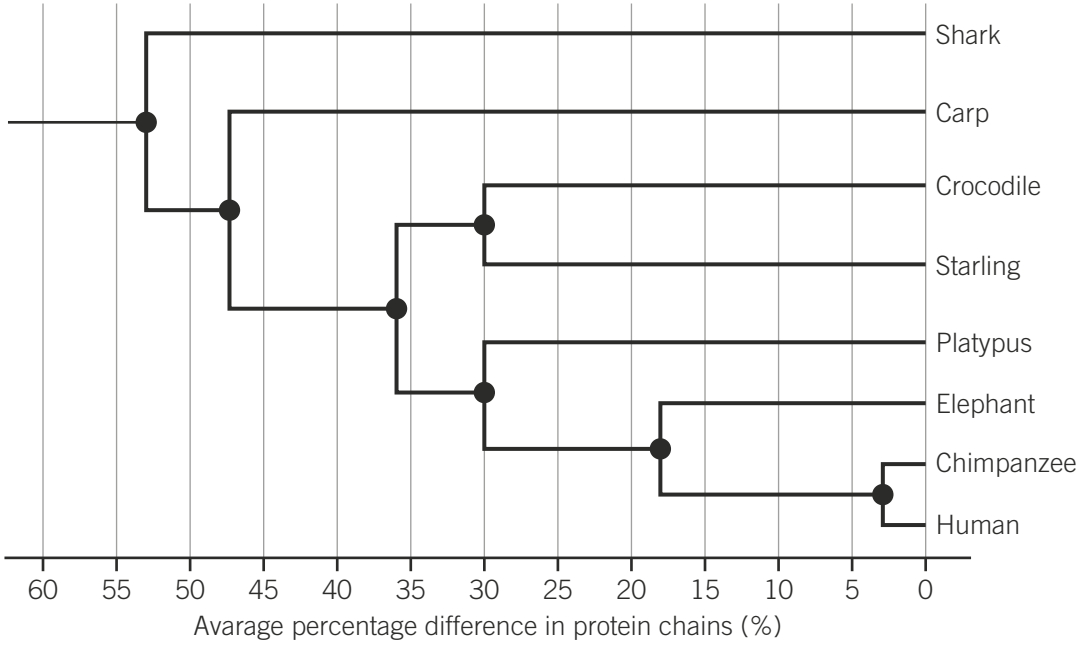


2 Darwin's work on natural selection focused on the beaks of finches living in the various habitats of the Galapagos Islands.
   a Are the beaks of Darwin's finches homologous structures? Explain.
   b Explain the pattern of evolution that these types of structures support.

3 Many flightless birds, such as the emu and the ostrich, have wings despite being unable to fly. Explain what these types of structures tell us about the ancestors of the emu and the ostrich.

4 Molecular homology techniques involve the analysis of DNA and amino acid sequences to determine how closely related species are. Explain why it is possible to determine the amino acid sequence of a protein from the DNA sequence, but it is not possible to determine the DNA sequence from the amino acid sequence.

Use the phylogenetic tree below to answer Questions 5–7.



5 What pattern of evolution is supported by the phylogenetic tree? Explain.

6 Is the human more closely related to the platypus or the starling? Use data to support your answer.

7 Explain how the variation in the protein chains of the species shown in this phylogenetic tree may have arisen.

8 What will happen to the mtDNA of a mother who gives birth to sons only?

9 Use the information below to construct a phylogenetic tree.
- Species K is a direct ancestor of species J and species L, and these two species diverged about 8 million years ago.
- About 5 million years ago, species J diverged to give species M and N.
- Species M became extinct about 3 million years ago.
- About 2 million years ago, species L diverged to produce species P and T.
- Species T and N are still alive today.
- Species P became extinct about 500 000 years ago.

Chapter 10 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

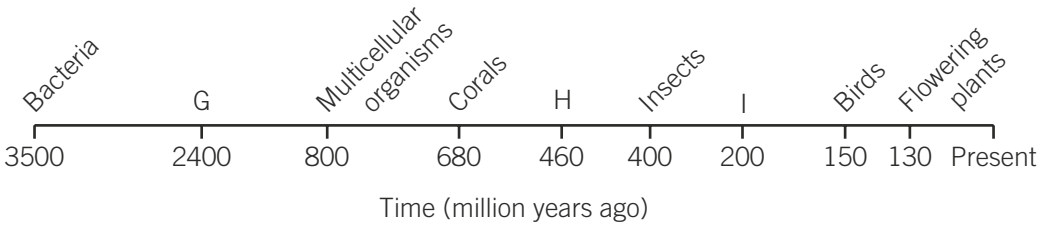
Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
10A.1	Explain the geological time scale as a dating system that shows the history of life on Earth	1☐
10A.2	Identify that the geological time scale is divided into eras and periods according to major changes in biodiversity/complexity as inferred from fossil evidence	2☐
10A.3	Describe the consequences that changing conditions on Earth (e.g. temperature, natural disasters, atmospheric composition) had on biodiversity at the time	11a☐
10A.4	Describe the role of extinction in evolution.	11a☐, b☐
10B.1	Define fossil, and identify the different forms in which fossils can be preserved as (petrified, cast, mould, carbon impression, trace)	14☐
10B.2	Explain the process that results in fossilisation.	3☐
10B.3	Describe the environmental conditions that must be present for fossilisation to occur.	3☐
10B.4	Use an example to explain the significance of transitional fossils in demonstrating the evolution of related species.	16a☐
10B.5	Distinguish between relative and absolute dating with reference to index fossils and radiometric dating.	4☐, 5☐, 15☐
10B.6	Analyse half-life graphs of various isotopes to determine the absolute age of a specimen.	ITB☐
10C.1	Distinguish between the different types of structural morphology and explain how they can be used to determine relatedness between species	17☐
10C.2	With the use of examples, describe the significance of vestigial structures as evidence of evolution	16c☐
10C.3	Explain how molecular homology (DNA and amino acid sequences) can be used as an indication of relatedness between species	8☐, 18☐
10C.4	Analyse phylogenetic trees for evidence of relatedness between species	10☐

Multiple-choice questions

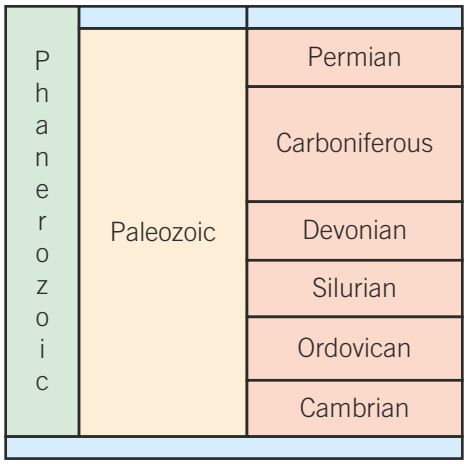
1 The timeline below shows the evolution of life on Earth, based on fossil record evidence. Three key groups of organisms are identified by the letters G, H and I.



Which option correctly identifies the groups of organisms represented by G, H and I?

	G	H	I
A	Prokaryotes	Mammals	Dinosaurs
B	Protists	Reptiles	Mammals
C	Cyanobacteria	Fish	Mammals
D	Eukaryotes	Dinosaurs	Non-flowering plants

2 The following diagram shows a section of the geological time scale.



What do the various divisions of time represent?

A when all the mass extinctions on Earth have occurred
B major changes in Earth's biodiversity according to the fossil record
C changes in the distribution and layout of Earth's continents
D the development of a new strata in sedimentary rock

3 The chance of an ammonite becoming fossilised is decreased by

A the rapid burial of remains in sediment.
B the presence of scavengers at the time of its death.
C the presence of a hard shell.
D low oxygen levels to protect against decay.

4 Which characteristics of a fossil are required for it to be useful as an index fossil that can be used to date the relative age of geographically isolated rock strata?

A The species was present for a short period of time over a narrow geographical range.
B The species was present for a short period of time over a wide geographical range.
C The species was present for a wide period of time across a narrow geographical range.
D The species was present for a wide period of time across a wide geographical range.

5 The desert bandicoot was declared extinct in 1943 by the International Union for Conservation of Nature.
The petrified skeleton of a desert bandicoot was recently found buried deep in sedimentary rock of the Northern Territory. These remains were dated at approximately 15 000 years old.
Which of the following methods was the most likely method used by scientists to date the remains?

A uranium–lead dating of the skeletal remains
B comparison of the bones with those of another bandicoot fossil of known age
C analysis of the ratio of carbon-14 to nitrogen-14 in the skeletal remains
D relative dating of the rock strata the fossil was found in

6 Two species show evidence of homologous structures.

A These structures would perform the same function in the organism.
B The structures are identical.
C The species have very different ancestors.
D The species have diverged from a common ancestor.

7 A vestigial structure is a

A reduced structure with no apparent function but had a function in a common ancestor.
B structure that has a different form in another organism due to environmental selection pressures.
C structure that has a similar form in another organism due to environmental selection pressures.
D structure that plays an important role in an organism crucial to its survival.

8 By analysing the DNA sequences of various primates, scientists have been able to make comparisons with human DNA to determine their degree of relatedness. The results of this investigation are summarised in the table.

Primate	% difference compared to human DNA
Chimpanzee	1.7
Gorilla	1.8
Orangutan	3.3
Gibbon	4.3
Rhesus monkey	7.0
Spider monkey	10.8

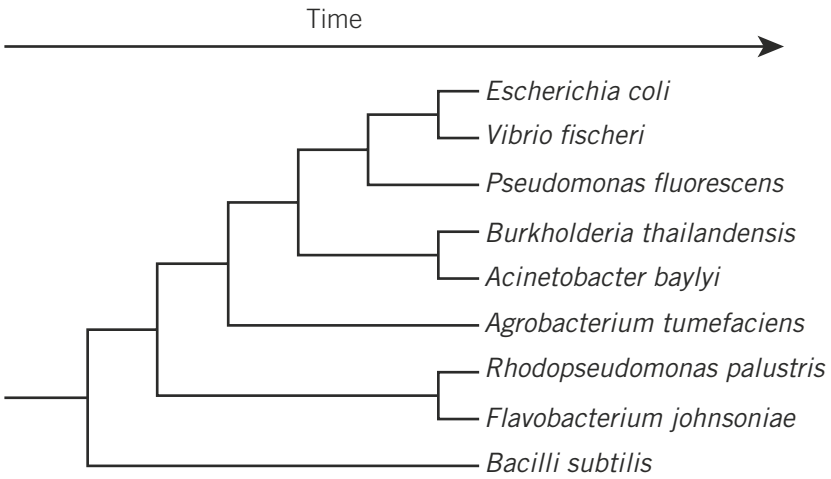
Based on the data in the table, which of the following primates diverged from a common ancestor with humans most recently?

A Rhesus monkey
B spider monkey
C orangutan
D gorilla

9 Mitochondrial DNA is inherited from

A both parents equally.
B the paternal grandfather.
C the maternal grandmother.
D mothers to daughters only.

10 Shown below is a phylogenetic tree of different bacterial species, constructed according to molecular and structural homology.



This information shows that

A *Escherichia coli* shares a more recent common ancestor with *Pseudomonas fluorescens* than with *Vibrio fischeri.*

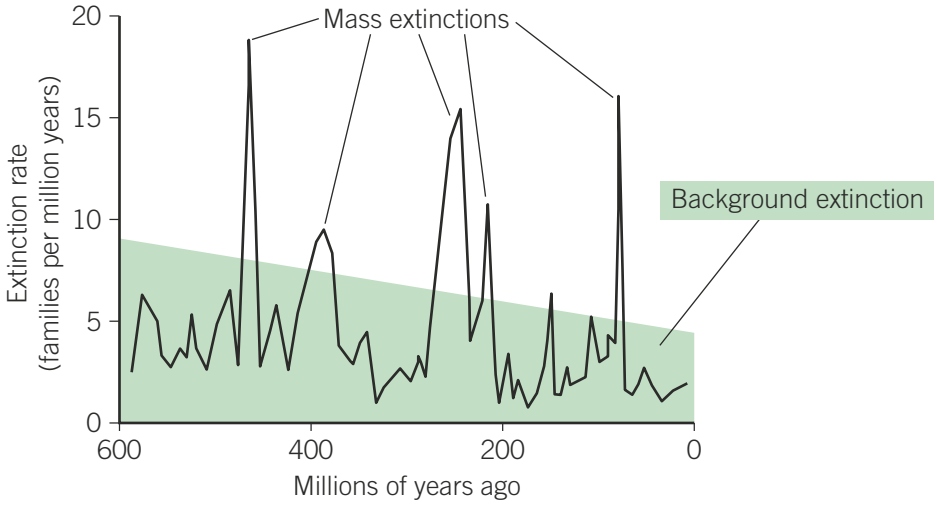
B *Flavobacterium johnsoniae* shows a higher rate of DNA conservation with *Rhodpseudomonas palustris* than with *Bacillis subtilis.*

C All nine species of bacteria diverged from each other at the same time.

D *Acinetobacter baylyi* is an older species of bacteria than *Agrobacterium tumefaciens.*

Short-answer questions

11 All five mass extinctions that have occurred throughout Earth's geological history have been the result of a catastrophic event(s) that resulted in significant environmental change.



a Explain the significance of mass extinctions in the diversification of new species as the planet's biodiversity is rebuilt. (1 mark)

b The background extinction rate, or 'normal' extinction rate as it is sometimes known, also plays an important role in the maintenance of Earth's species. Explain why this is the case. (1 mark)

12 The earliest evidence of a seed-producing plant comes from a plant fossil known as *Elkinsia polymorpha* or 'seed fern'. This fossil belongs to the Devonian period and has been dated at 400 million years old. The development of seeds allowed plant life to move away from the coastline and inhabit areas further inland. Explain why this is the case. (1 mark)

13 By the end of the Proterozoic eon, an abundance of soft-bodied multicellular organisms existed, yet there is little evidence of their time on Earth in the fossil record. Explain this observation. (1 mark)

14 Consider the image below, of extinct cave lion paw prints.

a Identify the type of fossil that these paw prints are best classified as. (1 mark)

In 2015, palaeontologists in Russia discovered two frozen cave lion cubs that had been encased in ice for at least 30 000 years. Both cubs were found in remarkable condition as a result of the permafrost conditions that dominated the ice age that they were born into.

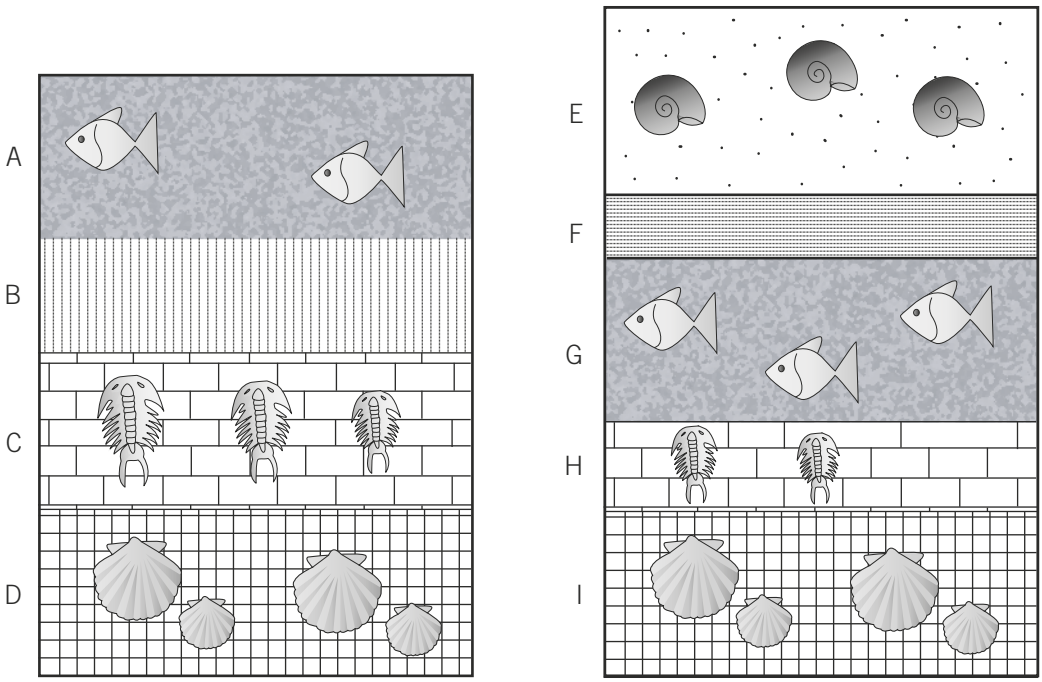
b What type of fossil would the cave lion cubs shown at right be classified as? (1 mark)

c Describe the fossilisation process that would have occurred from the death of the cubs to the discovery of their frozen remains. (3 marks)

d Despite their tiger-like appearance, genetic analysis has revealed that the cubs are more closely related to modern lions than tigers. Briefly explain one molecular homology technique that could have been used to draw this conclusion. (1 mark)

e Draw a simple phylogenetic tree that shows the relationship of extinct cave lions to modern-day lions and tigers. (2 marks)

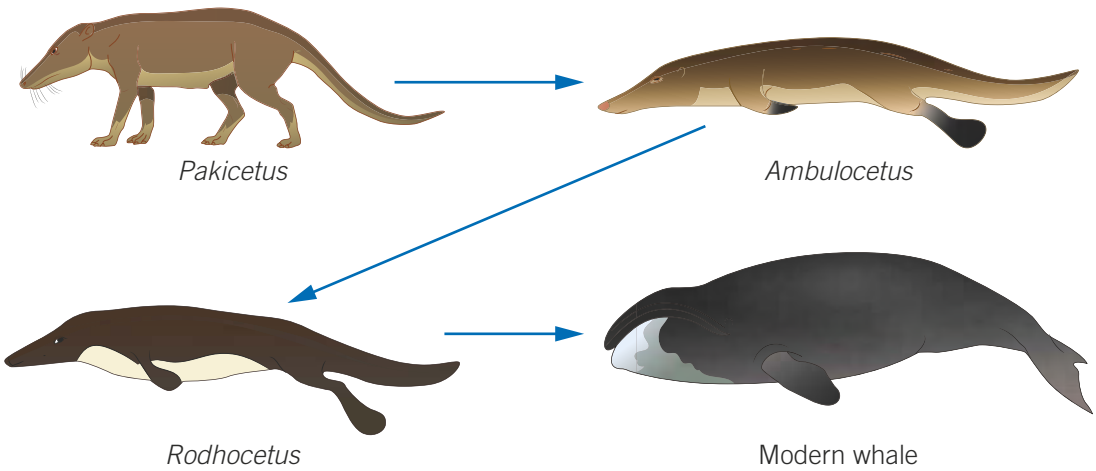
15 Examine the rock strata from two geographically distant locations, shown in the diagram below.



Rock strata from location 1 Rock strata from location 2

Make three inferences on the relative age of the fossils based on the information provided in the diagram. (3 marks)

16 Fossil evidence suggests that modern toothed whales evolved from a terrestrial ancestor known as *Pakicetus*. This theory is supported by the transitional fossil *Ambulocetus*, which links *Pakicetus* (terrestrial) to *Rodhocetus* (aquatic).

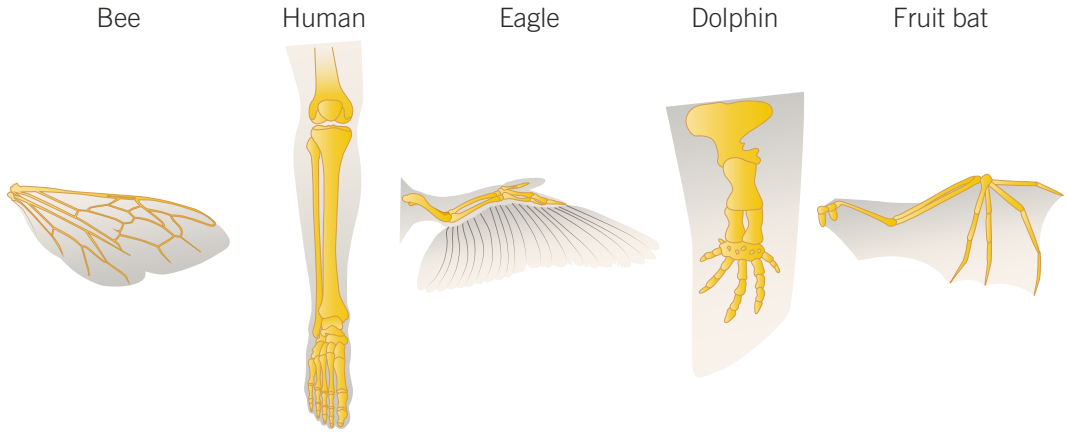


a What features would *Ambulocetus* need to show in order to be classified as a transitional fossil? (1 mark)

b Modern whales (e.g. the baleen whale) still show evidence of a pelvis and small hind leg bones. What name is given to this type of structure? (1 mark)

c Explain how the type of structure named in part **b** supports the theory of evolution. (2 marks)

17 Examine the arms/wings of the various animals below.



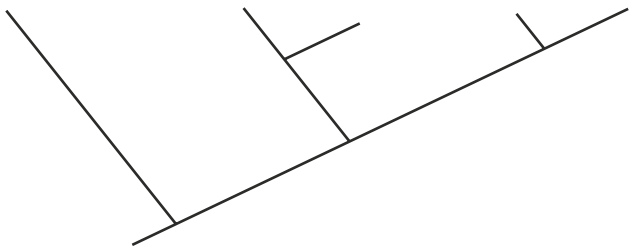
a Identify the animals that show homologous structures. (1 mark)

b Explain the role that the environment has played in shaping the homologous structures. (1 mark)

18 Cytochrome c is an important protein for cellular respiration. The table below shows the number of amino acid differences in a common sequence for cytochrome c from six species of vertebrate.

	E. ferus	*D. polylepis*	*G. gallus*	*A. forsteri*	*E. africanus*
E. ferus	0	21	11	13	1
D. polylepis		0	18	17	20
G. gallus			0	3	10
A. forsteri				0	12
E. africanus					0

a Use the data in the table to place each species on the phylogenetic tree below. (2 marks)



b Justify your placement of the species that is least related to the others and your placement of the species that are the most closely related. (2 marks)

c The human and the Rhesus monkey differ in one of the 104 amino acids that make up cytochrome c. In humans, amino acid 59 is isoleucine but in the Rhesus monkey it is threonine.

Is it accurate to conclude that in the human and in the Rhesus monkey the DNA sequence that codes for all 103 matching amino acids is identical? Explain. (2 marks)

UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES?

CHAPTER 11

HUMAN EVOLUTION

Introduction

Over the past century, palaeontologists have attempted to uncover the ancestral lineages that will provide definitive evidence for the development and evolution of modern humans, *Homo sapiens*. In 1924, key fossilised remains were uncovered that demonstrated the transition from apes to *Homo sapiens* some 3 million years ago. Since then, new discoveries have been a regular occurrence, especially in recent decades. These include more fossils of already known species, as well as new species previously unknown to paleontologists. With the assistance of some of the forms of evidence for evolution outlined in Chapter 10, the story of the evolutionary pathway for the rise of modern humans from their ape-like ancestors continues to be refined. Additionally, the classification of key structural features and the possibility of interbreeding between different species continues to drive scientists to question what they thought they understood previously. It also allows scientists to predict the placement of not only these new species in the evolutionary timeline, but our own position in this wide-branching phylogenetic tree.

This chapter explores the key characteristics shared by ancestral human species with *Homo sapiens*. It studies the migration patterns and timing of ancestral human populations from Africa and the evidence used to confirm these predictions. In doing so, a specific focus on the arrival of the ancestors of Aboriginal and Torres Strait Islander peoples in our Oceania region is examined. It then concludes by looking at key evidence for the major trends in human evolution and how this evidence is used to reinterpret the classification of known and newly discovered species.

Curriculum

Area of Study 2 Outcome 2
Human change over time

Study Design	Learning intentions – at the end of this chapter I will be able to:
• The shared characteristics that define mammals, primates, hominoids and hominins • Evidence for major trends in human evolution from the genus *Australopithecus* to the genus *Homo*: changes in brain size and limb structure	**11A Classification and characteristics of modern humans** **11A.1** Understand the difference between mammals, primates, hominoids and hominins **11A.2** Classify organisms as mammals, primates, hominoids and/or hominins **11A.3** Identify and explain the characteristics that define each of the classification levels **11A.4** Identify skeletal structural changes in hominins that support bipedalism

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Study Design	Learning intentions – at the end of this chapter I will be able to:
	11A.5 Compare and explain how structural changes have enhanced bipedalism and hominin survival for the *Australopithecus* and *Homo* genuses **11A.6** Identify skull structural changes that indicate changes in brain size **11A.7** Compare and explain skull changes in the *Australopithecus* and *Homo* genuses **11A.8** Recall the names, key features and approximate time of existence of different hominin species
• Ways of using fossil and DNA evidence (mtDNA and whole genomes) to explain the migration of modern human populations around the world, including the migration of Aboriginal and Torres Strait Islander populations and their connection to Country and Place	**11B Migration of modern humans** **11B.1** Recall the use of fossil and DNA evidence to classify organisms **11B.2** Explain the difference between nuclear and mtDNA, including the advantages and disadvantages of using each **11B.3** Apply knowledge of evidence to explain migration patterns of different human populations **11B.4** Apply knowledge of evidence to explain the migration of Aboriginal and Torres Strait Islander populations **11B.5** Identify examples of Aboriginal and Torres Strait Islander peoples connection to Country and Place
• The human fossil record as an example of a classification scheme that is open to differing interpretations that are contested, refined or replaced when challenged by new evidence, including evidence for interbreeding between *Homo sapiens* and *Homo neanderthalensis* and evidence for new putative *Homo* species	**11C Classifying newly discovered hominins and evidence for interbreeding between species** **11C.1** Recall types of evidence used to interpret hominin evolution **11C.2** Apply knowledge of hominin evolution to refine the evolutionary lineage of *Homo sapiens* and other hominin ancestors **11C.3** Recall evidence for the interbreeding of *Homo sapiens* and *Homo neanderthalensis* **11C.4** Apply understanding of the relationship of *Homo sapiens* with other hominin species **11C.5** Interpret evidence and information to appropriately classify newly discovered *Homo* species

Glossary

Bipedalism
Connection to Country
Foramen magnum
Genus
Hominin
Hominoid
Homologous structure
Interbreed
Molecular clock
Molecular homology
Primate
Putative
Species
Taxonomy

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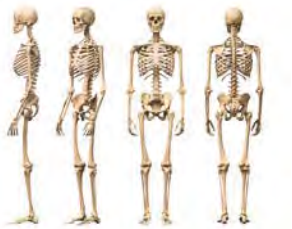
Concept map

11A Classification and characteristics of modern humans

Key features of modern humans compared to other primates and how these are used to help classify species

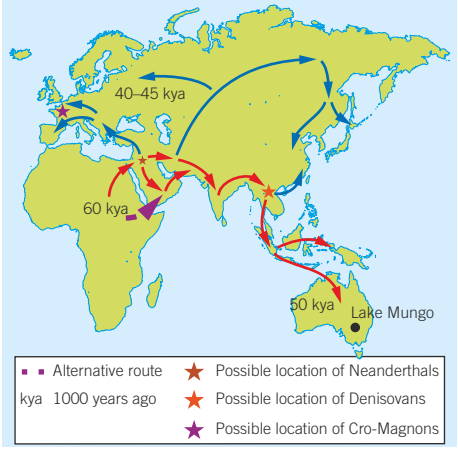


Use of fossil and DNA evidence

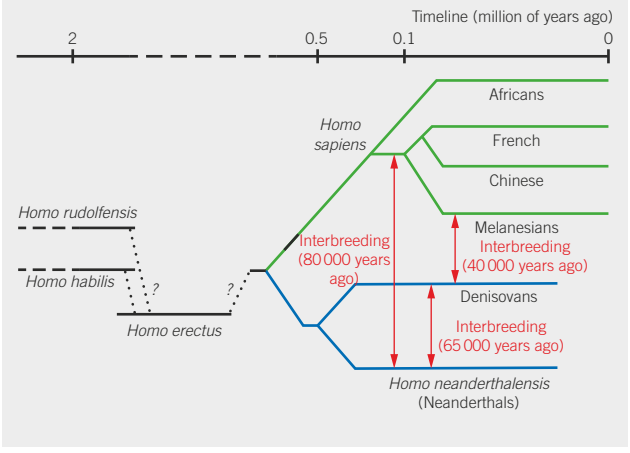
How ancestors of modern hominins migrated from Africa to other regions, including the arrival of Aboriginal peoples from ancestors in Asia

A closer look at interbreeding between different hominin species

11B Migration of modern humans



11C Classifying newly discovered hominins and evidence for interbreeding between species



See the Interactive Textbook for an interactive version of this concept map interlinked with all concept maps for the course.

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11A Classification and characteristics of modern humans

Study Design:
- The shared characteristics that define mammals, primates, hominoids and hominins
- Evidence for major trends in human evolution from the genus *Australopithecus* to the genus *Homo*: changes in brain size and limb structure

Glossary:
Bipedal
Bipedalism
Foramen magnum
Genus
Hominin
Hominoid
Homologous structure
Molecular phylogeny
Primate
Species
Taxonomy

ENGAGE

Have modern humans stopped evolving?

As you learnt in Section 10A, many species have appeared and disappeared over the past 4.6 billion years. This includes the many ancestral species of modern humans over the past 7 million years, a very short time span in the context of life on Earth. Small groups of these species were separated and isolated from their original group and, over time, as a result of mutations and natural selection, were not well adapted to their changing environment. Consequently, they were unable to survive and became extinct.

LINK 10A CHANGES IN BIODIVERSITY OVER TIME

With developments in technology and transportation, migration (gene flow) has become cheaper, more convenient and more frequent. Does this reduction in geographical isolation of populations preclude our species from undergoing further evolution?

Many scientists suspected that this was the case. However, recent research suggests otherwise. This research involved mapping the human genome. It indicated that there were more than 300 regions within our DNA that continue to change. Examples include changes in pigmentation of the skin in populations in northern Europe and Asia, as well as changes to eye colour in northern European populations.

LINK 9A MUTATIONS
LINK 9B EVOLVING AND NON-EVOLVING POPULATIONS

Figure 11A–1 Changes in the pigmentation of the skin and eye colour are noticeable in individuals from Asia (left) and northern Europe (right).

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In fact, over the past 10 000 years, it is estimated from cultural and technological developments that humans have evolved approximately 100 times faster than at any time in the previous 7 million years. One of the main reasons for this is the rapid rate at which our living conditions have changed. With the world's human population growing rapidly, the need for more efficient methods of agriculture and construction has grown too. While cities grow and encroach on land that was previously agricultural or wild, and humans live in closer proximity to other humans and to animals, new diseases continue to emerge. In areas where there is poor hygiene, infrastructure and education, the chances of survival of individuals is dramatically affected. Scientists work to develop vaccines and improve methods of agriculture and sanitation, to increase the survival of all people, rich and poor. Human evolution is no longer dependent on biological change alone. It is now influenced dramatically by both cultural and technological advancements.

8A EMERGENCE, RE-EMERGENCE AND DISEASE CONTAINMENT LINK

7E ACTIVE AND PASSIVE IMMUNITY LINK

EXPLAIN

Classification of modern humans

Taxonomy the process of identifying, naming and grouping organisms

Homologous structure a structure within a group of species that performs a different function yet has the same underlying structure

Molecular phylogeny comparison of nucleotide sequences of genes and amino acid sequences of proteins, from which evolutionary relationships can be inferred

There are approximately 8.7 million species of organisms on Earth. To classify all these organisms, scientists place them into eight categories, in a heirarchy based on the similarities and differences between them. In biology, this hierarchy is known as a taxonomic series.

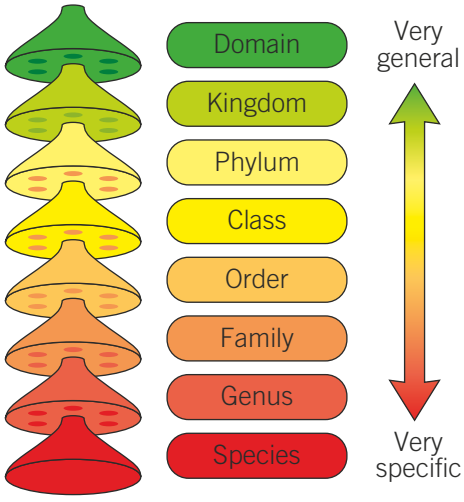

Figure 11A–2 The classification system for living organisms

10C EVIDENCE OF EVOLUTIONARY RELATIONSHIPS LINK

The **taxonomy** built up by scientists in the past centuries grouped organisms according to their physical features, using predominantly their **homologous structures**. Remember from Section 10C that homologous structures are found in species that share a recent common ancestor and have a similar morphological structure but vary depending on the organism's environment and selection pressures. However, in the past few decades this approach has been superseded by the new scientific discipline of **molecular phylogeny**, which compares nucleotide sequences of genes and amino acid sequences of proteins, from which evolutionary relationships can be inferred. In some cases this supports the previous taxonomy, but in others it leads to considerable revisions. The extraction of DNA from fossils, preserved organisms and existing species is revolutionising the field of taxonomy.

VIDEO 11A–1 TAXONOMY OF MODERN HUMANS

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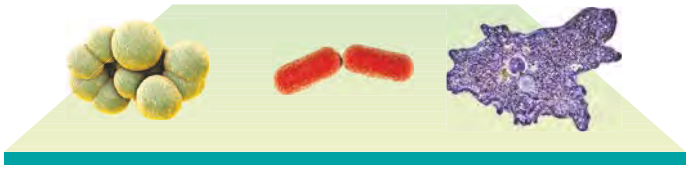
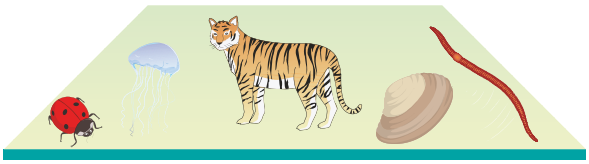
The focus of this section is the evolution of humans. Table 11A–1 outlines the taxonomy of humans, from the broadest level, domain, through to their specific genus and species.

Table 11A–1 The taxonomy of modern humans, from domain to species

Hierarchical level	Notes
All living things There are three domains: Bacteria, Archaea and Eukarya.  Bacteria Archaea Eukarya	• Grow, use energy and reproduce
Domain: Eukarya Humans are in Eukarya. Within the Eukarya domain, there are four kingdoms: Protista, Fungi, Plantae and Animalia. 	• Composed of eukaryotic cells containing a nucleus and membrane-bound organelles • DNA present as linear chromosomes • Somatic cells divide by mitosis and organisms reproduce sexually or asexually
Kingdom: Animalia Humans are in Animalia. Within the Animalia kingdom, there are about 35 phyla, but most common and familiar animals fall into seven: Porifera, Cnidaria, Platyhelminthes, Annelida, Mollusca, Arthropoda and Chordata. 	• Animals that are multicellular with specialised cells • Cells with a plasma membrane but no cell wall or chloroplast (so no photosynthesis)
Phylum: Chordata Humans are in Chordata with vertebrates and sea squirts (tunicates) as well as lancelets. The Chordata phylum has five classes: fish, amphibians, reptiles, birds and mammals. 	• Organisms with support for the body and nervous system that in most members forms a backbone (spine) • Post anal tail (present in embryonic development)

LINK 1A PLASMA MEMBRANE
LINK UNIT 1
LINK UNIT 2
LINK UNIT 1
LINK 5A PHOTOSYNTHESS

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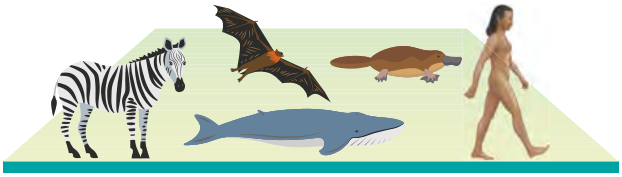
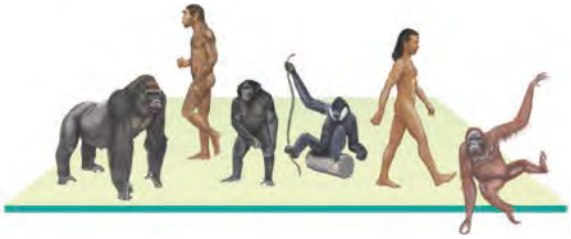
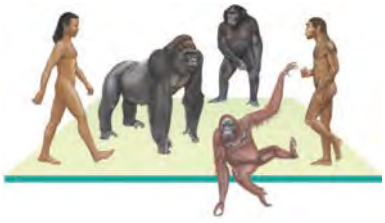
Table 11A–1 *Continued*

Hierarchical level	Notes
Class: Mammals Humans are mammals. There are many orders within the Mammalia class. 	• Warm blooded • Bear live young • Females have mammary glands that produce milk • Specialised teeth (incisors, canines, molars and pre-molars) • Three middle ear bones • Lower jaw consists of a single bone • Have fur, hair or hair follicles
Order: Primates Humans are in the Primates order. Primates include: • humans • great apes • gibbons • monkeys • lemurs and lorises. 	• Dextrous hands and feet with five digits (pentadactyly) that can grasp or curl (in some cases with opposable thumb and/or big toe) • Flexible shoulder (arm swinging) and hip joints (leg movement) • Flattened nails that are sensitive to touch • Forward-facing eyes • Relatively larger regions of the brain responsible for hand–eye coordination and 3D colour vision
Superfamily: Hominoids The Hominoidea superfamily includes humans, great apes and gibbons. Note that monkeys, lemurs and lorises are not hominoids. 	• No tail • Molars with five cusps (not four) • Broad and flattened rib cage
Family: Hominidae The Hominid family includes humans and great apes. Note that gibbons are not hominids. 	• Partially or fully erect (bipedal) posture allowing hands to manipulate food, care for young or, in some cases, use tools

10B FOSSILS LINK

10C EVIDENCE OF EVOLUTIONARY RELATIONSHIPS LINK

Primate
the order consisting of all current and extinct humans, apes and monkeys, characterised by dextrous hands with opposable thumbs and a relatively large, developed brain

Hominoid
the superfamily consisting of all current and extinct humans and apes

Table 11A–1 *Continued*

Hierarchical level	Notes
Subfamily: Hominins The Hominin subfamily includes only humans, both current species and ancestral species that were **bipedal**. It consists of the following genera: • *Sahelanthropus* • *Paranthropus* • *Ardipithecus* • *Australopithecus* • *Homo.* The last two will be investigated more in this section. Note that the great apes are not hominins. 	• Bipedal – this separates humans from other great apes
Genus: *Homo* • Modern and ancestral humans dating from approximately 2 million years ago to today 	
Species: *Homo sapiens* 	• Modern humans, *Homo sapiens*

Hominin
the subfamily consisting of all current and extinct bipedal primates

Bipedal
able to walk on two legs or upright; *bi* = two, *pedal* = foot/feet

Genus
a group of related organisms that share a recent common ancestor

Species
a group of organisms that can interbreed, producing fertile and viable offspring

NOTE

In Table 11A–1 there are some key glossary terms that you need to be familiar with. You also need to know the features that distinguish organisms in each of the groups in this table. Before continuing further in this chapter, it is important that you re-read the information in the table. Throughout this section, the term 'hominin' is used extensively. Hominins include all organisms that are capable of extended periods of bipedalism. This includes not only modern humans, *Homo sapiens*, but also ancestral species of the genuses *Australopithecus* and *Homo*, which will be referred to extensively in this chapter.

