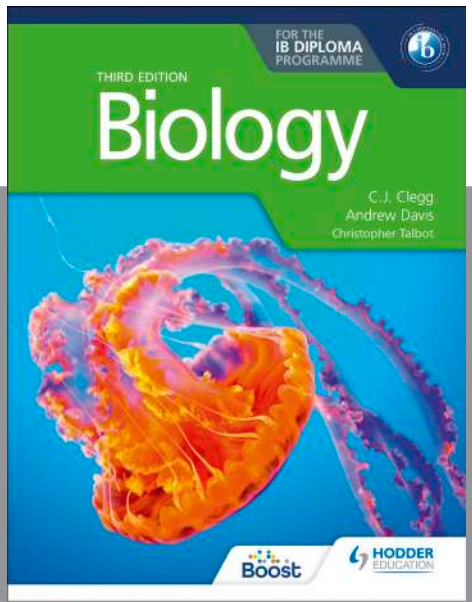
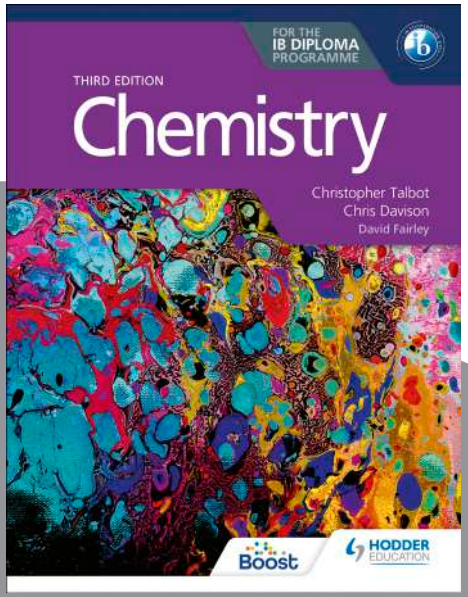
NEW 3RD EDITIONS

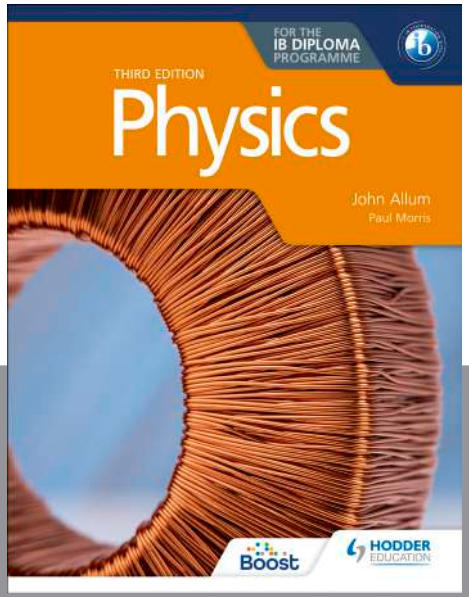
FOR THE IB DIPLOMA PROGRAMME

Biology, Chemistry and Physics for the IB Diploma

Trust experienced and best-selling authors to navigate the new syllabuses confidently with these co-published coursebooks that encompass inquiry-based, conceptually-focused teaching and learning.







Coursebooks developed in cooperation with the International Baccalaureate®

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HODDER EDUCATION

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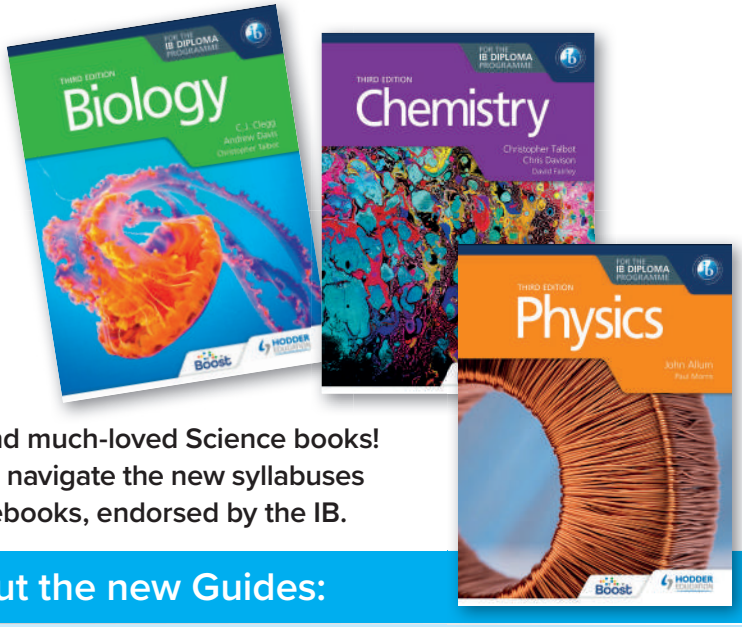


Dear IB Science educator,

We're really excited to be publishing for the new IB Sciences: Biology, Chemistry and Physics Guides for first teaching 2023, and first assessment 2025.

We are now going into third editions of our bestselling and much-loved Science books! Let our trusted, experienced, and expert authors help you navigate the new syllabuses confidently with Hodder Education's co-published coursebooks, endorsed by the IB.

We asked our authors what they like about the new Guides:

Andrew Davis

Andrew Davis has taught biology for over 20 years. He is the author of several IB textbooks and digital teaching and learning resources for Diploma and MYP, including *Biology for the MYP 4&5: By Concept.*

> ***"This new IB Biology syllabus has many exciting changes, with greater integration of concepts, content, and skills. The reorganization of content into Themes, each based around two linked concepts, enables students to gain a greater appreciation of interconnections within the subject.***
>
> ***The new syllabus offers greater flexibility for how the course is delivered. Each Theme follows the same path through four levels of organization: molecules, cells, organisms and ecosystems, giving the course a logical structure, which enables students to scaffold their understanding. The course can be taught by Theme, or by level of organization, or a combination of both."***

Chris Talbot has taught chemistry, biology and TOK at schools in Singapore for over 20 years. He is the author of numerous science textbooks, including *Chemistry for the MYP 4&5: By Concept.*

> ***"I like the division into Structure and Reactivity, especially in the context of Organic chemistry.***
>
> ***I particularly like the emphasis on fundamental chemical concepts, principles and facts and their integration and linking across traditional chemistry topics."***

Chris Talbot

Chris Davison

Chris Davison graduated with a PhD in Organic chemistry and taught at Oundle School before joining Wellington College where he teaches DP Chemistry and runs practical and theoretical based extension lessons.

> ***"I like that the new Guide has been designed to show the interdependence of the different areas of chemistry, inorganic, organic and physical. The topics fit under two broad titles, Structure, and Reactivity, and new linking questions highlight where subject matter both builds on and leads to other areas of the Guide.***
>
> ***The new Guide includes fossil fuels, biofuels and fuel cells – areas which are appealing and relevant to students and which only appeared in the option module previously."***

John Allum taught physics to pre-university level in international schools for more than thirty years (as a head of department). He has now retired from teaching, but lives a busy life in a mountainside village in South East Asia. He has also been an IB examiner for many years.

> ***"It is much more diverse than previously, providing students and teachers with many opportunities for variety, expanding beyond the limitations of just pure physics.***
>
> ***The removal of the Options and some of the more difficult content makes the course more manageable."***

John Allum

hoddereducation.com/ib-dp-science

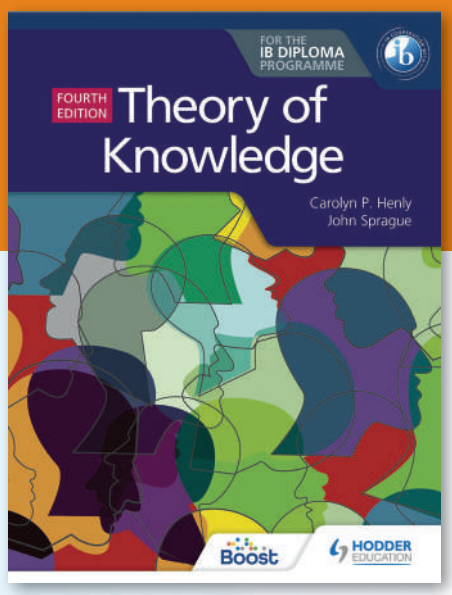
Our new co-published coursebooks support the new Guides by:

- Providing **guiding questions** at the start of each chapter along with a list of learning outcomes, each of which is mapped to the relevant assessment objective.
- Integrating **conceptual understanding** into all units, to ensure that a conceptual thread is woven throughout the course, making the subject more meaningful. This helps students develop clear evidence of synthesis and evaluation in their responses to assessment questions.
- Stimulating **creativity, curiosity, and critical thinking** with 'Inquiry', 'Tools', 'Approaches to Learning (ATL)' and 'Theory of Knowledge (TOK)' features throughout.
- Building the skills and techniques covered in the **Tools** (Experimental techniques, Technology and Mathematics). These skills are directly linked to relevant parts of the syllabus so they can be explored during delivery of the course. These skills also provide the foundation for practical work and internal assessment. They support the application and development of the inquiry process in the delivery of the new course.
- Supporting the **Inquiry** process with the new Inquiry feature, which focuses on aspects of the Inquiry cycle skills: Inquiring and designing, Collecting and processing data, Concluding and evaluating.
- Integrating **Theory of Knowledge** into your lessons and providing opportunities for cross-curriculum study with TOK links and Inquiries that provide real-world examples, case studies and questions. For Biology and Chemistry, the TOK links are written by the author of our bestselling TOK coursebook, John Sprague. For Physics the links are written by Paul Morris, our MYP by Concept series and Physics author, who has taught IB Physics for over 20 years and has also examined TOK.
- Developing **ATL** skills with a range of engaging activities with real-world applications.
- **Creating opportunities** for students to design investigations, collect data, develop manipulative skills, analyse results, collaborate with peers and evaluate and communicate their findings.
- Providing **Top tips** and **Common mistakes** to help ensure students' understanding is accurate and they are able to apply this effectively in their studies.
- **Improving performance** with short and simple knowledge-checking questions, a mixture of questions from past exam sessions and author-written exam-style questions and hints to help avoid common mistakes.
- Developing **International mindedness** by exploring how the exchange of information and ideas across national boundaries has been essential to the progress of science and illustrates the international aspects of science.
- Providing **Nature of science** boxes that encourage thinking, exploring ethical debates and learning how scientists work in the 21st century.
- Guiding students with the **IB Learner Profile** icon to help them develop as Thinkers, Risk-takers and Communicators.
- Creating opportunities for conceptual discussions and comparisons with **linking questions** at the end of each chapter.

We asked two of our expert authors what they are most proud of and what they enjoyed writing.

John Sprague

John Sprague is the author of our bestselling *Theory of Knowledge* coursebook.



"Much like how the IB Diploma is a real "programme" in the sense that all of its moving parts mesh together, Hodder Education is working to incorporate a genuine collaborative and unified vision among its author team. We've brought together writers from the science specialists and DP Core to provide opportunities for students to experience the integrative approach to knowledge that the IBDP captures. We believe that every new publication provides us an opportunity to show our readers how the construction and transfer of knowledge is a collaborative adventure."

Chris Clegg is an experienced teacher and examiner of Biology and has written many internationally-respected textbooks for pre-university courses. He was encouraged to write by his colleague and mentor at his school, textbook writer and teacher D.G. Mackean in the 1970s, and became his co-author on numerous books. He eventually took over the biology coursebook mantle from Don in the 1980s.

C. J. Clegg

"I'm proudest of the figures and diagrams which I conceived to help students to better understand complex ideas. As a reader said after the first edition of Biology for the IB Diploma, "what rockets this book above others are the brilliant illustrations in the text. They are detailed, well-annotated and ultimately support independent learning."

I gave careful thought to my choice of language and phrasing so as to be clear and precise as a means of helping students in their understanding of the subject."

To learn more about our IB DP Science series visit **hoddereducation.com/ib-dp-science**

Yours Faithfully,

Hodder Education International Team

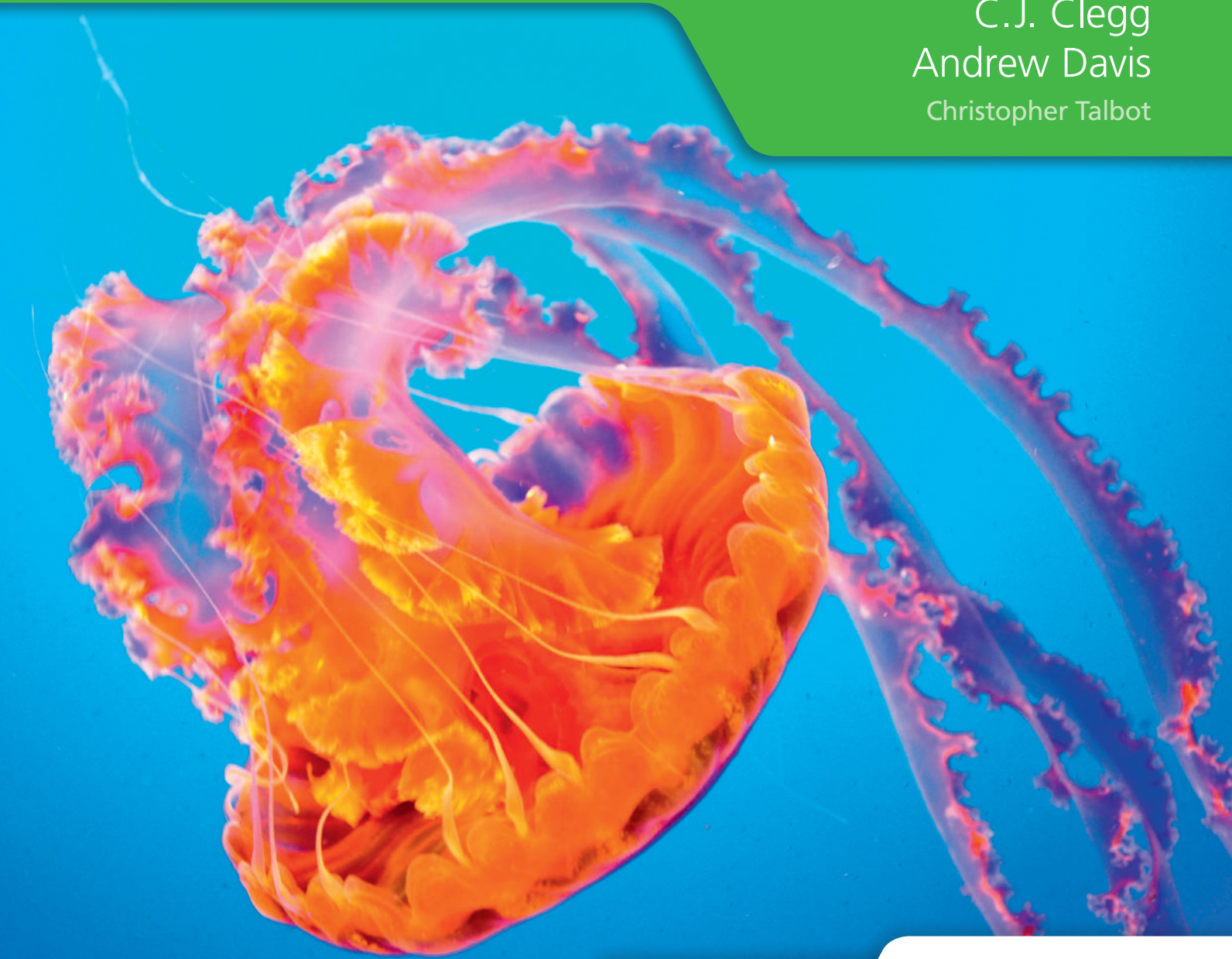
SAMPLE PAGES

FOR THE IB DIPLOMA PROGRAMME

THIRD EDITION

Biology

C.J. Clegg
Andrew Davis
Christopher Talbot



Boost

HODDER EDUCATION

Contents

Each of the four themes covered in this book is broken down into four levels of organization. These four levels are colour coded as follows:

1 Molecules
2 Cells
3 Organisms
4 Ecosystems

C Interaction and interdependence

D Continuity and change

Free online content

Go to our website www.hoddereducation.co.uk/ib-extras for free access to the following:

- Practice exam-style questions for each chapter
- Glossary
- Answers to self-assessment questions and practice exam-style questions
- Tools and Inquiries reference guide
- Internal Assessment – the scientific investigation

Introduction

Welcome to *Biology for the IB Diploma Third Edition*, updated and designed to meet the criteria of the new International Baccalaureate (IB) Diploma Programme Biology Guide. This coursebook provides complete coverage of the new IB Biology Diploma syllabus, with first teaching from 2023. Differentiated content for SL and HL students is clearly identified throughout.

The aim of this syllabus is to integrate concepts, topic content and the nature of science through inquiry. This book comprises four main themes, each made up of two broad integrating concepts:

- **Theme A**: Unity and diversity
- **Theme B**: Form and function
- **Theme C**: Interaction and interdependence
- **Theme D**: Continuity and change

Each theme is then further divided into four levels of biological organization. In this coursebook, each level is colour coded as follows:

1 Molecules **2 Cells** **3 Organisms** **4 Ecosystems**

About the authors

Chris Clegg is an experienced teacher and examiner of biology and has written many internationally respected textbooks for pre-university courses. He was encouraged to write by his colleague and mentor at his school, textbook writer and teacher D.G. Mackean, in the 1970s and became his co-author on numerous books. He eventually took over the biology coursebook mantle from Don in the 1980s.

Andrew Davis has taught biology for over 20 years. He is the author of several IB textbooks, including *Biology for the IB Diploma Study and Revision Guide, IB Diploma: Internal assessment for Biology: Skills for success*, and *Biology for the MYP 4 & 5: By Concept*. He is also author of online teaching and learning resources: *Biology for the IB Diploma Teaching and Learning* and *Biology for the IB MYP 4 & 5 Dynamic Learning*.

IB advisors

Chris Talbot graduated in Biochemistry from the University of Sussex in the United Kingdom. He has Masters Degrees in Life Sciences (Chemistry) and in Science Education from the National Technological University in the Republic of Singapore. He has taught IB Chemistry, IB Biology and Theory of Knowledge (TOK) in a number of local and international schools in Singapore. He is the author of numerous science textbooks, including *Chemistry for the MYP 4&5: By Concept*.

John Sprague has been teaching TOK for 20 years, in the UK, Switzerland and Singapore. Previously Director of IB at Sevenoaks School in the UK, he now teaches philosophy and TOK at Tanglin Trust School, Singapore.

The 'In cooperation with IB' logo signifies that this coursebook has been rigorously reviewed by the IB to ensure it fully aligns with the current IB curriculum and offers high-quality guidance and support for IB teaching and learning.

How to use this book

The following features of this book will help you to consolidate and develop your understanding of biology, through concept-based learning:

Guiding questions

- There are two guiding questions at the start of every chapter, as signposts for inquiry.
- These questions will help you to view the content of the syllabus through the conceptual lenses of both the themes and the levels of biological organization.

SYLLABUS CONTENT

- This coursebook follows the order of the contents of the IB Biology Diploma syllabus.
- At the beginning of each chapter is a list of the content to be covered, with all subsections clearly linked to the content statements and showing the breadth and depth of understanding required.

Key terms

◆ Definitions appear throughout the margins of this coursebook to provide context and to help you understand the language of biology. There is also a glossary of all key terms at **www.hoddereducation.co.uk/ib-extras**.

Common mistake

These detail some common misunderstandings and typical errors made by students, so that you can avoid making the same mistakes yourself.

Top tips!

This feature includes advice relating to the content being discussed and tips to help you retain the knowledge you need.

Concepts

The four themes that underpin the IB Biology Diploma course (A Unity and diversity, B Form and function, C Interaction and interdependence, and D Continuity and change) are integrated into the conceptual understandings of all the units to ensure that a conceptual thread is woven throughout the course.

Conceptual understanding therefore enhances your overall understanding of the course, making the subject more meaningful. This understanding assists you in developing clear evidence of synthesis and evaluation in your responses to questions asked in the assessment, and helps you make connections across the course.

Concepts are explored in context and can be found throughout the chapter.

Tools

The skills and techniques you must experience are encompassed within the Tools, which are integrated into the biology content to be practised in context. The skills in the study of biology can be assessed through internal and external assessment.

Inquiry

The application and development of the Inquiry process is supported throughout this coursebook, in close association with the Tools. The skills in the study of biology can be assessed through internal and external assessment.

WORKED EXAMPLES

These provide a step-by-step guide showing you how to answer the kind of quantitative questions that you might encounter in your studies and in the assessment.

TOK

Links to Theory of Knowledge (TOK) allow you to develop critical-thinking skills and deepen biology understanding by discussing the subject beyond the scope of the curriculum.

Links

Due to the conceptual nature of biology, many topics are connected. The Links feature states where relevant material is covered elsewhere in the coursebook. They may also help you to start creating your own linking questions.

Nature of science

Nature of science (NOS) is an overarching theme in the biology course that seeks to explore conceptual understandings related to the purpose, features and impact of scientific knowledge. It can be examined in biology papers. NOS explores the scientific process itself, and how science is represented and understood by the general public. It covers 11 aspects: Observations, Patterns and trends, Hypotheses, Experiments, Measurements, Models, Evidence, Theories, Falsification, Science as a shared endeavour and The global impact of science. It also examines the way in which science is the basis for technological developments and how these new technologies, in turn, drive developments in science.

ATL

Approaches to learning (ATL), including learning through inquiry, are integral to IB pedagogy. ATL activities are designed to get you to think about real-world applications of biology.

Going further

Written for students interested in further study, this optional feature contains material that goes beyond the IB Diploma Guide.

LINKING QUESTIONS

These questions are listed at the end of each chapter. They are designed to strengthen your understanding by making connections across the themes. The linking questions encourage you to apply broad, integrated and discipline-specific concepts from one topic to another, ideally networking your knowledge. Practice answering the linking questions first, on your own or in groups. Sample answers and structures are provided online at **www.hoddereducation.co.uk/ib-extras**. The list in this coursebook is not exhaustive; you may encounter other connections between concepts, leading you to create your own linking questions.

Self-assessment questions appear throughout the chapters, phrased to assist comprehension and recall, but also to help familiarize you with the assessment implications of the command terms. These command terms are defined in the online glossary. Practice exam-style questions for each chapter allow you to check your understanding and prepare for the assessments. The questions are in the style of those in the examination so that you get practise seeing the command terms and the weight of the answers with the mark scheme. Practice exam-style questions and their answers, together with self-assessment answers, are on the accompanying website, IB Extras: **www.hoddereducation.co.uk/ib-extras**

Skills are highlighted with this icon. Students are expected to be able to show these skills in the examination, so we have explicitly pointed these out when they are mentioned in the Guide.

International mindedness is indicated with this icon. It explores how the exchange of information and ideas across national boundaries has been essential to the progress of science and illustrates the international aspects of biology.

The **IB learner profile** icon indicates material that is particularly useful to help you towards developing the following attributes: to be inquirers, knowledgeable, thinkers, communicators, principled, open-minded, caring, risk-takers, balanced and reflective. When you see the icon, think about what learner profile attribute you might be demonstrating – it could be more than one.