As you can see in Table 11A–1, following on from the subfamily, the two most specific levels of the hierarchical classification system are genus and species. A genus is a group of closely related organisms that share a very recent common ancestor, whereas a species is a particular group of organisms within a genus. A species can be defined as a group of individuals that are capable of interbreeding to produce viable, fertile offspring (as you learnt in Section 9D). The easy way to remember this is that **gen**us refers to '**gen**eric', whereas **spec**ies refers to an organism's '**spec**ific' name. Each species has its own unique and universal name. The name of modern humans is *Homo sapiens*, which translates as 'wise man'. After being stated in full, such scientific names can be abbreviated within the same piece of text provided there's no danger of confusion. The abbreviation is the initial of the genus plus the full specific name, so here we can now write *H. sapiens*.

LINK **9D** EMERGENCE OF A NEW SPECIES

11C CLASSIFYING NEWLY DISCOVERED HOMININS AND EVIDENCE FOR INTERBREEDING BETWEEN SPECIES
LINK
10C EVIDENCE OF EVOLUTIONARY RELATIONSHIPS
LINK

One of the main reasons that scientists use such a detailed classification system is to predict characteristics of newly discovered organisms that are similar to other organisms already in that group. With human evolution in particular, as you will discover in Section 11C, new fossils are constantly being uncovered that reveal new species. This allows scientists to compare them to existing hominins, as well as forcing us to rethink our current classification scheme. This scheme now includes genetic evidence as a basis for comparing species. For this reason, an older classification scheme that initially had humans in their own Family, separate from the great apes, was abandoned. Genetic comparisons showed that humans and chimpanzees are more closely related than chimpanzees and gorillas. This evidence also confirmed that there is approximately a 7% difference in the genomes of apes and monkeys. This relationship is shown below, with percentage comparisons.

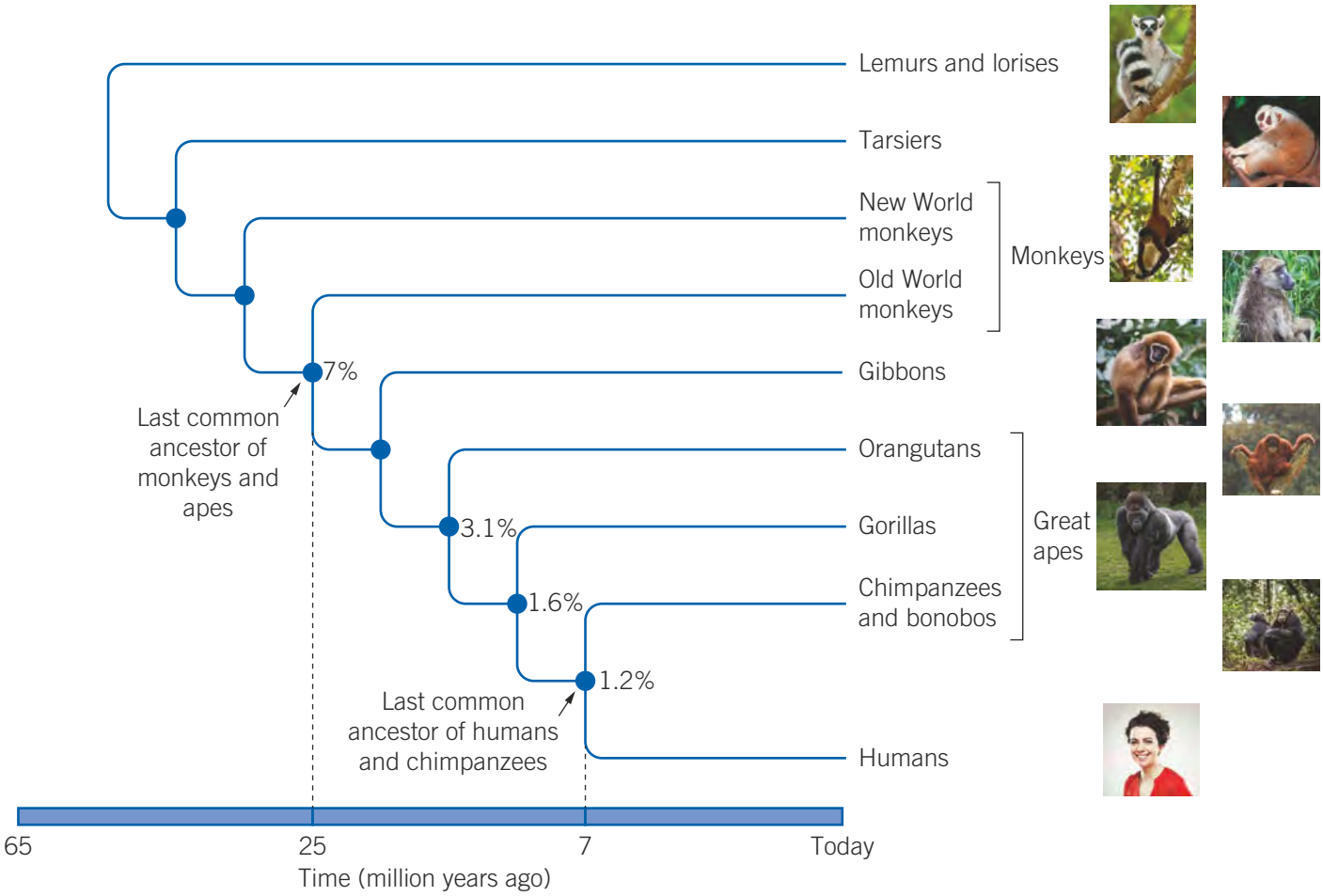


Figure 11A–3 Phylogenetic tree showing the approximate time (in millions of years) since different species of monkeys and apes diverged from each other, including percentages of genetic differences between some of these

Check in questions – Set 1

WORKSHEET 11A–1 THE EVOLUTION OF HOMININS

1 Identify three characteristics that all mammals have in common.
2 Identify three characteristics that all primates have in common.
3 What is the key feature that separates hominins from hominoids?
4 Write down the hierarchical levels of classification for modern humans, including their official genus and species name.

Characteristics of hominins

Two main characteristics in the evolution of hominins are the focus of the Study Design:

- change in limb structure
- increase in brain size.

Each of these changes is outlined in this section, through examining some of the key *Australopithecus* and *Homo* species. In addition, the importance of these changes and how they assisted the survival of hominins in changing environmental conditions is explored.

Figure 11A–4 Artist's rendition of the major *Australopithecus* and *Homo* species

From *Australopithecus* to *Homo*

Three major genera of hominins have existed since 7 million years ago (mya):

- early hominins (7–4.4 mya – not covered in this course)
- *Australopithecus* (4.4–1.4 mya)
- *Homo* (2 mya – current).

Over time, there have been changes in limb structure and the shape of the skull, to what we now refer to as distinctly hominin features.

Figure 11A–5 shows the fossilised remains (sometimes digitally reconstructed, as not all skeletal bones were uncovered) of key Australopithecines and *Homo* species. Look closely at how certain skeletal features have changed over time. These specific anatomical features are further summarised in Figure 11A–6 (page 472) and Figure 11A–9 (page 476).

NOTE

The early hominins referred to in the paragraph above consist of two genera: *Sahelanthropus* (existed 7–6 mya) and *Ardipithecus* (6–4.4 mya). Evidence discovered for these hominins indicates that they were bipedal but had a smaller brain volume, similar to that of chimpanzees. According to scientists, chimpanzees had recently split from the hominin line, at approximately 7 mya. This evidence indicates that bipedalism appeared prior to any increase in brain size.

Figure 11A–5 The evolution of some key *Australopithecus* and *Homo* species and their time of existence. Note that the timescale on the left is more compressed than on the right. Some key features and behaviours associated with specific species are also outlined.

***Australopithecus afarensis* ('Lucy')**

Time: 3.9–2.9 mya

Location: Africa

Many fossils discovered of this species, including footprint, indicating big toe aligned with other toes. Also had features such as strong arms suggesting it was a good climber.

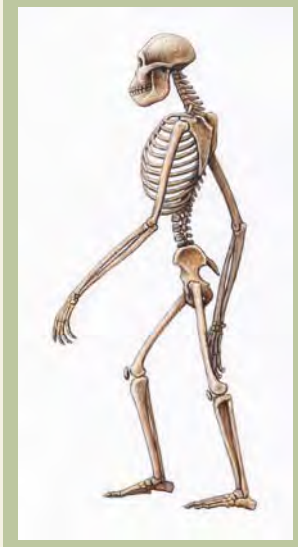
Australopithecus africanus

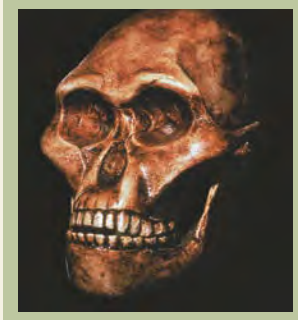
Time: 3.3–2.1 mya

Location: Africa (possibly also Europe and Asia)

Features resembling both apes and humans, such as bowl-shaped pelvis (indicating bipedalism) but long curled fingers and toes (indicating they climbed trees).

Teeth size suggested a mostly vegetarian diet.





Homo habilis

Time: 2.4–1.4 mya

Location: Africa

Name means 'handy man' – was believed to be first stone toolmaker (now disproved). Tools were used for scraping or cutting flesh off dead animals.

Features suggest they were a transitional species between *Australopithecus* and other species of *Homo*.

Homo erectus

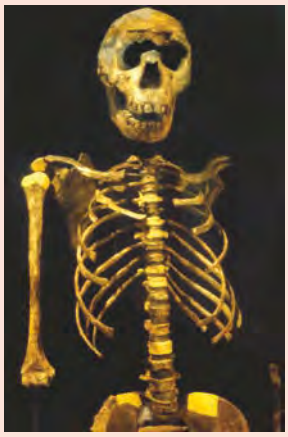
Time: 1.8–0.1 mya

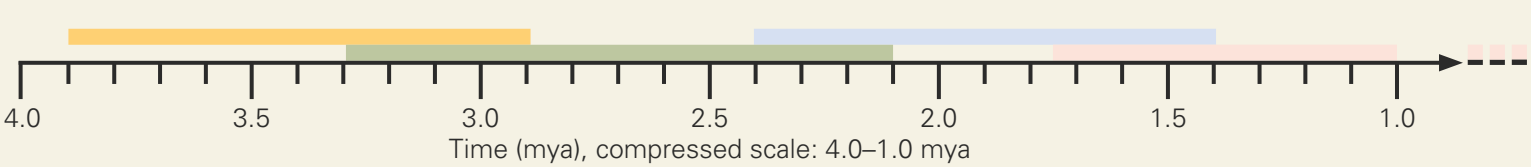
Location: Africa, Europe and Asia (mainly China and Indonesia)

Fossils found outside of Africa suggest they were first to leave.

More advanced stone tools and used fire (potentially not controlled yet) for cooking or warmth.

Lived communal lives. The species was in existence for the longest of hominins to date, surviving for nine time longer than humans have been in existence.





LINK 11C CLASSIFYING NEW DISCOVERIES OF HOMININS AND EVIDENCE FOR THE INTERBREEDING BETWEEN SPECIES

Homo heidelbergensis

Time: 0.7–0.2 mya

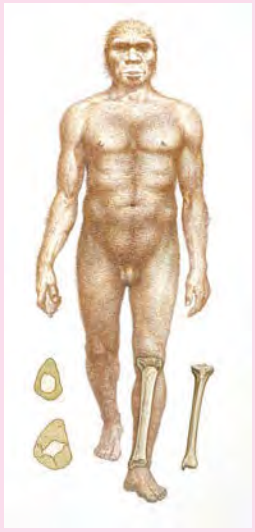
Location: Africa, Asia and Europe

An intermediate species between *Homo erectus* and *H. sapiens*.

Potentially first hominins to move into Europe. Therefore believed to be the direct ancestors of Neanderthals.

Fossils discovered in China. Therefore believed to be the direct ancestors of *H. sapiens*, making *H. heidelbergensis* the last common ancestor of *H. neanderthalensis* and *H. sapiens*.

Built shelter and hunted large animals with spears.



Homo neanderthalensis

Time: 0.4–0.03 mya

Location: Europe, Middle East, Russia, northern Africa

Short stocky bodies for heat conservation (shorter limbs) as they lived in cooler climates.

Not a direct ancestor of *H. sapiens*, as they coexisted.

More advanced tools and demonstrated controlled use of fire.

Other evidence indicates they were the first to wear clothes, communicate orally, bury dead and exhibit symbolic behaviour.

DNA comparisons indicate similarity with Denisovans (diverged ~400 000 years ago). Also confirms interbreeding occurred between Denisovans and modern humans (discussed further in Section 11C).

Homo denisova

Time: 0.12–0.04 mya

Location: Siberia

DNA comparisons indicate similarity to Neanderthals (diverged ~400 000 years ago) and modern humans (diverged ~600 000 years ago). Also confirms that interbreeding occurred with both Neanderthals and modern humans (discussed further in Section 11C).

Homo floresiensis

Time: 0.1–0.02 mya

Location: Indonesia (island of Flores)

Nicknamed the 'hobbit'. Smallest known *Homo* genus, with brain volume one-third the size of *H. sapiens*.

Believed to have evolved from isolated group of *H. erectus*.

Used stone tools and demonstrated controlled use of fire.

Coexisted with *H. sapiens*.

Homo sapiens

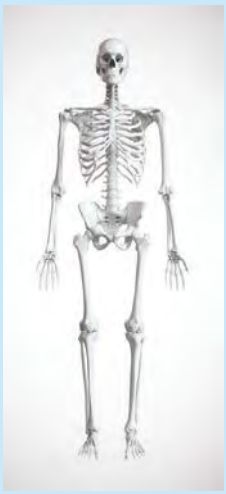
Time: 0.2 mya–present

Location: global

Only living hominin species alive today.

Advanced tool development, complex speech and communication.

Transitioned from hunting and gathering to agricultural farming approximately 10 000 years ago, coinciding with rapid acceleration of cultural and technological evolution.



1.0 0.5 0

Time (mya), expanded scale: 1.0–present

Bipedal locomotion

Bipedalism the characteristic of being bipedal, i.e. walking upright on two legs

Foramen magnum hole in the base of the skull through which the spinal cord enters/exits the skull

Structural changes

The development of **bipedalism** in humans is what distinguishes us from other primates that are not able to permanently walk upright and instead are quadrupedal. A number of skeletal changes that aid in bipedalism have occurred and are crucial in supporting this form of locomotion. Some of these, such as a change in the position of the hole in the base of the skull, the **foramen magnum**, are not intuitively obvious. These changes are outlined for both modern humans and other primates (gorillas) in Figure 11A–6. Note the structural changes in different body parts and how these are significantly different. Species that appear between these two organisms in the evolutionary timeline – for example, *Australopithecus* – have features that show a transition between the different structures.

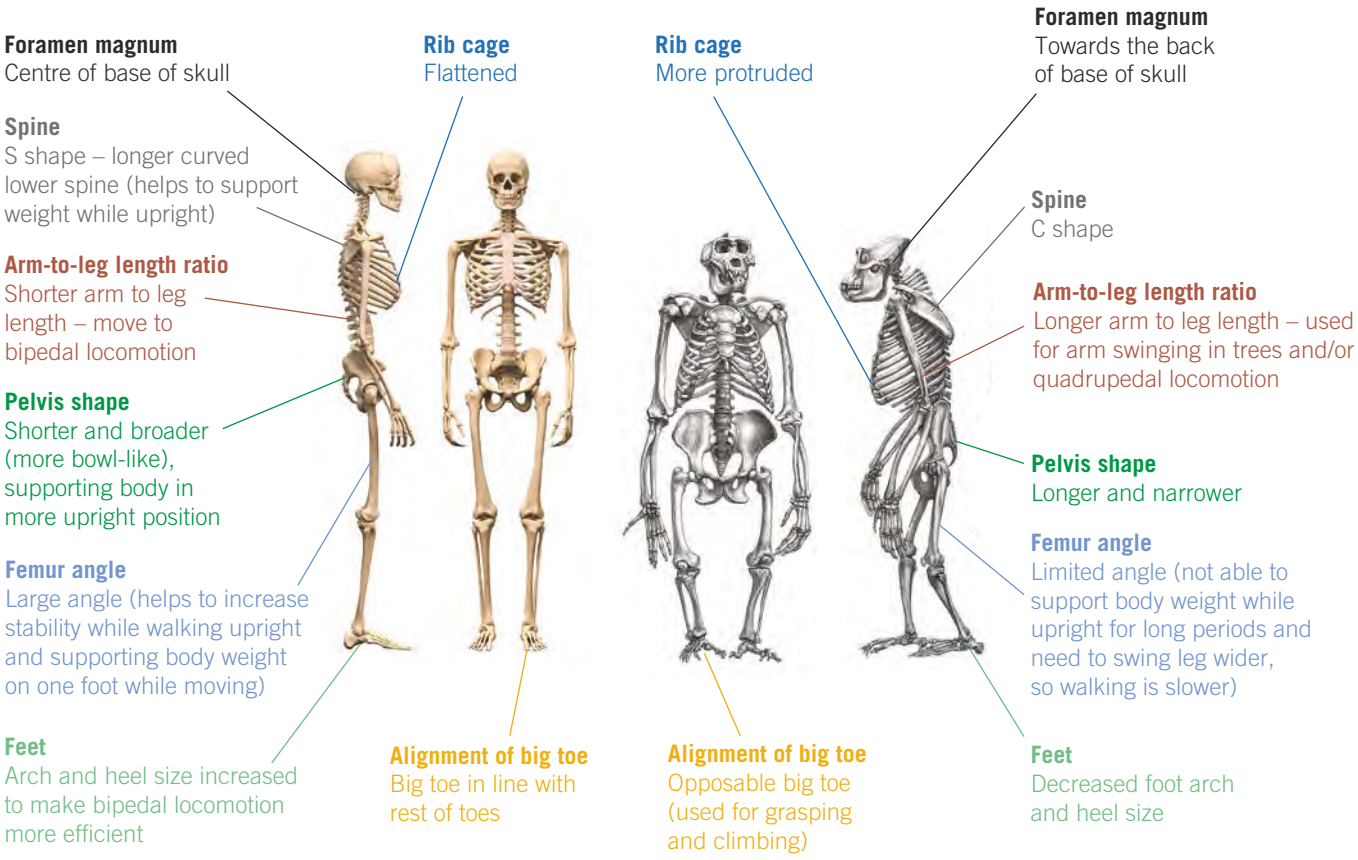


Figure 11A–6 Comparison of skeletal structure in humans (left) and gorillas (right), showing changes in humans that assist with bipedalism

As you can see in Figure 11A–6, a significant number of adaptations occurred during the transition from quadrupeds to bipeds. For this course, the focus is primarily on the change in limb structure that assists with bipedalism in hominins. Therefore, the following four features from Figure 11A–6 are important:

- arm-to-leg length ratio
- femur angle
- alignment of the big toe
- other foot features (arch and heel size).

If you look back at Figure 11A–5, you should see more clearly the changing nature of these features in the transition from *Australopithecus* to *Homo.*

Advantages of bipedalism

The structural changes leading to bipedalism occurred in the evolution of the *Homo* genus over several million years. These changes resulted in significant advantages in their survival in different environments and with changing selection pressures. These are outlined in Table 11A–2.

Table 11A–2 Advantages of bipedalism

Advantage	Description of advantage
Helps keep the body cool (thermoregulation)	Upright stature exposes less body surface area to the sun and raises it higher away from the hot ground and into cooler air currents, in hot and dry daylight hours, especially in open habitats such as a savannah.
Able to visually scan the environment	Standing taller allows observation for food (including prey), water or potential predators
Frees the hands	Makes it possible to manipulate or carry objects or offspring while moving, and hold tools or weapons (*Homo* genus only) Aids taking care of young

LINK UNIT 1

The most significant advantage outlined in Table 11A–2 is the freeing of the hands. Many articles and texts state that, for hominins, this was to make greater use of tools. However, available evidence suggests that not all bipedal individuals, such as *Australopithecus* and earlier ancestors, used tools. In fact, the development and use of tools has only been definitively attributed to the *Homo* genus, approximately 2.6 mya. So when we talk about hominins being able to manipulate 'objects', it is only in the case of *Homo* that we mean 'tools'.

It is hypothesised that this freeing of the hands subsequently led to the increase in size and development of the brain, which is explained further below.

NOTE

It is important to understand that the real reason(s) for hominins becoming bipedal are not fully known. However, fossil evidence, both biological (skeletal hominin, other animal and plant remains) and cultural (tools, fire pit, and so on), has influenced scientists' knowledge of the likely environment at the time for different ancestral species, leading to some of the reasons listed in Table 11A–2.

Changes in the skull

Change in brain volume

Over time, the size of the brain has increased. This is observed when looking at the average volume in cubic centimetres (cm^3, which is equivalent to volume in mL). As can be seen in Figure 11A–7, the average size of the brain of a chimpanzee is 300 cm^3, compared to that of a modern human at 1300 cm^3.

NOTE

As you know from Section 10B, the tissue that makes up the brain would not have fossilised. Therefore, the average volume of the brain for each species is estimated based on internal casts (called endocasts) of the skulls uncovered.

LINK 10B FOSSILS

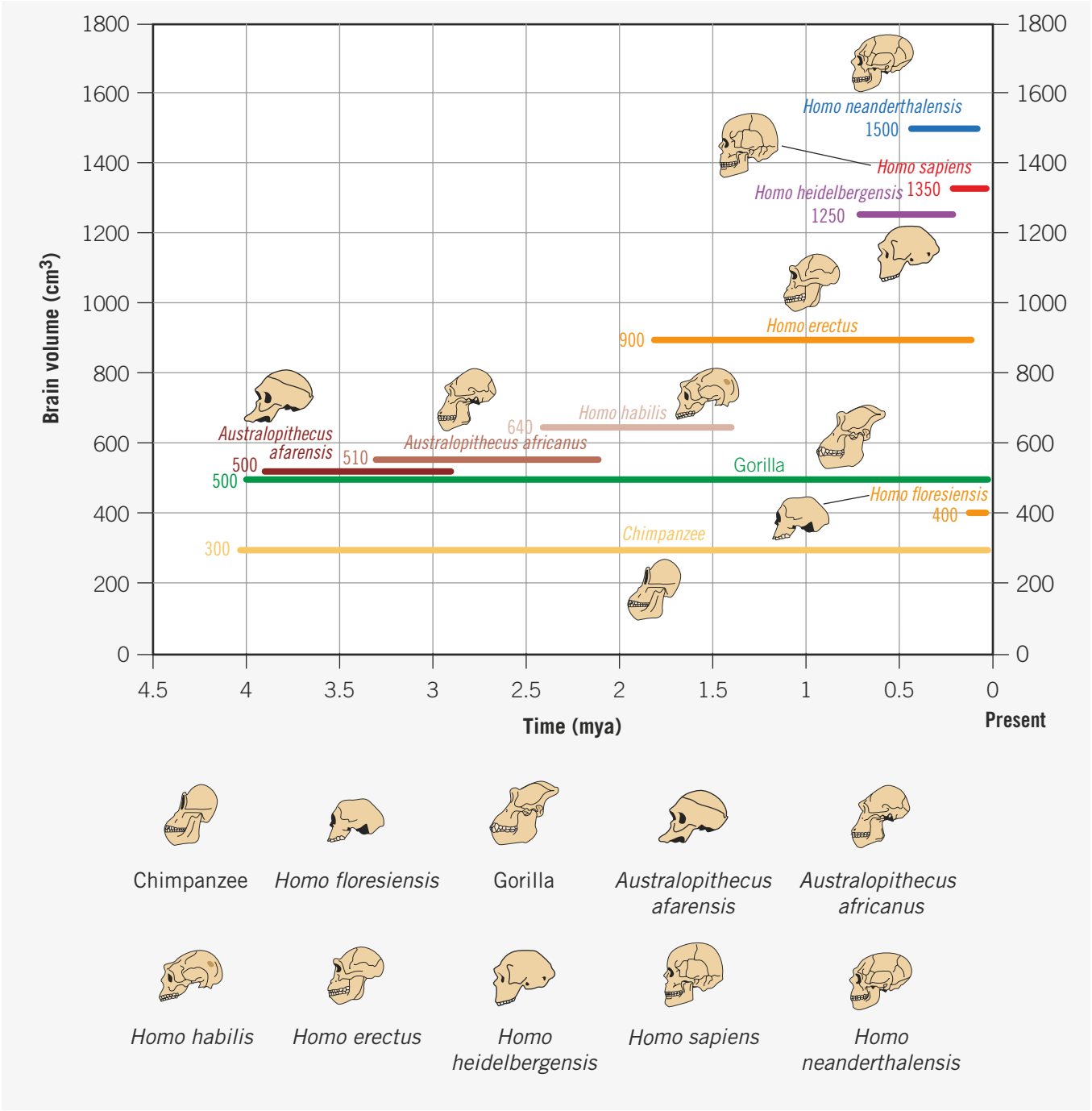


Figure 11A–7 Difference in the average size of the brain of apes compared with other hominins over the past 7 million years

Figure 11A–7 provides a visual representation of the general increasing trend in the size of the brain for hominin species over time. There are two obvious outliers in this data: *Homo neanderthalensis* and *Homo floresiensis*. For a considerable time, before the roles of different regions of the brain were understood, scientists equated an increase in brain size with an increase in intelligence.

In Figure 11A–7, note that *Homo neanderthalensis* has a much larger brain volume than that of modern humans. However, although Neanderthals were well-adapted hominins with cultural understanding and capabilities, they are not believed to have been as intelligent as *Homo sapiens*.

Also, *Homo floresiensis* had a very small brain volume, comparable to that of apes, yet they were clearly hominin, from other observed skeletal structures such as arm-to-leg length ratio, pelvis shape and alignment of the big toe. Remains of cultural evidence discovered with their fossils indicates that they were able to use tools.

Therefore, although scientists often compare the general trend of increasing brain size of hominins, it is also important to look at the expansion of key parts of the brain (Figure 11A–8) and the folding of four key regions.

Table 11A–3 Development of four key regions of the brain in hominins

Region	Significance of this region
Occipital lobe	Increased vision over greater distances
Cerebral cortex (neocortex)	Processing of visual information Depth of planning Memory Problem solving and reasoning Linked to regulatory genes involved in development
Broca's area (in the frontal lobe)	Language/speech
Cerebellum	Fine movements and sensations (particularly for the fingers)

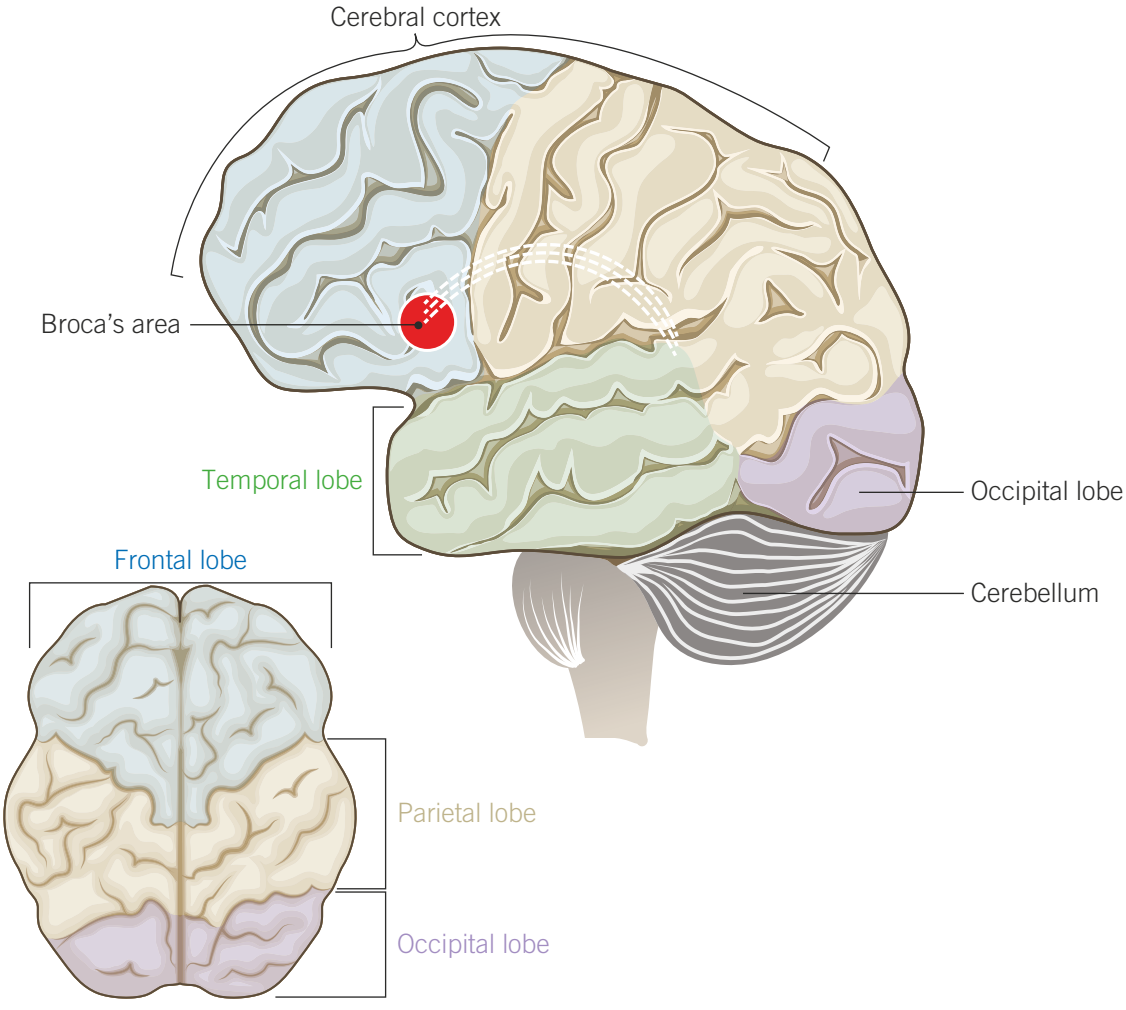


Figure 11A–8 Image of the brain, highlighting the two key regions that have developed over the past 7 million years in humans: the cerebral cortex and Broca's area

Changes in other parts of the skull

Structurally, the shape and size of other parts of the skull changed with the development of the brain. Like Figure 11A–6, in which skeletal features that assist with bipedalism were compared for humans and gorillas, Figure 11A–9 compares the skull of modern humans with that of gorillas. Again, you can infer the general trend of this change from the apes, through *Australopithecus* and earlier *Homo* species, leading to what we now observe today with *Homo sapiens*. These changes are outlined in Figure 11A–9.

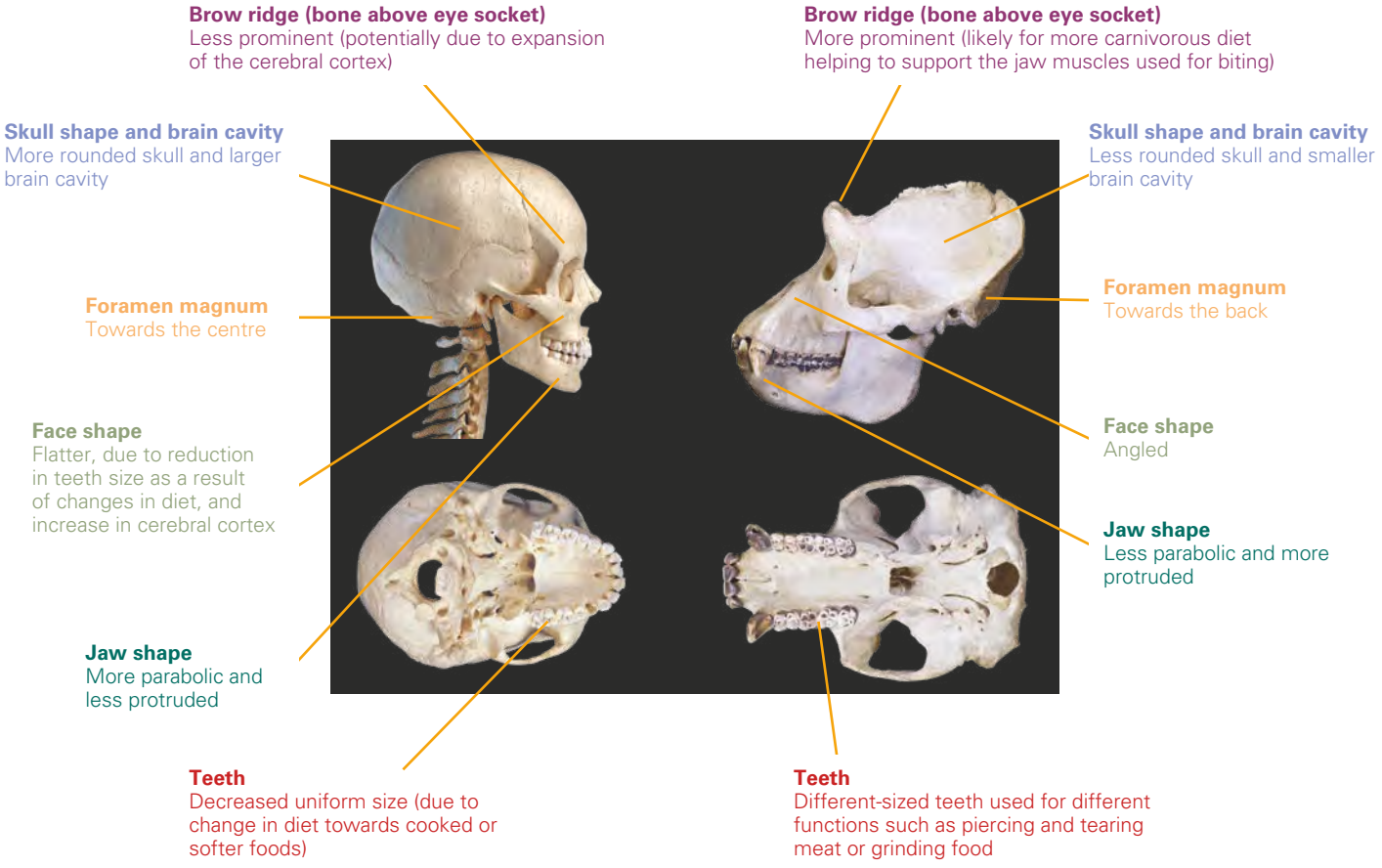


Figure 11A–9 Comparison of the structural changes observed in other parts of the skull from apes to modern humans

Check in questions – Set 2

WORKSHEET 11A–2 STRUCTURAL CHANGES IN HOMININS

1 Outline the general trend in brain size over the past 7 million years.
2 Identify four changes in the human skeletal structure that have occurred with the move to bipedalism.
3 Identify four changes in the human skull that have occurred over the past 7 million years.

Summary of the evolution of bipedalism and brain size

In hominins, the evolutionary change in size and development of the brain was independent of the evolution of bipedalism. However, it is thought that hominins becoming bipedal likely led to an increase in the size of the brain. The biggest advantages of bipedalism were:

- the hands were freed, resulting in a further expansion of the cerebellum. This is the area of the brain responsible for fine movement and sensation of the fingers. Ultimately, this led to the development and use of tools by the *Homo* genus.
- standing taller led to an increase in size of the occipital lobe. This region assists with increased vision over greater distances.

11A SKILLS

Which features of particular hominin species must be memorised?
It is important that you are able to identify the following about the key species outlined in this section:

- Names of *Australopithecus* species (e.g. *A. afarensis*, *A. africanus*)
- Names of *Homo* species (e.g. *H. habilis*, *H. erectus*, *H. neanderthalensis*, *H. sapiens*)
- The genus *Australopithecus* existed prior to the genus *Homo*
- Potential ancestors of certain species, based on the timeline on pages 470–471.
- That *Homo sapiens* are the only hominin species alive today
- That the use of tools is specifically assigned to the *Homo* genus.
- That particular species that go against the general trend in structural features over time. For example, brain size in both *H. neanderthalensis* and *H. floresiensis*. But you do not need to memorise specific brain volumes for each of these.
- The location of certain species that existed outside Africa, namely *H. neanderthalensis* in Europe and *H. floresiensis* in Indonesia.

Typically, specific information and context for a particular species is provided in the stem material at the beginning of a question, or throughout the different parts of a question. Therefore, you are not expected to memorise the specific dates in which each of these species existed. However, you might be asked to use the information provided to determine when a particular species existed and some of the structural features it displays. In Sections 11B and 11C, you will use your knowledge of the evidence for evolution (covered in Sections 10B and 10C) to analyse the migratory patterns of hominins and whether interbreeding occurred between any of these co-existing species.

It is recommended that you revise the times of existence of these species and some of their key features, by completing a short set of cue cards. You might use these to try and create your own phylogenetic tree based on their structural similarities, as shown in the figures and timeline on pages 470–471.

Relating the evolution of key structural changes to natural selection
It is important to realise that these changes in limb structure leading to bipedalism and the changes in brain size and development of key regions of the brain are all due to natural selection, which you learnt about in Section 9B. Of course, these adaptations would have arisen as a result of spontaneous mutations initially occurring in specific individuals.

A typical question may be:

Question

Why did bipedalism evolve in a specific population?

Or

Why was bipedalism an important adaptation in a specific ancestral hominin species?

To answer these questions, you need to be able to refer to all the key points you learnt previously, including these:

- There was initially variation (as a result of mutation) in the population. For example, some individuals were bipedal, some quadrupedal, or some able to maintain the bipedal locomotion for longer periods of time.

VIDEO 11A–2 SKILLS: MEMORISING PARTICULAR HOMININ SPECIES

11B MIGRATION OF MODERN HUMANS

11C CLASSIFYING NEW DISCOVERIES OF HOMININS AND EVIDENCE OF INTERBREEDING BETWEEN SPECIES

10B FOSSILS

10C EVIDENCE OF EVOLUTIONARY RELATIONSHIPS

VIDEO 11A–3 SKILLS: RELATING STRUCTURAL CHANGES TO NATURAL SELECTION

9B EVOLVING AND NON-EVOLVING POPULATIONS

9A MUTATIONS

- **Bipedalism was a selective advantage for this species in a particular environment with certain selection pressures.**
- Those bipedal individuals who were were favoured survived and reproduced, passing on the alleles enabling bipedalism to their offspring.

The important aspect to consider here is the second dot point above in bold. What was the environment like, and what was present in the environment that resulted in individuals who displayed bipedalism having a selective advantage?

To answer this, you must consider what the environment would have been like for ancestors of humans who were not bipedal. It is likely that they lived in a richly dense forest environment, abundant with trees. Perhaps a species was pushed out of this environment due to a lack of food. As a result, they were required to explore new environments, moving out to the savannah where there were open plains and few trees. Finding water and food may have required greater distances to be covered, which was difficult on all fours. The sun may have been intense, and bipedal individuals, who had less of their body's surface area exposed to the sun, were able to keep their body temperature lower. Once having found food or other resources, bipedal individuals would have been able to carry these while moving, unlike individuals who were quadrupeds. If you extrapolate this further, these bipedal individuals were therefore able to provide for their tribe. They became more sexually appealing to members of that tribe, meaning they reproduced, and there was more likelihood of their alleles being passed to the next generation.

UNIT 2 LINK

Even though reference to specific environments was not covered in this section, you can use your knowledge of adaptations, evolution and the structural features of hominins to predict why these features flourished in the first place.

VIDEO 11A–4 SKILLS: MAKING COMPARISONS

Making comparisons in responses to questions

When answering questions that require you to compare the structural differences between hominins, it is important to write your answers in a way that clearly shows a comparison between the two species.

For example:

Question

With reference to the skull shown below, identify two reasons why you know this is a *Homo erectus* individual and not an individual of the *Homo sapiens* species.

Incorrect answer

- Large brow ridge
- Small brain cavity

What this answer fails to do is make a comparative statement between the *Homo erectus* and *Homo sapiens* species mentioned. The answer simply lists what is observed in the image of the fossilised skull. A better answer would be:

Correct answer

- Has a larger brow ridge than *Homo sapiens,* which has a smaller brow ridge.
- Has a smaller brain cavity (and therefore a smaller brain volume) than *Homo sapiens,* which has a larger brain cavity.

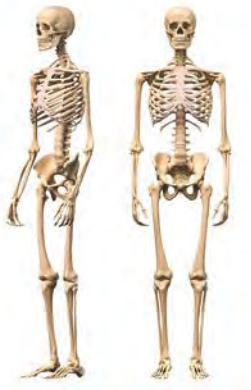
In this improved answer, terms such as 'larger' and 'smaller' are comparative, whereas just stating that a feature is 'large' or 'small' does not make a comparison. Additionally, the answer continues to make reference to how each structure is distinctly different in *Homo sapiens*, further emphasising the differences observed in this skull.

When answering questions that require you to compare the features of different hominins (or primates), or to analyse the general trend in features, it is important that you make the comparisons clear in your responses.

Section 11A questions

1 The taxonomy for modern humans has many levels of classification to separate them from other organisms based on physical characteristics. One of these levels of classification is the hominoids.
   - **a** What features distinguish the hominoids from the broader classification of primates?
   - **b** Which apes make up the hominoids?
   - **c** What species (or family of apes) are separated from the hominoids in order to produce the hominids family?

2 Modern humans, *Homo sapiens,* are classified as hominins.
   - **a** What is the major difference between hominids and hominins? Explain, referring to examples of species in each classification category.
   - **b** Using the figure provided below, identify five observable characteristics that distinguish *Homo sapiens* from all other primates, including the other hominids.
     Note: As outlined in the Skills section, ensure your answers make a comparative statement.



   - **c** For the characteristics you identified in part **b** that structurally support the major difference you identified in part **a**, provide a brief explanation as to how each feature supports the difference.

3 Shown in the figure are two skeletons, one of a hominin and the other of a hominoid.

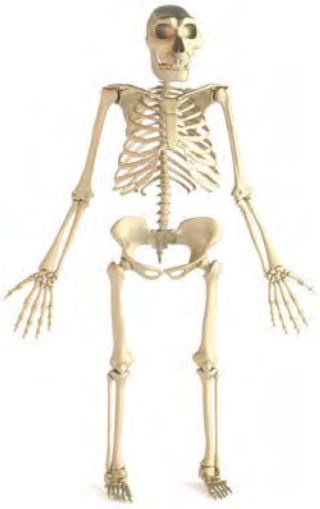
a Define hominin.

b Which is the skeleton (left or right) of the hominin individual?

c Looking at only the skull of each, identify two features that confirm your choice for part **a**.

d Both fossils in this figure are dated at 100 000 years ago. Explain how it is possible for them to be the same age, yet look so different from one another.

The figure below shows the digital reconstruction of an Australopithicine skeleton from fossilised remains that were dated to approximately 3.8 million years ago.



e Which species of *Australopithecus* is this likely to represent: *A. afarensis* or *A. africanus*? Explain your answer.

Note: You may need to use the timeline provided on pages 470–471.

f Two scientists were debating whether this species had the capability to use tools. Freddy was arguing for use of tools whereas Georgie was arguing against the use of tools. Outline one feature of the remains shown in the figure that could support each of these scientist's answers.

g Would the features of this fossil be more closely related to modern humans or great apes? Use one piece of evidence to support your answer.

h Outline how this *Australopithecus* species would have had a selective advantage over other species it coexisted with in an environment that changed from dense tree-filled forest to dry savannah with long grass.

Migration of modern humans

Study Design:
Ways of using fossil and DNA evidence (mtDNA and whole genomes) to explain the migration of modern human populations around the world, including the migration of Aboriginal and Torres Strait Islander populations and their connection to Country and Place

Glossary:
Connection to Country
Molecular clock
Molecular homology

ENGAGE

A species that disproved the migration patterns of hominins

In 2003, scientists uncovered a hominin fossil on the island of Flores in Indonesia, and named it *Homo floresiensis*. Initial dating techniques placed the age of this species at 100 000–60 000 years ago, indicating that it coexisted with *Homo sapiens*. This date of 60 000 years also made it a critical new discovery, as it was one of the most recent hominin species to become extinct.

Figure 11B–1 Digital reconstruction of *Homo floresiensis* from fossilised remains discovered on the island of Flores, Indonesia

However, this species proved to be unlike previously known hominins at the time. It did not follow the trend in anatomical features seen in previous hominin fossils uncovered, and those outlined in Section 11A. It had a small body – only 1 metre tall, considerably shorter than *Homo erectus* (average height 1.6 metres) and *Homo sapiens* (average height 1.65 metres). Its skull was also quite small, even for its small body size, with estimates of a brain volume of 400 cm^3, similar to that of chimpanzees. Despite this smaller brain size, evidence also suggested that they made and used stone tools, controlled fire and hunted wild animals. Scientists believe their short stature was due to island dwarfism – a fast-acting version of natural selection as a result of no gene flow occurring in an island environment in which predation and limited food were selection pressures. This dwarfism, and potentially their small brain size, was a way to reduce unnecessary energy usage to survive, allowing for reproduction.

In 2016, further dating undertaken on newly found lower jaw and teeth fossils indicated that these were 700 000 years old and were from an earlier form of *Homo floresiensis*.

The island of Flores is separated from mainland Asia, and even during periods of low sea levels, the crossing would have been close to 25 kilometres. Even though there are other examples of organisms worldwide that have reached islands by swimming or floating on debris, exactly how and when *H. floresiensis* arrived at the island remains a mystery.

This section explores some of the forms of evidence scientists use to help classify newly discovered remains, and which species are direct ancestors of these, as well as the migratory patterns of hominins from Africa.

LINK **11A** CLASSIFICATION AND CHARACTERISTICS OF MODERN HUMANS

LINK **9B** EVOLVING AND NON-EVOLVING POPULATIONS

LINK **10B** FOSSILS

LINK **10C** EVIDENCE OF EVOLUTIONARY RELATIONSHIPS

EXPLAIN

Forms of evidence used to classify newly discovered hominins

The information below, about different forms of evidence used to help classify newly discovered hominins as either a new, unique species or belonging to an existing species, is important to understanding the migration of ancestral hominins (covered in this section), as well as the potential interbreeding between species (covered in Section 11C).

11C CLASSIFYING NEWLY DISCOVERED HOMININS AND EVIDENCE FOR INTERBREEDING BETWEEN SPECIES LINK

Molecular homology

Two forms of **molecular homology** are used to assess the similarity between newly discovered hominin fossils and current existing species:

- mitochondrial DNA (mtDNA)
- nuclear DNA.

10C EVIDENCE OF EVOLUTIONARY RELATIONSHIPS LINK

Recall from Section 10C that these sequences are used to compare the relatedness between different species. For human evolution, there are advantages in using both nuclear and mitochondrial DNA. These will be covered in the 11C Skills section.

Comparing the DNA sequences of specific genes, chromosomes or whole genomes allows scientists to determine the number of differences between two species. The measure of the number of spontaneous mutations that have accumulated in a gene sequence is based on the assumption that mutations occur at a known constant rate and therefore an approximate amount of time can be calculated since the two species diverged from a common ancestor. This is referred to as the **molecular clock**.

11C CLASSIFYING NEWLY DISCOVERED HOMININS AND EVIDENCE FOR INTERBREEDING BETWEEN SPECIES LINK

In Section 11C, you will see how the use of DNA sequences, primarily mtDNA sequences, have also been used to determine whether interbreeding between species occurred.

Fossils

10B FOSSILS LINK

Recall from Section 10B that fossils can be used to confirm evidence of the relatedness between species through comparative anatomy, namely *homologous structures*. Comparing these similar structures allows scientists to infer other features of hominins that may not have been recovered from fossil evidence. Fossil evidence may not be restricted to that of deceased organisms, but can also include the existence of tools, evidence of fire-pits, agricultural practices or artwork. These also provide an understanding of the behaviour and capabilities of hominins during certain time periods. If remains are well preserved, DNA samples from cells can be obtained, to compare the molecular homology of these species with species whose genomes are already fully or partially mapped. The sedimentary layers surrounding the fossil can also be dated using radiometric techniques, providing an absolute age of that layer, and therefore the fossil.

Molecular homology the analysis of DNA and amino acid sequences as evidence of evolutionary relationships

Molecular clock using the predicted mutation rate of DNA (or amino acid) sequences to determine the approximate time at which two species diverged

However, as you will also recall, interpreting the fossil record is difficult because the record is largely incomplete. This is especially true for hominin evolution. The current fossil record for hominins is largely composed of skulls, rather than structures that directly indicate whether hominins were bipedal or quadrupedal. As such, scientists have used the skulls to indicate more than just

Figure 11B–2 A fossilised skull of *Homo neanderthalensis*

11C CLASSIFYING NEWLY DISCOVERED HOMININS AND EVIDENCE FOR INTERBREEDING BETWEEN SPECIES LINK

size of the brain, but also cultural and technological capabilities. Looking at the type, size and arrangement of teeth can inform scientists about a particular hominin's diet. From this, they can infer things such as their level of cultural evolution and ability to use tools or control the use of fire. As you will see from the three examples presented in Section 11C, this jigsaw is slowly being put together.

The two waves of human migration

From current fossil and mtDNA evidence, scientists know that hominins evolved in Africa. From the timeline of hominin evolution on pages 470–471, the *Australopithecus* genus (and species present prior to this but not discussed) existed solely in different regions of Africa. Early members of the *Homo* genus, such as *Homo habilis*, also appeared only in Africa.

In terms of the movement of ancestral hominins out of Africa, scientists' theories include the following:

- A group of *Homo erectus* or a group from an unknown species of the *Homo* genus similar to *Homo habilis* were the first to leave Africa, 2–1.75 mya. These hominins had a larger brain volume (approximately 600 cm^3), were capable of tool use and had a diet that included meat, so a wider variety of foods could be consumed.
- Other hominins, possibly still undiscovered, moved from Africa into parts of Asia as early as 1 mya. There was also movement of these hominins back into Africa.
- A group of *Homo heidelbergensis*, proposed as the ancestor of *Homo neanderthalensis*, due to their stocky body shape and size, moved out of Africa into Europe as early as 900 000 years ago. With changing environments, this species underwent evolutionary changes that led to the rise of *H. neanderthalensis* by approximately 300 000 years ago.

What about the migration of *Homo sapiens*? How did we come to exist on different continents? One theory proposed by scientists is the Out of Africa theory, which states that *H. sapiens* arose in Africa and then moved out of Africa to other continents in two waves at different times.

The key piece of evidence to support this theory is that current humans have very little genetic diversity. The greatest diversity would be found in Africa, where *Homo sapiens* first appeared, as there has been more time for spontaneous mutations to accumulate.

The first wave: 160 000 to 100 000 years ago

Fossil evidence of *Homo sapiens* is limited, due to the short time frame in which we have existed. However, from fossilised remains of our species, scientists have observed the following key points in relation to the first migratory wave of *Homo sapiens:*

- *Homo sapiens* fossils in East Africa have been dated to approximately 160 000 years ago.
- Evidence suggests that migration into northern parts of Africa occurred, likely along the eastern coastline.
- *Homo sapiens* reached what is now referred to as the Middle East approximately 100 000 years ago.

Scientists know four species existed by 100 000 years ago: *H. neanderthalensis* in Europe, *H. denisova* in Russia, *H. floresiensis* in Indonesia and *H. sapiens* in Africa and the Middle East, as mentioned above.

The second wave: 60 000 to 40 000 years ago

WORKSHEET 11B–1 EVIDENCE FOR HUMAN MIGRATION

There is little to no fossil evidence of *H. sapiens* outside Africa between 100 000 and 60 000 years ago. From fossils found in Europe and Asia dated to approximately 40 000 years, two hypotheses have been suggested. These are shown in Table 11B–1 with supporting evidence.

Table 11B–1 Two hypotheses about the second wave of modern human migration, and their supporting evidence

Hypothesis	Evidence
1 After the first wave (160 000–100 000 years ago), modern humans migrated further north-west from the Middle East into Europe, and south-east into Asia	Stone tools found in the following regions with dates: United Arab Emirates – 80 000 years ago Yemen – 80 000 years ago India – 74 000 years ago However, no hominin remains have as yet been found with the stone tools.
2 A second group of modern humans moved from regions of Africa into Europe and Asia, extending beyond the Middle East where the first wave of modern humans reached.	Fossils of hominins, called Cro-Magnons, found in a French cave. Comparisons of the skeletal structures to today's modern human populations indicates they were more similar to modern Africans than modern Europeans.

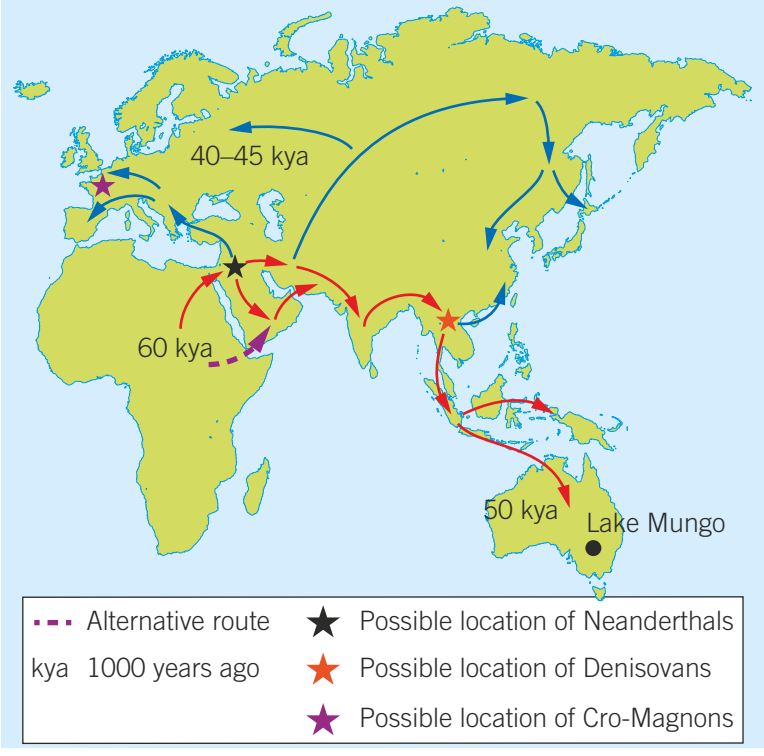


Figure 11B–3 The proposed migration of modern humans and times during the second wave of migration

This second wave is also what led to the arrival of Indigenous populations in the Oceania region, including the arrival of Aboriginal peoples' ancestors, approximately 50 000 years ago. However, recent evidence suggests this arrival could have been even earlier, at around 65 000 years ago.

Check-in questions – Set 1

1 List two key forms of evidence that have been used to determine the migratory patterns of hominins.
2 The migration of modern humans was believed to have occurred in two waves.
   a What are the proposed times of these two waves?
   b What is one piece of evidence for each wave that supports the theory that migration occurred at those times?

Other evidence supporting the migration of *Homo sapiens*

In addition to the evidence of skeletal remains and comparisons of mtDNA, dated to approximately 40 000 years ago and coinciding with the second wave of human migration, palaeontologists noticed an increase in evidence of art and culture, including carvings and cave art or paintings.

This is an indicator of the cultural evolution of particular species, providing clues to the way they lived, the social interactions they had with each other and their interactions with their environment.

Figure 11B–4 An example of some of the earliest cave art discovered in Indonesia. The art depicts a dwarf buffalo species, which still exists in that country today.

Connection to Country

A population's interaction with their environment can also be referred to as **Connection to Country**. This was (and still is) an important way of life for Indigenous populations in the Pacific region, specifically that of Aboriginal and Torres Strait Islanders.

Connection to Country the relationship between people and their indigenous land or environment

It is understood that Aboriginal Australians have the longest unbroken record of ancient art. For their ancestors, creating art would have been a way of forming a greater connection between each other, and also a way to define their unique identity among other populations.

The transmission of this information and key cultural practices within and between generations would not have been possible without an increase in brain volume and the development of key regions of the brain. Therefore, the use of language, in both a verbal and an artistic sense, is a key component of Aboriginal and Torres Strait Islanders' way of life. Current generations have continued to inherit this cultural understanding and the practices of their ancestors, continuing to add to this record.

Consider how the ancestors of these Indigenous populations would have used the key developments in hominin evolution (outlined in Table 11B–2) to enhance their survival and way of life.

Figure 11B–5 An example of artwork found in Quinkan country, Queensland, featuring images of humans, dingoes and eels. This work has been dated to approximately 15 000 years ago.

Table 11B–2 Key cultural and technological developments that support Aboriginal and Torres Strait Islander survival

Cultural development	Impact on Connection to Country/way of life
Controlled use of fire	Cooking food extracts more nutrients Burning of landscape promotes growth of new grass, which then attracts other animals (e.g. kangaroos), which can then be hunted
Language	Sharing of stories and practices between members of the same generation and between generations Forms of art and paintings (as outlined previously)
Development and use of tools	Increasing complexity of tools improved the ease of practices such as: • hunting – use of spears, clubs and boomerangs • agriculture – repeated aeration of the soil through tilling, loosening it for seed germination and root penetration.
Ritualistic behaviour	Burial rites, wearing of jewellery

LINK 11A CLASSIFICATION AND CHARACTERISTICS OF MODERN HUMANS

VIDEO 11B–1 ARRIVAL OF INDIGENOUS AUSTRALIANS

LINK UNIT 2

Figure 11B–6 Location of Lake Mungo

Key locations for ancestors of Aboriginal peoples

Lake Mungo

One key location is Lake Mungo, in the south-western region of New South Wales. Three bodies were uncovered here between 1969 and 1974. The first and most famous, called Mungo Lady, was later determined to have been ritualistically buried. Five years later, a male skeleton was uncovered, and called Mungo Man. Mungo Man had also been ritualistically buried. These two individuals and the nature of their burials are among the earliest evidence of this cultural practice in the world.

These are the oldest human remains discovered in Australia, having been dated at 42 000–40 000 years old. It is believed that Aboriginal peoples would have arrived at the lake at around this time and found a rich diversity of plant and animal life. Evidence indicates that the lake was thriving, with fish, shellfish and yabbies, and the high vegetation around the lake, including eucalyptus trees, would have attracted many birds, frogs, reptiles and other mammals. Evidence also indicates that the Aboriginal population in this area built fires, developed tools and painted their bodies.

In 2003, geologists uncovered 460 footprints in a clay pan around the lake. This was the largest collection to date globally of fossilised footprints in a single find, and some of the oldest – dating estimated them to be from 20 000 years ago.

Today, the Paakantji, Mutthi Mutthi and Ngyimpaa people continue to maintain a close connection to the now World Heritage-listed Willandra Lakes, which include Lake Mungo. An important Connection to Country for Aboriginal Australians and part of their communal history was the return of the remains of Mungo Lady (in 1992) and Mungo Man (in 2017).

Figure 11B–7 The return of Mungo Man in 2017 to the Paakantji, Mutthi Mutthi and Ngyimpaa people (left) was marked with a traditional Aboriginal ceremony (right).

Devil's Lair

Another region of significance is Devil's Lair in Western Australia. The cave gets its name from the discovery of Tasmanian Devil fossils in the 1970s. Since then, other artefacts have been found, such as bone points and beads, and plant remains. Again, this indicates the cultural evolution of the ancestors of the Noongar people living in the region some 40 000 years ago.

Figure 11B–8 The archaeological site at Devil's Lair in Western Australia

Figure 11B–9 Bone points, like that found in caves of Devil's Lair

Summary of the arrival of modern humans in Australia

Estimates of the exact time of arrival of the ancestors of Aboriginal peoples keep changing. New discoveries indicate that it could have been 65 000 years ago.

Much of this section has presented evidence and information from a Westernised viewpoint, that modern humans arose in Africa and migrated outwards from there. However, Aboriginal peoples traditionally believe that they have been present in Australia since the time of creation, and the knowledge of their history is ingrained in their blood and Country.

The Dreaming is a system of belief of many Aboriginal and Torres Strait Islander peoples, and a way to confirm their origin. It is a complex cultural concept, worldview and system of laws that explains the creation of everything and the purpose of life. Different Aboriginal peoples and cultures have different Dreamings with their own name in the local language. Some Dreamings state that 'all-powerful beings roamed the landscape and laid the moral and physical groundwork for human society' (Evidence of First Peoples, National Australia Museum). Prior to the Dreaming, the land was flat. When it was moulded, it gave life to the 'first people' and their culture. The Westernised view, that the ancestors of Aboriginal peoples arrived in Australia 65 000 years ago, diminishes their Connection to Country.

Check-in questions – Set 2

1 Name two locations within Australia that have been key archaeological sites in the discovery of ancestral remains of Aboriginal peoples.
2 Explain what is meant by the term Connection to Country.
3 List two examples of cultural evolution and explain how they helped Aboriginal peoples to survive on the land.

11B SKILLS

VIDEO 11B–2 SKILLS: USING INFORMATION FROM QUESTION STEM

Using information provided in the question stem

For this topic in particular, the context required in your responses will come from the information provided in the question stem. An example of this is shown with the article below.

First Australians arrived in an incredible planned migration with over 1300 people

Corey J.A. Bradshaw, Laura S. Weyrich, Michael Bird & Sean Ulm, The Conversation, 18 June 2019

The size of the first population of people needed to arrive, survive, and thrive in what is now Australia … took more than 1000 people .... But this was no accidental migration, as our work shows the first arrivals must have been planned.

Our data suggest the ancestors of the Aboriginal, Torres Strait Islander, and Melanesian peoples first made it to Australia as part of an organised, technologically advanced migration to start a new life.

Changing coastlines

The continent of Australia that the first arrivals encountered wasn't what we know as Australia today. Instead, New Guinea, mainland Australia, and Tasmania were joined and formed a mega-continent referred to as Sahul.

This mega-continent existed from before the time the first people arrived right up until about 8000–10 000 years ago. ...

When we talk about how and in what ways people first arrived in Australia, we really mean in Sahul.

We know people have been in Australia for a very long time – at least for the past 50 000 years, and possibly substantially longer than that.

We also know people ultimately came to Australia through the islands to the northwest. Many Aboriginal communities across northern Australia have strong oral histories of ancestral beings arriving from the north.

But how can we possibly infer what happened when people first arrived tens of millennia ago?

It turns out there are several ways. We can look indirectly at:

- *where* people most likely entered Sahul from the island chains we now call Indonesia and Timor-Leste
- *how* many people were needed to enter Sahul to survive the rigours of their new environment.

...

It turns out the northern route connecting the current-day islands of Mangoli, Buru, and Seram into Bird's Head (West Papua) would probably have been easier to navigate than the southern route from Alor and Timor to the now-drowned Sahul Shelf off the modern-day Kimberley.

While the southern route via the Sahul Shelf is less likely, it would still have been possible.

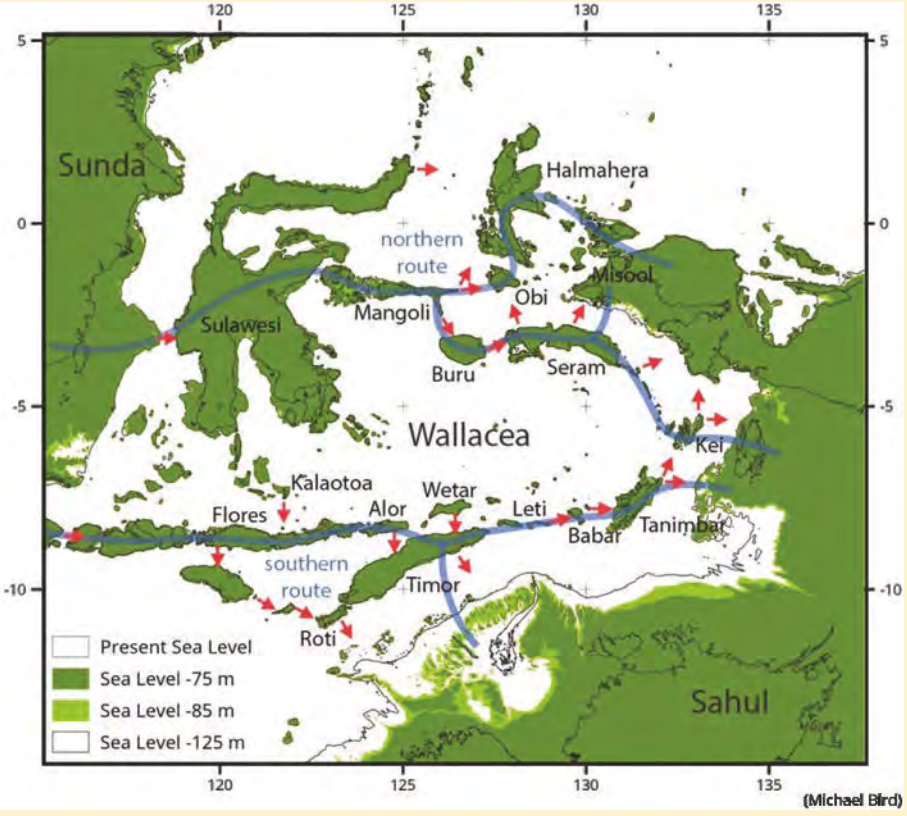


Figure 11B–10 Modelled routes for making landfall in Sahul. Sea levels are shown at –75 m and –85 m. Potential northern and southern routes indicated by blue lines. Red arrows indicate the directions of modelled crossings.

Next, we extended these demographic models to work out how many people would have had to arrive to survive in a new island continent, and to estimate the number of people the landscape could support.

We applied a unique combination of:

1. fertility, longevity, and survival data from hunter-gatherer societies around the globe
2. 'hindcasts' of past climatic conditions from general circulation models (very much like what we use to forecast future climate changes)
3. well-established principles of population ecology.

Our simulations indicate at least 1300 people likely arrived in a single migration event to Sahul, regardless of the route taken. Any fewer than that, and they probably would not have survived – for the same reasons that it is unlikely that an endangered species can recover from only a few remaining individuals.

Alternatively, the probability of survival was also large if people arrived in smaller, successive waves, averaging at least 130 people every 70 or so years over the course of about 700 years.

A planned arrival

Our data suggest that the peopling of Sahul could not have been an accident or random event. It was very much a planned and well-organised maritime migration.

Our results are similar to findings from several studies that also suggest this number of people is required to populate a new environment successfully, especially as people spread out of Africa and arrived in new regions around the world.

The overall implications of these results are fascinating. They verify that the first ancestors of Aboriginal, Torres Strait Islander, and Melanesian people to arrive in Sahul possessed sophisticated technological knowledge to build watercraft, and they were able to plan, navigate, and make complicated, open-ocean voyages to transport large numbers of people toward targeted destinations.

Our results also suggest that they did so by making many directed voyages, potentially over centuries, providing the beginnings of the complex, interconnected Indigenous societies that we see across the continent today.

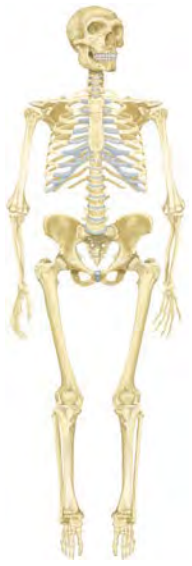
These findings are a testament to the remarkable sophistication and adaptation of the first maritime arrivals in Sahul tens of thousands of years ago.

This extract provides information indicating the approximate time of arrival of Indigenous populations in Australia and surrounding islands of the Melanesian area. It also presents information about possible routes they would have taken from south-eastern parts of Asia. And it provides evidence to support the reasoning given.

For questions about this extract, you would need to use information from the extract to support your answers. You therefore do not need to memorise details of this section. This section does not present all the information currently proposed about the migration of Aboriginal and Torres Strait Islanders to our region. It does, however, provide a succinct overview of the hypotheses in general, based on examples of evidence related to this course.

Section 11B questions

1 The skeleton shown on the right is that of the extinct *Homo neanderthalensis*.



   a Identify two features of this skeleton that would be similar to features observed in a fossil of an *Australopithecus* species.
   b Identify two features of this skeleton that would be different from features observed in a fossil of an *Australopithecus* species.

It is known that Neanderthals lived in the cold climates of Europe and Asia from approximately 200 000–30 000 years ago. Using the understanding of the migratory movements of *Homo sapiens* out of Africa, it is likely that these two species coexisted.

   c Which wave of migration, 1 or 2, is likely to have brought *Homo sapiens* to Europe?
   d Provide evidence and relevant times to support your answer to part **c**.

The occurrence of these waves of modern human migration did not result in the arrival of Neanderthals in Europe, as they were already present for a considerable time before this.

   e Provide a reason to suggest how Neanderthals came to be in Europe.
   f Fossil evidence found with remains of Neanderthal skeletons includes primitive tools believed to resemble sewing equipment. Referring to the information provided, outline why this would have been an important tool and cultural skill for Neanderthals.

2 Explain why, when fossils are uncovered, it is not always possible to use molecular homology to compare these species with other species.

3 In 2003, on the island of Flores in Indonesia, a hominin fossil was discovered. Using relative dating methods, the hominin was dated to between 38 000 and 18 000 years old. This fossil was short, only 1 metre tall, and had a small cranial capacity, similar to that of chimpanzees. However, the fossil also contained features that suggested it was capable of bipedalism. The fossil was also uncovered with evidence of stone tools, fireplaces used for cooking and cave paintings.

   a Their short stature and smaller cranial volume go against the trend observed in other hominins over time. Explain why this reduction in size and cranial capacity might have occurred.
   b Do you think their existence in Indonesia, and therefore arrival before 38 000 years ago, would have coincided with the arrival of the ancestors of Indigenous peoples in Australia? Provide reasons to support your answer.
   c From the information provided, identify one piece of cultural evidence discovered in Indonesia that was also discovered in Australia.
   d What is the importance of this form of cultural evolution for present-day Aboriginal and Torres Strait Islander peoples?
   e Provide examples of two ways in which Aboriginal and Torres Strait Islander peoples demonstrated a Connection to Country. In your answer, also clearly articulate what is meant by the term 'Connection to Country'

4 Evidence from the genomes of modern human populations indicates that Europeans, East Asians and Aboriginal peoples contain a very small percentage of Neanderthal DNA in their genomes. What could this suggest to scientists about the timing and route of migration of the ancestors of Aboriginal peoples to Australia?

Classifying newly discovered hominins and evidence for interbreeding between species

Study Design:
The human fossil record as an example of a classification scheme that is open to differing interpretations that are contested, refined or replaced when challenged by new evidence, including evidence for interbreeding between *Homo sapiens* and *Homo neanderthalensis* and evidence for new putative *Homo* species

Glossary:
Interbreed
Putative

10C EVIDENCE OF EVOLUTIONARY RELATIONSHIPS LINK

ENGAGE

Who was Mitochondrial Eve?

As you learnt in Section 10C, mitochondrial DNA is inherited mainly from the maternal line. It therefore provides an easy way for scientists to trace the direct lineage of the maternal ancestor to descendants through the number of mutations between these sequences.

Scientists can therefore trace mitochondrial DNA back to one common ancestor, whom they refer to as Mitochondrial Eve. This name is taken from the biblical Adam and Eve. It is suspected that Mitochondrial Eve lived between 200 000 and 100 000 years ago in southern regions of Africa. Mitochondrial Eve would not have been the first *Homo sapiens* present, but every previous female lineage eventually had no more female offspring, meaning that this passing on of that specific mitochondrial DNA ceased. Therefore, all current *H. sapiens* alive today can trace their mitochondrial DNA back to Mitochondrial Eve.

11B MIGRATION OF MODERN HUMANS LINK

As you will recall from Section 11B, since the time of Mitochondrial Eve, modern humans have gone through two waves of migration, spreading from regions within Africa to other continents. In this time, different Indigenous populations have arisen, and distinct ethnic groups are now present on each continent. Genetically, these groups have gone through significant changes due to very different selection pressures and environments, as well as genetic bottlenecks.

9B EVOLVING AND NON-EVOLVING POPULATIONS LINK

In 2010, scientists discovered the fossil remains of a man in southern Africa who is believed to have been the closest known relative of Mitochondrial Eve. His ancestors are believed to have diverged from this earliest lineage related to Mitochondrial Eve. He died some 2300 years ago, around 315 BC.

Scientists hope to continue to uncover remains and compare their mitochondrial and nuclear genomes, gaining more information about the migration of modern humans, as well as a clearer understanding of how different groups of hominins mixed and interbred with each other. This is explored further in this section, with some key examples.

EXPLAIN

Can different hominin species interbreed?

In Section 11A, you learnt about some of the different hominins that lived during the past 4 million years. As you will learn in this section, analysis of their DNA indicates that interbreeding occurred between some of these species. From the understanding you developed in Section 9D, how is it possible that different species thousands of years ago were able to produce viable, fertile offspring?

LINK 11A CLASSIFICATION AND CHARACTERISTICS OF MODERN HUMANS

LINK 9D EMERGENCE OF A NEW SPECIES

LINK 10C EVIDENCE OF EVOLUTIONARY RELATIONSHIPS

LINK UNIT 2

This question stems from a common misunderstanding of what defines a biological species.

Palaeontologists often use structural morphology to classify species as being distinctly different and therefore different species. However, it is more likely that genetic similarities between species give a more accurate indication of whether they can interbreed. This is commonly seen with members of the *Canis* genus, which includes wolves and dogs. Both have 39 pairs of chromosomes. This means that they can **interbreed**, likely as a result of the chromosomes being able to pair up during meiosis. The same could have been the case for different classified species of humans, such as *H. sapiens, H. denisova, H. neanderthalensis* (and even *H. erectus*), as is discussed in this section.

Interbreed mate with an organism of another species (sometimes used between different genetic groups or populations)

Figure 11C–1 Wolves (left) and dogs (right) are members of the *Canis* genus and are capable of interbreeding to produce fertile offspring, likely due to them having the same number of chromosomes, 39 pairs.

It is therefore important to realise that views are constantly changing and being contested or refined as new evidence and analysis shed new light on what scientists currently understand about human evolution.

VIDEO 11C–1 INTERBREEDING BETWEEN HOMININS

Hominins that were capable of interbreeding with modern humans

Recent discoveries of hominin fossils, as well as earlier discoveries of Neanderthal fossils in the mid-19th century, along with analysis of their DNA sequences, have confirmed scientists' understanding that there was interbreeding between species. These discoveries and analysis have also helped clarify scientists' understanding of the relationships between different species of hominins, when they were likely to have coexisted in a similar environment, and the migratory patterns of modern humans (explored in Section 11B).

LINK 11B MIGRATION OF MODERN HUMANS

The key examples presented below indicate how our understanding of the lineage of ancestors of *Homo sapiens* continues to be altered and adjusted in response to new discoveries and analysis.

Homo neanderthalensis

Fossilised remains of Neanderthals uncovered in Europe and the south-west regions of Asia indicate that they lived in these locations. However, no fossils have been uncovered in Africa.

Comparing the nuclear DNA genomes of Neanderthals with modern humans shows that they are 99.7% identical. Comparing the mtDNA of Neanderthals with populations of modern humans on different continents reveals a:

- 1–4% similarity between Neanderthals and non-African populations of modern humans
- 0% similarity between Neanderthals and African populations of modern humans.

This confirms that interbreeding occurred between Neanderthals and non-African populations of *H. sapiens* after *H. sapiens* had migrated from Africa. It was likely to have occurred during the second wave of human migration, between 80 000 and 40 000 years ago. This is shown in Figure 11C–2.

11B MIGRATION OF MODERN HUMANS LINK

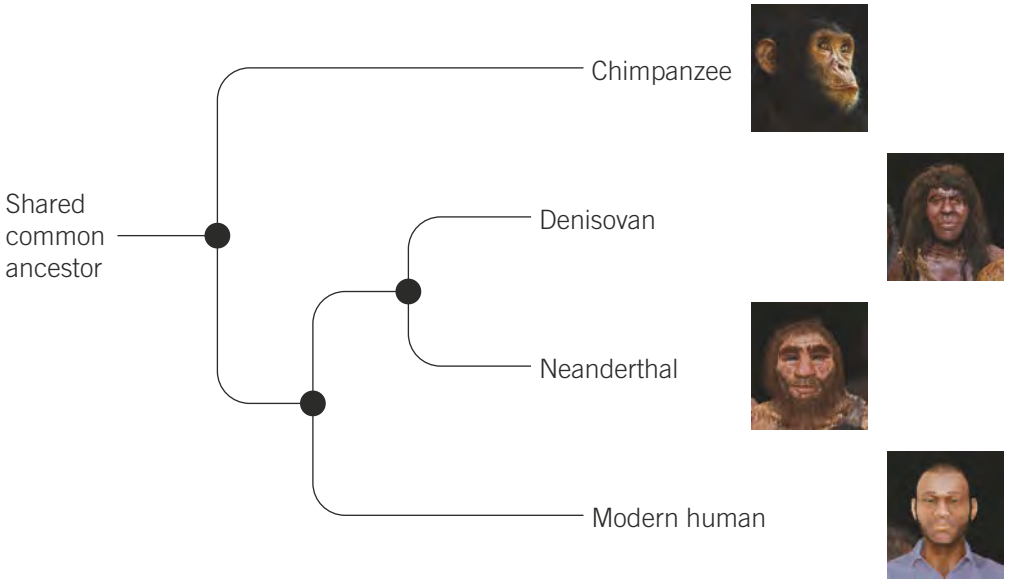


Figure 11C–2 This simple phylogenetic tree shows that Denisovans and Neanderthals share a recent common ancestor, and are also similar to modern humans. These four species all share a common ancestor that can be traced back to approximately 7 million years ago.

However, comparison of the number 21 chromosome between Neanderthals and modern humans, from two Neanderthal fossils discovered in Europe in 2016, indicates that this interbreeding may actually have occurred earlier than previously thought, possibly as early as 100 000 years ago, likely towards the end of the first wave of human migration in the Middle East.

Scientists theorise that there would have actually been more than one point in time when this interbreeding between the two species occurred.

Many questions remain unanswered. For example, why did Neanderthals become extinct, even though modern humans, with whom they coexisted, survived and continued to thrive? Did the two species compete? Or were there other reasons, such as changing environmental climate or *H. sapiens* having greater cognitive capabilities, such as problem solving and forward planning?

Check-in questions – Set 1

1 Define interbreeding.
2 Current-day African populations do not share any similarities in their DNA with Neanderthals. Explain why this is the case.

Homo denisova

The *Homo denisova* species was classified based on fossil evidence of only a finger bone and two teeth, discovered in Denisova Cave, Siberia in 2010.

The shape of the finger bone indicated that this species was robust and similar to Neanderthals. The mtDNA, which was well preserved thanks to the very cold and dry environment within the cave, further confirmed that this was a distinct species, although it had a close similarity to both *H. neanderthalensis* and *H. sapiens*.