Tools and Inquiry

Skills in the study of biology

The skills and techniques you must experience through this biology course are encompassed within the tools. These support the application and development of the inquiry process in the delivery of the course.

Tools

- **Tool 1:** Experimental techniques
- **Tool 2:** Technology
- **Tool 3:** Mathematics

Inquiry process

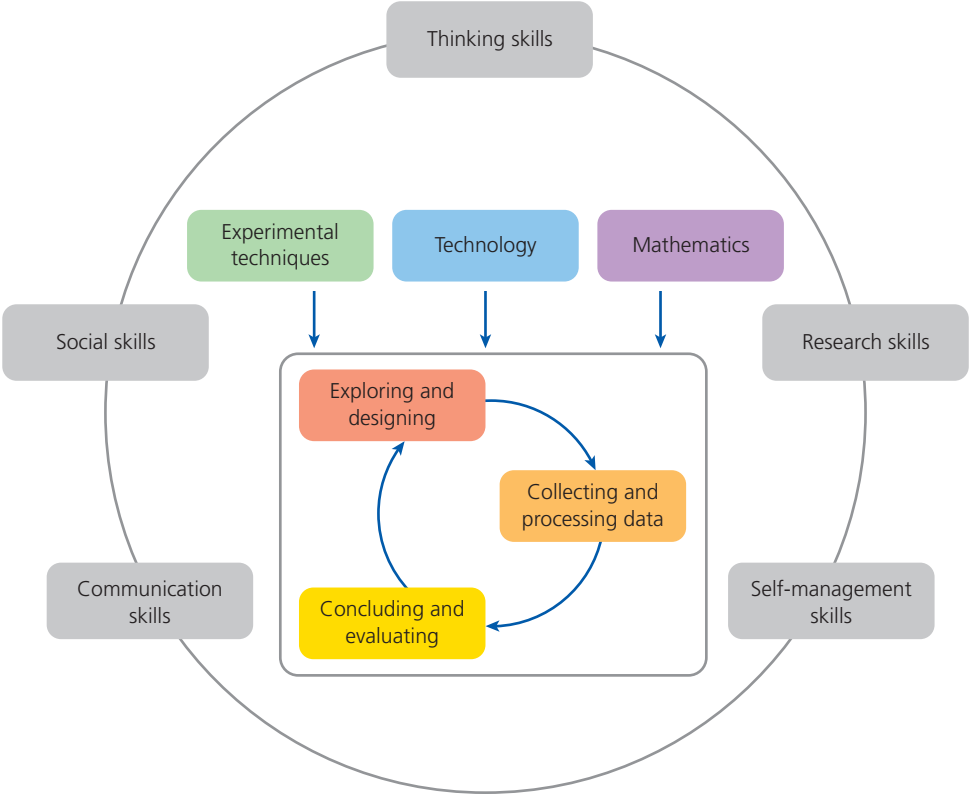
- **Inquiry 1:** Exploring and designing
- **Inquiry 2:** Collecting and processing data
- **Inquiry 3:** Concluding and evaluating

Throughout the programme, you will be given opportunities to encounter and practise the skills; and instead of stand-alone topics, they will be integrated into the teaching of the syllabus when they are relevant to the topics being covered.

You can see what the Tools and Inquiry boxes look like in the *How to use this book* section on page vi.

The skills in the study of biology can be assessed through internal and external assessment.

The approaches to learning provide the framework for the development of these skills.



Skills for biology

From IB Diploma Programme Biology Guide, page 28

Visit the following website to view the online glossary chapter covering all of the Tools and Inquiries:
www.hoddereducation.co.uk/ibextras

Tool 1: Experimental techniques

Skill	Description
Addressing safety of self, others and the environment	Recognize and address relevant safety, ethical or environmental issues in an investigation.
Measuring variables	Understand how to accurately measure the following to an appropriate level of precision: • mass • volume • time • temperature • length. Make careful observations, including the following: • counts • drawing annotated diagrams from observation • making appropriate qualitative observations • classifying.
Applying techniques	Show awareness of the purpose and practice of: • paper or thin layer chromatography • colorimetry or spectrophotometry • serial dilutions • physical and digital molecular modelling • a light microscope and eyepiece graticule • preparation of temporary mounts • identifying and classifying organisms • using a variety of sampling techniques/using random and systematic sampling • karyotyping and karyograms • cladogram analysis.

Tool 2: Technology

Skill	Description
Applying technology to collect data	• Use sensors. • Identify and extract data from databases. • Generate data from models and simulations.
Applying technology to process data	• Use spreadsheets to manipulate data. • Represent data in a graphical form. • Use computer modelling. • Carry out image analysis.

Tool 3: Mathematics

Skill	Description
Applying general mathematics	• Use basic arithmetic and algebraic calculations to solve problems. • Carry out calculations involving: decimals, fractions, percentages, ratios, proportions, frequencies (including allele frequencies), densities, approximations and reciprocals. • Calculate measures of central tendency: mean, median and mode. • Apply measures of dispersion: range, standard deviation (SD), standard error (SE), interquartile range (IQR). • Use and interpret scientific notation (for example, 3.5×10^6). • Use approximation and estimation. • Calculate scales of magnification. • Calculate rates of change from graphical or tabulated data. • Understand direct and inverse proportionality between variables, as well as positive and negative correlations between variables. • Calculate and interpret percentage change and percentage difference. • Distinguish between continuous and discrete variables. • Calculate the actual size from a micrograph that has a scale bar. • Apply Simpson's reciprocal index. • Apply the Lincoln index. • Apply the chi-squared test. • Apply the *t*-test.
Using units, symbols and numerical values	• Apply and use SI prefixes and units or non-SI metric units. • Express quantities and uncertainties to an appropriate number of decimal places.
Processing uncertainties	• Understand the significance of uncertainties in raw and processed data. • Record uncertainties in measurements as a range (±) to an appropriate precision. • Express ranges, degrees of precision, standard error or standard deviations as error bars. • Express measurement and processed uncertainties to an appropriate number of decimal places or level of precision. • Apply the coefficient of determination (R^2) to evaluate the fit of a trend line. • Interpret values of the correlation coefficient and identify correlations as positive or negative. • Apply and interpret appropriate tests of statistical significance (for example, chi-squared test).
Graphing	• Sketch graphs, with labelled but unscaled axes, to qualitatively describe trends. • Construct and interpret tables, charts and graphs for raw and processed data including bar charts, histograms, scatter graphs, line and curve graphs, logarithmic graphs, pie charts and box-and-whisker plots. • Plot linear and non-linear graphs showing the relationship between two variables with appropriate scales and axes. • Draw lines or curves of best fit. • Interpret features of graphs including gradient, changes in gradient, intercepts, maxima and minima. • Draw and interpret uncertainty/error bars. • Extrapolate and interpolate graphs. • Design dichotomous keys. • Represent energy flow in the form of food chains, food webs and pyramids of energy. • Represent familial genetic relationships using pedigree charts.

Inquiry process

Inquiry 1: Exploring and designing

Skill	Description
Exploring	• Demonstrate independent thinking, initiative and insight. • Consult a variety of sources. • Select sufficient and relevant sources of information. • Formulate research questions and hypotheses. • State and explain predictions using scientific understanding.
Designing	• Demonstrate creativity in the designing, implementation and presentation of the investigation. • Develop investigations that involve hands-on laboratory experiments, databases, simulations, modelling and surveys. • Identify and justify the choice of dependent, independent and control variables. • Justify the range and quantity of measurements. • Design and explain a valid methodology. • Pilot methodologies.
Controlling variables	Appreciate when and how to: • calibrate measuring apparatus • maintain constant environmental conditions of systems • choose representative random samples and minimize sampling errors • set up a control run where appropriate.

Inquiry 2: Collecting and processing data

Skill	Description
Collecting data	• Identify and record relevant qualitative observations. • Collect and record sufficient relevant quantitative data. • Identify and address issues that arise during data collection.
Processing data	• Carry out relevant and accurate data processing.
Interpreting results	• Interpret qualitative and quantitative data. • Interpret diagrams, graphs and charts. • Identify, describe and explain patterns, trends and relationships. • Identify and justify the removal or inclusion of outliers in data (no mathematical processing is required). • Assess accuracy, precision, reliability and validity.

Inquiry 3: Concluding and evaluating

Skill	Description
Concluding	• Interpret processed data and analysis to draw and justify conclusions. • Compare the outcomes of an investigation to the accepted scientific context. • Relate the outcomes of an investigation to the stated research question or hypothesis. • Discuss the impact of uncertainties on the conclusions.
Evaluating	• Evaluate hypotheses. • Identify and discuss sources and impacts of random and systematic errors. • Evaluate the implications of methodological weaknesses, limitations and assumptions on conclusions. • Explain realistic and relevant improvements to an investigation.

Tables from IB Diploma Programme Biology Guide, pages 28–32

A1.1 Water

Concept: Unity and diversity

Common ancestry has given living organisms many shared features while evolution has resulted in the rich biodiversity of life on Earth.

Concept: Unity

All living organisms require water to exist. Enzymes – biological molecules that increase the rate of chemical reactions – need to be dissolved in water to work. Water provides a chemically stable medium for life processes to operate.

Guiding questions

- What physical and chemical properties of water make it essential for life?
- What are the challenges and opportunities of water as a habitat?

SYLLABUS CONTENT

This chapter covers the following syllabus content:

- A1.1.1 Water as the medium for life
- A1.1.2 Hydrogen bonds as a consequence of the polar covalent bonds within water molecules
- A1.1.3 Cohesion of water molecules due to hydrogen bonding and consequences for organisms
- A1.1.4 Adhesion of water to materials that are polar or charged and impacts for organisms
- A1.1.5 Solvent properties of water linked to its role as a medium for metabolism and for transport in plants and animals
- A1.1.6 Physical properties of water and the consequences for animals in aquatic habitats
- A1.1.7 Extraplanetary origin of water on Earth and reasons for its retention (HL only)
- A1.1.8 The relationship between the search for extraterrestrial life and the presence of water (HL only)

Water: the medium for life

The Earth is covered mainly by water and so appears a mostly blue planet when viewed from space. Approximately 71% of our planet's surface is water, with 97% found in oceans and only 3% as fresh water. Evidence from the geological record indicates that water has existed on Earth for 3.8 billion years. The Earth formed an estimated 4.5 billion years ago, so water has existed on its surface for most of its history. The first cells originated in water, where the oceans blocked harmful ultraviolet radiation from the Sun, allowing the first life to evolve. Water remains the medium in which most processes of life occur.

Water forms a large proportion of living organisms – between 65% and 95% by mass of most multicellular plants and animals (about 80% of a human cell consists of water). Despite this, and the fact that water has some unusual properties, water is a substance that is often taken for granted. As we will see in this chapter, the properties of water allow life to exist at a range of scales – from the smallest bacteria to the tallest tree – and without water life would not exist on Earth.

ATL A1.1A

Freshwater is a limited resource globally. Work in a group to produce an informative poster on the threats to freshwater sources and the solutions available for providing sufficient, clean drinking water for all.

◆ **Covalent bond**: a bond between atoms in which pairs of electrons are shared.

◆ **Polar molecule**: a molecule where there is an unequal distribution of electrical charge: one end is slightly positive and the other end is slightly negative.

◆ **Hydrogen bond**: a weak attractive intermolecular force; a hydrogen atom in a molecule is attracted to an electronegative atom, such as oxygen, in a different molecule.

Hydrogen bonds

The water molecule consists of one atom of oxygen and two atoms of hydrogen combined by sharing pairs of electrons (**covalent bonding**). However, the molecule is V-shaped rather than linear. The nucleus of the oxygen atom draws electrons (negatively charged) away from the hydrogen nuclei (positively charged) with an interesting consequence. Although overall the water molecule is electrically neutral, there is a net negative charge on the oxygen atom and a net positive charge on the hydrogen atoms. The water molecule therefore carries an unequal distribution of electrical charge within it. This arrangement is known as a **polar molecule** (Figure A1.1.1).

With water molecules, the positively charged hydrogen atoms of one molecule are attracted to negatively charged oxygen atoms of nearby water molecules, causing attractive forces called **hydrogen bonds** (Figure A1.1.1). These intermolecular forces are weak compared to covalent bonds, yet they are strong enough to hold water molecules together and to attract water molecules to charged particles or to a charged surface. Hydrogen bonds largely account for the unique properties of water. We will examine these properties next.

1 **Distinguish** between ionic and covalent bonding.

one oxygen atom combines with two hydrogen atoms by sharing pairs of electrons (covalent bond)

↓

the oxygen nucleus draws electrons (negatively charged) away from the hydrogen nucleus (positively charged)

↓

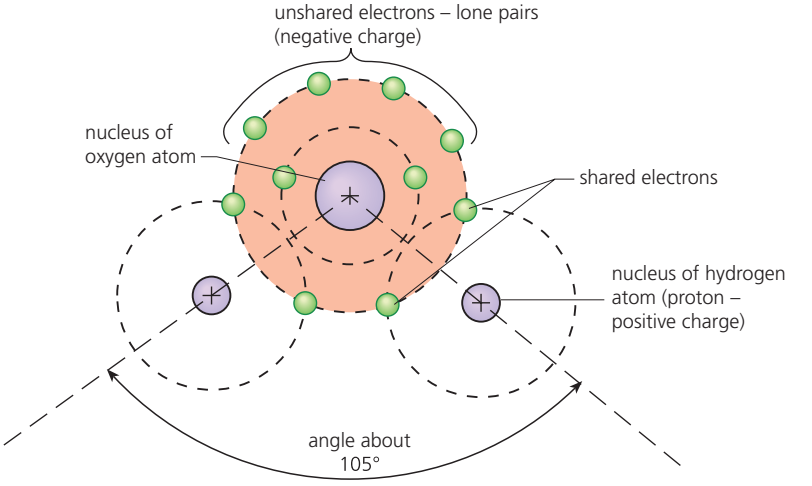
the water molecule carries an **unequal distribution of electrical charge**, even though overall it is electrically neutral

↓

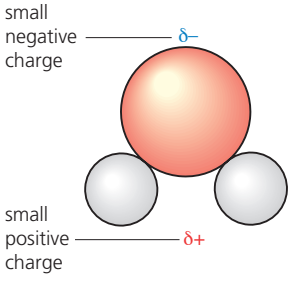
polar water molecule

↓

there is electrostatic attraction between the positively charged region of one water molecule and the negatively charged region of a neighbouring one, giving rise to weak bonds or intermolecular forces called **hydrogen bonds**



Note the water molecule is V-shaped, not linear



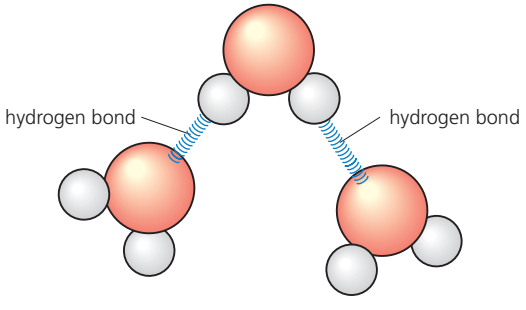


Figure A1.1.1 The water molecule and the hydrogen bonds it forms

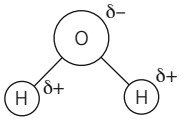


Figure A1.1.2 The polarity of water

Figure A1.1.2 (left) shows how to indicate polarity in a water molecule.