LINK 10B FOSSILS

In the same Siberian cave, scientists also uncovered fossilised remains of both *H. neanderthalensis* and *H. sapiens*. Comparisons of mtDNA sequences showed that the mtDNA genomes of the Denisovans were 17% identical to the Neanderthals, suggesting that at some point these two species successfully interbred.

Further comparison of the nuclear DNA genomes of *H. denisova* and certain modern-day populations of humans has provided definitive evidence that they share approximately 4–6% of this DNA. These populations are the Melanesians (inhabitants of Indonesia, Papua New Guinea, Solomon Islands, Vanuatu, Fiji and New Caledonia), as well as Aboriginal and Torres Strait Islander peoples. Therefore, there was likely interbreeding at some point with ancestors of these species as they migrated through the southern and south-eastern parts of Asia, before they arrived in their current island locations. This is thought to have been around 45 000 years ago (Figure 11C–4).

LINK 11B MIGRATION OF MODERN HUMANS

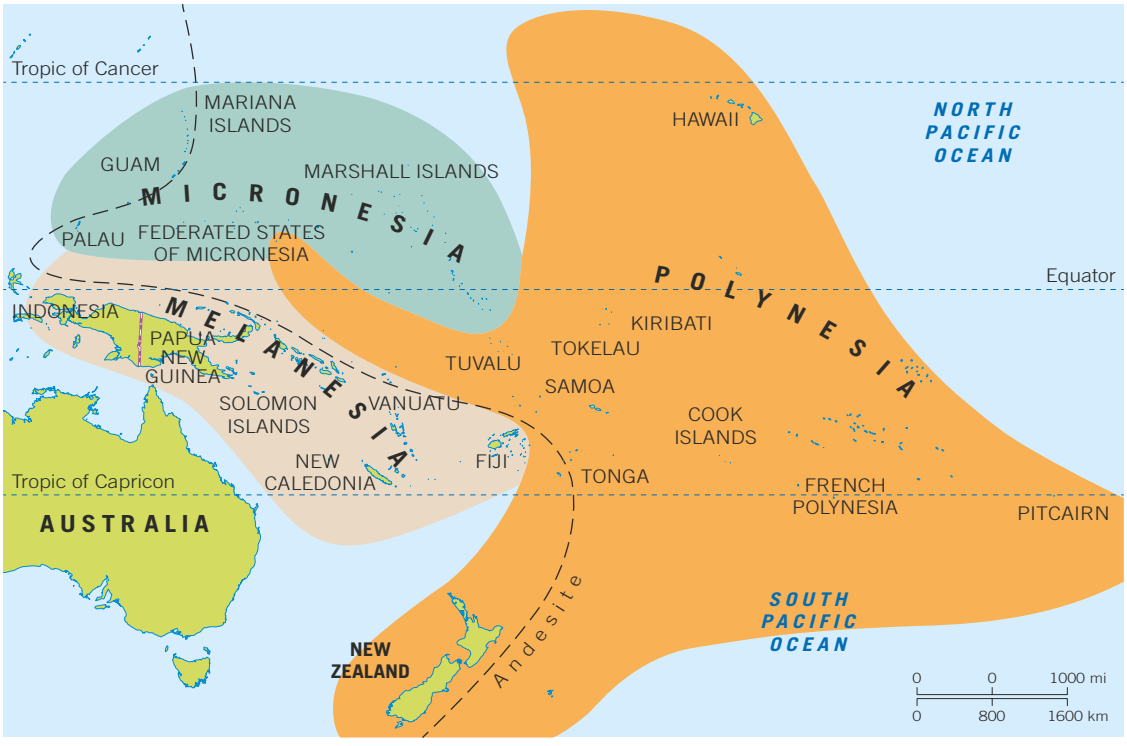
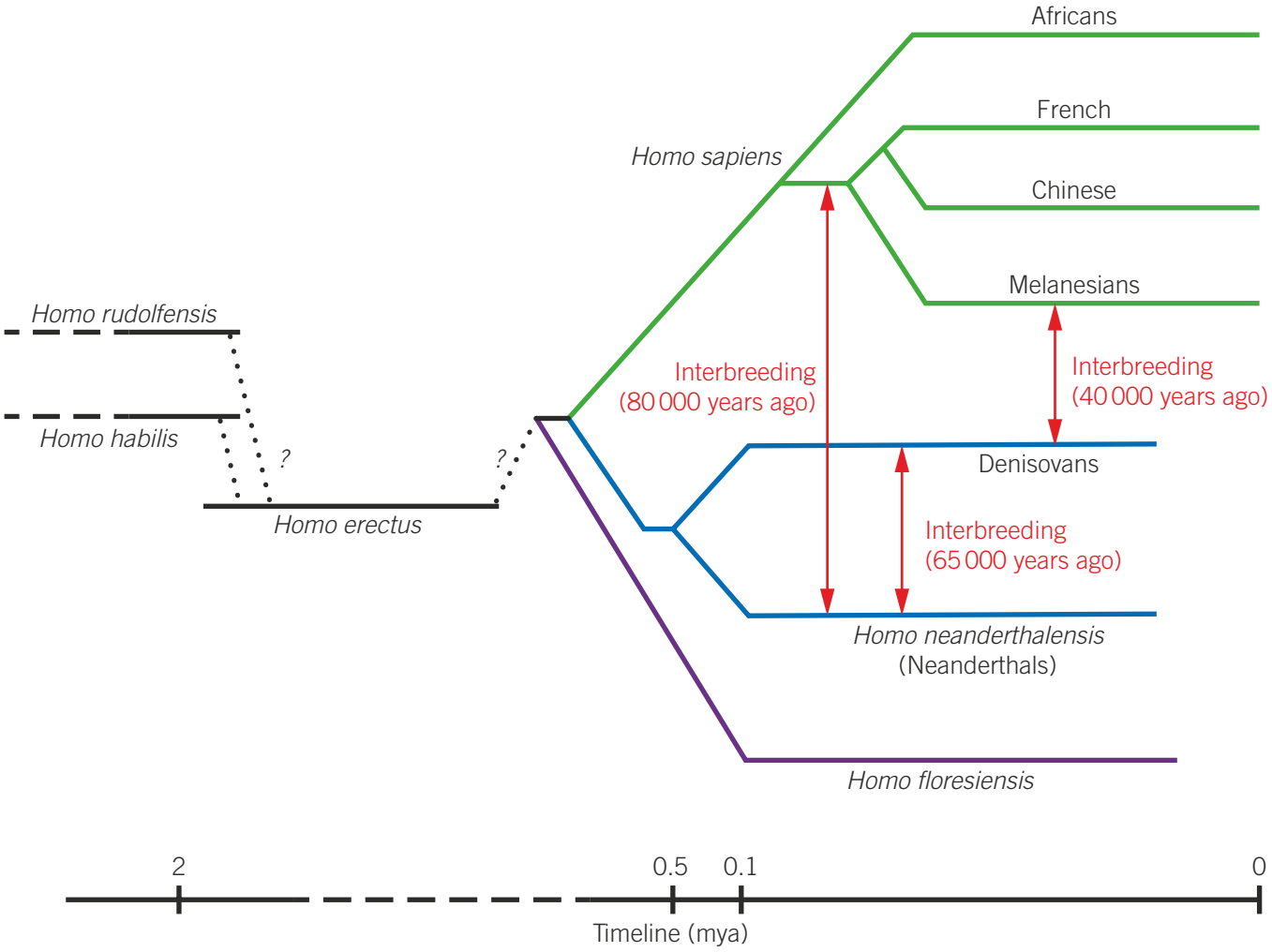


Figure 11C–3 The Pacific region north and north-east of Australia, including areas of Micronesian, Polynesian and Melanesian populations. It is the Melanesian genomes that have shown similarity to the Denisovan genome.

9D EMERGENCE OF A NEW SPECIES LINK
11B MIGRATION OF MODERN HUMANS LINK

Further comparisons of the genomes of other Asian populations, including Chinese, show that *most* of these populations do not contain any signs of Denisovan DNA. However, there is evidence of Neanderthal DNA in their genomes, indicating that there was interbreeding between their ancestors approximately 80 000 years ago. Therefore, it is possible that even within this second wave of human migration out of Africa, there may have been multiple movements of ancestral humans through different regions. These postulated migratory patterns (or gene flow, which you learnt about in Sections 9D and 11B) are constantly being updated and refined as scientists analyse new evidence.



Not drawn to scale. Not all lineages in the hominin family tree are shown.

Figure 11C–4 Phylogenetic tree showing the estimated timeline and likely interbreeding between different species. It also shows that interbreeding occurred between Denisovans and Neanderthals approximately 65 000 years ago, and that *Homo floresiensis* diverged earlier (but does not indicate any evidence of interbreeding that may have occurred between this species and modern humans).

Homo naledi

In 2013, palaeontologists discovered bone fragments from 15 specimens in the Rising Star Cave in Johannesburg, South Africa. It was classified as a member of the genus *Homo*, due to features that indicated bipedalism, and given the species name *naledi*, meaning 'star'. These fragments also exhibited features that resembled the *Australopithecus* genus. A comparison of these features is outlined in Table 11C–1.

Figure 11C–5 Facial reconstruction of *Homo naledi* from fossilised fragments found in the Rising Star Cave, Johannesburg, in 2013

Table 11C–1 *Australopithecus*-like and *Homo*-like features in the remains of *Homo naledi* uncovered in South Africa

Homo-like features	*Australopithecus*-like features
Big toe aligned with rest of toes, suggesting they were bipedal	Pelvis not as bowl-shaped
Similar dextrous hands suggesting tool use and manipulation of objects	Long curved fingers, suggesting they were tree climbers
Shorter arm-to-leg length ratio	Large shoulder blades and wider rib cage
Skull shape	Small skull size

From the fossils uncovered, there was much contention about exactly when *Homo naledi* lived, mostly due to the fact that when this fossil was discovered, radiometric dating was not undertaken. As a result, some scientific groups believed that features resembling both the Australopithicines and more modern humans placed *H. naledi* in the evolutionary timeline as a transitional species. However, other scientific groups believed that this species would have lived more recently, as use of tools is a more complex skill that relied on a larger and more developed brain.

LINK 10B FOSSILS

In 2017, radiometric dating of the flowstone (calcite and other minerals that form when water falls down the walls or flows along the floor of a cave) surrounding the fossil dated this to 236 000 years. However, there was no flowstone below the fossil, so some of the other sedimentary rock and teeth remains were dated, arriving at an oldest possible date of 335 000 years. This confirmed that *H. naledi* existed more recently and may have coexisted with *H. sapiens* for some time during the latter parts of their existence, as shown in Figure 11C–6. This figure also indicates the other possible placement of this species in our evolutionary timeline, based on the differing views of various scientific groups.

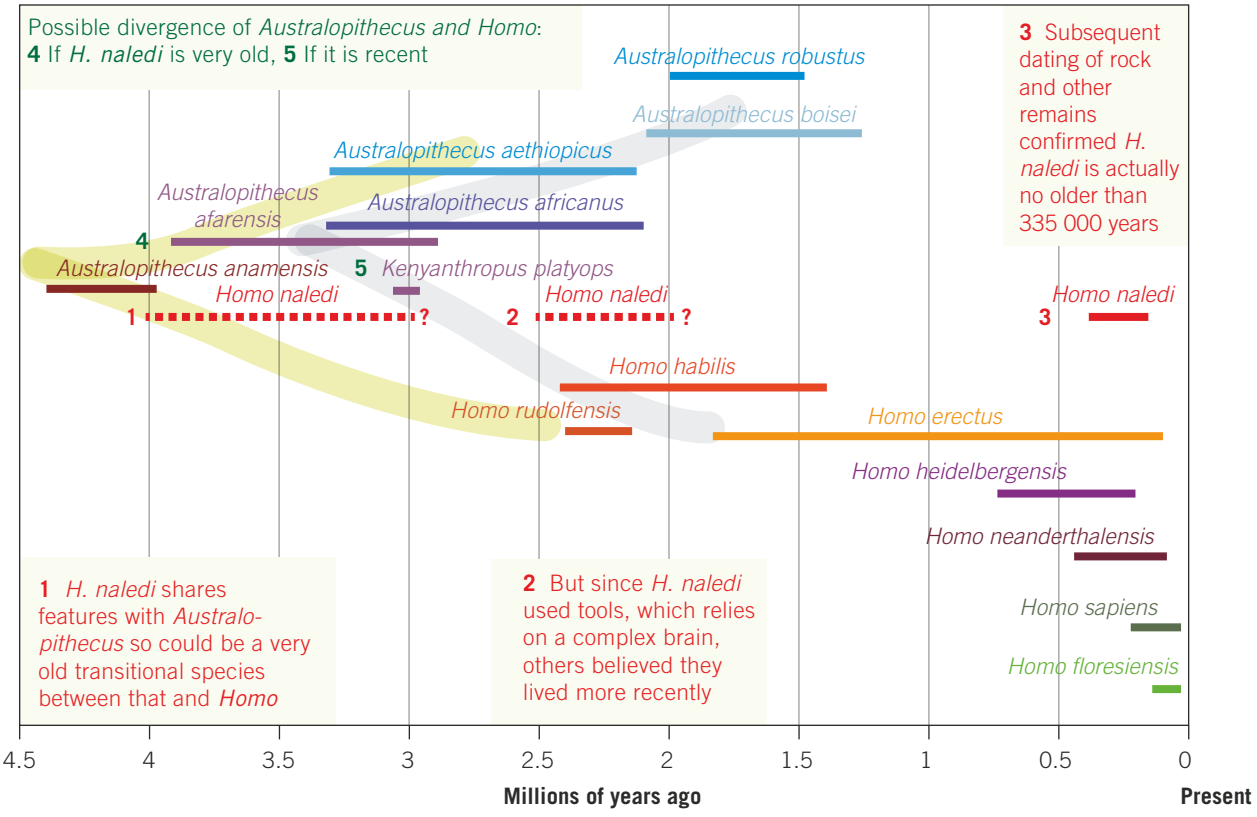


Figure 11C–6 Simplified timeline of the evolution of hominins in the past 4 million years, with three possible timelines for the placement of *Homo naledi* (shown in red) based on differing views presented by different scientific groups

The placement of the bones in the cave also supports this later date, as it indicated that the cave may have been used as a burial site, suggesting more complex behaviour and thought, unseen in the *Australopithecus* genus, with a similar small brain size.

No analysis of the *H. naledi* genome has been done yet to indicate whether there was interbreeding between *H. naledi* and other species, including *H. sapiens*.

Check-in questions – Set 2

WORKSHEET 11C–1 INTERBREEDING BETWEEN DIFFERENT HOMININ SPECIES

1. Give two examples of countries located in the Melanesian region.
2. *Homo denisova* share similarities in DNA sequences with modern Indigenous populations of Melanesians. Identify where and when interbreeding was likely to have occurred.
3. With which other species of hominin do the Denisovans share similarities in their DNA?
4. What evidence suggested to scientists that fossilised remains of *Homo naledi* were in fact a member of the *Homo* genus, and not the *Australopithecus* genus?

Summary

Putative a term used to describe something that is expected or assumed to have existed, without any current direct proof

The information presented in this section is just a small sample of the structural and molecular evidence for relationships and interbreeding patterns between these species and both modern humans and known ancestral species. There is undoubtedly more of the story to be told and this will continue to develop and be rewritten as evidence of new **putative** species is uncovered.

11C SKILLS

VIDEO 11C–2 SKILLS: ADVANTAGES OF EVIDENCE FOR EVOLUTION

Understanding the advantages of different pieces of evidence for evolution

A key aspect of this section is the use of different forms of evidence for evolution. The most common examples studied in this section include:

- fossils that demonstrate homologous structures with other members of the same or a different genus
- molecular-based comparisons using both nuclear and mitochondrial DNA.

10B FOSSILS LINK

10C EVIDENCE FOR EVOLUTION LINK

Each of these forms of evidence has assisted the development of scientists' understanding of both the migration of hominins and their interbreeding capabilities.

Whether comparing mitochondrial DNA or nuclear DNA between different hominins, each method has its advantages. Some of these are outlined in Table 11C–2.

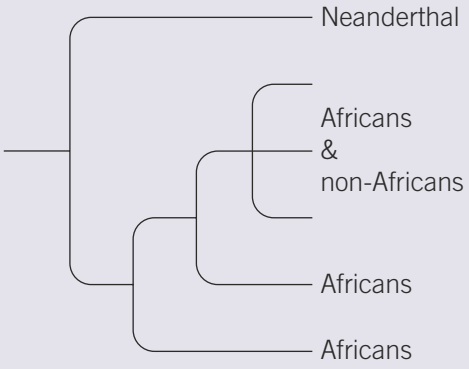
Table 11C–2 Advantages of using mitochondrial DNA and nuclear DNA in human evolution studies

Mitochondrial DNA	Nuclear DNA
Inherited from maternal line mainly, so uninterrupted track of direct ancestors	Contains DNA inherited from both parents
High copy number as there are often many mitochondria within each cell	Larger genome so more DNA regions/genes to compare
Faster rate of mutation, so can directly assess the number of differences between even closely related species	

It is vital that you are able to use evidence presented in the information that accompanies a question to make assumptions about whether interbreeding between particular hominins did or did not occur. For example, consider the following information and subsequent question.

Information

The mtDNA of various living human populations has been analysed. The mtDNA of these populations was then compared to the extinct hominin species, *Homo neanderthalensis*. A phylogenetic tree based on these mtDNA comparisons was developed and is shown in the figure below.



The phylogenetic tree shows that there is no similarity between Neanderthal mtDNA and the mtDNA of various current African populations. It also shows that there is a great deal of variation in the mtDNA of African populations, further emphasising that modern humans evolved in Africa. The mtDNA of other human populations, like Europeans and Chinese, was also analysed. Although not shown in the figure above, a small percentage, approximately 1–3%, of their mtDNA was similar to Neanderthals.

Question

Does the information presented above indicate that ancestors of current-day African populations interbred with Neanderthals? Explain your answer.

To answer this question, you need to be able to identify some important points:

1 The location of the Neanderthals' existence, which was mostly in Europe.
2 The lack of similarity between the mtDNA in Neanderthals and current African populations.
3 The small percentage of similarity between the mtDNA of Neanderthals and populations found outside Africa today.
4 What comparing DNA sequences between different organisms or species allows scientists to determine.

Answer

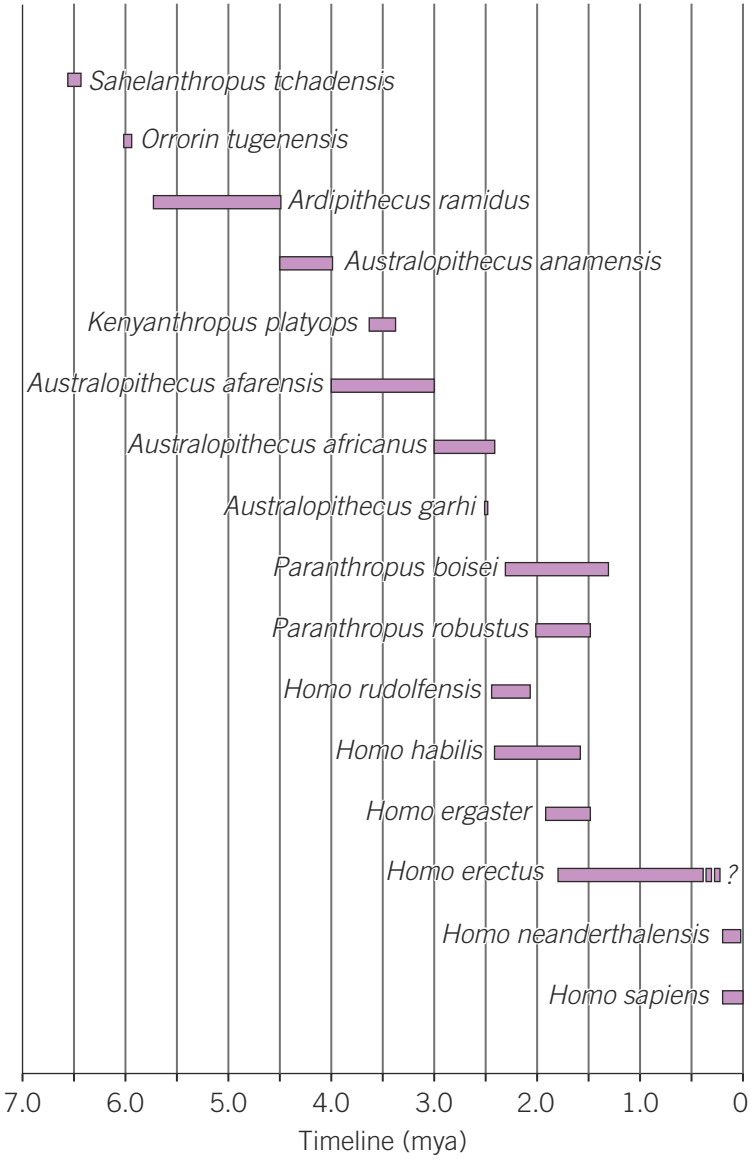
The information indicates that there was no interbreeding between Neanderthals and ancestors of current African populations. This is due to the migration of ancestors of modern humans from Africa to regions such as Europe after Neanderthals had inhabited that area. These ancestors of modern humans then remained or moved to regions further into Europe, but not back to Africa, where those populations continued to evolve independently.

You can see from this answer that the information you have learnt in Sections 11B and 11C is very much intertwined, and you may be required to use knowledge from both sections in your answer. Further questions analysing this can be found in the end-of-chapter questions.

LINK **11B** MIGRATION OF MODERN HUMANS

Section 11C questions

1 The diagram below shows the evolution of hominins. The coloured horizontal bars next to each fossil show the time period (displayed on the *x*-axis) during which each hominin existed.



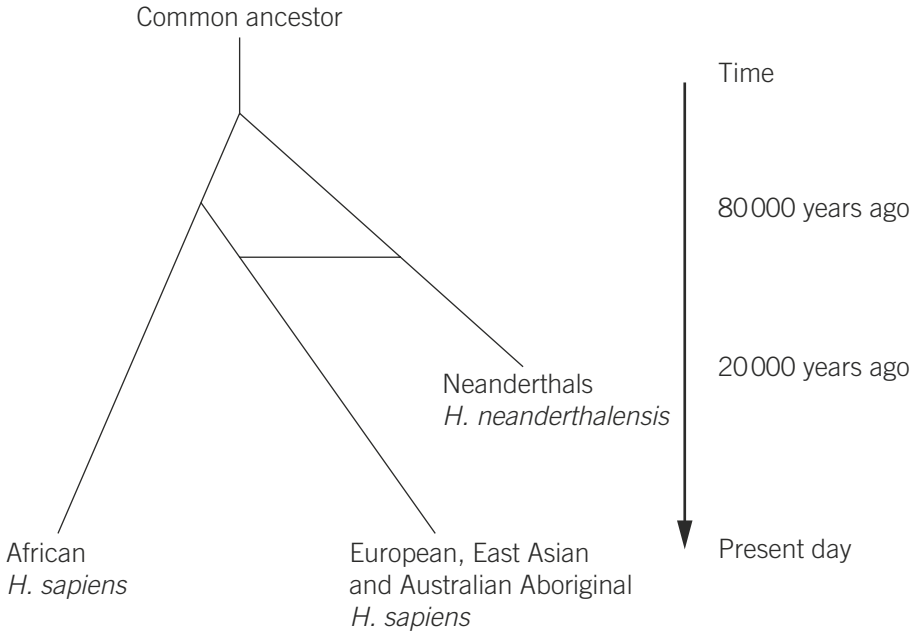
a What is the oldest hominin species shown in the diagram, and how long ago did it exist?

b Based on the diagram, identify two other hominin species that *Homo habilis* coexisted with between 2.0 and 2.5 mya.

c *Homo sapiens* coexisted with *Homo neanderthalensis* and some interbreeding may have occurred. Explain how DNA analysis could show that interbreeding may have occurred.

d *Homo neanderthalensis* and *Homo sapiens* were the last two existing hominins until *H. neanderthalensis* became extinct. State a reason that explains the extinction of the Neanderthals.

2 Evidence indicates that between 100 000 and 60 000 years ago, modern humans who had moved out of Africa lived in close proximity to the Neanderthals in parts of the Middle East and Europe.
Scientists developed the phylogenetic tree pictured below to represent the interaction between Neanderthals and current-day populations living in different regions.



When scientists compared the DNA of Neanderthals with that of modern-day Europeans, East Asians and Aboriginal Australians, they found a small percentage of similarity between them.

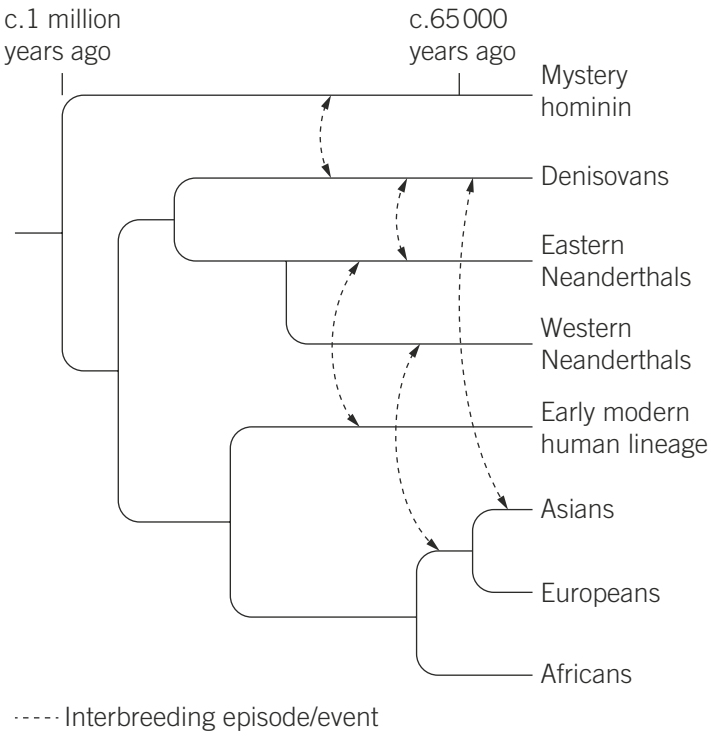
a Explain the reason for this small percentage of similarity between the DNA sequences observed and how long this has likely been present within members of these populations.

b Which type of DNA do you think was used for this analysis, mtDNA or nuclear DNA?

c Provide reasons to support your choice of DNA in part **b**, including a reason *not* to use the type of DNA you didn't select.

d Why is there no evidence of Neanderthal DNA in current African populations? Explain by referring to the migration patterns of *Homo sapiens*.

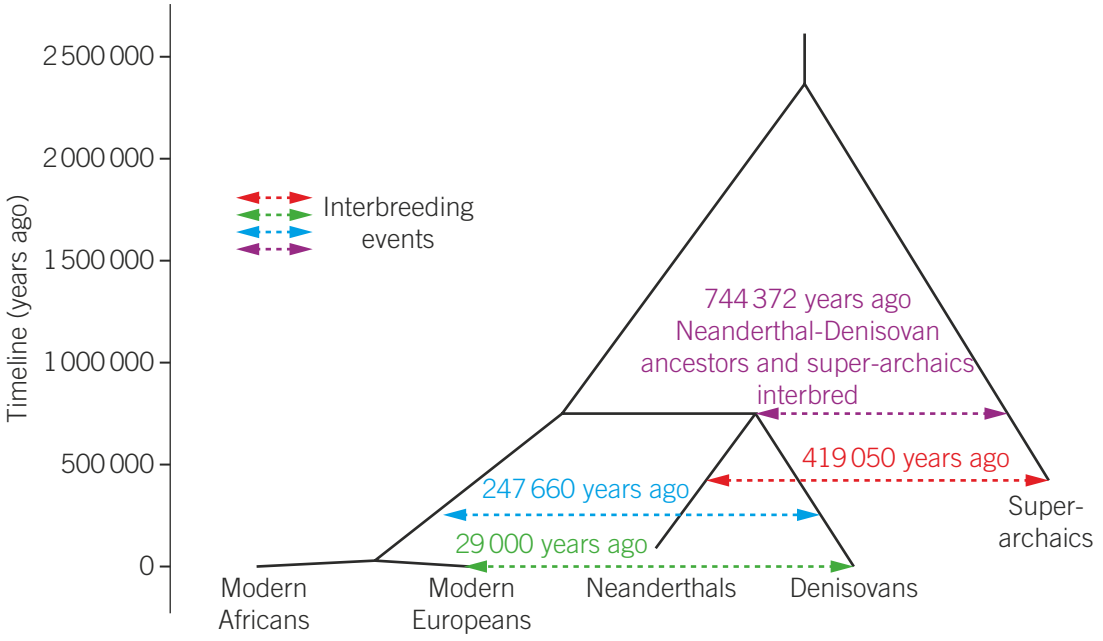
e How does this affect the definition of a species you learnt about in Section 9D?



3 The phylogenetic tree shown on the left indicates the divergence of different species, as well as when and if interbreeding occurred between these different hominins. (Note: the timeline is not drawn to scale.)

- **a** According to this phylogenetic tree, how many interbreeding events have occurred?
- **b** Which two species would be expected to be most similar? Explain.
- **c** Would you expect any similarities in the DNA sequences between Denisovans and Neanderthals? Explain.
- **d** According to this phylogenetic tree, how many speciation events occurred?

Another group of scientists proposed another phylogenetic tree for the interbreeding that occurred between Neanderthals, Denisovans and ancestors of modern humans. This is shown below.



- **e** How is this phylogenetic tree different from the first one provided in this question?
- **f** Is the 'super-archaics' hominin likely to be a member of the *Homo* genus or the *Australopithecus* genus? Explain.
- **g** Upon closer inspection of the Denisovans in this phylogenetic tree, what do you notice as being incorrect about their existence? Use your understanding of the definition of hominins and your knowledge of the approximate timelines for different species to support your answer.

Chapter 11 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
11A.1	Understand the difference between mammals, primates, hominoids and hominins	11a☐
11A.2	Classifying organisms as mammals, primates, hominoids and/or hominins	3☐, 11b☐
11A.3	Identify and explain the characteristics that define each of the classification levels	11c☐, 12d☐
11A.4	Identify skeletal structural changes in hominins that support bipedalism	11d☐, e☐, f☐, 12a☐, b☐
11A.5	Compare and explain how structural changes have enhanced bipedalism and hominin survival for the *Australopithecus* and *Homo* genuses	2☐, 11f☐
11A.6	Identify skull structural changes that indicate changes in brain size	1☐, 11f☐, 12a☐, 16b☐, f☐
11A.7	Compare and explain skull changes in the *Australopithecus* and *Homo* genuses	11f☐, 12b☐, c☐, d☐
11A.8	Recall the names, key features and approximate time of existence of different hominin species	5☐, 12c☐, 14b☐, c☐, 15b☐
11B.1	Recall the use of fossil and DNA evidence to classify organisms	13b☐, 14d☐, 15a☐, 17a☐
11B.2	Explain the difference between nuclear and mtDNA, including the advantages and disadvantages of using each	13d☐, 17b☐
11B.3	Apply knowledge of evidence to explain migration patterns of different human populations	6☐, 7☐, 13g☐, h☐, 14a☐
11B.4	Apply knowledge of evidence to explain the migration of Aboriginal and Torres Strait Islander populations	18a☐, b☐
11B.5	Identify examples of Aboriginal and Torres Strait Islanders' Connection to Country and Place	9☐, 10☐, 18c☐
11C.1	Recall types of evidence used to interpret hominin evolution	4☐, 15b☐, c☐
11C.2	Apply knowledge of hominin evolution to refine the evolutionary lineage of *Homo sapiens* and other hominin ancestors	13c☐
11C.3	Recall evidence for the interbreeding of *Homo sapiens* and *Homo neanderthalensis*	14e☐

Success criteria – I am now able to:		Linked question
11C.4	Apply understanding of the relationship of *Homo sapiens* with other hominin species	6☐, 7☐
11C.5	Interpret evidence and information to appropriately classify newly discovered *Homo* species	7☐, 16a☐

Multiple-choice questions

1 *Homo neanderthalensis* fossils have a larger brain case than *Homo sapiens* fossils found from around the same time. However, it is believed that *H. sapiens* out-competed *H. neanderthalensis*, benefitting from more complex thought processes in relation to food gathering and distribution of labour, to the point of extinction of *H. neanderthalensis*. Select the best explanation for this from the options below.

A *H. neanderthalensis* skeletons were bigger overall than *H. sapiens.*

B *H. neanderthalensis* had a smaller brain case to jaw ratio than *H. sapiens.*

C Despite greater overall brain size, *H. neanderthalensis* had a less developed cerebral cortex than *H. sapiens.*

D *H. neanderthalensis* spent too much time thinking about hunting and not enough time thinking about raising their young.

2 The fossil popularly known as Lucy is one example of an early hominin. This fossil included a skull and other bones of the body, such as the pelvis and some leg bones. Compared with a modern human, which of the following features would Lucy be expected to show?

A larger brain capacity

B smaller teeth

C narrower pelvis

D longer legs

3 Key elements in classifying an ape fossil as hominin include

A location of the foramen magnum.

B presence of pre-molar teeth.

C presence of a lower jaw made of a single bone.

D presence of an opposable digit.

4 Which of the following pieces of information would be *least* reliable in determining that two hominin species are closely related?

A The DNA base sequence of the two species differ by 1%.

B The two species share an analogous structure.

C The amino acid sequence of a peptide hormone is the same in both species.

D Analysis of the fossil remains shows that both species closely resemble an extinct species.

5 Which of the following correctly identifies the species, structural feature, functional ability and resulting cultural evolution?

A *Homo habilis*, central foramen magnum, bipedal, use of basic stone tools

B *Pan troglodytes*, uniform teeth, grinding seeds, use of stone tools

C *Australopithecus afarensis*, large brain case, complex language, use of fire

D *Homo neanderthalensis*, large brain case, complex thought, cave drawings

6 Modern non-African *Homo sapiens* have a small percentage of Neanderthal DNA as a result of ancestral interbreeding, whereas modern African *H. sapiens* have no Neanderthal DNA. This interbreeding between the two hominins was initially though to have occurred around 65 000 years ago. Palaeantologists have found *H. sapiens* DNA in the genomes of 100 000-year-old Neanderthal remains. From this study, it would be reasonable to assume that

A Neanderthals are the ancestors of modern humans.

B there was an earlier wave of migration of *H. sapiens* from Africa that occurred prior to 100 000 years ago.

C the ancestors of modern African populations migrated from Europe back to Africa before 65 000 years ago.

D approximately 100 000 years ago, the Neanderthals interbred with *H. sapiens* within Africa, before the Neanderthals then migrated to the Middle East and Europe.

Use the information below to answer Questions 7 and 8.

A new species of ancestral hominin was recently identified in the Philippines from a discovery of a total of seven teeth and six small bone fragments, one of which was part of the femur. This minimal evidence suggested that it was a new species that had not been discovered previously by scientists. They named it *Homo luzonensis*. It was dated to approximately 65 000–50 000 years ago.

7 Which of the following statements supports the current understanding of the migration patterns of ancestors of modern humans?

A The dating of this fossil provides further evidence of the migration of modern humans from Africa to south-eastern Asia from 100 000 years ago.

B This species would be closely related to *Homo neanderthalensis,* which existed in regions of Europe at a similar time.

C This species would be the ancestor of Indigenous Australians, as the natural progression from the Philippines to Australia would have occurred.

D This species would share some DNA with all hominin species uncovered in Africa.

8 From the information provided and your knowledge of Unit 4, which of the following dating techniques could have been used on this fossil?

A comparison of vestigial structures

B carbon-14 dating

C use of index fossils present with the fossilised fragments of bone

D absolute dating using stratigraphy

Use the information below to answer Questions 9 and 10.

Fossils of early Aboriginal peoples were discovered in sedimentary layers near Lake Mungo in New South Wales. These bones were dated to 40 000 years ago. Comparisons of these bones to those of *Homo floresiensis* found on the island of Flores in Indonesia show that these early Aboriginal Australians were much larger in body size and had more signs of cultural evolution. These signs of cultural evolution also provide scientists with an understanding of 'Connection to Country'.

9 Evidence for this cultural evolution of Aboriginal peoples would include

A footprints, indicating that they were bipedal.

B fragments of hand bones, indicating that they had an opposable thumb.

C the discovery of bones from many individuals in one location.

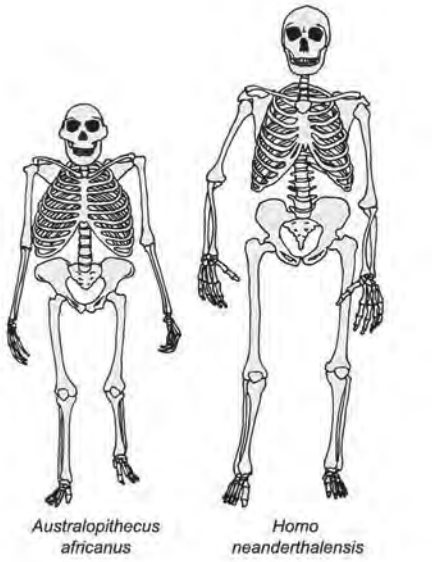
D tools that would have been used for artwork, such as pots.

10 Which of the following is *least* likely to be an example of how early Aboriginal Australians demonstrated their Connection to Country?

- **A** burning of the landscape to promote new growth of vegetation, which attracts other animals
- **B** footprints leading towards and away from Lake Mungo
- **C** the presence of artwork or paintings, and stories shared between generations
- **D** aeration of the soil, loosening it for further seed germination

Short-answer questions

11 The figure shows two hominins, *Australopithecus africanus* and *Homo neanderthalensis*. Both species are now extinct.



VCE Biology Sample Exam (2017), Section B, Q. 8; © VCAA

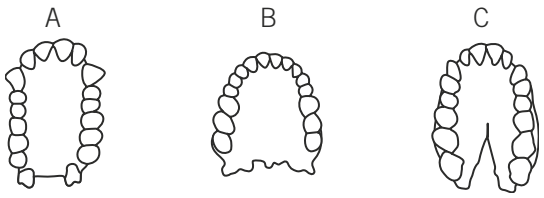
Both hominins can also be classified as members of the Hominid and Primate taxonomy groups.

- **a** Define hominin. (1 mark)
- **b** Give an example of another organism in each of the classification groups Hominids and Primates. (1 mark)
- **c** Using the skeletons of the two species, identify a feature common to all primates. (1 mark)
- **d** Using the skeletons of the two species, identify a feature common to all hominids. This should be a different feature from your answer to part **c**. (1 mark)
- **e** Using evidence from the skeletal structures of these two species, were they bipedal or quadrupedal? (1 mark)
- **f** Using evidence from their skeletons, describe how two features have changed over time and the advantage they gave to the more recent of the two species. (3 marks)

12 Over time, significant changes in the locomotion of hominins and their brain volume occurred.

- **a** Outline the general trend observed for each of these. (2 marks)
- **b** Using your understanding, which of the two changes appeared first? Explain. (2 marks)

With a change in brain volume came other changes to regions of the skull. One of these changes was observed in the size and arrangement of the teeth. The figure below shows an inside view of the bottom jaw of three species: a chimpanzee, *Australopithecus africanus* and *Homo sapiens*.