Top tip!

You need to be able to represent two or more water molecules and hydrogen bonds between them. Delta (δ) symbols indicate a small charge.

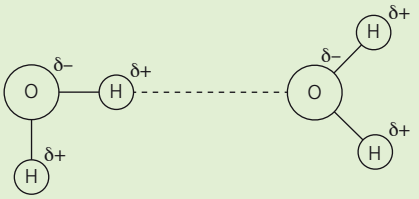


Figure A1.1.3 Hydrogen bonds between water molecules; the dashed line between the oxygen and hydrogen atoms represents a hydrogen bond

Common mistake

A common mistake is suggesting that hydrogen bonding occurs within water molecules. Do not confuse intra- (within) and inter- (between) molecular bonding. Covalent bonding acts **within** a water molecule; hydrogen bonds are formed **between** water molecules.

2 **List** the important properties of water that are due to its polar nature.

Tool 2: Technology

Using computer modelling

Computer modelling allows scientists to explore how the structure of water is essential for maintaining its properties and, therefore, in maintaining life.

Ruth Lynden-Bell and co-workers at Queen's University Belfast used computer simulations to model changes in water's properties. The bond angle in water molecules is 104.5°: they found that if this was changed to 90°, or if the hydrogen bonds were about 15% weaker, the three-dimensional network of hydrogen bonds – crucial to the liquid's unique properties – would be severely disrupted or fall apart.

TOK

The central principle of homeopathy is that water can retain a 'memory' of substances previously dissolved in it, even after any number of serial dilutions. Such claims about the 'memory of water' are categorized as 'pseudoscientific', meaning that while the theories or ideas might look as if they follow the scientific method as normally applied by expert scientists, they do not.

What are the criteria that can be used to distinguish scientific claims from pseudoscientific claims?

The scientific method uses hypothesis, observations and falsification to develop new scientific ideas. This means that scientists set out to challenge hypotheses and look for evidence that might prove them false. If researchers only seek more and more *confirmation* of their ideas, rather than trying to find how their ideas might be false, it is possible that their results could be biased. The results may appear well established, but really the research is either irrelevant or ignores false results. One characteristic of 'pseudoscience' is that it only looks for evidence that supports its claims.

Confirmation bias

Confirmation bias refers to the tendency to search for, interpret and favour information or data in a way that confirms your pre-existing beliefs or hypotheses. You may be guilty of this when you use an internet search engine to settle an argument and only look for results that confirm what you already think.

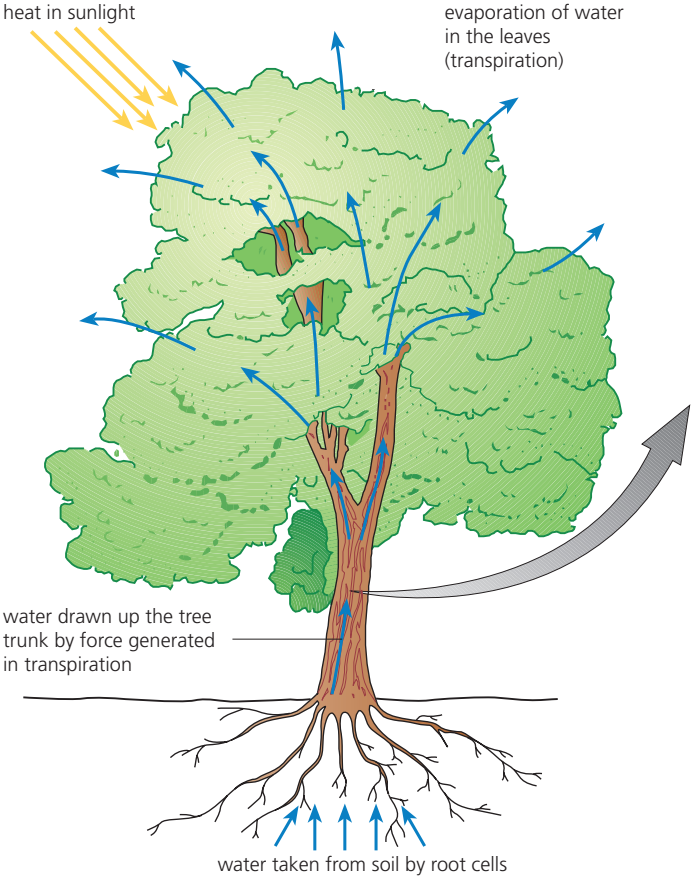
The concept of water having 'memory' of what it has previously encountered contradicts current scientific understanding of physical chemistry. Another characteristic of pseudoscientific theories is that they are at odds with well-established scientific findings; they are wildly surprising. The responsible scientific approach is therefore to replicate the tests to see whether the same results are found. With the cooperation of Benveniste's own team, a group from *Nature* tried to repeat Benveniste's findings but failed, ultimately showing that there was no evidence that water had any sort of chemical 'memory'. Subsequent investigations did not support Benveniste's findings. Given the scientific evidence, then (as opposed to anecdotal evidence), there is no reason to believe that water has a chemical memory.

◆ **Cohesion**: force by which individual molecules of the same type attract and associate ('stick together').

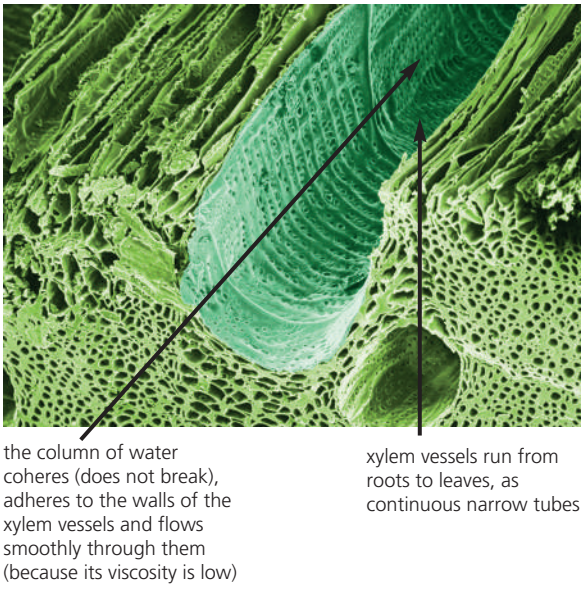
◆ **Surface tension**: property of the surface of a liquid that allows it to resist an external force, due to the cohesion between water molecules.

Cohesion of water molecules and the consequences for organisms

Cohesion is the force by which individual molecules of the same type attract and associate (stick together). Water molecules stick together because of hydrogen bonding. These bonds continually break and reform with surrounding water molecules, although at any one moment a large number are held together by their hydrogen bonds. Cohesive forces allow water molecules to be drawn up xylem vessels in plants by the evaporative loss of water from the leaves (Figure A1.1.4). Compared with other liquids, water has extremely strong cohesive properties that prevent it 'breaking' under tension.



Water can be drawn up to a great height without the column breaking or pulling apart.



■ **Figure A1.1.4** Water is drawn up a tree trunk through xylem vessels: cohesive forces stop the water column from breaking and help draw water up the tree

Link

The transport of water from roots to leaves during transpiration is covered in Chapter B3.2, page XX.

Common mistake

A common mistake is suggesting that hydrogen bonds are strong – this is not the case. A single hydrogen bond is a weak interaction. It is only because there are many hydrogen bonds in water that they collectively exert large cohesive forces.

Related to the property of cohesion is the property of **surface tension**. The outermost molecules of water form hydrogen bonds with the water molecules below them. This gives water a very high surface tension (Figure A1.1.5), higher than any other liquid except mercury. The water molecules on the surface have no neighbouring water molecules above and therefore exhibit stronger attractive forces upon their nearest neighbours on and below the surface. Water's strong surface tension allows it to form almost completely spherical droplets.