- **c** Identify which species matches each of the structures A, B and C. (1 mark)
- **d** From your answer to **c**, justify the reason for the *Homo sapiens* teeth appearing the way they do. **c**. Explain how the biological and cultural evolution of *H. sapiens* led to this appearance. (3 marks)

13 Scientists uncovered the fossilised remains of a finger bone in a Siberian cave. The finger bone was well preserved due to the cold dry climate within the cave. Scientists then analysed nuclear DNA extracted from the bone cells. This analysis confirmed that the species was very closely related to both Neanderthals and modern humans, but genetically different enough to be classified as a new species. They named this species *Homo denisova*, also called 'Denisovans'.

Comparing this DNA to various modern human populations provided some intriguing results. The Denisovan genome was on average 4% similar to the genome of native members of Vanuatu.

However, scientists have not uncovered other evidence of this species in this region of the Pacific or in the south-eastern parts of Asia.

a Aside from the cold dry climate, what other conditions would have been required in the cave for the fossilised remains of this Denisovan to have formed? Name two of these. (2 marks)

b Why do you think scientists needed to use DNA analysis to confirm whether this species was an existing known hominin or a new hominin? (1 mark)

c Given that no other Denisovan remains have been discovered in the Pacific region, explain how modern-day people from Vanuatu contain a small percentage of Denisovan DNA. (2 marks)

d Explain why analysis of nuclear DNA, not mtDNA, was conducted. (3 marks)

One theory of the ancestory of the Denisovans and Neanderthals is that they diverged from their common ancestor, *Homo heidelbergensis*. Approximately 350 000 years ago, a small group of *H. heidelbergensis* individuals left Africa and moved into the region now known as the Middle East. Part of this group then moved north-west into Europe and over time became the Neanderthals. The other part of this group moved east into Asia and over time became the Denisovans. The *H. heidelbergensis* that remained in Africa eventually gave rise to *Homo sapiens* approximately 130 000 years ago, and these did not leave Africa until around 60 000 years ago.

e What is the name of the process that resulted in the appearance of both Neanderthals and Denisovans from *Homo heidelbergensis*? (1 mark)

f Explain the process you identified in part **e** with reference to the Denisovans specifically. (6 marks)

g The information states that modern humans appeared in Africa around 130 000 years ago. From this information, which wave of modern human migration would this appearance of *Homo sapiens* have correlated to? Explain your answer. (2 marks)

h Using the information presented in this question, which hominins would have been found at the following locations 100 000 years ago? Justify each answer with supporting information.

i Vanuatu (2 marks)

ii Europe (2 marks)

iii Africa (2 marks)

14 Recently discovered fossilised partial skulls from Ethiopia have raised new questions about early human evolution. This has led to different theories about the origins of *Homo sapiens*. One of these theories is illustrated in the following diagram.

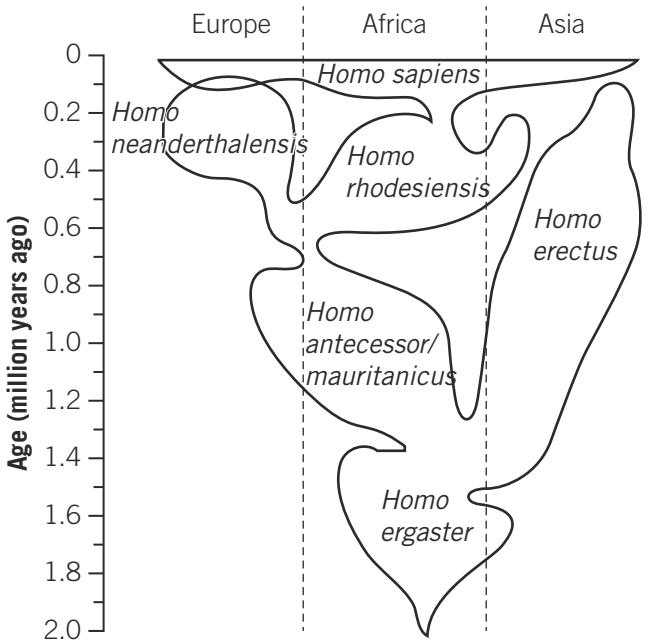
a Identify the species that shows the greatest geographical distribution. (1 mark)

b How does the data indicate that the *Homo* genus originated in Africa? (2 marks)

c Using the data provided, analyse the relationship between *Homo neanderthalensis* and *Homo sapiens*. (2 marks)

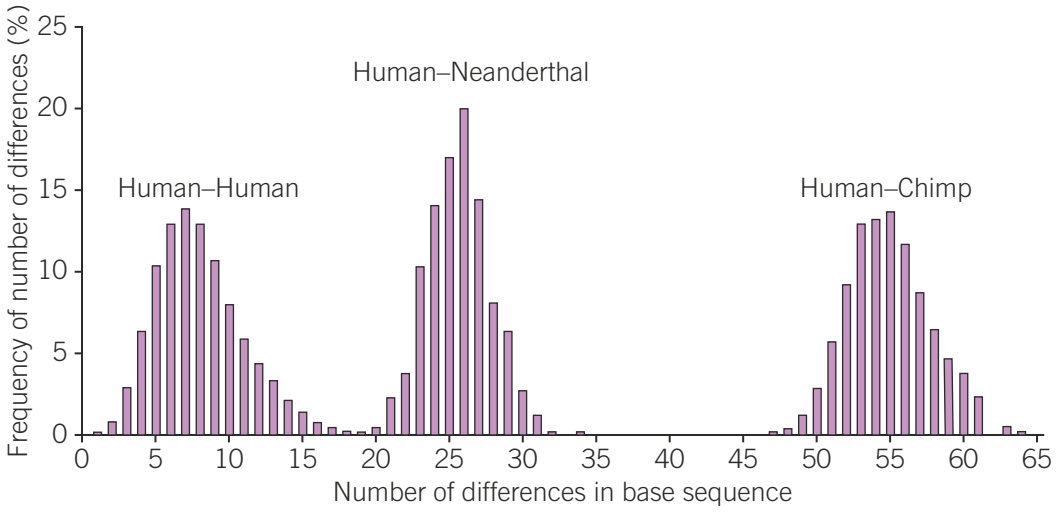
d Evaluate whether *Homo antecessor/mauritanicus* or *Homo erectus* would likely have provided the most fossil evidence. Explain your answer. (2 marks)

e What evidence from this diagram suggests that people with non-African heritage carry some Neanderthal DNA? Write a hypothesis to account for this finding. (2 marks)



15 The evolution of groups of living organisms can be studied by comparing the base sequences of their DNA. If a species becomes separated into two groups, differences in base sequence between the two species accumulate gradually over long periods of time. The number of differences can be used as an evolutionary clock.

Samples of DNA were recently obtained from fossil bones of a Neanderthal (*Homo neanderthalensis*). A section of the DNA from the mitochondrion was chosen for study, as it shows a high level of variation in base sequence between different individuals. A section of the Neanderthal mitochondrial DNA was sequenced and compared with sequences from 994 humans and 16 chimpanzees. The bar chart below shows how many base sequence differences were found among humans, between the humans and the Neanderthal, and between humans and chimpanzees.



a The number of differences in base sequence between pairs of humans varied from 1 to 24. State the number of differences shown by the highest percentage of pairs of humans. (1 mark)

b Humans and Neanderthals are both classified in the genus *Homo* and chimpanzees are classified in the genus *Pan*. Discuss whether this classification is supported by the data in the bar chart. (3 marks)

c Data suggests that humans and Neanderthals diverged 550 000–700 000 years ago. If a sample of mitochondrial DNA was obtained from a fossil bone of *Australopithecus*, predict, providing your reason, how many base sequence differences there would be between it and human DNA. (2 marks)

16 Palaeontologists hit the jackpot when they are able to find fossils of new putative species of hominins. The table below provides a sample of primate fossils that were well preserved, enabling palaeontologists to determine an approximate body mass and brain volume. The table also shows the approximate dates (in million years ago, mya) of when these species existed.

Body mass, brain volume and date of existence for various hominin fossils

Species	Date existed (mya)	Body mass (kg)		Average brain volume (cm^3)
		Male	Female	
Australopithecus afarensis	3.5	45	29	440
Australopithecus africanus	2.5	41	30	450
Australopithecus aethiopicus	2.4			400
Homo habilis	1.8	52	32	575
Homo ergaster	1.8	58	52	800
Homo erectus	0.5	60	55	1100
Homo neanderthalensis	0.05	80	65	1550
Homo sapiens	0	58	49	1400

a Define putative. (1 mark)

b Identify, giving a reason, which hominin species is the least closely related to *Homo sapiens*. (2 marks)

c Suggest one reason why there is no data provided for the body mass of males and females for *Australopithecus aethiopicus*. (1 mark)

d Is there a correlation between body weight and brain volume? Explain by referring to the data provided. (2 marks)

e Draw a graph that shows the timeline for the existence of these hominins and their change in brain volume. Your graph should be the correct type of graph (e.g. line or column) and be fully labeled. (3 marks)

f Using the data provided in the table, explain why brain volume alone is not an accurate indicator of the intelligence of a species. (2 marks)

17 One hypothesis used to explain how modern human beings arose is the Out of Africa hypothesis. This states that *Homo sapiens* arose in Africa and then migrated to other regions. Evidence for this includes the following:

- Transitional fossils of forms of hominins from ancestral to modern humans were found only in Africa.
- Variation in mitochondrial DNA is greater in African populations.

a Choose one of the pieces of evidence above and explain how that statement supports the hypothesis. (1 mark)

b Why is analysis of mitochondrial DNA, rather than nuclear DNA, often useful in determining evolutionary relationships? (1 mark)

18 Refer to the article 'First Australians arrived in an incredible planned migration with over 1300 people' at the end of Section 11B.

a Using information from the article, describe the route taken by ancestors of Aboriginal and Torres Strait Islander peoples before first arriving at Australia. (2 marks)

b What evidence from the article supports the migration of the ancestors of Aboriginal and Torres Strait Islander peoples along the route provided in your answer to part **a**? (1 mark)

UNIT 4

HOW DOES LIFE CHANGE AND RESPOND TO CHALLENGES?

CHAPTER 12

SCIENTIFIC INVESTIGATIONS

Introduction

Scientific investigation (practical) skills and understanding are fundamental to the daily work of a scientist. As a component of your assessment for Units 3 and 4, you will be required to design or adapt an investigation related to cellular processes and/or how life changes and responds to challenges. You will be required to conduct this as a practical investigation, followed by the production of a scientific poster, as this is a key requirement for school-assessed coursework (SAC) in Unit 4. Many VCAA exam questions focus on practical understanding and skills. This is an opportunity for you to develop your knowledge and skills in this area.

This chapter outlines how to plan, conduct and present the results of a scientific investigation, and provides useful tips and examples along the way. The digital resource contains additional information about alternative formats for demonstrating your understanding of cellular processes or how life changes and responds to challenges, which may be used as the basis for different assessments. These include:

- preparing an article for scientific publication
- preparing an oral, multimedia or visual presentation.

Curriculum

Area of Study 3 Outcome 3

Study Design	Learning intentions – at the end of this chapter I will be able to:
Investigation design • Authentication of generated primary data through the use of a logbook	**12A Investigative design** **12A.1** Document investigations appropriately using a logbook
• Biological concepts specific to the selected scientific investigation and their significance, including definitions of key terms	**12A.2** Define key terms related to scientific skills

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Characteristics of the selected scientific methodology and method, and appropriateness of the use of independent, dependent and controlled variables in the selected scientific investigation	**12A.3** Define controlled experiment **12A.4** List the features that a controlled experiment includes **12A.5** Distinguish between a positive and a negative control **12A.6** Understand the meaning of single variable exploration **12A.7** Define and identify independent, dependent and controlled variables **12A.8** Select and use equipment and procedures appropriate to an investigation
Scientific evidence • The nature of evidence that supports or refutes a hypothesis, model, or theory	**12A.9** Distinguish between an aim and a hypothesis **12A.10** Construct aims and questions for investigations **12A.11** Formulate hypotheses and predict possible outcomes
Investigation design • The accuracy, precision, reproducibility, repeatability and validity of measurements	**12A.12** Define reproducibility, repeatability and validity, and distinguish between these **12A.13** Design an investigation that accounts for accuracy and precision and is valid **12A.14** Design an experiment that is fully reproducible by others
• The health, safety and ethical guidelines relevant to the selected scientific investigation	**12A.15** Determine potential ethical issues with investigation design **12A.16** Identify how bias can be minimised in an investigation **12A.17** Follow clear guidelines for health and safety when undertaking practical investigations
Investigation design • Techniques of primary quantitative data generation relevant to the selected scientific investigation	**12B** **Scientific evidence** **12B.1** Define qualitative and quantitative **12B.2** Distinguish between qualitative and quantitative data **12B.3** Include appropriate units of measurement for quantitative data
Scientific evidence • Authentication of generated primary data through the use of a logbook **Investigation design** • The accuracy, precision, reproducibility, repeatability and validity of measurements	**12B.4** Analyse generated primary data to determine whether it is accurate and/or precise, and define these terms

Study Design	Learning intentions – at the end of this chapter I will be able to:
• Ways of organising, analysing and evaluating primary data to identify patterns and relationships including sources of error and uncertainty	**12B.5** Transform primary data into an appropriate format of results (table, flow chart, bar and/or line graph) **12B.6** Identify trends in data **12B.7** Define the different types of errors (random and systematic) **12B.8** Identify sources of error and outliers from primary data
• The nature of evidence that supports or refutes a hypothesis, model or theory	**12B.9** Use evidence to determine whether an investigation supports or discounts a hypothesis
• Assumptions and limitations of investigation methodology and/or data generation and/or analysis methods	**12B.10** Identify areas for improvement in investigation design and analysis to increase accuracy and precision and reduce the likelihood of errors
Science communication • Conventions of science communication: scientific terminology and representations, formulas, standard abbreviations and units of measurement • conventions of scientific poster presentation, including succinct communication of the selected scientific investigation and acknowledgements and references	**12C Scientific communication** **12C.1** Appropriately communicate all aspects of a scientific investigation **12C.2** Apply correct abbreviations to biological terminology **12C.3** Present a scientific poster in the mandated format
• The key findings and implications of the selected scientific investigation	**12C.4** Justify conclusions and evaluate whether evidence supports or refutes the hypothesis **12C.5** Interpret investigation outcomes in terms of broader biological concepts

Glossary

Aim
Accuracy
Conclusion
Continuous data
Control group
Controlled variable
Dependent variable
Discrete data
Hypothesis
Independent variable
Introduction
Line of best fit
Method
Negative control
Outlier
Placebo
Positive control
Precision
Qualitative data
Quantitative data
Random error
Reliability
Repeatability
Reproducibility
Single-variable exploration
Systematic error
Title
True value
Validity

Investigative design

Study Design:

Investigation design

- Authentication of generated primary data through the use of a logbook
- Biological concepts specific to the selected scientific investigation and their significance, including definitions of key terms
- Characteristics of the selected scientific methodology and method, and appropriateness of the use of independent, dependent and controlled variables in the selected scientific investigation

Scientific evidence

- The nature of evidence that supports or refutes a hypothesis, model or theory

Investigation design

- The accuracy, precision, reproducibility, repeatability and validity of measurements
- The health, safety and ethical guidelines relevant to the selected scientific investigation

Glossary:

Aim
Control group
Controlled variable
Dependent variable
Hypothesis
Independent variable
Introduction
Method
Negative control
Placebo
Positive control
Reliability
Repeatability
Reproducibility
Single-variable exploration
Title
Validity

ENGAGE

Scientific breakthroughs

Scientists ask questions about things that interest them, trouble them or puzzle them. They plan for new investigations and adjust the practical investigations they are currently undertaking. They work both individually and in teams to share knowledge for the greater good of humans and the existence and survival of many other species. Some of the greatest discoveries in the past two centuries have come from this process, and include:

Figure 12A–1 Professor Ian Frazer at work in his biomedical laboratory in Brisbane

- the discovery of the structure of DNA by James Watson, Francis Crick, Rosalind Franklin and Maurice Wilkins in 1953
- the discovery of penicillin by Alexander Fleming in 1928 and its subsequent development as a drug by the Australian Howard Florey Institute
- Gregor Mendel's published work with pea plants in 1865 after eight years of growing thousands of pea plants and tracking their progeny and traits, to discover the laws of Mendelian inheritance
- the discovery of the structure of insulin and other large biological molecules by Dorothy Hodgkin through her leadership and encouragement of a team of scientists, going on to collaborate with other laboratories to develop mass production of the hormone and inspire the development of related diabetes drugs
- the development of a vaccine against the virus that causes cervical cancer, in Australia by a team of scientists led by Professor Ian Frazer in 2006. This was the first vaccine against cancer.

EXPLAIN

Structuring a logbook – investigative design

These discoveries were possible not only due to the persistence and determination of the scientists but also, no doubt, through their effective use of a logbook. A logbook allows a scientist to keep track of the specific dates of their investigation ideas, planning, questions, important results, errors and any modifications required, thereby maintaining a record of all work conducted. A requirement of the VCE is that each student must maintain a printed logbook of practical activities in Units 1–4 for assessment purposes. The need for a printed logbook, rather than an online or digital logbook, removes the likelihood of any tampering with results. Of course, this will depend on your school and your teacher's authentication practices, so it is best to work within your school's guidelines.

12C SCIENTIFIC COMMUNICATION LINK

In this section, you will learn how to develop a logbook for your own practical investigation and the importance of each section in contributing to the end product. This is usually in the form of a scientific poster (outlined in Section 12C).

An important function of an effective logbook is that it clearly demonstrates the development of the ideas that come from your research and investigation, including changes in direction, equipment failures and so on, as well as your collaboration and discussion with peers. This is demonstrated in the example below, which shows two versions of the same investigation:

- **Investigation 1** is the initial version. The ideas that are highlighted for the investigation are not perfect but they show the initial planning coming together.
- **Investigation 2** is the final version. Adjustments have been made to the initial investigation to improve the validity and reliability of the results (terms explored in more detail later).

The investigation involved finding the optimal temperature for an enzyme. The transition between Investigation 1 (initial ideas) and Investigation 2 (final product), as well as tips on what to include in each of the key sections, are included to assist you with your own logbook development.

Title
the research question under investigation; includes information about what is being tested

Logbook

Title

1 *What temperature do enzymes work at?*

2 *What is the optimal temperature for the enzyme catalase to break down hydrogen peroxide into oxygen and water?*

Notes
The title should include reference to the variables being changed (IV) and measured (DV), along with enough detail for the reader to decide whether they want to continue reading.

VIDEO 12A–1 LOGBOOK EXAMPLE

Introduction

1. Enzymes are essential to help reactions proceed more efficiently, in order to sustain life. They are made of proteins, which are coded for by an organism's DNA, and they are specific to the substance they act on.

2. The enzyme catalase reacts with the substrate hydrogen peroxide (H_2O_2), breaking it down into water and oxygen (products) in a catabolic reaction. Catalase is primarily found in the liver and is important in protecting the organism from damage caused by hydrogen peroxide, which is constantly produced by mammals.

The rate of reaction between an enzyme and the substrate can be affected by many things, such as concentration of enzyme and substrate, pH level and inhibitors. It can also be affected by temperature. Every enzyme has an optimal temperature, at which its rate of reaction is highest with the most successful collisions with the substrate. The general rule for this is that, as temperature increases, the rate of reaction increases until optimal temperature is reached. Any further increase in temperature will result in a dramatic drop in rate of reaction and may lead to denaturation of the enzyme. Denaturation is an irreversible process caused by the hydrogen bonds being broken, destroying the characteristic 3D structure of the protein and therefore changing its active site so it cannot bind to the substrate.

The normal functioning temperature for a human is 37°C, which is similar to that of a lamb, where the liver containing catalase was obtained from. If the temperature decreases in the lamb, then there is less kinetic energy and therefore fewer successful collisions between the enzyme and substrate, leaving the lamb vulnerable to damage occurring from the peroxide. Similarly, if the temperature increases to a certain point, the enzyme denatures again, leaving it vulnerable.

Aim

1. To determine the temperature at which catalase functions most efficiently.

2. To determine the optimal temperature for the enzyme catalase, by measuring the height of bubbles (oxygen gas) produced during the breakdown of the substrate hydrogen peroxide.

Hypothesis

1. That the catalase will function best at 37°C.

2. That as the temperature to which the lamb liver is exposed increases, so too will the rate of reaction, producing a greater height of oxygen bubbles until the optimal temperature of 37°C (normal temperature of a live lamb) is reached. Any further increase in temperature will lower the rate of reaction due to the 3D conformational shape of the catalase denaturing.

In the introduction (to a poster) it can also be appropriate to present a labelled diagram of the concept/idea being investigated.

The aim includes explicit reference to the independent and dependent variable.

The hypothesis is a prediction of what you think will occur. It does not have to be correct, but should be supported by knowledge of the theory, which is the difference between investigations 1 and 2.

Introduction
a detailed but succinct explanation of the reason for undertaking an investigation; includes key biological concepts, aim and hypothesis

LINK UNITS 1&2

Aim
the main purpose of an investigation and what you hope to achieve

Hypothesis
a prediction of the outcomes, which are testable experimentally and form the basis of the methodology

NOTE

Scientific theories and models are built up from hypotheses. Therefore evidence in support of a hypothesis is evidence in support of models or theories that encompass the hypothesis.

Independent variable
the variable for which quantities are changed by the experimenter

Independent variable

1. The temperature of the catalase
2. The temperature to which the enzyme catalase from lamb is exposed (0°C, 10°C, 20°C, 30°C, 37°C, 40°C, 50°C, 60°C)

The independent variable should be specific and list all quantities or changes being investigated. It should also list the control group, if one is included in the investigation.

Dependent variable
the variable that changes in response to changes in the independent variable; the experimenter measures these changes

Dependent variable

1. The breakdown of hydrogen peroxide
2. The volume of oxygen produced (as bubbles) from the breakdown of hydrogen peroxide, measured in millimetres using a ruler.

State how the dependent variable will be measured in your experiment. This could be using an instrument and units of measurement, or even a simple scale of effects, but always give the meaning of each number in the scale (e.g. 0 = no symptoms, 5 = extreme symptoms) must always be given.

Controlled variable
anything kept constant, or monitored, so it does not affect the independent and dependent variables, and therefore the validity of experimental results

Controlled variables

1. Environmental conditions
   Equipment
   Catalase
2. Same mass of liver in each experiment (2 g)
   Same surface area to volume ratio of the lamb liver
   Same volume of hydrogen peroxide used (50 mL)
   Same concentration of hydrogen peroxide used (3% solution)

There will always be more than one controlled variable in any investigation. List as many as you can for your investigation. (When answering questions on this, usually only two are required.)
The word ‘same’ is used for each variable, to show that the variables have been kept constant, and so the results are valid.

WORKSHEET 12A–1 IDENTIFYING VARIABLES

Control group

1 The control group is the normal body temperature.

2 As this experiment is trying to determine the optimal temperature of the catalase enzyme from lamb liver, there is not really a control group included. A negative control could be included, which would be a set-up where no lamb liver is added to the hydrogen peroxide, to prove that the reaction is caused by the catalase present in the liver.

If possible, a control group should be included in an investigation. If the investigation has to do with drug administration in humans, then a **placebo** is included. Including a control group gives a known result for comparison with the experimental groups. You may need to account for the difference between a **positive control** and a **negative control**.

Method

1
1 Place hydrogen peroxide in a flask.
2 Add enzyme to flask.
3 Allow experiment to run.
4 Measure the maximum height of bubbles in the test tube.

2
1 Set up the equipment (conical flask, rubber stopper with two openings: one for syringe and one for tubing, beaker and test tube).
2 Cut liver into equal-sized pieces of equal mass (2 g).
3 Place the liver into a beaker of 150 mL of water at a temperature of 0°C. (Note: An ice water bath will be required for this.) Leave for 3 minutes.
4 Transfer the liver into a separate empty conical flask.
5 Using the syringe, inject 50 mL of 3% hydrogen peroxide solution into the flask.
6 Using a ruler, record the height of the oxygen bubbles (in millimetres) produced in the test tube. Alternatively, the displacement of water in the upside down test tube could be measured in millilitres.
7 Repeat steps 1–6 two more times at the same temperature.
8 Repeat steps 1–7, heating the liver to a different temperature each time (10°C, 20°C, 30°C, 37°C, 40°C, 50°C and 60°C). Liver is to be heated to required temperature as per step 3 in a water bath set at specific temperatures to be tested.

The method must contain enough information for the investigation to be repeated by yourself (repeatability) and others (reproducibility). Therefore it must include specific quantities of any substances and specific equipment used. This helps to account for validity.
The investigation also needs to be repeated, ensuring there is a large sample of results – the larger the sample, the more reliable the results.
The method should also include the set-up for the control group (if there is a control group).

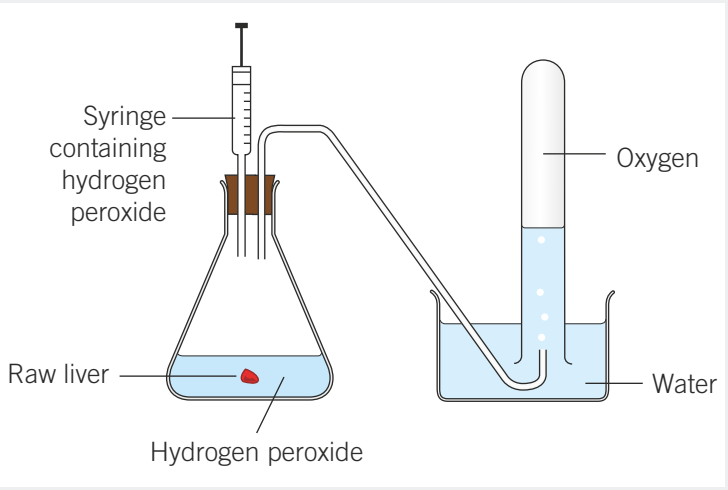
Control group
the set-up or group in an experiment that does not receive treatment; it is used as the 'standard of comparison'

Placebo
a substance that has no therapeutic effect but may have a psychological effect

Positive control
a control group that receives a treatment with a known response that can then be compared to the experimental group(s)

Negative control
a control group that isn't expected to produce a result

Method
a series of numbered steps describing the procedure



Safety and ethical considerations

1 Wearing of lab coat, safety glasses and gloves.
There are no ethical issues to consider.

2 For this experiment, wearing of personal protective equipment (lab coat, safety glasses) is required, especially when handling chemicals such as hydrogen peroxide, and particularly if diluting this from a higher concentration stock solution. It is important to ensure that hands are washed thoroughly after the investigation.
Ethical issues:
The lamb liver was obtained from a verified butcher that only obtains meat from farms that practise correct handling of animals and processing of different body parts following death. When alive, these animals were treated well and given access to sufficient food and water and suitable environments for exercise.

Most schools have access to an online risk assessment program which allows you to input equipment and any chemicals specific to your own investigation and generate safety requirements needing to be followed. With the use of living organisms, ethical integrity when using data and reporting on outcomes should be clearly considered and outlined.

Repeatability recording of results produced when the experiment is repeated in one lab by one operator under the same conditions

Repeatability

1 Repeat experiment three times to determine the temperature at which catalase works best.

2 This experiment was conducted three times for each of the eight temperatures tested, to increase the sample size and ensure reliability of results.

Without repeating your experiment, your results are potentially valid but not reliable (representative of normal conditions). It is important in any investigation to have a large sample size.

Reproducibility

1. Repeat experiment three times to determine the temperature at which catalase works best.

2. The experiment could be repeated by other investigators using a different concentration of hydrogen peroxide or a different mass of liver containing catalase. Results could then be compared, based on the similarity of experimental set-up and aim of the investigation.

Others getting the same result as you after conducting the same investigation further strengthens the validity and **reliability** of your results. It is worth finding out whether others have done your experiment previously, or getting your peers to perform your experiment using your method to see if they get the same results. It is important not to confuse reproducibility and repeatability.

Reproducibility when the same results are obtained for the same experiment by different operators using different equipment

Reliability the extent to which an experiment always yields the same results under the same conditions

Validity

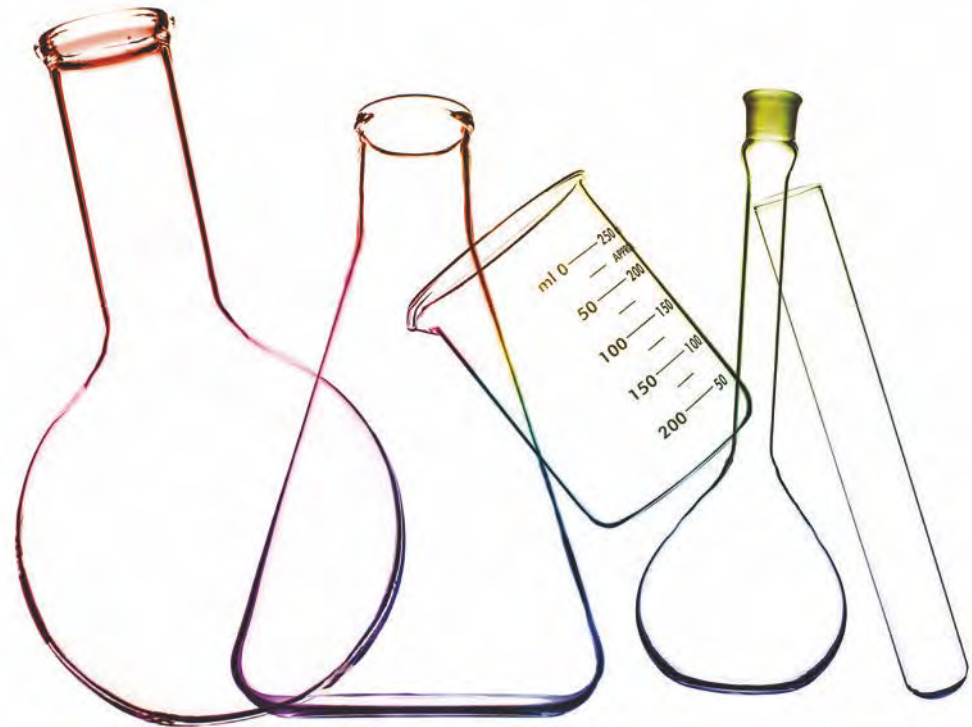
1. All variables are controlled in this experiment, and therefore it is a valid test.

2. This experiment contains one independent variable (temperature at which the liver is incubated) and one dependent variable (height of oxygen bubbles, measured in millimetres), where all other factors in the experimental design have been controlled (concentration and volume of hydrogen peroxide, mass and surface area to volume ratio of the liver, etc). Hence it is a single-variable exploration.

It is crucial to ensure that the independent variable is the only aspect changing in your investigation (**single-variable exploration**), and that all other variables are controlled. If this is not done or noted, then your work is unable to be accepted by your peers, teacher or the broader community.

Validity the extent to which all variables in the experiment have been controlled, so that the independent variable is the only factor that changes

Single-variable exploration an investigation that contains only one independent and one dependent variable



Section 12A questions

1 What features does a controlled experiment include?

2 Define the following terms: independent variable, dependent variable, controlled variable.

3 What is the difference between a positive control group and a negative control group?

4 Compare validity and reliability.

5 Outline the difference between repeatability and reproducibility.

6 Explain what single-variable exploration means.

7 Two scientists are conducting an investigation on photosynthesis. Michael says that a suitable control group would be placing the plant in question in a dark cupboard away from any light. However, Simar says that the control group should be the volume of water given to each plant in the experiment.
   - **a** Explain why Michael is correct.
   - **b** What was the mistake Simar made in her comment?
   - **c** Design an experiment to test the question: 'Does light cause photosynthesis?' In your answer include the following: independent variable, dependent variable, controlled variables, control group, method.

8 The Biol fish has recently startled scientists by displaying the ability to change its colour when placed in an alkaline (high pH) environment. The Biol fish is usually bright red. However, it has been reported that the fish turns blue when placed in alkaline water. From this report, scientists devised experiments to test this claim. They constructed four identical ponds. Three of the ponds contained water of pH 9, and the fourth contained water of pH 7 (neutral). The scientists then released 300 fish into each pond into each pond:

   Pond 1 (pH 9) – 300 Biol fish
   Pond 2 (pH 9) – 300 Biol fish
   Pond 3 (pH 9) – 300 Biol fish
   Pond 4 (pH 7) – 300 Biol fish

   Using what you have learnt in this chapter, write a method for this experiment. Ensure you mention all relevant variables in your response.

9 A group of brilliant Biology students have developed a drug that they think can cure Parkinson's disease. A large drug company has reviewed their design and deemed it fit to undergo clinical trials. Design an experiment that could be used to test this drug on human patients. Include all necessary information.

10 Patients who have received an organ transplant will need to take immunosuppressant drugs to lower the chance of organ rejection.
   Medical scientists have developed a new immunosuppressant drug 'X', which is thought to prevent organ rejection in patients who have received new organs.
   Design an experiment to test the effectiveness of this new drug X on 1000 mice that have received an organ transplant. In your experimental design, be sure to include:
   - the independent variable
   - the dependent variable
   - at least two controlled variables
   - a control group
   - the expected outcomes.

Scientific evidence

Study Design:

Investigation design

- Techniques of primary quantitative data generation relevant to the selected scientific investigation

Scientific evidence

- Authentication of generated primary data through the use of a logbook
- Ways of organising, analysing and evaluating primary data to identify patterns and relationships including sources of error and uncertainty
- The nature of evidence that supports or refutes a hypothesis, model or theory
- Assumptions and limitations of investigation methodology and/or data generation and/or analysis methods

Glossary:

Accuracy
Conclusion
Continuous data
Discrete data
Line of best fit
Outlier
Precision
Qualitative data
Quantitative data
Random error
Systematic error
True value

Structuring the logbook – scientific evidence

This section discusses how to represent the results of your investigation in your logbook, including drawing up tables and graphs, analysing the data collected, commenting on any errors, ensuring the precision and accuracy of the data, and noting areas for improvement.

Before recording your results, it is important to understand the difference between **qualitative data** and **quantitative data**. Qualitative data is *descriptive* – this means it is in the form of words, not numbers. For example, it could be the appearance of something (e.g. 'cloudy' or 'clear') or colour (e.g. 'red' or 'yellow'). Quantitative data is *numerical* – this means it is in the form of numbers, based on counting or measuring. For example, it could be temperature (e.g. 100°C) or symptoms recorded on a scale of 0 to 10 (e.g. 0 for no pain and 10 for intense pain).

Qualitative data
data that is descriptive (not numeric)

Quantitative data
data that is measured and represented numerically

Knowing this difference is important when determining what type of graph should be used to represent the data you have generated.

1 **Results – table**

	Temperature					
	10	20	30	40	50	60
Trial 1	7	15	16	20	30	3

2 **Results – table**

Title: Changes in temperature of catalase activity and resulting height of oxygen bubbles produced

Temperature (°C)	Height of oxygen bubbles (mm)			
	Trial 1	Trial 21	Trial 3	Mean
0	0.8	1.0	0.9	0.9
10	2.0	2.5	2.7	2.4
20	8.0	9.2	8.8	8.7
30	12.5	11.1	13.6	12.4
37	16.4	15.8	17.3	16.5
40	20.1	18.2	17.8	18.7
50	8.8	9.3	9.9	9.3
60	1.0	1.2	0.6	0.9

A results table should include a title with both variables (independent and dependent) mentioned.

All columns/rows must be labelled with an appropriate heading and relevant units.

It is good to include an average, or a percentage, from multiple repeats. The averages should be rounded to the same number of decimal places as the data, one decimal place in this case.

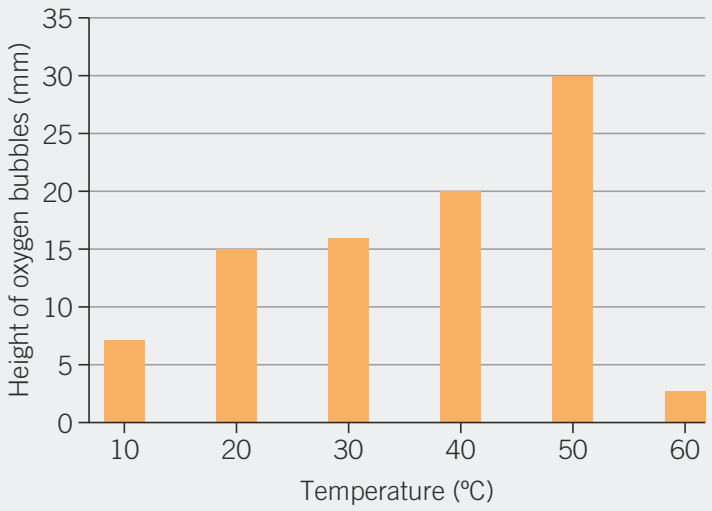
The table can be drawn by hand or generated digitally.

WORKSHEET 12B–1 REPRESENTING DATA

Continuous data data that is measurable and continuous, with infinite possible values; best represented by a line graph

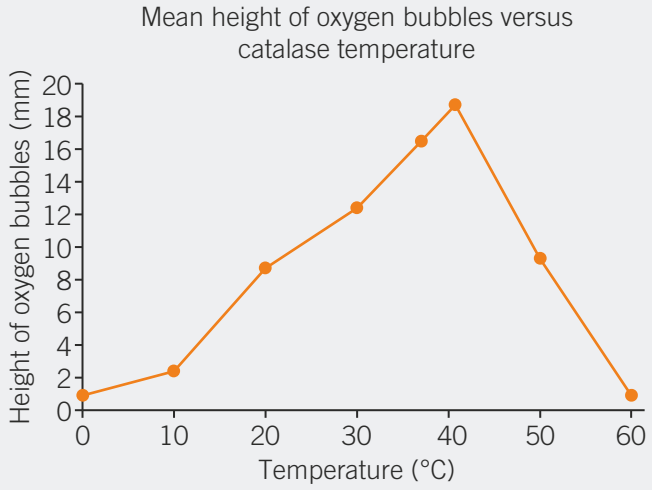
Discrete data data that is countable and in discrete categories; contains distinct or separate values; best represented by a bar graph

1 **Results graph – Investigation 1**



Graphs should include a main title and have both *x*- and *y*-axes labelled with units (if required). The *x*-axis is for the independent variable and the *y*-axis is for the dependent variable.
The correct type of graph must be used. **Discrete data** (based on counting), should be represented as a bar graph.
Continuous data (based on measuring, e.g. changing time, temperature, concentration, pH), should be represented as a line graph.
Can be hand drawn or digital.

2 **Results graph – Investigation 2**



Accuracy how close the measurements are to the 'true' value of the quantity being measured

True value the value or range of values that would be obtained if the quantity could be measured perfectly

Accuracy

1 *This experiment is accurate, as data was generated using correct experimental procedure.*

2 *To fully determine the accuracy of this experiment, the results would need to be compared to investigations done previously, or compared with the known optimal temperature of the lamb liver catalase at approximately 40°C. There is also not a definitive* ***'true' value*** *for the height of oxygen bubbles for each temperature in this experiment – this is evident from the variations in recordings of the three trials at each temperature.*

The accuracy of an experiment is not always known. It depends on how unique your investigation is. Most experiments you perform will have been completed by others previously, so you are just reproducing results. In those cases, a true value is known and you can compare your own results to them.

Systematic errors

1. Each time the liver was transferred from the water bath to the set-up, its temperature changed, which could have affected the results.
2. For liver that was initially kept at temperatures below room temperature (0°C, 10°C and 20°C), transferring it from the water bath to the conical flask resulted in a slight increase in temperature. Additionally, the way the experiment was set up meant that the oxygen produced from the reaction needed to travel from the conical flask through the tubing and into the test tube. This would not be an accurate representation of all the oxygen causing bubbles to be formed, as some of the oxygen each time would remain in the conical flask, thereby affecting the results for the investigation at each temperature.