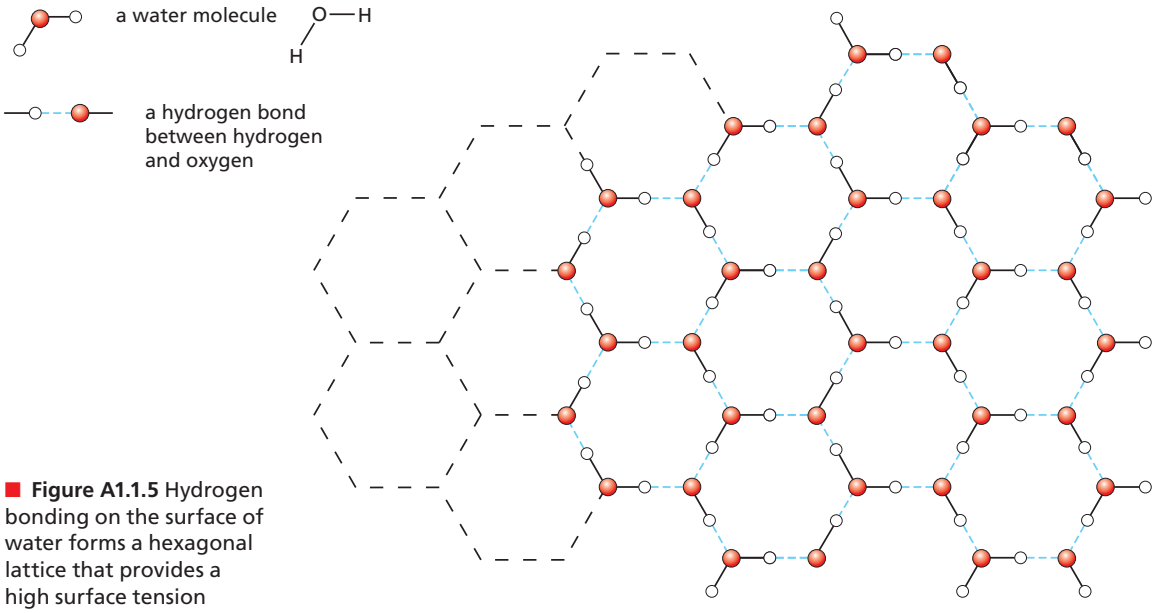
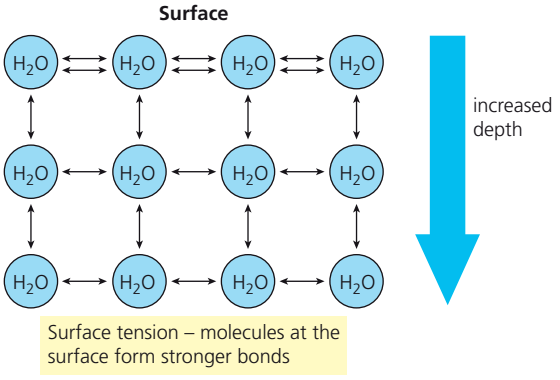


Figure A1.1.5 Hydrogen bonding on the surface of water forms a hexagonal lattice that provides a high surface tension

Within a body of liquid, there is no net force on a molecule because the cohesive forces exerted by the neighbouring molecules all cancel out (see Figure A1.1.5). However, for a molecule on the surface of the liquid, there is a net inward cohesive force since there is no attractive force acting from above. This inward net force causes the molecules on the surface to contract and to resist being stretched or broken. Thus, the surface is under tension, hence the name 'surface tension'.

The surface tension of water is exploited by insects that 'surface skate' (Figure A1.1.6). The insect's waxy cuticle prevents the wetting of its body, and the mass of the insect is not great enough to break the surface tension.

a



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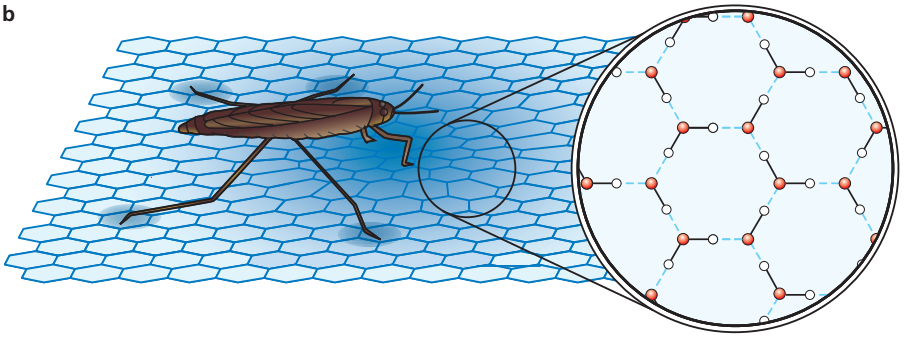


Figure A1.1.6 a) A pond skater moving over the water surface; b) the surface tension supports the pond skater – the surface is depressed but the hydrogen bonds hold it together

ATL A1.1B

What other examples of surface tension are there? How does the knowledge of surface tension help you understand everyday phenomena and experiences? For example:

- Why are droplets of water pulled into a spherical shape?
- Why is it better to wash in hot water rather than cold water?
- Why are soaps and detergents used to clean clothes?

You could use the website below or other sources to research other examples of surface tension and why the property is useful to know about.

www.usgs.gov/special-topics/water-science-school/science/surface-tension-and-water#overview

◆ **Viscosity**: a measure of a fluid's resistance to flow

Below the surface, water molecules slide past each other very easily. This property is described as low **viscosity**. Consequently, water flows readily through narrow capillaries, tiny gaps and pores.

Top tip!

The diffusion of molecules through a solvent, such as water, is inversely proportional to the viscosity of the solvent. Temperature affects the viscosity of liquids, for example, the viscosity of water at 25 °C is approximately half that than when the temperature is 4 °C. As we will see in Theme B2.1, the diffusion rate of molecules is extremely important for the processes that are needed to sustain life.

Inquiry 1: Exploring and designing

Designing

Surface tension is one of water's most important properties. It causes water to collect in drops.

Design an investigation to show the properties of water's surface tension using a paper clip.

Use the following equipment:

- drinking glass
- water
- liquid dishwashing detergent
- paper clips
- piece of paper towel.

◆ **Adhesion**: the force by which individual molecules stick to surrounding materials and surfaces.

◆ **Hydrophilic**: attracted to water; e.g. hydrogen bonds are readily formed between a molecule and water.

Adhesion of water and the impacts for organisms

Adhesion is the force by which individual molecules cling to surrounding materials and surfaces. Materials and substances with an affinity for water are described as **hydrophilic** (page Xx). Water adheres strongly to most surfaces and can be drawn up long columns, for example through narrow tubes such as the xylem vessels of plant stems, without danger of the water column breaking (Figure A1.1.4). It should be noted that cohesion is a far more significant force in xylem transport and explains how tensions can be resisted. Adhesion is only significant when air-filled xylem vessels refill with aqueous sap under positive pressures, which is something that happens only rarely (no more than once a year). Figure A1.1.7 shows both adhesive and cohesive forces at work in a xylem vessel.

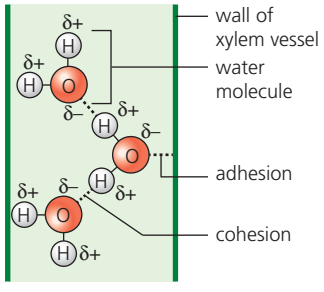


Figure A1.1.7 Adhesive and cohesive forces supporting a column of water in a xylem vessel

Common mistake

The terms 'cohesion' and 'adhesion' are sometimes treated as if their meanings are interchangeable, but this is not the case. If they were, we would have one word for these forces rather than two! Cohesion ('co' means 'together') is attraction between water molecules, while adhesion ('ad' means 'toward') is attraction to a surface.