A systematic error cannot be improved by repeating measurements, having a larger sample size or taking a mean. As the results are always out by a consistent value, they will always be inaccurate. Therefore, a change in the method or equipment must occur.

Systematic error when the readings obtained from measurements differ from the 'true' value consistently in one direction every time

Random errors

1. There were no random errors because the experiment was only conducted once.
2. Random errors were present in this experiment, as it is clear that there are differences in measurements between the three trials at each temperature. This could have been due to human errors in measuring the concentrations of hydrogen peroxide or in cutting and weighing pieces of lamb liver. It could also be due to fluctuations in the temperature of the water bath away from the set temperature when heating the 2 g of liver. This is equally likely to be higher or lower at different times if not monitored correctly.

The effect of a random error can be reduced by repeating measurements, having a larger sample size and/or taking a mean. As results are equally likely to be high or low, averaging can improve the precision of results. For most experiments in a laboratory where multiple trials are conducted, you will need to account for the effect of these on your investigation. You may need to also account for **outliers** by repeating measurements.

Random error an unpredictable variation in the readings obtained, due to variables not all being controlled (extraneous variables), and resulting in the readings being higher or lower than expected

Outlier a reading that is very different from other results obtained for the same measurement

Precision

1. The results were not precise as they increased up to 50°C before decreasing at 60°C.
2. The three results at 0°C were similar, but none of the results at other temperatures were similar for all three trials. However, an average of the three trials was taken, to improve the precision of the results as much as possible.

Precision can be achieved in your own investigations by ensuring that you have a large sample size (repeats) and take a mean. This is more easily identifiable with continuous data measurements.

Precision how close all the measurements are to each other

WORKSHEET 12B–2 ERRORS IN EXPERIMENTAL DATA

Discussion

The discussion is the most crucial aspect of the investigation. Use your logbook to write down any notes about what you plan to address in this section. The discussion should include the following key content:

- *Interpret and evaluate the trends and patterns in your data* – use a graph if possible, as this will clearly show the relationship between the independent and dependent variables. It is important here to also quote relevant data from the table or graph when referring to trends in the data.
- *Acknowledge any deviations (outliers) in the data from the results that were expected* – relate the results to the relevant biological theory and key terms. This is very important, as this outlines how well you understand your results and can interpret them based on your knowledge of the theory. Therefore, use the knowledge in previous chapters of this book to help with the respective topic you are investigating.
- State whether the data you collected *supports or doesn't support the hypothesis*.
- Identify *any limitations in the data or the method* – refer to random and systematic errors, and accuracy and precision, as discussed earlier in this chapter.
- Suggest *future improvements to the investigation* if it were to be performed again – this means referring to experimental errors, not human errors (e.g. incorrect measuring of volume or not timing with a stopwatch appropriately). If your experiment is performed correctly, there should be no human errors.

NOTE

Avoid terms such as 'proved', 'disproved', 'correct' and 'incorrect' in relation to your hypothesis, as it is unlikely that you can be this certain from a single investigation. Instead, use terms such as 'supported', 'not supported', 'indicated' and 'suggested'. If your results contradict your predictions, this would warrant repeating the experiment, if you have time. If you cannot repeat the experiment, then your report should include a discussion of flaws in the design or method, and suggestions for how the investigation could be altered to minimise or eliminate these.

Conclusion

Conclusion a summary of what you can deduce from the results of the investigation, including whether the tested hypothesis was supported

The main purpose of the **conclusion** is to briefly summarise the position of the experiment in the wider understanding of the biological topic(s). You need to state the important overall trend of the data (referring specifically to data from your results) and whether or not the results support the tested hypothesis. The conclusion should also assess whether the results of the experiment have contributed new information to what is known about the topic, and any further investigations that need to be undertaken. The conclusion should not introduce any information that has not already been discussed in the results and discussion section.

For example, in the experiment described in this chapter, the conclusion might read like this:

> In conclusion, the results indicate that the optimal temperature of the catalase enzyme in lamb liver is 40°C as shown by the highest mean height of oxygen bubbles produced, 18.7 mm. This is compared to the predicted optimal temperature of 37°C, which produced an oxygen bubble height of only 16.5 mm. As such, the results do not support the hypothesis. However, whether this was completely true should be further explored by conducting the investigation at smaller increments of temperature, to find the exact optimal temperature.

Check-in questions – Set 1

1 What is the difference between qualitative data and quantitative data?
2 What is the appropriate method of representing continuous data?
3 Compare random and systematic errors, including what results from each type of error.
4 What is the difference between accuracy and precision?

12B SKILLS

When completing work within this 'scientific skills' section, you will draw upon some very important skills. Many of these you will have learnt in previous years of studying science, but it is particularly important to highlight some here, to ensure that you maximise your performance on any given assessment.

VIDEO 12B–1 SKILLS: RECORDING RESULTS IN A TABLE

Recording results in a table

When constructing and recording your results in a table:

- Rule the table in pencil, so any amendments can be made easily.
- Give each column a clear heading, including both the quantity and the unit it is measured in. Do not enter the units in the table along with each numerical value – the units go in the heading only.
- The independent variable is usually placed in the first column, with the dependent variable to follow, in the other columns.
- Organise the results appropriately. For example, if your experiment involved testing an increasing concentration of a solute solution, your results should start with the lowest concentration and continue to the highest concentration.
- If recording quantitative results, all values should have the same number of decimal points.
- Include results for all repeats in the table, and the mean (average) calculated for these.
- Any results that are outliers should be recorded again (repeat the measurement). If there is no time to repeat the experiment, include the outliers but ignore them when calculating the mean.
- Give the table an overall title. This should include mention of both the independent and dependent variables.
- In most cases, data from a table also needs to be displayed as a graph: a line graph for continuous data, a bar graph for discrete data.

The top table below highlights errors in the representation of data. A corrected version is shown in the table below it.

Units are included in body of table, not column heading

Results not in increasing order

Results do not all have the same number of decimal places

Title of table missing

Column has no heading (only units)

No mean calculated for the two repeats

This result (3.31) does not seem to match the others, so it is a potential outlier. (Therefore, in the correct table below, it is not included in the mean of the two trials.)

Concentration of solution	Initial mass of 10 potato discs (g)		
	Trial 1	Trial 2	%
0.0 M	2	3.31	+10
0.8 M	2.05	2.13	+4
0.4 M	2.08	1.97	-4
0.2 M	2.05	1.95	-5
0.6 M	2	2	-5

Effect of increasing solution concentration on mass of potato disc

Concentration of solution (M)	Initial mass of 10 potato discs (g)		
	Trial 1	Trial 2	Mean
0.0	2.00	3.31	2.00
0.2	2.05	1.95	2.00
0.4	2.08	1.97	2.03
0.6	2.00	2.00	2.00
0.8	2.05	2.13	2.09

VIDEO 12B–2 SKILLS: DRAWING GRAPHS

Drawing graphs

When constructing a graph:

- Use pencil, as this will allow you to make any amendments easily.
- Put the independent variable on the *x*-axis (horizontal axis) and the dependent variable on the *y*-axis (vertical axis).
- Fully label both axes and include units (units should be the same as the results table if headings are correct there).
- The scale on the axes should have increasing values spaced at equal intervals, and it should be easy to read values between these intervals. Do not extend the scales too far beyond the recorded data values. *Note*: You do not have to begin your scale at 0.
- Make the graph as large as possible, so it is easy to read precise values.

When drawing *line graphs*:

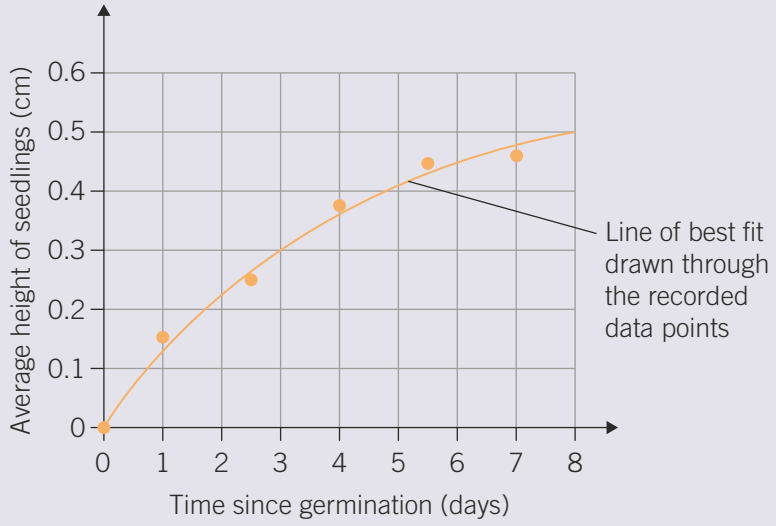
- Use a line graph to represent continuous data.
- Plot data points as crosses (×) or dots (•). If using dots, be sure to draw them large enough so that they are not covered by the line.
- Draw a **line of best fit**. This does not need to go through the first and last point, nor does it need to be a straight line (both common mistakes made by students).

Line of best fit a line on a graph that shows the general trend of the data points; the distance to the points above the line should equal the distance to the points below the line

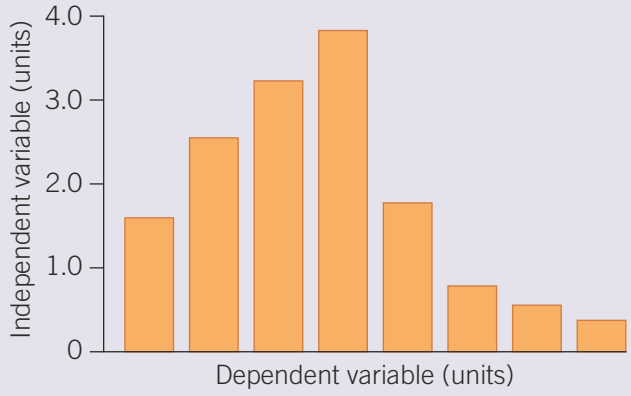
When drawing *bar graphs*:

- Use a bar graph to represent discrete data, and draw the bars with gaps between them.
- However for a histogram, a special type of bar graph showing the distribution of numerical data, the rules are different. It displays the frequency of values on the vertical axis that fall into defined ranges called 'bins' marked on the horizontal axis.

Line graph



Bar graph



Ordering your discussion

When choosing an order for all the points to talk about in your discussion, follow this sequence:

1. Describe the overall trend (overall relationship between the independent and dependent variable).
2. Describe any changes in the gradient of the graph, particularly focusing on sharp changes and where they occur.
3. Quote data from the graph.
4. Explain the results using your scientific knowledge of the key concepts studied in your investigation.
5. Identify sources of errors in the experiment. Begin this section with any *systematic errors* and how these affected the accuracy of your measurements due to limitations in the apparatus, experimental technique or experimental design. Do not include human errors (e.g. incorrectly measuring an exact volume of solution). Then identify any *random errors* and how these affected the precision of your measurements. You would include human errors here.
6. Identify areas for improvement. Focus on how to reduce the errors mentioned in step 5. This could include things such as:
   - using better techniques for measuring the dependent variable
   - using equipment that is more likely to keep controlled variables constant and therefore making your data valid, as well as more precise and accurate
   - repeating the investigation to increase reliability.

VIDEO 12B–3
SKILLS: ORDERING YOUR DISCUSSION

Section 12B questions

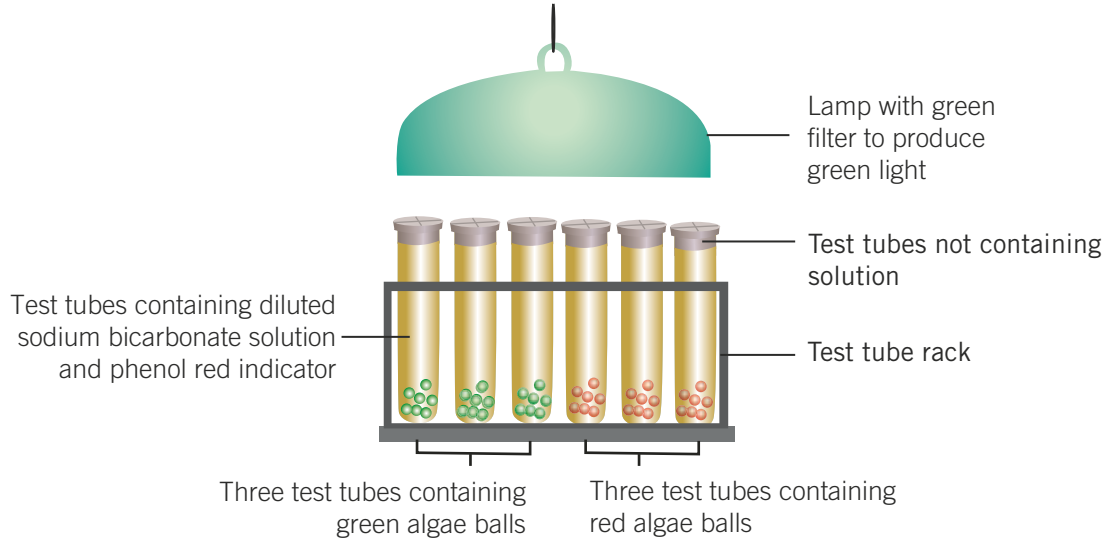
1 A student is investigating the effect of solute (sucrose concentration) on the rate of osmosis in potato cells. They cut 50 potato discs in total and placed 10 discs in a Petri dish covered with 50 mL of the sucrose solution at varying concentrations (0 M, 0.2 M, 0.4 M, 0.6 M and 0.8 M). The initial mass (in grams) of the potato discs was recorded and compared to the final mass (in grams) of the potato discs after 24 hours, allowing the student to calculate the percentage change in mass of the potato discs. The following results were produced.

Concentration of sucrose solution (M)	Initial mass of 10 potato discs (g)	Final mass of 10 potato discs (g)	Change in mass (final – initial) (g)	Percentage change in mass (%)
0.0	2.10	2.31	+0.21	+10
0.2	2.05	2.13	+0.08	+4
0.4	2.08	1.97	–0.11	–5
0.6	2.05	1.95	–0.10	–5
0.8	2.10	2.00	–0.10	–5

a Identify the independent variable and the dependent variable in this investigation.
b What would be two controlled variables required in this experiment? Explain by stating:
  i how you would control these variables
  ii the effect that these variables would have on the rate of osmosis if they were not controlled.
c What is the purpose of a control group?
d Does this experiment have a control group? If yes, identify which set-up it is. If no, identify and explain what would be an appropriate control group.
e Are the results of this investigation qualitative or quantitative? Explain.
f Describe whether the results of this investigation are reliable (use information in the question and results table to assist you).
g Use the information from the table to construct a graph of the results. Be sure to draw the appropriate type of graph.
h By looking at the table of results above, what could be done to improve the precision of these results?
i Using the graph you drew for part **g**, in which concentration of sucrose solution, 0.2 M or 0.6 M, is the concentration of free water molecules higher?

2 Erin was performing an experiment based on one completed previously by other researchers. Their experiment confirmed that the survival of algae at different water depths was dependent on the algae's colour. Red algae, which contains a pigment called phycoerythrin, allowed the algae to absorb more light in the green wavelength. This allowed the algae to survive at increased water depths compared to green algae, which contains the pigment chlorophyll and absorbed light of the blue and red wavelengths better.
Like the previous researchers, Erin grew her own algal jelly balls, one set containing green algae and the other set containing red algae. She placed each of these groups of jelly balls into different test tubes and submerged them in a diluted sodium hydrogen carbonate solution (providing carbon dioxide and water to the algae). She also added three drops of phenol red indicator, which can detect changes in pH due to different

concentrations of carbon dioxide. In low concentrations of carbon dioxide, the indicator remains red; in high concentrations of carbon dioxide, the indicator changes to yellow. Erin measured the rate of photosynthesis using a stopwatch to record the time for a colour change to occur in any of the test tubes.
A diagram of Erin's experiment is shown here:



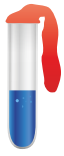
a If Erin managed to get the same results as those of the previous researchers, does this refer to 'reproducibility' or 'repeatability'?
b List three controlled variables that Erin would include in her experiment.
c State the independent variable in Erin's investigation.
d State a possible dependent variable in Erin's investigation and how this could be measured.
e Is the measurement of the dependent variable you identified in part **d** classified as qualitative or quantitative?
f What results from this investigation would support the results of the previous researchers?
g If the light in Erin's experiment was changed from green to blue, what results would she expect to see? Explain your answer.
h Erin's laboratory partner, Adrianne, suggested that they should also set up an identical experiment but keep the test tubes and their contents in the dark. Explain why this is a good suggestion.

3 Yeast, a microscopic, single-celled organism, belongs to the group of organisms known as fungi. Dry yeast is composed of living dormant (in a state of inactivity) yeast cells. Yeast enzymes chemically break down sugars into products that the cell can use for energy. Yeast can convert disaccharides like sucrose into monosaccharides (glucose) and use the glucose for anaerobic respiration (fermentation).
A method for an investigation is described in detail below. The scientists wish to observe alcoholic fermentation of yeast, which is carried out in the absence of oxygen (anaerobic conditions). The materials they will use include: 2 large test tubes, 2 balloons, 10% sucrose solution, warm distilled water (45°C), distilled water at room temperature.

Method

1 Rinse the test tubes and place them on a rack.
2 Label one test tube 'sucrose' and the other test tube 'water'.
3 Prepare the balloons by inflating and deflating each balloon 3–5 times.
4 Place a teaspoon of yeast in each test tube.
5 Add warm water to each test tube to a depth of 2 cm.
6 To the test tube labelled 'sucrose' add sucrose solution until the test tube is half-filled. Seal the test tube with a deflated balloon that you prepared in Step 3. With your palm sealing the top, shake the test tube until the yeast has dissolved, then place in the rack and observe.
7 To the test tube labelled 'water' add water (room temperature) until the test tube is half-filled. Seal the test tube with the other deflated balloon that you prepared in Step 3. With your palm sealing the top, shake the test tube until the yeast has dissolved, then place in the rack and observe.
8 Record your observations of both test tubes by drawing them in a table, as shown below.
9 Observe the test tubes again after 30 minutes, and draw them again.

The results of the investigation are displayed in the table below.

Time	Observations	
	Test tube with sucrose solution	Test tube with distilled water
At the start of incubation		
After 30 minutes		

a Write a suitable hypothesis for this investigation.
b Write the word equation for the alcoholic fermentation of yeast.
c Why was it necessary to inflate and deflate the balloons several times before the start of the experiment?
d What is the purpose of having a test tube with distilled water instead of sucrose?
e Predict what the results might be if boiling water was added to the yeast first, before the sucrose solution.
f State two purposes of the balloon in the experiment.
g Is this investigation valid? Use your knowledge of cellular respiration and your experimental knowledge to explain.
h Is this investigation reliable? Explain.
i Design an experiment to investigate the effect of different concentrations of sucrose solutions on rate of alcoholic fermentation.

Scientific communication

Study Design:
Science communication

- Conventions of science communication: scientific terminology and representations, formulas, standard abbreviations and units of measurement
- Conventions of scientific poster presentation, including succinct communication of scientific investigation and acknowledgements and references
- The key findings and implications of the selected scientific investigation

ENGAGE

Communicating your work

Think about how you've probably presented your experimental work in Science in previous years. You would have written up your planning stages and presented your results in a typical scientific report. While this is still done even at university, would you want to read a scientist's research presented to you in this way? Probably not. So scientists often communicate their findings in the form of a scientific poster. In the past few decades, posters have become a popular way to showcase new investigations and findings to the scientific community, nationally and internationally.

Producing a scientific poster is a skill and is not as easy as you might think. It often requires a lot of thought and preparation, as well as the ability to keep your ideas concise.

EXPLAIN

Scientific poster template

A key part of the School Assessed Coursework (SACs) in Units 3 and 4 Biology is a self-designed practical investigation presented as a poster, using the following template:

VIDEO 12C–1 SCIENTIFIC POSTER EXAMPLE

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

Methodology and method

Results</td><td>Communication statement reporting the key finding of the investigation as a one-sentence summary</td><td>Discussion

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

This template is the same as the one provided in the VCAA Study Design. You'll notice from the template that the heading of each section correlates closely with the information that is recorded in your logbook from Sections 12A and 12B. This should therefore highlight the importance of the progression of information that is recorded there, as it will need to be transferred in a more succinct fashion onto your poster. The VCAA has also stipulated that this should not exceed 600 words. The centre of the poster occupies approximately one-quarter of the page and needs to be one sentence summarising the main outcomes of your investigation, thereby answering your investigation question (title). This should be as engaging as possible, to encourage any reader to want to read the other sections of your poster.

u might be wondering where the aim and hypothesis from your logbook should be cluded on the poster. They are included in the introduction, following a brief explanation of e reasons for conducting your investigation, and link to the relevant biological concepts.

The poster can be produced either electronically or in hard copy format and may even be done in a different structure to this in Unit 1. It will of course depend on what your own school chooses as the preferred format, and you should therefore follow this.

Purpose of the scientific poster

The scientific poster's main goal is to get your message, the findings of your investigation, across to everyone. It is designed not just for those with a scientific derstanding of the investigation conducted, but for those with a non-scientific ckground as well. The reason for setting it out in the template proposed above is for aximum impact and visual appeal. There are no large blocks of text that are not inviting read. It is organised into columns, to help your readers follow the information. Including able of results and a graph breaks up the text and avoids large blank areas. Lastly, e limitation on word count is so that the text is clear and concise. Your investigation ould therefore be explicit and not too broad or overly complicated, so the results can be plained concisely.

the poster, you should also include the sources of information (references) that you ed when planning and conducting the investigation. You can also acknowledge those io supported your investigation, such as peers, your teacher or others. How to correctly mplete references is explored in the Units 1&2 Interactive Textbook, Section 9C, aocument 9C-2.

DOCUMENT 12C–1 PREPARING AN ARTICLE FOR SCIENTIFIC PUBLICATION

DOCUMENT 12C–2 PREPARING AN ORAL, MULTIMEDIA OR VISUAL PRESENTATION

Other methods for presenting an investigation

In addition to presenting your results in the form of a poster, you should be able to discuss your investigation with your peers, teacher, family or anyone from a non-scientific background. Most of those in the scientific community prefer to have individual discussions with other experimenters about their work, rather than reading it from a poster. Talking about their work with others also gives the experimenter a chance to discuss aspects of their investigation that have not been included in the final presentation, and to answer any follow-up questions.

Therefore, other methods for presenting the purpose and results of your investigation include oral presentation, multimedia presentation, visual representation or even an article for a scientific publication. Tips and suggestions for these are included in the digital resources.

Section 12C questions

1 Where should you draw information from to complete your scientific poster?
2 What is included in the introduction section of the scientific poster?
3 What are the key sections of a scientific poster?
4 What is the purpose of the communicating statement in the centre of the poster?
5 What are some other ways of presenting the results of an investigation in Biology, and in general to the scientific community?

Chapter 12 review

Summary

Create your own set of summary notes for this chapter on paper or in a digital document. A model summary is provided in the Teacher Resources which can be used to compare with yours.

Checklist

In the Interactive Textbook, the success criteria are linked from the review questions and will be automatically ticked when answers are correct. Alternatively, print or photocopy this page and tick the boxes when you have answered the corresponding questions correctly.

Success criteria – I am now able to:		Linked question
12A.1	Document investigations appropriately using a logbook	ITB☐
12A.2	Define key terms related to scientific skills	13d☐
12A.3	Define controlled experiment	13i☐
12A.4	List the features that a controlled experiment includes	13i☐
12A.5	Distinguish between a positive and negative control	ITB☐
12A.6	Understand the meaning of single variable exploration	13i☐
12A.7	Define and identify independent, dependent and controlled variables	1☐, 2☐,11a☐, 14b☐, 15b☐, d☐, 16a☐, d☐, e☐
12A.8	Select and use equipment and procedures appropriate to an investigation	7☐, 14d☐
12A.9	Distinguish between an aim and a hypothesis	3☐
12A.10	Construct aims and questions for investigations	11b☐
12A.11	Formulate hypotheses and predict possible outcomes	13h☐, 14b☐
12A.12	Define reproducibility, repeatability and validity, and distinguish between these	12☐, 13e☐, f☐, 14b☐
12A.13	Design an investigation that accounts for accuracy and precision and is valid	4☐, 14c☐, f☐
12A.14	Design an experiment that is fully reproducible by others	7☐, 14f☐
12A.15	Determine potential ethical issues with investigation design	ITB☐
12A.16	Identify how bias can be minimised in an investigation	13e☐, f☐, 15c☐
12A.17	Follow clear guidelines for health and safety when undertaking practical investigations	ITB☐
12B.1	Define qualitative and quantitative	ITB☐
12B.2	Distinguish between qualitative and quantitative data	13a☐, g☐
12B.3	Include appropriate units of measurement for quantitative data	6☐, 13g☐
12B.4	Analyse generated primary data to determine whether it is accurate and/or precise, and define these terms	13b☐

Success criteria – I am now able to:		Linked question
12B.5	Transform primary data into an appropriate format of results (table, flow chart, bar and/or line graph)	4☐, 14a☐, 16b☐, c☐
12B.6	Identify trends in data	13b☐, 14c☐, 15e☐
12B.7	Define the different types of errors (random and systematic)	13c☐
12B.8	Identify sources of error and outliers from primary data	4☐, 8☐
12B.9	Use evidence to determine whether an investigation supports or discounts a hypothesis	4☐, 16f☐, g☐
12B.10	Identify areas for improvement in investigation design and analysis to increase accuracy and precision and reduce the likelihood of errors	13h☐, 15f☐
12C.1	Appropriately communicate all aspects of a scientific investigation	5☐, 6☐
12C.2	Apply correct abbreviations to biological terminology	ITB☐
12C.3	Present a scientific poster in the mandated format	ITB☐
12C.4	Justify conclusions and evaluate whether evidence supports or refutes the hypothesis	9☐, 10☐, 14e☐, 15e☐
12C.5	Interpret investigation outcomes in terms of broader biological concepts	ITB☐

Multiple-choice questions

1 An investigation is performed to observe the different organelles present in plant and animal cells, using an electron microscope. What could be the dependent variable?

A the type of microscope used
B the type of cell observed under the microscope
C the organelles observed under the microscope
D the size of the cell

2 Aside from the independent and dependent variables in an investigation, what other type of variable is included to ensure it is valid?

A changed
B controlled
C control group
D precise

3 A hypothesis is best described as

A a statement describing what the investigation hopes to determine.
B a series of steps involved in planning an investigation.
C clear ethical guidelines for how any living organisms should be handled in the experiment.
D a prediction of what will be observed.

The following information relates to Questions 4–6.
A sphygmomanometer is an instrument used to measure blood pressure.
The table below shows measurements from four sphygmomanometers, W, X, Y and Z.
Sphygmomanometer W is known to be accurate.

Sphygmomanometer	Pressure (mmHg)				
W	20.00	20.00	20.00	20.00	20.01
X	19.91	20.23	20.13	19.42	19.59
Y	19.00	20.40	19.50	20.10	21.00
Z	19.00	19.00	19.00	19.00	19.00

4 Which statement is correct?
- **A** The measurements from sphygmomanometer Z are more precise but less accurate than the measurements from sphygmomanometer Y.
- **B** The measurements from sphygmomanometer Z indicate a random error and are more accurate than the measurements from sphygmomanometer X.
- **C** The measurements from sphygmomanometer X indicate a systematic error.
- **D** The measurements from sphygmomanometer X have the same degree of precision as the measurements from sphygmomanometer Y.

5 Which of the following is *least* likely to improve the quality of the data in this investigation?
- **A** more blood pressure measurements recorded for each sphygmomanometer
- **B** more sphygmomanometers being tested
- **C** all groups using the same sphygmomanometer, W
- **D** taking an average of the combined results of all groups

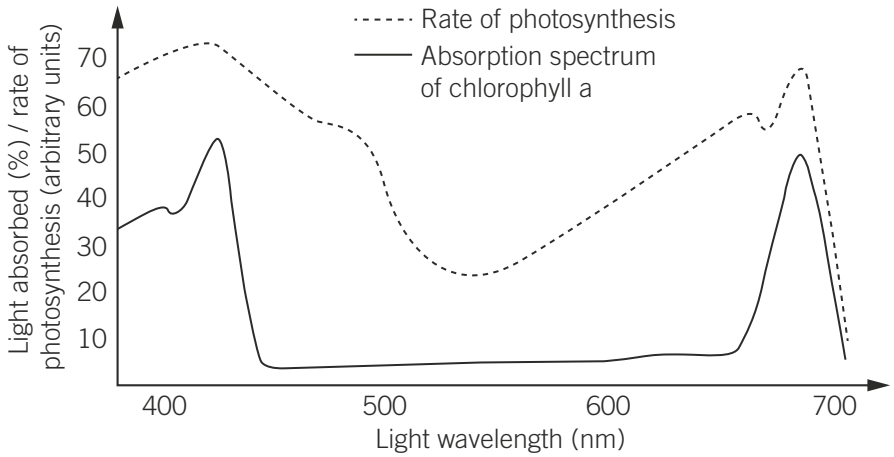
6 The reasons sphygmomanometer W was included as a reference for accuracy for the investigation was to
- **A** remove any possible random errors from the results.
- **B** allow the pressure to be measured accurately.
- **C** ensure the other instruments were precise.
- **D** ensure the experiment generated qualitative data.

7 Which of the following statements would be appropriate in the Methods section of a logbook?
- **A** Exactly 50 mL of solution was measured using a measuring cylinder.
- **B** Exactly 50 mL of solution was measured using a beaker.
- **C** Solution was measured and placed in a tube.
- **D** Add solution to a measuring cylinder.

8 A systematic error
- **A** can be minimised by increasing the sample size and taking a mean.
- **B** is equally likely to be quantitatively higher or lower.
- **C** affects the precision of results.
- **D** occurs consistently in one direction.

9 A conclusion should *not*
- **A** summarise the key findings of an investigation.
- **B** state key data from results.
- **C** introduce new information relevant to the investigation.
- **D** state whether or not the hypothesis was supported.

10 The graph below compares the absorption spectrum of chlorophyll a with the rate of photosynthesis of a plant upon exposure to a range of light wavelengths.



From this graph, it can be concluded that chlorophyll a is not the only pigment involved in photosynthesis. Evidence that supports this conclusion includes the fact that

A the rate of photosynthesis remains high when the plant is exposed to light wavelengths between 450 nm and 650 nm. The percentage of light absorbed by chlorophyll a over these wavelengths is low.

B the rate of photosynthesis is low when the plant is exposed to light wavelengths such as 450 nm. Absorption of light by chlorophyll a is higher at these wavelengths.

C the rate of photosynthesis and the percentage of light absorbed by chlorophyll a are equal at 650 nm.

D between wavelengths 450 nm and 700 nm, the percentage of light absorbed by chlorophyll a is constant.

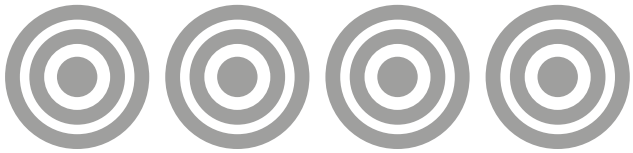
Short-answer questions

11 a Copy and complete the table. (4 marks)

Hypothesis	Independent variable	Dependent variable	Controlled variables (×2)
That intelligence of a group of students is affected by the amount of oxygen inhaled	Amount of oxygen inhaled	Intelligence	Same number of students in each group Same gender
That watching television while doing schoolwork decreases performance on the task			
That 'watering' lemon trees with urine makes them grow faster			
That antacid tablets dissolve faster in warmer water			

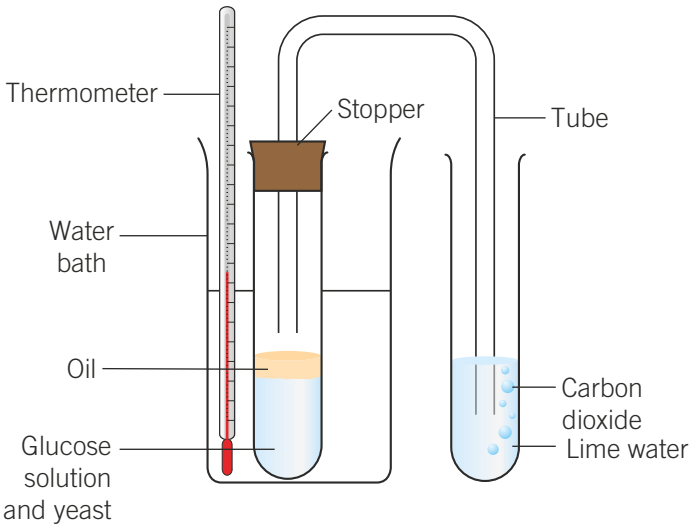
b For each of the hypotheses in the table, write an aim that would be appropriate for that investigation. (4 marks)

12 Using the standard bullseye/dartboard-style shown below, draw separate diagrams that clearly show data that is:



a accurate and precise.
b accurate but not precise.
c precise but not accurate.
d neither accurate nor precise. (4 marks)

13 A scientist decided to investigate the effect of temperature on yeast to determine the temperature at which yeast is best able to perform anaerobic cellular respiration. The experiment was set up as shown below and the cloudiness of the limewater (caused by production of carbon dioxide) was measured. The results are shown in the table.



Temperature of water (˚C)	Cloudiness of limewater solution*
10	0
20	1
30	2
40	3
50	1
60	0
70	0

*0 = no change, 1 = partly cloudy, 2 = cloudy, 3 = very cloudy

a What type of graph should be drawn to represent these results? (1 mark)
b Draw a graph for the data shown in the table. Include axis titles and units. (4 marks)
c Describe the trend observed in the results. (2 marks)
d Using your understanding of the effect of temperature on the rate of reactions, describe the difference observed in the results at 20°C and 30°C. (2 marks)
e Suggest ways in which the scientist could ensure that the investigation results are valid. (3 marks)
f Suggest ways in which the scientist could ensure that the investigation results are reliable. (1 mark)
g Are the results of this experiment qualitative or quantitative? Explain. (3 marks)
h Before conducting this investigation, the scientist hypothesised that, as the temperature increased, the rate of anaerobic cellular respiration would also increase. Is the hypothesis supported by the results? Explain your answer. (2 marks)
i Identify the features of this experiment that make it a controlled experiment. In your answer, outline what a controlled experiment is. (4 marks)

14 A Year 12 student designed an experiment to investigate the effect of light intensity on the rate of photosynthesis in *Elodea*, a freshwater pond weed. The experiment involved exposing sections of *Elodea* to varying light intensities for one hour. Light intensity was measured in arbitrary units, and the amount of oxygen gas produced was recorded and used to indicate the rate of photosynthesis. The student's results are shown in the table.

Light intensity (arbitrary units)	Oxygen production (µL)
0 (no light)	1
10	6
20	11
30	37
40	51
50	49
60	7
70	No data
80	1
90	1
100	1

a From the table of results provided, plot a graph of oxygen production versus light intensity. (6 marks)

b i Before starting their experiment, the student wrote a hypothesis. Give an example of the hypothesis they may have written. (1 mark)

ii Identify the independent and dependent variables of the experiment. (2 marks)

iii To ensure the experiment was valid, the student made a list of factors that needed to be controlled. State two factors that the student would have included in their list. (2 marks)

c i The student was unable to collect data for a light intensity of 70 arbitrary units. They used the graph to estimate the amount of oxygen produced at this intensity. Write their estimate. (1 mark)

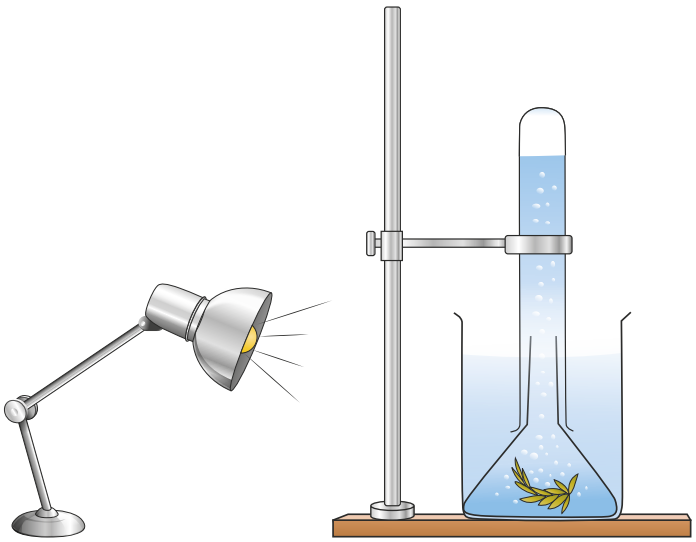
ii Consider your answer to part **i**. Is this likely to be accurate? Give reason(s) for your answer. (1 mark)

d Explain why the student used the amount of oxygen gas produced by *Elodea* as an indicator of the rate of photosynthesis. Include a balanced chemical equation. (2 marks)

e Write a conclusion based on what the student's results suggest about the relationship between light intensity and the rate of photosynthesis. (2marks)

f The student then decided to repeat the experiment using different sections of *Elodea*. Why was this a good idea? (1 mark)

15 Fatima investigated how changes in light intensity affected oxygen (O_2) and carbon dioxide (CO_2) levels in the water surrounding an *aquatic plant* and an *aquatic snail*. She used three digital probes linked to a computer, a closed water chamber and a lamp in the experimental set-up shown below.



a Name the cellular process(es) being investigated in Fatima's experiment. (1 mark)

b Identify the dependent variable and the independent variable in this investigation. (2 marks)

Fatima turned on the lamp. Before placing the snail and the plant in the chamber, she measured the light intensity, carbon dioxide and oxygen levels for 3 minutes. The following results were recorded.

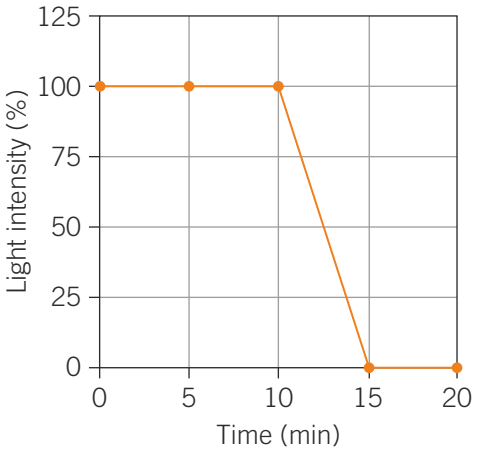
Time (min)	Light intensity (%)	Carbon dioxide (%)	Oxygen (%)
1	50	0.05	22.1
2	100	0.05	22.2
3	100	0.05	22.2

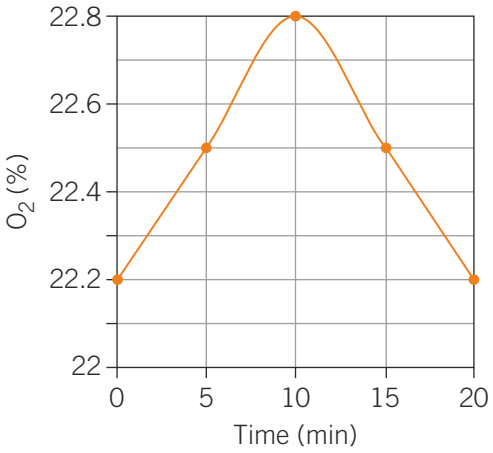
c Referring to the data, explain why Fatima recorded for 3 minutes and not just 1 minute. (1 mark)

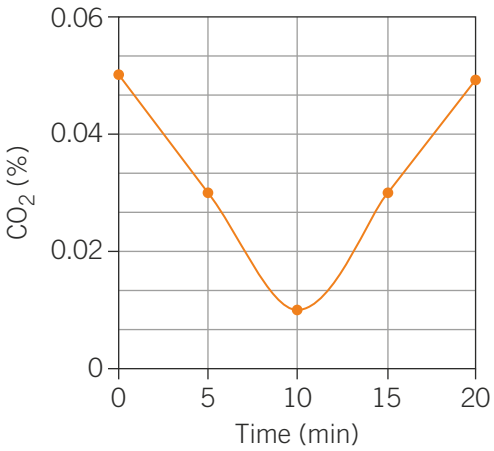
After the initial 3-minute period, Fatima quickly placed the aquatic snail and plant in the chamber and began recording the data from the digital probes. After 10 minutes, she switched the lamp off. She recorded the data using the digital probes for a further 10 minutes. She repeated the experiment once every day for the next 6 days with the same set-up.

d Other than repeating the entire experiment, identify two control measures Fatima should have included in her experimental design. Explain how each of these control measures could affect the results if not kept constant. (4 marks)

Fatima constructed the following graphs from the averaged results of the seven experiments.







e i Using the graphical data, describe the changes in the levels of carbon dioxide and oxygen with the changes in light intensity. (2 marks)

ii What conclusion do you think Fatima can draw from this investigation? You should refer to each of the following in your response:

- the cellular processes named in part **a**
- the variables identified in part **b**
- the evidence collected during Fatima's experiment. (4 marks)

f Explain how Fatima could increase the reliability of this experiment. (2 marks)

16 Germination is the process in which a juvenile (young) plant emerges from a seed when environmental conditions are suitable. Some environmental conditions that can influence the ability of the juvenile plant to germinate are temperature, pH, physical abrasion and water availability.

Jenny loves avocados and wants to grow an avocado plant. On a recent trip to Queensland, she noticed that avocado trees were more abundant in this region than in Canberra, which has much colder weather. She started to think about how temperature might affect the germination of an avocado seed.

She set up an experiment to test the hypothesis 'that germination time of avocado seeds decreases as environmental temperature increases'.

a What is the independent variable in Jenny's experiment? (1 mark)

b Is the data for the independent variable going to be discrete or continuous? Explain by referring to the difference between the two types of data. (2 mark)

c Would a bar or line graph be more suitable for displaying this data? (1 mark)

d What is the dependent variable in this experiment? (1 mark)

e List three variables that would be important to control in this experiment. (3 marks)

Jenny divides 10 avocado seeds equally between two pots. She subjects one pot to high temperature and one pot to low temperature.

f Describe the results that would support her hypothesis. (1 mark)

g Describe results that would *not* support her hypothesis. (2 marks)

Unit 4 Revision exercise

Multiple-choice questions

1 Antigenic shift is to _____ as antigenic drift is to _____.

A swapping viral genes; random mutation

B swapping genomes; random mutation

C random mutation; swapping viral genes

D random mutation; swapping genomes

2 Which statement best describes sympatric speciation?

A Speciation that occurs when some members of a species become geographically isolated.

B Speciation that occurs when some members of a species become reproductively isolated from the rest of the species.

C Speciation that occurs without reproductive isolation.

D Speciation that occurs without geographic isolation.

3 Gene flow

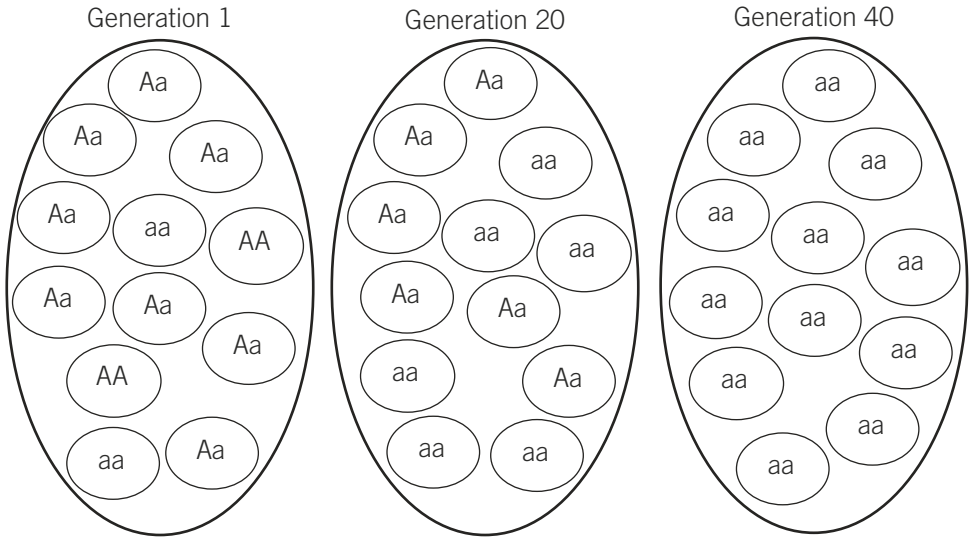
A is the movement of alleles between populations.

B is the migration of members of one population to another.

C is a random change in allele frequency.

D only occurs between members of different species.

4 Consider the following gene pool over 40 generations.



One could conclude that, over the 40 generations,

A new alleles have appeared via random mutation.

B generation 1 has two alleles of equal frequency.

C individuals with Aa had a selective advantage.

D the genetic diversity of the gene pool is increasing over time.

5 A student took 1000 sandflies of the same species from a population and divided them into two equal populations, living in different tanks. One of the populations lived on monosaccharide-based food, and the other lived on polysaccharide-based food. After many generations, the two populations of flies were reintroduced to each other. The flies were then tested to see which flies they preferred to mate with.

The 'monosaccharide flies' preferred to breed with 'monosaccharide flies'. However, if 'monosaccharide flies' were not available, they would breed with 'polysaccharide flies'. Similarly, the 'polysaccharide flies' preferred to breed with other 'polysaccharide flies', but would still mate with 'monosaccharide flies'.

From the results, it is reasonable to conclude that

A two new species of sandflies had evolved.

B the sandflies had undergone genetic drift and consequently were unable to make gametes.

C the sandflies had developed different mating rituals due to their different diet and this prevented their breeding.

D reproductive isolation had begun to occur due to the geographic isolation of the two populations.

6 Which of the following statements correctly distinguishes between a vector and a pathogen?

A A pathogen can be cellular or non-cellular, but a vector can only carry non-cellular pathogens.

B A vector is an animal that carries a pathogen, while a pathogen is a disease-causing agent.

C Both the vector and the pathogen will show symptoms of the disease.

D Vectors can also be referred to as carriers, while pathogens are also known as microbes.

7 Of all the different types of pathogens, which of the following combinations of characteristics applies only to pathogenic fungi?

A eukaryotic, heterotrophic, presence of rigid cell wall

B eukaryotic, autotrophic, presence of rigid cell wall

C prokaryotic, heterotrophic, presence of rigid cell wall

D prokaryotic, autotrophic, presence of rigid cell wall

8 The accumulation of oxygen in Earth's atmosphere began around 2.3 billion years ago. This was significant, as

A it allowed for the formation of Earth.

B all this oxygen resulted in the development of the ozone layer.

C it encouraged the development of Earth's biodiversity.

D the oceans were able to spread further to support life.

9 Which of the following best describes a pair of homologous structures?

A the forearm of a human and the foreleg of a horse

B the dorsal fins of a shark and a dolphin

C the lack of eyes in both a star-nosed mole and a Kaua'i cave wolf spider

D the wings of a dragonfly and the wings of a pigeon

10 A feature that hominins and primates have in common is

A bipedalism.

B opposable thumbs.

C a parabolic jaw shape.

D a long arm-to-leg ratio.

11 Phagocytosis involves

A the assistance of a T-helper cell in identifying foreign material.

B a macrophage engulfing bacteria and forming a phagosome.

C the use of antigens produced by plasma cells.

D the identification of a cell containing a MHC I marker.

12 The inflammatory response is part of the second line of defence, which involves interactions between different cellular components of the immune system and chemical signalling. Key indicators of an inflammatory response are redness, swelling, heat (in the area of inflammation) and pain. Which of the following statements about the inflammatory response is true?

A Histamine is released from dendritic cells to cause vasodilation.

B Complement proteins cause the redness that appears during an inflammatory response.

C Increased fluid leaving the capillaries in the site of infection results in swelling.

D Viruses are the only pathogens that can cause an inflammatory response.

13 Veins and lymphatic vessels both have valves. What is the purpose of the valves?

A Valves maintain enough pressure within the lymphatic vessel to cause lymph to flow towards the lymphatic capillary.

B Valves assist in preventing the lymph from flowing backwards.

C Valves assist the body in increasing the lymphatic vessels' efficiency in absorbing fats from the small intestine.

D Valves are vestigial structures, just like an appendix, and no longer have any function other than providing evidence of ancestral relationships.

14 Which option is *not* a strategy for preventing the spread of a disease?

A Maintain social distancing and wear protective coverings such as masks.

B Ensure access to clean drinking water and appropriate sewage treatments.

C Rely on herd immunity to develop during a pandemic.

D Use quarantine practices for people, animals and food.

15 Monoclonal antibodies are an immunotherapy that uses the natural immune response of an individual. An example is rituximab, which is used to treat B cell lymphomas (a type of blood cancer). Which of the following statements about rituximab is true?

A The antigen-binding site of the monoclonal antibody would be complementary to the B cell causing the lymphoma.

B Rituximab would cause an increased proliferation of the B cells causing the lymphoma.

C Rituximab would cause proliferation into plasma B cells and memory B cells.

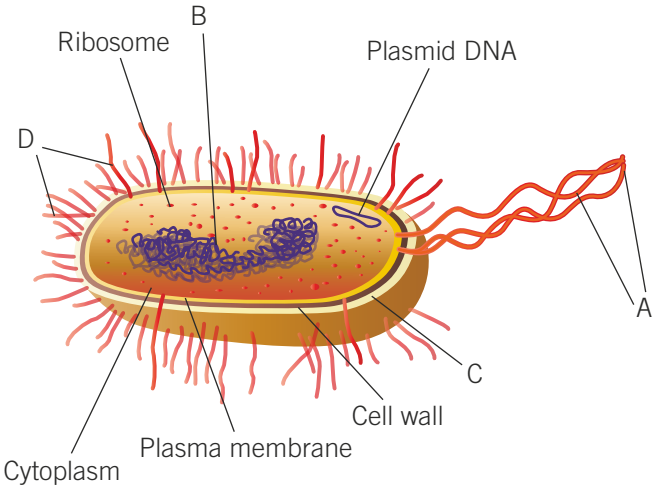
D Rituximab would be composed of protein, displaying quaternary structure with different antigen-binding sites.

Short-answer questions

16 Tuberculosis (TB) is a bacterial disease caused by *Mycobacterium tuberculosis*. The antibiotic streptomycin was first used with great success. However, a few years later, resistant strains of *M. tuberculosis* began to appear. New antibiotics were developed, but resistance developed again. Now, different strains of *M. tuberculosis* are classified according to their level of resistance: multi-drug-resistant TB, extensively drug-resistant TB and totally drug-resistant TB.

a Would the pathogen responsible for tuberculosis be classified as a cellular or non-cellular pathogen? (1 mark)

Examine the image of a bacterial cell below.



b Identify the key features labelled A–D in the diagram, and explain the role that each plays in the onset of bacterial disease. (4 marks)

c Explain what is meant by bacterial resistance. (1 mark)

d How could scientists determine whether the drug resistance observed is genetically based? (2 marks)

e Natural selection is the reason for the development of resistance in *Mycobacterium tuberculosis*. However, perhaps a more accurate term would be 'artificial selection', as the antibiotics are administered by doctors. Do you agree? Give reasons for your answer. (2 marks)

f How does a new allele, like that of resistance, come into existence? And how does its presence in the gene pool affect the population's genetic diversity? (2 marks)

Below is a DNA sequence from the gene coding for a ribosomal protein in a tuberculosis bacterium. This is the gene where, when mutations occur, resistance to streptomycin results.

TACTTTAAGAGCTTATCG ← template strand

ATGAAATTCTCGAATAGC ← coding strand

g Write the mRNA sequence that would be transcribed from this DNA sequence. (1 mark)

*Use the following table, which shows part of the genetic code, to answer part **h**.*

Second base

First base	U	C	A	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
A	AUU, AUC, AUA: Ile AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn AAA, AAG: Lys	AGU, AGC: Ser AGA, AGG: Arg	U C A G

h The ninth base on the template strand of the sequence above is substituted by nucleotide C.

i What type of mutation is this? (1 mark)

ii Explain the effect this mutation will have on the amino acid sequence of the protein produced. (1 mark)

i The eleventh base pair of the sequence is deleted. This is a frameshift mutation. Explain what 'frameshift mutation' means and why it can have such a major impact. (2 marks)

j Explain the effect that this mutation will have on the amino acid sequence of the protein produced. (1 mark)

Drug-resistant TB continues to be a public health crisis. The best estimate is that, in 2017, worldwide, 558 000 people developed TB that was resistant to rifampicin, the most effective drug. Of these, 82% had multiple-drug-resistant forms of TB. Among cases of multiple-drug-resistant forms of TB in 2017, 8.5% were estimated to have extensively drug-resistant TB.

k Give reasons why bacterial resistance causes ongoing challenges for treatment strategies against bacteria. (2 marks)

17 Bacterial infections are usually associated with the humoral immune response. Humans can develop immunity against TB by activating their humoral response.

a What line of defence is the humoral response involved in? (1 mark)

b What are four components of the humoral response? (2 marks)

c Provide a reason why bacterial infections are usually associated with the humoral response. (1 mark)

d Explain how the humoral response assists with immunological memory of a pathogen. (2 marks)

e Explain why a person would not have an effective humoral response against a new strain of TB. (2 marks)

18 Koala (*Phascolarctos cinereus*) populations in southern Australia have a history of genetic bottlenecks. Their numbers decreased rapidly last century as their fur became a target for hunters, and then, as their habitats were destroyed or fragmented and predators increased, their numbers decreased even further. Consequently, this species became extinct in South Australia, and almost extinct in Victoria.

To try to prevent koala extinction, small numbers of koalas were relocated to French Island (Victoria) and Kangaroo Island (South Australia). The conservation program was deemed successful as the populations increased in size. Scientists took blood samples from the koalas and compared the distribution of unique DNA sequences, called microsatellites, which are scattered through the koalas' chromosomes. The results indicated very low genetic diversity within the populations.

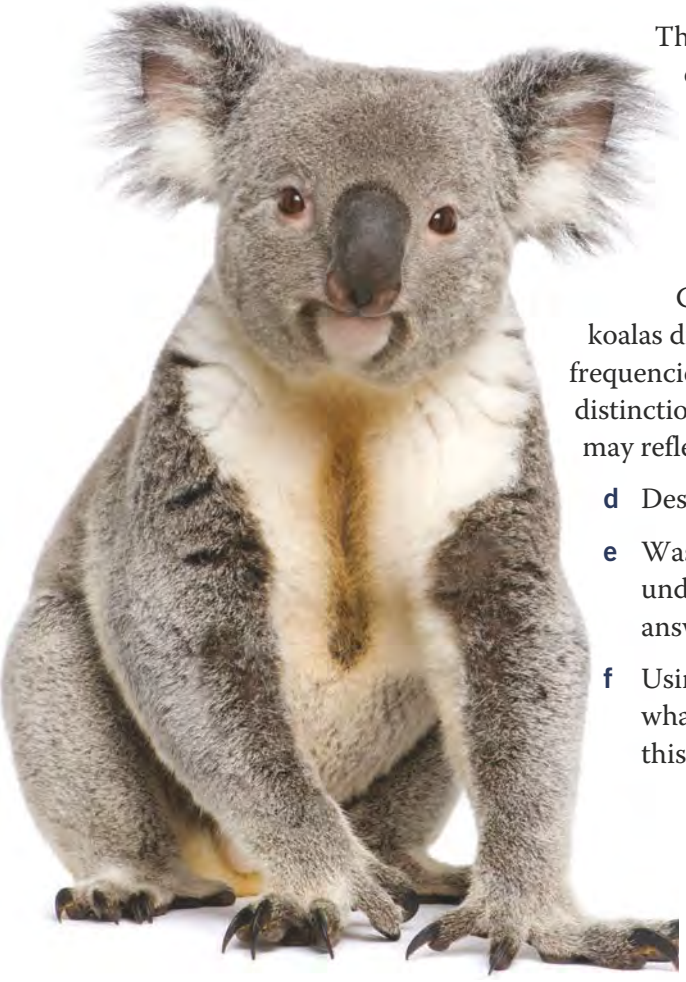
a Explain how a genetic bottleneck may lead to a decrease in genetic diversity. (2 marks)

Despite the koalas' lack of genetic diversity, the population size has been increasing steadily over many generations.

b Suggest one reason for the koalas' success despite the lack of genetic diversity within the gene pool of the population. (1 mark)

c In order to re-establish populations on mainland Australia, some scientists have suggested that breeding individuals from French and Kangaroo Island should be released into Victoria. Give one reason for this suggestion. (1 mark)

As part of a larger study of the genetic structure of koala populations in southern Australia, scientists undertook a survey of mitochondrial DNA restriction fragment length polymorphism (mtDNA-RFLP) variability. Genomic DNA of 91 koalas from five populations was examined using 23 different restriction enzymes.



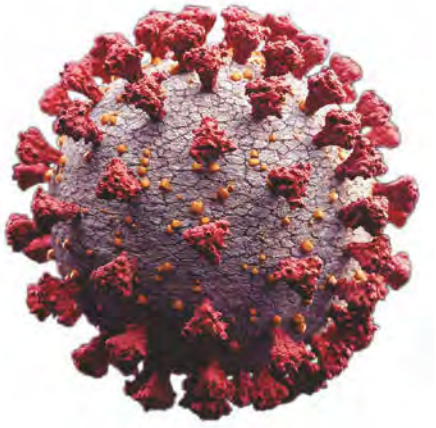
The French Island koala populations, and populations established predominantly from French Island immigrant koalas, showed groups of alleles that were inherited together from a single parent. The mtDNA data was therefore consistent with the interpretation that the koala translocation program has resulted in similar allele frequencies among the populations involved. South Gippsland is not recorded as having received translocated koalas directly, and has significantly different mtDNA-RFLP allele frequencies than all other populations examined. The fact that this distinction was not previously observed in nuclear allele frequencies may reflect predominantly male-mediated dispersal in koalas.

d Describe the role of restriction enzymes in this study. (1 mark)

e Was the work undertaken in this study valid? Use your understanding of experimental variables to support your answer. (2 marks)

f Using evidence from the information to support your answer, what was the advantage of using mtDNA over nuclear DNA in this study? (3 marks)

19 The year 2020 will be known as the year when COVID-19 took hold of the world. This highly infectious disease is the result of a newly discovered coronavirus that is thought to have crossed the species barrier from bats to humans. By mid-2021 this novel coronavirus (pictured here) had caused more than 3.5 million deaths around the world.



a What feature(s) of this virus are detected as non-self by a host's immune system? (1 mark)

b Being a virus, COVID-19 is considered to be a non-cellular pathogen. Identify another example of a non-cellular pathogen that is capable of infecting animals. (1 mark)

c How does the virus use the host cell as part of its replication process? (2 marks)

d A virus compromising a cell can lead to the activation of a cell-mediated response. Outline the key steps that occur to activate this adaptive immune response. (3 marks)

e Outline how MHC I markers play a role in the cell-mediated response. (1 mark)

f Explain how there is crossover between the cell-mediated and humoral responses during the activation process. (3 marks)

20 Whooping cough is a bacterial infection caused by *Bordetella pertussis*. It is spread when someone coughs or sneezes and you breathe it in. The bacteria target the lung tissue and airways, causing violent coughs, reducing the individual's ability to breathe.

a Aside from bacteria, identify another example of a cellular pathogen. (1 mark)

The best form of prevention for whooping cough is vaccination programs.

b Some people are scared of getting vaccinated, because it involves injection by needle. Outline the importance of the needle. (2 marks)

c Vaccination programs are used to build up immunity within the community, in order to achieve herd immunity. Why is herd immunity important for a community? (1 mark)

d Vaccinations are an artificial active form of immunity. How does this compare to a natural passive form of immunity? (2 marks)

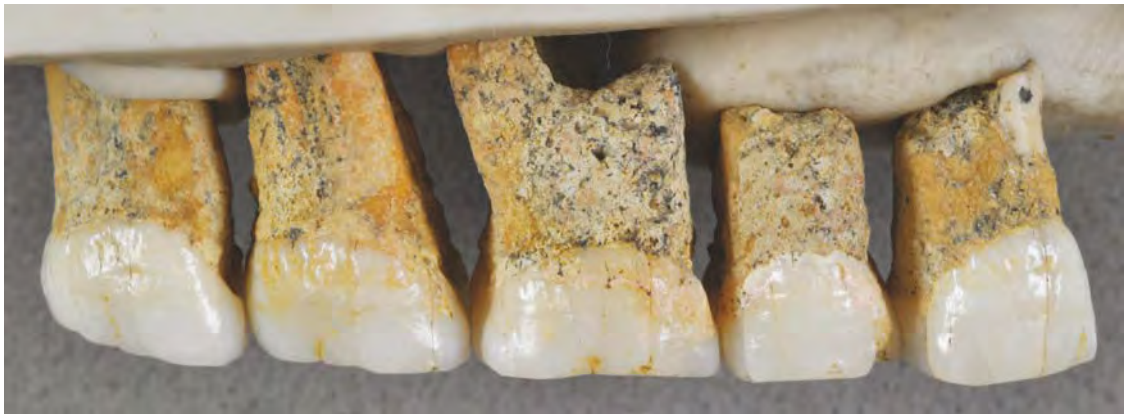
e How does a mother with a newborn baby provide natural passive immunity to her baby? (2 marks)

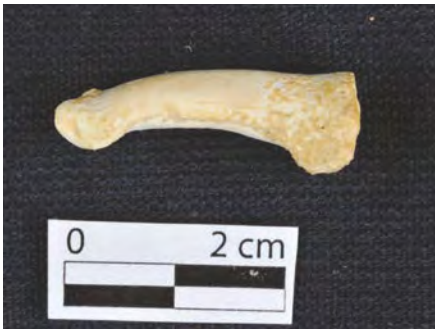
f The whooping cough vaccination program is usually administered at 6 weeks, and at 4, 6 and 18 months of age. At the second administration of the vaccine, polio antigens are added. Graph the antibody levels for the whooping cough and polio over the life of the program. (3 marks)

21 Read the following extract from *National Geographic* and answer the questions below.

In 2019, scientists announced they had discovered a new species of hominin, called *Homo luzonensis*, in the Philippines, initially uncovered in 2007 from a cave on the island of Luzon. Similar to that of *Homo floresiensis* discovered in Indonesia, it was a small bodied hominin that lived approximately 50 000 to 67 000 years ago. The hominin was identified from fossilised remains that consisted of only 7 teeth and 6 small bones. However, Aida Gomez-Robles, a palaeontologist from University College London, was very hesitant to say that this finding confirmed a new species. The small size of the teeth indicate a more modern hominin, however, a foot bone was more similar to that of an Australopithecine. Attempts to extract DNA from the fossilised remains were unsuccessful.

Some of the teeth and one of the bones are shown here.





a Describe two conditions that would have led to the formation of the *Homo luzonensis* fossil. (2 marks)

Yousuke Kaifu, a scientist from Tokyo's National Museum of Nature and Science, was quoted as saying '[the discovery] further highlights remarkable diversity of archaic hominins once present in Asia, in a way beyond my expectation'.

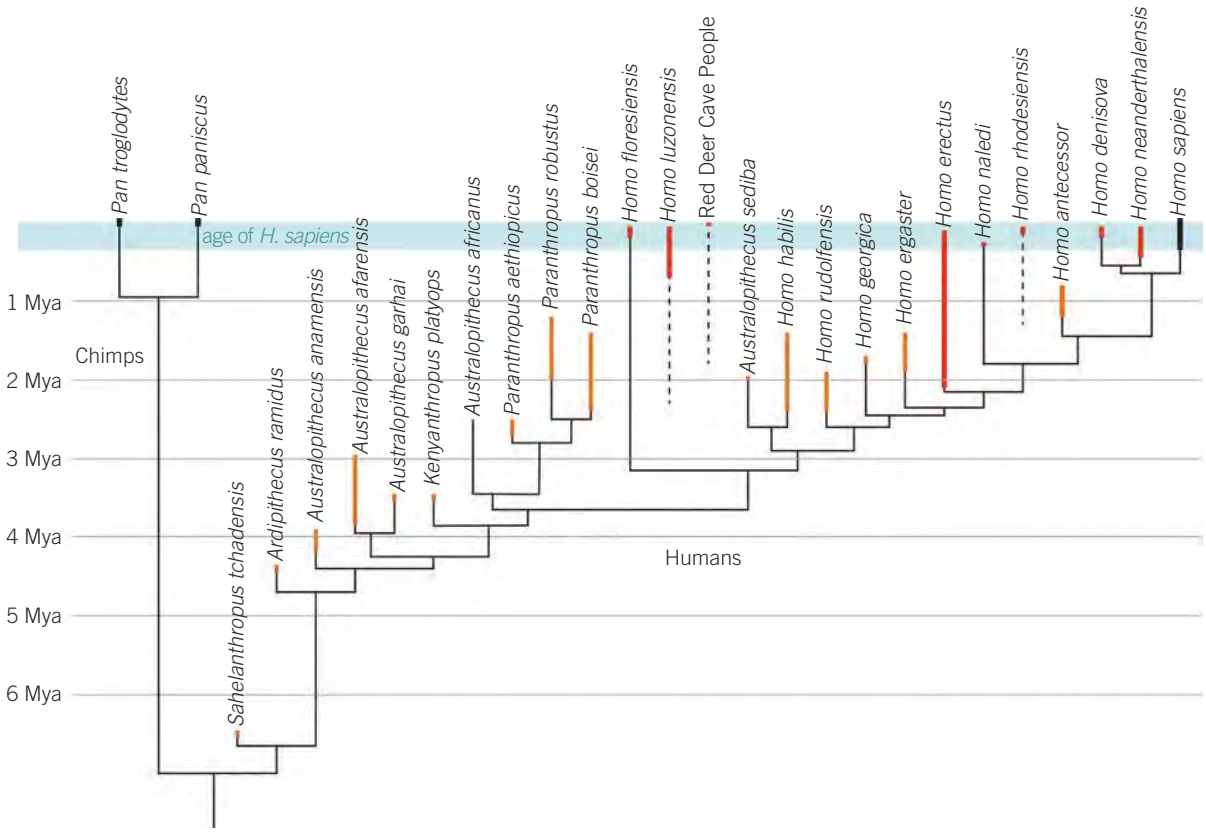
b Why was the palaeontologist hesitant to confirm that this discovery did indicate a new species of hominin? (1 mark)

c Why is it impossible to determine whether there was any interbreeding with other hominin species that lived in Asia at a similar time? (1 mark)

d How does the discovery of *Homo luzonensis* confirm the diversity of hominin ancestors present in Asia? (2 marks)

e Does the estimated timing of existence for *Homo luzonensis* add confirmation to the time of arrival of ancestors of Aboriginal and Torres Strait Islander peoples? (2 marks)

f The exact placement of *Homo luzonensis* in the hominin phylogeny is currently under debate. The mixture of both *Australopithecus* and *Homo* features makes it difficult to accurately classify from the limited evidence currently available.



Explain the structural features that *Homo luzonensis* would show for it to be more closely related to each of the following. Use features different to those already presented in the information provided.

i the *Australopithecus* genus (1 mark)

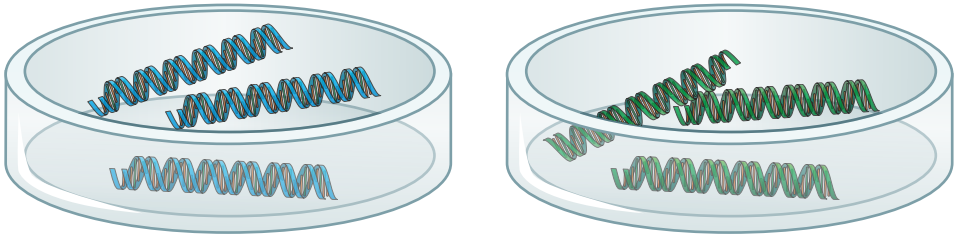
ii the *Homo* genus (1 mark)

g If more fossilised remains can be uncovered in the future, explain how the concept of a 'molecular clock' could be used to determine relatedness between *Homo luzonensis* and other hominins. (2 marks)

h Identify one structural feature that the common ancestor of *Homo luzonensis* and other hominins would share with its diverging groups. Explain your answer. (2 marks)

22 Historically, a technique used to compare the similarity of a DNA sequence of different species was DNA hybridisation. This technique relies on heating the double-stranded DNA until it unwinds and the two strands separate (dissociate). When the temperature drops, two complementary strands will pair up again, due to hydrogen bonding between complementary nucleotides to re-form a double-stranded DNA molecule. The steps in this method are indicated below.

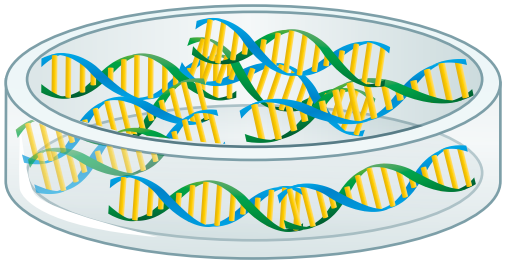
Step 1 DNA is extracted from tissues of two species. The DNA is cut up using restriction enzymes to investigate a specific gene or region of a gene.



Species 1 Species 2

Step 2 The double-stranded DNA from each of the two species is separated by heating. The temperature at which the DNA strands separate, known as the melting temperature or T_m, is recorded.

Step 3 The separated strands of the two species are then mixed together and will recombine to some extent as a hybrid DNA molecule. Pairing of the single strands will occur where the two strands have complementary nucleotides. Where they are different, the strands will not pair up. Therefore, the greater the similarity between the DNA of the two organisms, the greater the level of hybridisation that will occur.



Step 4 The level of similarity is measured by reheating the hybrid molecule. The T_m needed to dissociate this hybrid DNA is recorded. When the degree of complementary base pairing is lower, there are fewer hydrogen bonds to break and therefore a lower temperature is required. The higher the temperature, the greater the similarity between the two species. It is assumed that the greater the genetic similarity, the closer the two species are in evolutionary terms.

In an experiment, scientists wanted to use DNA hybridisation to test whether humans were more closely related to chimpanzees or bonobo monkeys.

a What are two controlled variables that would need to be considered in this experiment? (2 marks)

b What is the dependent variable in this experiment? (1 mark)

c What would be an appropriate control group for this experiment? Outline what data is expected from this and how this will act as a standard of comparison for the experimental groups. (2 marks)

d What would be the expected results from this experiment? And what does it indicate about the evolutionary relatedness of these species? (2 marks)

e Provide one reason why this technique has been replaced over time with the aid of digital technology that can compare the sequence of nucleotides of different species. Use scientific investigation terminology to support your answer. (2 marks)

f This was just one form of molecular homology that could be used to compare the relationship between different species. Identify another molecular comparison, other than comparing nucleic acid sequences, that could be used. (1 mark)

Glossary

absolute dating
determines the actual age of the specimen being analysed

accuracy
how close the measurements are to the 'true' value of the quantity being measured

activation energy
the minimum amount of energy required for a reaction to proceed

active immunity
when an individual's adaptive immune response is activated

active site
the region of an enzyme where the substrate binds for a chemical reaction to take place

active transport
the net movement of substances from a region of low substance concentration to a region of high substance concentration using a protein carrier; requires energy to be input

adaptations
the behavioural, physiological and structural features of an organism that help them survive in an environment

adaptive immune response
response of the vertebrate immune system to a specific antigen, which typically results in immunological memory

adaptive radiation
the rapid diversification of a large number of related species from a single ancestral species

ADP (adenosine diphosphate)
a compound composed of adenosine and two phosphate groups that can store energy when another phosphate group is added, forming ATP

aerobic cellular respiration
cellular respiration that occurs in the presence of oxygen and involves the transformation of the chemical energy stored in glucose into ATP; includes the Krebs Cycle and the electron transport chain, which occur in the mitochondria

agglutination
where antibodies join to the pathogen's antigens, joining more than one pathogen together

aim
the main purpose of an investigation and what you hope to achieve

allele
an alternative form of a gene

allele frequency
the proportion of a particular allele within a population

allergen
any substance that causes an allergic reaction

allopatric speciation
a form of speciation that occurs when a geographical barrier physically divides a population; the two sub-populations accumulate so many genetic differences that they become reproductively isolated and are considered different species

allosteric site
a binding site on an enzyme, where molecules other than the substrate may bind

amino acid
the monomer that forms polypeptide chains and proteins

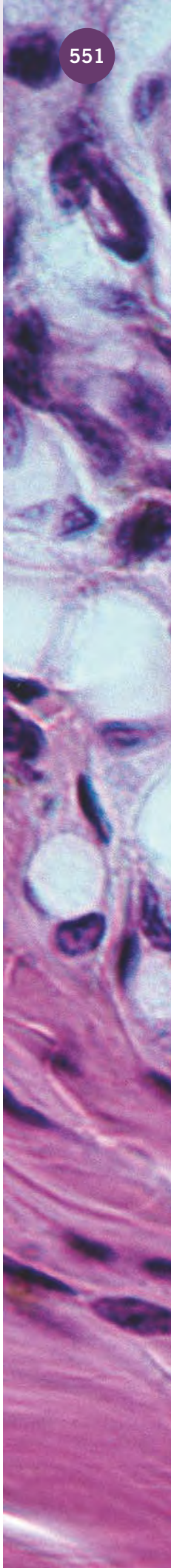
amphibians
a class of tetrapods that are semi-terrestrial, with young living in water before moving to land as adults; examples are frogs, toads and salamanders

anabolic
describes a type of chemical reaction that requires energy and involves constructing molecules from simpler components

anaerobic cellular respiration
cellular respiration that occurs in the absence of oxygen and involves the transformation of the chemical energy stored in glucose into 2 ATP; the products depend on the type of organism carrying out the process

aneuploidy
when a cell has one more or one less chromosome than expected, usually due to non-disjunction

antibiotic
a substance that inhibits the growth of bacteria; an example is penicillin



antibody
a protein that has a Y shape containing two identical arms with an antigen-binding site specific to an antigen (or allergen); also referred to as immunoglobulins (Ig)

anticodon
a set of three bases on transfer RNA that are complementary to codons in messenger RNA

antigen
a unique marker on the surface of cells or viruses that is used in the identification of self from non-self

antigenic drift
mutations altering viral surface antigens, making the virus unrecognisable to the host's immune system; can result in an epidemic

antigenic shift
reassortment of genes on genomes from different viruses infecting the same host cell, altering viral surface antigens, resulting in novel strains that can cause pandemics

antigen-presenting cell (APC)
a specific type of white blood cell that uses phagocytosis to engulf a pathogen before displaying peptide fragments (epitopes) on its MHC Class II markers for detection by white blood cells

apoptosis
death and disintegration of a cell through a controlled process

arthropod
a type of animal that has no internal backbone, a segmented body and a hard, external covering known as an exoskeleton

ATP (adenosine triphosphate)
the main immediate source of chemical energy in a cell, powering most cellular processes; when a phosphate group is removed, energy is released and ADP is formed

ATP synthase
an enzyme responsible for catalysing the formation of ATP from ADP and P_i

attenuated
describes a pathogen or infectious agent that has been altered to remove its virulence

autoimmune disease
a disease in which the immune system acts abnormally and begins to attack the body's own cells (self cells)

autotroph
an organism that synthesises its own organic materials (food), by capturing energy and taking in inorganic compounds from its physical environment, to meet its energy needs (*auto* = self, *troph* = food)

background extinction rate
the normal extinction rate expected to occur over a period of time due to natural environmental factors

bacteria
unicellular, prokaryotic organisms that lack membrane-bound organelles

bacterial resistance
the ability of bacteria to survive and reproduce in the presence of an antibiotic that has been designed to slow their growth or kill them; arises by mutation and becomes widespread when antibiotics are over-used

bacteriophage
a virus that specifically infects bacteria

biodiversity
the variety of plant and animal life in an ecosystem at any given time

biofuel
fuel produced from biomass; usually liquid

biomacromolecule
a large biological polymer, such as a protein, a nucleic acid or a carbohydrate

biomass
organic material, including plant material, animal by-products, microbes and waste material; produced by many different industries

bipedal
able to walk on two legs or upright; *bi* = two, *pedal* = foot/feet

bipedalism
the characteristic of being bipedal, i.e. walking upright on two legs

bispecific monoclonal antibody
a monoclonal antibody that has two different antigen-binding sites

block mutation
a type of mutation that affects large sections of DNA, typically containing multiple genes; also called chromosome mutation

blood–brain barrier
a barrier of specialised epithelial cells in the brain and spinal cord that prevents pathogens and toxins reaching the neurons

blunt ends
short lengths of fully paired nucleotides in DNA resulting from a straight cut by a restriction enzyme

bottleneck effect
when a population is drastically reduced to low numbers by a random or chance event and the allele frequencies of the surviving population do not reflect the genetic diversity of the original population

brachiopod
a marine invertebrate that consists of a hinged upper and lower shell

bulk transport
the movement of large particles (solid or liquid) through the plasma membrane, requiring the input of energy (ATP)

C_3 plants
plants that fix CO_2 from the atmosphere to form 3-phosphoglycerate or PGA, which contains three carbon atoms; are better suited to cooler and temperate climates; examples are rice, wheat, soybeans and cotton

C_4 plants
plants that fix CO_2 to form malate, which contains four carbon atoms; better suited to grasslands; examples are maize and sugar cane

CAM plants
plants that fix CO_2 to form malate, which contains four carbon atoms; better suited to deserts; examples are cacti and pineapples

Cambrian explosion
a rapid increase in complex biodiversity within the fossil record that occurred at the beginning of the Cambrian period

capsid
protective protein coat that surrounds the genetic material of a virion

carrier protein
a transmembrane protein that binds to a specific substance (e.g. glucose) and changes shape to move that substance across the plasma membrane, releasing it to the other side

Cas9
an endonuclease (enzyme) that cuts DNA at a specific point determined by guide RNA (gRNA)

cast fossil
fossil formed when an organism decays, leaving an impression, which fills with minerals, resulting in a 3D object of the organism's external surface

catabolic
describes a type of chemical reaction that releases energy and involves breaking down molecules into simpler components

catalyst
a substance that increases the rate of a reaction by lowering the activation energy and providing an alternative reaction pathway

cell theory
the theory that living things are made up of at least one cell, and that these cells are the basic unit of life and came from pre-existing cells

cell wall (in plants)
a structure only in plants that surrounds the cell and provides support and protection

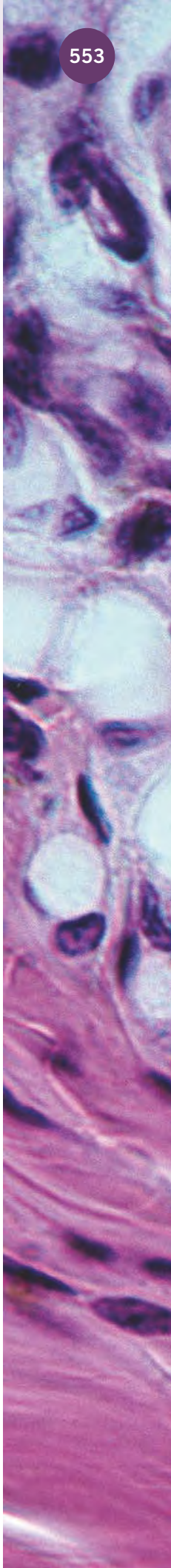
cellular pathogen
living organism that causes disease within a host

cellular respiration
a series of chemical reactions in which the organic compound glucose is broken down, producing various products (depending on presence or absence of oxygen) and energy stored in ATP

channel protein
a transmembrane protein that allows hydrophilic or polar substances to move across the plasma membrane from a region of high concentration to a region of low concentration

chlorophyll
the green pigment on the thylakoid membranes of the chloroplasts of green plants; absorbs light energy for photosynthesis

chloroplast
an organelle where photosynthesis occurs; contains chlorophyll



chordate
any animal that contains evidence of a spinal cord at some point in its development

chromosome abnormality
mutation that involves a whole chromosome, or a change in the number of chromosomes, which can be identified using a karyotype; examples are aneuploidy and polyploidy

cilia
short microtubule projections from a cell that move to provide motility (movement of the cell) or movement of fluid

clonal expansion
the proliferation of a lymphocyte that has been selected by an antigen

clonal selection theory
the scientific theory that a specific antigen activates a specific lymphocyte that has a complementary receptor

coding region
the introns and exons of a gene that are transcribed into pre-mRNA

codon
a set of three bases in mRNA that code for a specific amino acid

coenzyme
an organic molecule that contains carbon and bind to enzymes to help them to function; examples are NADP, NAD and FAD

competitive inhibition
the process of disrupting the function of an enzyme by blocking its active site with a molecule other than the substrate

complementary
the term used to describe the fact that a nitrogenous base can only pair with one other nitrogenous base (cytosine is complementary to guanine, adenine is complementary to thymine)

concentration gradient
the difference between the concentrations of a substance in two regions; if there is a large difference, the concentration gradient is steep

conclusion
a summary of what you can deduce from the results of the investigation, including whether the tested hypothesis was supported

condensation reaction
a reaction in which two molecules are joined to make a larger molecule, resulting in the loss of a smaller molecule as another product (in organisms, this is usually water)

conjugated monoclonal antibody
an antibody that has been combined with a radioactive substance or chemotherapy agent (a cytotoxin)

conjugation
the process by which genetic material is exchanged between a donor bacterium and a recipient bacterium; occurs through a tube via direct cell–cell contact; also known as horizontal gene transfer

Connection to Country
the relationship between people and their indigenous land or environment

contagious
describes a pathogen that is able to spread from an infected person to others

continuous data
data that is measureable and continuous, with infinite possible values; best represented by a line graph

control group
the set-up or group in an experiment that does not receive treatment; it is used as the 'standard of comparison'

controlled variable
anything kept constant, or monitored, so it does not affect the independent and dependent variables, and therefore the validity of experimental results

CRISPR
a section of DNA containing short repetitions of nucleotides, involved in bacterial defence against viruses

CRISPR-Cas9
an immune system in bacteria that uses CRISPR nucleotide sequences and the Cas9 DNA-cutting enzyme, also modified for use as a genome editing tool

crista
a fold in the inner membrane of a mitochondrion and site of the third stage of aerobic cellular respiration, the electron transport chain

cyanobacteria
a group of prokaryotic microorganisms that are capable of photosynthesis; recognised as the earliest form of life on Earth

cytokines
compounds released by cells as chemical signals to other cells

cytoplasm
all the contents inside the membrane of a cell, except the nucleus; includes organelles

cytosol
the liquid inside a cell, between the organelles (doesn't include the organelles)

defensins
proteins that are toxic to microbes

degenerate
describes a genetic code in which multiple codons code for the same amino acid; also referred to as redundant

denaturation
the process by which a protein loses its 3D conformational structure through breaking of hydrogen bonds, caused by an external stress such as high temperature or pH

dendritic cell
white blood cell with many folds and projections in its membrane, carries out phagocytosis and acts as an antigen-presenting cell to the adaptive immune system

dependent variable
the variable that changes in response to changes in the independent variable; the experimenter measures these changes

digestion
(in the context of restriction enzymes) a reaction using an enzyme to break down large molecules

discrete data
data that is countable in discrete categories; contains distinct or separate values; best represented by a bar graph

disease
any condition that affects the normal function of either a part of an organism or the complete organism

divergent evolution
where two or more species form from a single ancestral species over time

DNA ligase
an enzyme that joins two pieces of DNA at their sugar–phosphate backbone

DNA profiling
a method of DNA analyis in which regions of DNA from different individuals are analysed and compared

DNA standard
a DNA sample that contains fragments of DNA of known size that is used to compare the sizes of unknown DNA fragments in base pairs or kilo base pairs; also known as a DNA ladder

dormant
when a virus is present within the host but is inactive and therefore not currently causing symptoms associated with the disease

effector cell
a cell that has been activated to perform its role

embryo
an early stage of development in the womb

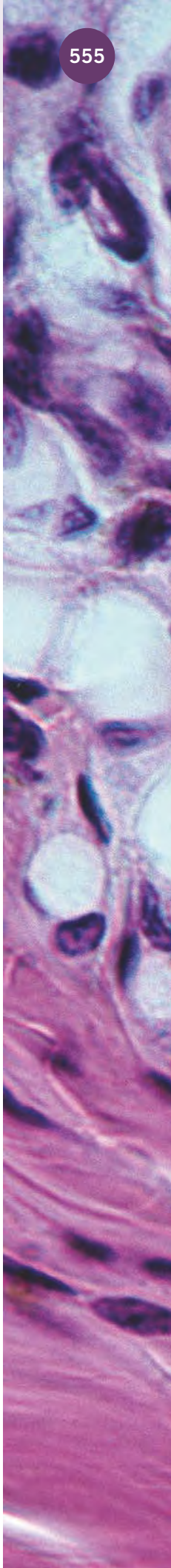
emerging infectious disease
a disease not yet seen in people, or a disease that is increasing in incidence or geographical range

endemic
the usual area where an organism is found

endocytosis
the movement of large particles (or a large quantity of small particles) into the cell without directly crossing the plasma membrane, using vesicles and ATP

energy shuttle
the cycling between the formation of ATP when energy is stored and the formation of ADP and P_i when energy is released; also known as the ATP–ADP cycle

enzyme
a type of protein, also referred to as a biological catalyst, that speeds up reactions within an organism by lowering activation energy



enzyme saturation
the point at which the rate of reaction reaches a maximum, with no further increase at a specific enzyme concentration

eon
a long period of time that consists of at least two eras

eosinophil
white blood cell that targets parasites

epidemic
the rapid spread of an infectious disease to a large number of people within a population

epidemic
when more than the expected number of cases of a disease occur in a community or region, during a given period of time (9)

epidemiologist
professional who studies the occurrence of diseases in a population

epidemiology
a branch of medicine based on the study of disease distribution and control

epitope
the specific region of an antigen that is recognised by the immune system

era
a subdivision of an eon

ethics
moral principles that guide our beliefs about what is right or wrong conduct

eukaryote
single-celled or multicellular organism whose cells include membrane-bound organelles; includes protists, fungi, plants and animals

evolution
a change in the allele frequencies of a population over time

exocytosis
the movement of large particles (or a large quantity of small particles) out of the cell without directly crossing the plasma membrane, using vesicles and ATP

exon
a region of a gene that contains genetic information that codes for the specific protein to be synthesised

facilitated diffusion
the net passive movement of a particular substance from a region of high concentration to a region of low concentration with the assistance of carrier proteins or channel proteins; also known as protein-mediated transport

fatality
the occurrence of death

fermentation
the process by which glucose is broken down in the absence of oxygen to produce 2 ATP; also called anaerobic cellular respiration

fertile
able to reproduce

fever
a rise in body temperature caused by infection

first line of defence
the first innate response; consists of physical, chemical and microbiota barriers

first-generation biofuels
a biofuel produced from edible feedstocks, e.g. starch and glucose from plants like corn and sugar cane

flagella
long microtubules projecting from a cell that move to provide motility (movement of the cell) or movement of fluid

fluid mosaic model
a model that represents the plasma membrane as a combination (mosaic) of phospholipids, proteins, cholesterol and carbohydrates that gives the membrane its fluid nature

foramen magnum
hole in the base of the skull through which the spinal cord enters/exits the skull

fossil
the remains or traces of a pre-existing life form

fossil record
a record of organisms that once lived, through geological time, as documented by fossils

founder effect
when a small sample of a large population moves away to colonise a new area and becomes isolated; the allele frequencies of the founder population do not represent the genetic diversity of the larger original population

frameshift mutation
a type of point mutation that occurs when one base is inserted into or deleted from a gene, causing an incorrect reading of the codons due to a shift in the reading frame

fungi
a wide variety of eukaryotic organisms that include mushrooms, mould and yeasts

gel electrophoresis
a technique used to separate different-sized fragments of DNA (or protein)

gene cloning
the production of exact copies (clones) of a gene (DNA sequence) using various DNA manipulation techniques

gene editing
the insertion, removal or replacement of DNA within the genome of a living organism

gene expression
conversion of the code in DNA of a gene into a protein through protein synthesis

gene flow
the exchange of genetic information, specifically alleles, between populations

gene pool
the sum total of alleles present in a population of organisms

genetic diversity
the genetic variability within a species

genetic drift
a random change in allele frequency, occurring naturally in every population, due to chance events

genetic screening
DNA profiling to determine whether an individual is carrying a particular gene for a disorder

genetic transformation
the genetic alteration of a cell, resulting from taking up foreign DNA

genetically modified organism (GMO)
an organism that has had its genome altered

genome
the collection of all of the genes contained with the DNA of an organism

genome editing
(also referred to as gene editing) the insertion, removal or replacement of DNA within the genome of a living cell

genotype
the genetic make-up or the combination of alleles for a particular gene of an organism

genus
a group of related organisms that share a recent common ancestor

geological time scale
a scale dividing Earth's history into intervals according to the geological and biological events and conditions present at that time

glycolysis
the first stage of cellular respiration, where glucose is broken down into two pyruvate molecules in the cytosol, producing 2 ATP and 2 NADH; does not require oxygen

Golgi apparatus
an organelle consisting of layers that modifies and packages proteins

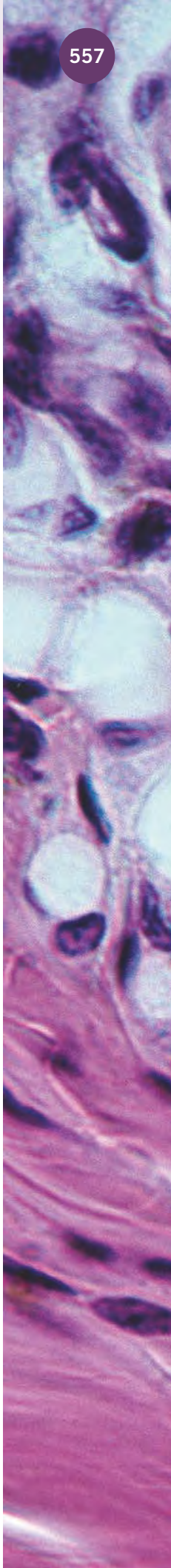
granum
(plural *grana*) a stack of thylakoid membranes inside the chloroplast of plant and algae cells

guide RNA (gRNA)
a specific RNA sequence that recognises the desired DNA and directs the Cas enzyme there to cut DNA

haemagglutinin
a glycoprotein embedded in the viral envelope of the influenza virus; plays an important role in the attachment and entry of the virus into the host cell

half-life
the time taken for 50% of a parent isotope to decay into its corresponding daughter isotope

herd immunity
when a large percentage of a population is immune to a disease (through vaccination), slowing the spread of the disease and protecting those who are not immune



heterotroph
an organism that ingests organic materials by feeding on autotrophs or on other organisms and their products, in order to make energy in the form of ATP (*heteros* = other, *trophe* = food)

heterotrophic
describes any organism that obtains its nutrients by feeding on organic matter

histamine
compound released by cells to start an inflammatory response

hominin
the subfamily consisting of all current and extinct bipedal primates

hominoid
a superfamily consisting of all current and extinct humans and apes

homologous chromosomes
chromosomes that have matching structural features (size, banding pattern, centromere location) and gene loci

homologous structure
a structure within a group of species that performs a different function yet has the same underlying structure

host
an organism that has been infected by a pathogen

hybridoma
a cell that is a result of combining of a B lymphocyte and a cancer cell

hydrophilic
water attracting or water soluble; dissolves readily in water

hydrophobic
water repelling; does not dissolve readily in water

hyphae
long, branching filaments that extend off the main body of the fungus and secrete digestive enzymes

hypothesis
a prediction of the outcomes, which are testable experimentally and form the basis of the methodology

immunological memory
the ability of the immune system to quickly and specifically recognise an antigen that the body has previously encountered and initiate a corresponding immune response

immunotherapy
a treatment that uses the activation or suppression of the immune system

independent variable
the variable for which quantities are changed by the experimenter

index fossil
a fossil that is used to date and correlate the strata within which it is found

indirect transmission
transmission of a pathogen from a location where it has been away from its host for a long time

infection
when a pathogen has breached the first line of defence and begun to replicate

infectious
able to be transmitted between hosts

infectious (communicable) disease
a disease that can be transmitted from one organism to another

inflammatory response
heat, pain, redness, swelling and loss of function as part of the innate immune response to harmful stimuli

inhibitor
a molecule that is involved in disrupting the function of an enzyme, either directly (competitive) or indirectly (non-competitive)

innate response
a non-specific defence against a pathogen

interbreed
mate with an organism of another species (sometimes used between different genetic groups or populations)

interstitial fluid
fluid that collects in spaces between cells and tissues

introduction
a brief but succint explanation of the reason for undertaking the investigation; includes key biological concepts, aim and hypothesis

intron
a region of a gene that contains sequences that do not code for the protein to be expressed

invertebrate
an organism that does not have a backbone

isotope
variant of an element that differs in the number of neutrons in the nucleus

karyotype
a pictorial representation of chromosomes that allows a geneticist to determine size, banding pattern, shape and number of chromosomes in an individual's somatic cell; allows the determination of diploid number, gender and chromosomal abnormalities

light dependent stage
the first stage of photosynthesis; occurs in the thylakoid membranes and involves the splitting of water using light energy

light independent stage
the second stage of photosynthesis; occurs in the stroma of the chloroplast and involves the use of carbon dioxide to create glucose; also called the Calvin Cycle or carbon fixation

limiting factor
any factor that slows down the rate of a reaction or process when there is not enough of it, for example, in photosynthesis, carbon dioxide, water, chlorophyll and light energy

line of best fit
a line on a graph that shows the general trend of the data points; the distance to the points above the line should equal the distance to the points below the line

lipophilic
dissolves easily in lipids; also called hydrophobic

lipophobic
does not dissolve readily in lipids; also called hydrophilic

lymph
colourless fluid that flows through the lymphatic system

lymphocyte
a type of white blood cell; includes B and T cells

lymphoid organ
organs involved in the production or function of lymphocytes

lysis
breakdown of the cell membrane

lysosome
an organelle containing enzymes that break down foreign matter or materials no longer required

macrophage
large white blood cell that carries out phagocytosis and may act as an antigen-presenting cell

malaria
a serious disease caused by the *Plasmodium* protozoan, which invades red blood cells when transmitted by mosquito vectors into the host

mass extinction
a loss of approximately three-quarters of all species that exist on Earth in a 'short' geological period of time

mast cell
white blood cell involved in the inflammatory response, releasing histamine, which triggers inflammation

matrix
the fluid component of a mitochondrion and site of the second stage of aerobic cellular respiration, the Krebs Cycle

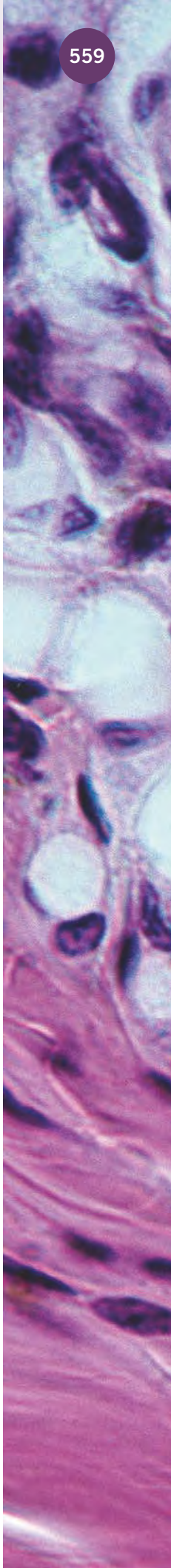
megafauna
large or giant mammals that were still living in the Quaternary period

mesophyll
the tissue forming the middle layer of leaves where the photosynthetic cells with chloroplasts are located

metamorphic rock
a type of rock that arises from the transformation of existing rocks

method
a series of numbered steps describing the procedure

MHC (major histocompatibility complex) marker
a protein that is found on the surface of cells and is used in the identification of pathogens in the immune response



MHC Class I marker
a type of protein marker on the surface of all nucleated cells that assists in the identification of self from non-self

MHC Class II marker
a type of protein marker on antigen-presenting white blood cells that is used in the activation of a specific immune response

missense mutation
a mutation in which the base change in the nucleotide sequence of the DNA changes the amino acid that is coded for; a type of substitution point mutation

mitochondrion
an organelle where respiration occurs, releasing energy (ATP)

molecular clock
using the predicted mutation rate of DNA (or amino acid) sequences to determine the approximate time at which two species diverged

molecular homology
the analysis of DNA and amino acid sequences as evidence of evolutionary relationships

molecular phylogeny
comparison of nucleotide sequences of genes and amino acid sequences of proteins, from which evolutionary relationships can be inferred

monoclonal antibody (mAb)
antibody made by cloning a unique parent immune cell, produced in large quantities in the laboratory as a drug targeting specific cells or substances

monomer
a molecule that forms bonds with other identical molecules as the repeating units that make up a polymer

mould fossil
an impression that forms from the decay of the organism within a rock

mutagenic agents
agents known to cause mutations; for example, radiation sources and chemicals; also called mutagens

mutation
a permanent change in the nucleotide sequence of a section of DNA

mycelium
a collection of hyphae

myeloma cell
an abnormal plasma cell used in the production of monoclonal antibodies

myriapod
a subphylum of mostly terrestrial arthropods including millipedes, centipedes and other 'many-legged' invertebrates

NAD^+
a coenzyme that accepts hydrogen ions and transfers them from one place to another during cellular respiration

$NADP^+$
a coenzyme that accepts and transfers hydrogen ions from one place to another during photosynthesis

naive
not yet activated

natural killer (NK) cell
white blood cell involved in the innate immune response; kills infected host cells and cancer cells

natural selection
an evolutionary process whereby those individuals in a population that have a particular set of alleles are best suited to the environment and will survive, reproduce and pass on their genetic information to the next generation

negative control
a control group that isn't expected to produce a result

neuraminidase
a glycoprotein embedded in the viral envelope of the influenza virus; plays an important role in the detachment of new viral particles from the host cell, freeing them to infect new host cells

neutrophil
white blood cell that carries out phagocytosis and kills pathgens with defensins

niche
the role that a species plays within its ecosystem

non-cellular pathogen
a disease-causing agent that lacks cellular structures and cannot replicate outside a host cell

non-competitive inhibition
the process of disrupting the function of an enzyme through a molecule binding to another site on the enzyme, which alters the shape of the active site in such a way that the substrate cannot bind

non-infectious (non-communicable) disease
a disease that cannot be transmitted from one organism to another

non-self antigen
an antigen on the surface of cells of an organism that is identified by the immune system as foreign to the organism and triggers an immune response when detected

nonsense mutation
a mutation that occurs when the base change in the nucleotide sequence of the DNA codes for a STOP codon, prematurely halting the production of the polypeptide; a type of substitution point mutation

normal flora
naturally occurring microorganisms that live in or on animals and plants and do not cause harm or an immune response

nucleoid
in a prokaryote, an irregularly shaped area where the genetic material is located

nucleotide
the monomer (building block) of nucleic acids which are joined together to form DNA or RNA (polymers); consists of a phosphate group, sugar and nitrogenous base

nucleus
double-membrane bound organelle that contains genetic material (DNA, RNA)

operator
a section of DNA code where the repressor protein can bind

operon
a series of genes under the control of a single promoter and operator

organelle
a compartment within a cell that performs specific functions

osmosis
the net passive movement of free water from a region of high free water concentration to a region of low free water concentration across a semi-permeable membrane until equilibrium is reached

outlier
a reading that is very different from other results obtained for the same measurement

palaeontologist
a scientist who studies fossils

palindrome
a sequence that reads the same in both directions

pandemic
an outbreak of infectious disease that occurs over a wide geographical area, affecting a large number of people

parasite
an organism that lives on or in another host organism and survives by feeding off its nutrients, causing the host organism harm

passive immunity
short-term immunity resulting in a person receiving antibodies from another person or animal; no memory

passive transport
the net movement of substances from a region of high substance concentration to a region of low substance concentration without the need for energy input; can also occur in non-living systems where there is no cell membrane

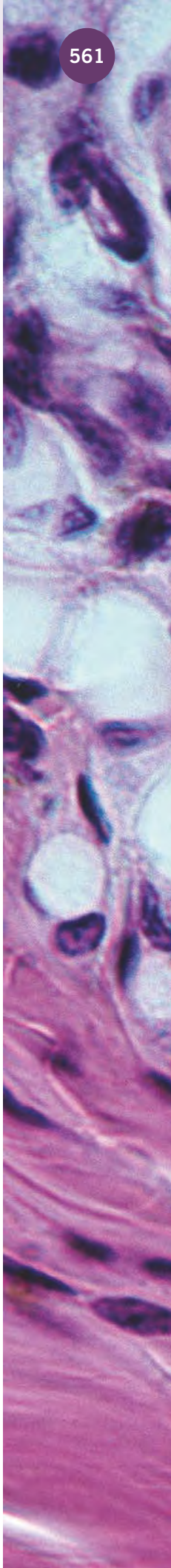
pathogen
a disease-causing agent

pathogenic bacteria
bacteria that cause harm and an immune response

peptide bond
a chemical bond between two amino acids

perforin
a protein that kills cells by making holes in their plasma membranes

period
a time interval characterised by specific rock layers; periods are subdivisions of eras



permeable
allows things to pass through; for example, a semi-permeable membrane only allows some substances to pass through

petrification
the replacement of an organism's organic matter with minerals, turning it into a stony material

PGA
3-phosphoglycerate, a 3C (three-carbon) compound formed when the enzyme Rubisco catalyses the attachment of a carbon from carbon dioxide to RuBP during the Calvin Cycle of photosynthesis

PGAL
glyceraldehyde-3-phosphate, is a 3C (three-carbon) sugar that leads to the formation of glucose and regenerates RuBP in the process to continue the Calvin Cycle

phagocytosis
a type of endocytosis in which a solid substance enters a cell via vesicle mediated transport

phagosome
a vesicle that engulfs a pathogen during phagocytosis

phenotype
a physical characteristic determined by genotype and environment

photolysis
the splitting of water using the light energy from the Sun

photorespiration
the series of reactions that occur as a consequence of Rubisco using O_2 as a substrate instead of CO_2; an inefficient process that cannot produce glucose

photosynthesis
a chemical reaction in which light energy is used to convert the inorganic compounds carbon dioxide (CO_2) and water (H_2O) into the organic compound glucose; occurs in the chloroplast (photo = light, synthesis = build or put together)

phylogenetic tree
a branching diagram used to represent the evolutionary relationships between species

phylogeny
a branch of science that studies the evolutionary relationships between a group of species

pili
hairlike structures found on the surface of some bacterial cells; assist in surface attachment and transfer of genetic material between bacterial cells; singular pilus

pinocytosis
a type of endocytosis in which a liquid or dissolved substance enters a cell via vesicle mediated transport

placebo
a substance that has no therapeutic effect but may have a psychological effect

plasma membrane
a membrane made up of two layers (known as a bilayer) of phospholipids that encloses the contents of a cell

plasmid
a circular piece of double-stranded DNA found naturally in bacteria

plasmodesmata
microscopic channels that connect the cell walls of plant cells, allowing communication and transport between the cells

Plasmodium
a genus of parasitic protozoans that are transmitted by the *Anopheles* mosquito, resulting in the disease malaria

plate tectonics
a scientific theory that focuses on the separation of Earth's crust into plates that move across the underlying mantle

point mutation
a change to one base in the nucleotide sequence of a section of DNA; includes base substitutions and frameshift mutations

polar
describes a molecule that has different charged sides ('poles') and dissolves in water, which is also a polar substance

polymer
a molecule made up of a large number of smaller, repeating units

polymerase chain reaction (PCR)
a technique used to amplify a sample (template) of DNA

polypeptide
a long chain of amino acids forming part of a protein

polyploidy
a condition in which an organism has more than two full sets of chromosomes in its cells; more common in plants than animals

population
a group of individuals of the same species living in the same region at a given time

positive control
a control group that receives a treatment with a known response that can then be compared to the experimental group(s)

precision
how close all the measurements are to each other

primate
the order consisting of all current and extinct humans, apes and monkeys, characterised by dextrous hands with opposable thumbs and a relatively large, developed brain

primer
synthetic single-stranded piece of DNA (or RNA) complementary to a specific sequence of nucleotides

prion
a pathogenic protein with a mutant structure that can trigger normal proteins to fold abnormally, resulting in disease

prokaryote
a single-celled organism that does not have membrane-bouind organelles; includes bacteria and archaea

promoter
the region of a gene at which RNA polymerase binds, to initiate transcription

protein carrier
a selective peripheral protein that uses ATP to move substances across a plasma membrane; also known as a protein pump

protein-mediated transport
when a transmembrane protein assists in the transport of a substance across a plasma membrane; also known as facilitated diffusion

proteome
the complete collection of proteins within an organism at a given time

protozoa
unicellular, eukaryotic organisms that belong to the kingdom Protista; singular *protozoan*

PrP^{C}
normal form of the protein associated with prions

PrP^{Sc}
disease-causing, mutant prion

putative
a term used to describe something that is expected or assumed to have existed, without any current direct proof

qualitative data
data that is descriptive (not numeric)

quantitative data
data is measured and represented numerically

radiometric dating
a method of absolute dating that uses the concept of isotope decay to determine the age of a geological sample

random error
an unpredictable variation in the readings obtained, due to variables not all being controlled (extraneous variables), and resulting in the readings being higher or lower than expected

rate
the speed at which a process occurs, or how quickly the reactants are used up and the products are created

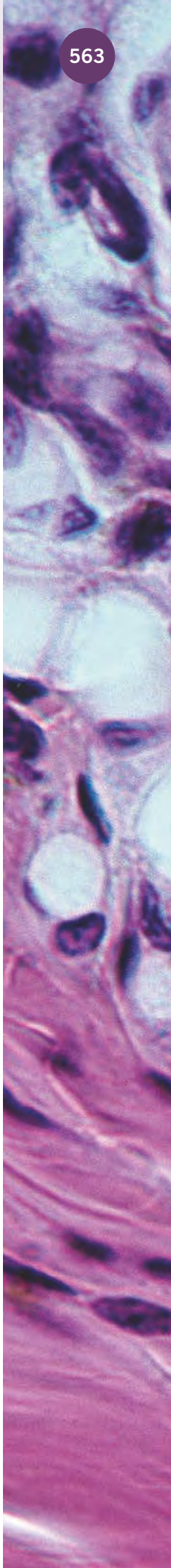
recognition (restriction) site
a specific sequence of nucleotides that is the location for a restriction enzyme to cut

recombinant DNA
DNA that has been artificially formed by combining DNA from different organisms

re-emerging infectious disease
a disease that appears again after having previously been eliminated

regulatory gene
a region of DNA that codes for a regulatory protein, which controls the expression of other genes

relative dating
determines the age of a specimen by comparing its placement with that of other fossils or the rock layers it is found in



reliability
the extent to which an experiment always yields the same results under the same conditions

repeatability
recording of results produced when the experiment is repeated in one lab by one operator under the same conditions

repressor
a regulatory protein that binds to DNA, inhibiting transcription

reproducibility
when the same results are obtained for the same experiment by different operators using different equipment

reproductive isolation
the inability of two groups of organisms to interbreed successfully; genetic isolation also ensures there is no gene flow between the two populations

reptiles
a class of air-breathing tetrapods with skin covered in scales, that lay eggs on land

reservoir
original or usual site of a disease in relation to its spread

restriction enzyme
a bacterially produced protein that cuts DNA at a specific sequence of nucleotides called a recognition site; also known as a restriction endonuclease

ribosome
a non-membrane bound organelle involved in synthesis of proteins

rice blast disease
a fungal infection of rice that results in characteristic lesions and spots throughout the plant's shoot system

rough endoplasmic reticulum
organelle that transports proteins in vesicles to the Golgi apparatus

Rubisco
RuBP carboxylase, an enzyme that catalyses the formation of PGA by fixing carbon dioxide to RuBP during the Calvin Cycle of photosynthesis

RuBP
ribulose bisphosphate, a 5C (five-carbon) compound that combines with carbon dioxide at the start of the Calvin Cycle of photosynthesis to form PGA

saponin
soapy compound that occurs naturally in plants; has anti-fungal and antimicrobial properties

sauropod
a large herbivorous dinosaur characterised by its long neck and tail, and four-legged stance

second-generation biofuels
a biofuel produced from non-edible feedstocks, e.g. cellulose and other fibrous plant materials derived from crop residues, straw and municipal waste

sediment
naturally occurring material that is formed through the effects of weathering and erosion

sedimentary rock
a type of rock that is formed from the accumulation of sediment into layers

selection pressures
the conditions or factors that influence allele frequencies in a population by contributing to the selection of which phenotypes survive in a given environment, e.g. availability of resources, environmental conditions, predators and disease

selective advantage
a trait or phenotype that provides a survival advantage

selective breeding or artificial selection
a process whereby humans intervene in the breeding of a species to keep desired features in a population that are economically beneficial or aesthetically pleasing, by selecting which organisms are to survive and reproduce

self-antigen
an antigen on the surface of cells of an organism that is identified by the immune system as belonging to the organism and therefore does not trigger an immune response

semi-permeable membrane
a membrane that only lets certain substances cross it; also called partially permeable, differentially permeable and selectively permeable

silent mutation
a mutation where the change in the nucleotide sequence doesn't change the amino acid that is coded for

simple diffusion
the net passive movement of a substance from a region of high concentration to a region of low concentration until equilibrium is reached; a form of passive transport, as it does not require energy

single-variable exploration
an investigation that contains only one independent and one dependent variable

smooth endoplasmic reticulum
organelle that synthesises and transports lipids

speciation
the evolutionary process of forming a new species from a pre-existing ancestral species

species
a group of organisms that can interbreed, producing fertile and viable offspring

spores (bacterial)
structures that bacteria form that aid in the survival of the organism under adverse environmental conditions

stakeholder
an individual or organisation who will be affected by the factor under consideration

sticky ends
short lengths of unpaired nucleotides in DNA resulting from a staggered cut by a restriction enzyme

stratigraphy
a branch of geology that uses the 'principle of rock succession' to examine the order and position of strata in connection with fossilised remains

stroma
the gel-like fluid inside a chloroplast which surrounds the grana; site of the light independent stage of photosynthesis

stromatolite
a structure that consists of layered deposits made by cyanobacterial colonies; fossilised stromatolites are among the earliest fossils known

structural gene
a region of DNA that codes for a protein which performs a specific function for a cell or organism

structural morphology
the study of an organism's features and form to determine the evolutionary relationship of species

substitution mutation
a type of point mutation that occurs when one nitrogenous base in a gene is replaced with another base; includes silent, missense and nonsense mutations

substrate
a molecule that binds to the active site of an enzyme and then takes part in a reaction; also referred to as a reactant

supereon
a period of geological time that consists of more than one eon

surfactants
molecules that reduce the surface tension of water and aqueous solutions

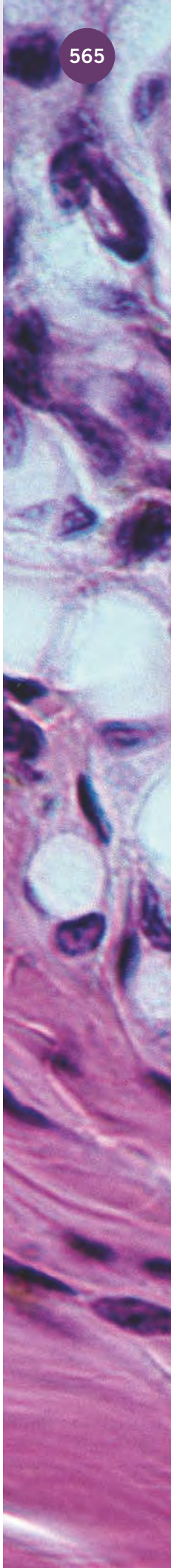
sympatric speciation
a form of speciation that occurs without the involvement of a physical barrier; often occurs in plants as a consequence of polyploidy

systematic error
when the readings obtained from measurements differ from the 'true' value consistently in one direction every time

taxonomy
the process of identifying, naming and grouping organisms

terminator
the region of a gene at which transcription stops and the RNA polymerase dissociates from the strand

terrestrial
describes any living organism that lives or grows on land



tetanus
a bacterial disease characterised by muscle stiffness and spasms

tetrapod
any vertebrate animal that has four legs or limb-like attachments, e.g. amphibians, reptiles, birds and mammals

theropod
a type of carnivorous dinosaur with short forelimbs that ran on powerful, hind legs

thylakoid membrane
disc-shaped interconnected membrane-bound compartments inside a chloroplast that make up the grana and are the location of the pigment chlorophyll, and therefore the site of the light dependent stage of photosynthesis

tinea
a common fungal infection that results in a red, flaky rash in the area of the body that is affected

title
the research question under investigation; includes information about what is being tested

tonicity
how the concentration of solutes dissolved in an extracellular solution determines the direction and rate of osmosis and therefore the volume of a cell

trace fossil
fossilised signs or remains of an organism's activity, e.g. tracks

transcription
the process through which DNA is converted to messenger RNA (mRNA) and the genetic code in the DNA is copied to the mRNA

transformed bacteria
bacteria that have taken up foreign DNA; in gene cloning, the foreign DNA is in the recombinant plasmid

transgenically modified organism (TMO)
a type of GMO that has had genetic material from a different species inserted into its genome

transitional fossil
a hybrid fossil that shows traits of both an ancestral group and a descendant group

translation
the process through which the information in mRNA is converted into a sequence of amino acids to synthesise a protein

transmission
how a pathogen is passed between hosts

true value
the value or range of values that would be obtained if the quantity could be measured perfectly

universal triplet code
the genetic coding system based on codons with three bases, shared by most organisms

vaccination
the administration of a vaccine to cause an adaptive immune response

vaccine
substance that contains an agent (usually an antigen or an attenuated version of the pathogen) that will induce an adaptive immune response when administered

vacuole
an organelle that stores substances; important in maintaining structure of plant cells

validity
the extent to which all variables in the experiment have been controlled, so that the independent variable is the only factor that changes

variable number tandem repeats (VNTRs)
region of a chromosome that shows variation between individuals in length and number of repeats of nucleotide sequences; also referred to as short tandem repeats (STRs) when 2–6 base pairs long

vasodilation
the widening of a blood vessel (especially capillaries), to increase blood flow

vector
(genetics) a DNA molecule used as a vehicle to carry foreign genetic material from one organism to another; (disease) a living organism that carries and transmits a pathogen from one organism to another

vertebrate
having a backbone

vesicle
an organelle that transports materials between organelles and within the cell

vesicle mediated transport
the movement of substances through the plasma membrane using membrane-bound structures within the cell

vestigial structure
a structure within an organism that is no longer functional but served a purpose in a common ancestor

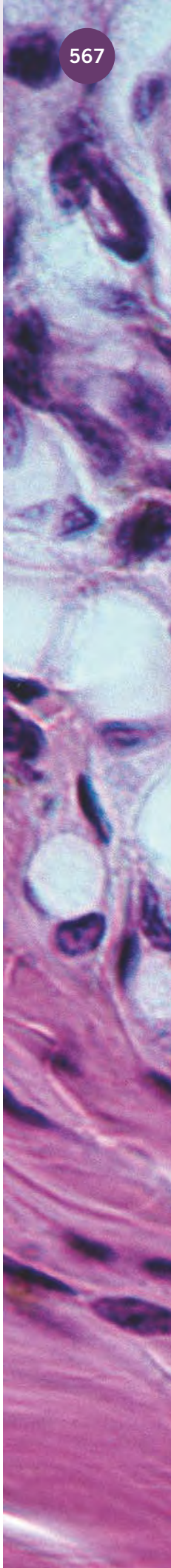
viable
able to survive

viral envelope
the lipid-based, outermost layer of the capsid on some types of viruses

virion
a single virus particle existing outside a host cell

virulence
how likely a pathogen is to cause harm/ disease

virus
a non-cellular pathogen that causes disease by taking over host cell machinery to rapidly produce identical virus copies, which further infect host cells, disrupting normal cellular function



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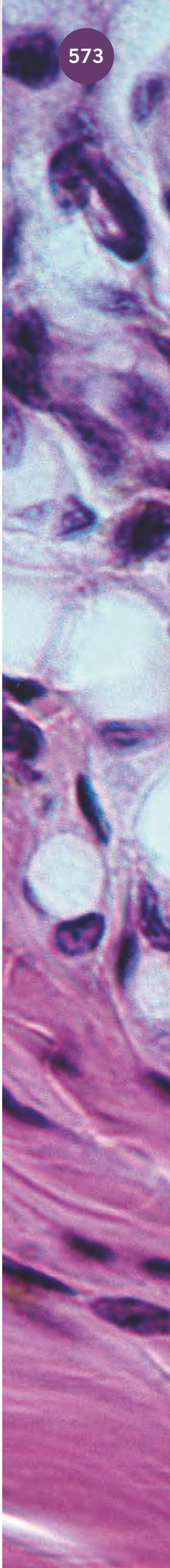

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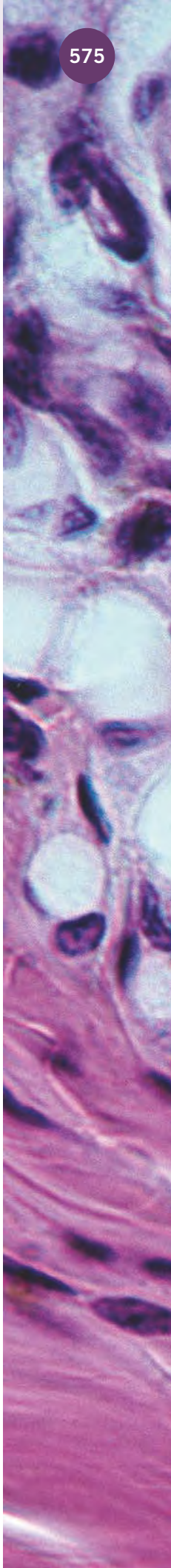

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