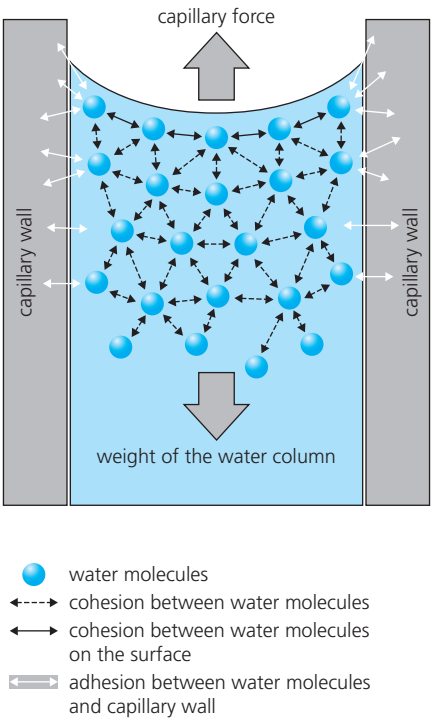


Figure A1.1.8 Channels in soils and spaces between cellulose fibres in the cell wall act as capillary tubes, drawing water through the plant

Capillary action in soils and plant cell walls

Soil contains many vertical, thin channels known as **capillary tubes**, in which plant roots are located. When water enters capillary tubes, adhesion between the water molecules and the wall of the capillary draws water up the small tube: this is called **capillary action**. In this way, plants bring water up from the water table to the roots when the ground becomes dry.

The cell walls of plants are made from a fibrous material called cellulose (see page XX). Cellulose is polar/hydrophilic to a certain degree. Fibrous materials can act like wicks, drawing water up into the material by capillary action (see Figure A1.1.8). Cell walls can draw water by capillary action from nearby xylem vessels, keeping water flowing through plant tissue. Cells that are directly exposed to the air, such as those found in the spongy mesophyll tissue of leaves (page XX), remain constantly wetted by capillary action into these cells. Water evaporates from the moist, blotting-paper-like cell walls of the mesophyll and then diffuses out of leaves through pores on the surface of the leaf (stomata), enabling water to be transported up the plant.

Solvent properties of water

Hydrogen bonds pull water molecules very close to each other because the potential energy of the hydrogen bonds is greater than the kinetic energies of the water molecules up to 100 °C (at atmospheric pressure). This is why water is a liquid at the temperatures and pressure that exist over much of the Earth's surface. As a result, we have a liquid medium with distinctive thermal and solvent properties.

- **Capillary tubes**: channels with a very small internal diameter.
- **Capillary action**: the tendency of a liquid to move up against gravity when confined within a narrow tube (capillary). Also known as capillarity.
- **Solute**: dissolved molecule or ion in a solution.
- **Solvent**: a liquid in which another substance can be dissolved.
- **Hydrophobic**: repelled by water.

Water is a powerful solvent for polar substances such as ionic substances like sodium chloride (Na^+ and Cl^-). All cations (positively charged ions) and anions (negatively charged ions) become surrounded by a layer of orientated water molecules (Figure A1.1.9).

There is a diverse range of hydrophilic molecules that dissolve in water, such as carbon-containing (organic) molecules with ionized groups (for example, amino acids have a negatively charged carboxyl group, $–COO^-$, and a positively charged amino group, $–NH_3^+$); soluble organic molecules like sugars dissolve in water due to the formation of hydrogen bonds with their slightly charged hydroxyl groups (–OH). Once they have dissolved, molecules or ions (the **solute**) are free to move around in water (the **solvent**) by diffusion and, as a result, are more chemically reactive than when in the undissolved solid.

On the other hand, non-polar substances are repelled by water, as in the case of oil on the surface of water. Non-polar substances are **hydrophobic**. The functions of some molecules in cells depends on them being hydrophobic and insoluble. For example, the cell membrane is made from phospholipids, the tails of which are hydrophobic and form the internal structure of the membrane.

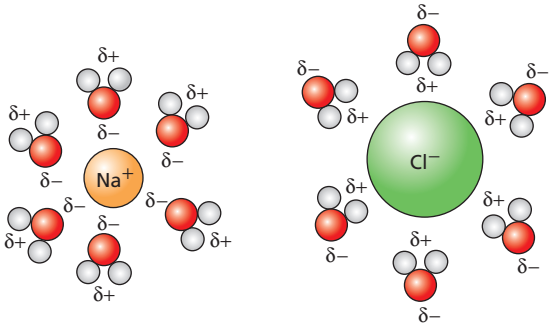


Figure A1.1.9 The hydration of sodium and chloride ions

Of the common gases, carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2), only carbon dioxide is particularly soluble in water; nitrogen and oxygen are only slightly soluble in water. Carbon dioxide is moderately soluble in water because a proportion of it undergoes a chemical reaction to form carbonic acid (H_2CO_3 (aq)), which immediately ionises or dissociates to form hydrogen ions, H^+ (aq), and hydrogencarbonate ions, HCO_3^- (aq).

Oxygen and nitrogen have low solubility in water because they are non-polar and do not form hydrogen bonds with water. In addition, they do not undergo dissociation or ionisation. One consequence of the poor solubility of oxygen in water is the evolution of respiratory pigments, for example haemoglobin, which greatly increase the oxygen-carrying capacity of blood relative to that of pure water.

Most enzymes catalyse reactions in aqueous solution. Enzymes require a certain level of water in their structures to maintain enzyme shape and stability, enabling them to function effectively. Most naturally occurring enzymes cannot form their active forms without being immersed in water. Hydrogen bonds often act as bridges between enzyme binding sites and their substrates. Although most enzymes act in aqueous solutions, sometimes an enzyme may be in a fixed position, such as within a cell membrane. In these cases, the location of the enzyme allows reactions to be localized to particular sites.

Link

The structure of cell membranes is covered in Chapter B2.1, page X–XX.

Link

For more on enzymes, see Chapter C1.1, page X–X.

3 In an aqueous solution of glucose, **state** which component is the solvent and which is the solute.

Physical properties of water and the consequences for animals in aquatic habitats

The physical properties of water depend on the hydrogen bonding between water molecules and include **buoyancy**, **viscosity**, **thermal conductivity** and **specific heat capacity**.

Buoyancy is the ability of any fluid (liquid or gas) to provide a vertical upwards force on an object placed in or on it. Objects float in water when their average density is less than water and sink when they are denser. The density of a substance is its mass per unit volume.

◆ **Buoyancy**: the ability of any fluid to provide a vertical upwards force on an object placed in or on it.

◆ **Thermal conductivity (*k*)**: the measure of how easily heat flows through a specific type of material.

◆ **Specific heat capacity**: the amount of energy required to raise the temperature of 1 kg of a substance by 1 °C.

Common mistake

Do not confuse the terms 'heat capacity' with 'specific heat capacity'. Heat capacity is the amount of heat required to change the temperature of a body by one degree. The amount of heat energy **per unit mass** is needed to calculate the specific heat capacity. Unlike heat capacity, the specific heat capacity is therefore independent of mass or volume.

The differences between these properties in air and water are shown in Table A1.1.1.

Table A1.1.1 Physical properties of water and air at 20 °C and 1 atm pressure (for air, molar mass is a weighted average, since molar masses can only be calculated for pure substances)

	Water	Air
density, ρ ($kg\,m^{-3}$)	998.21	1.204
thermal conductivity, k ($W\,m\,K^{-1}$)	0.598	0.02154
specific heat capacity, c_p ($J\,kg^{-1}\,°C^{-1}$)	4 184	1 007
dynamic viscosity, η ($kg\,m^{-1}\,s^{-1}$)	1.002×10^{-3}	1.825×10^{-5}

For example, at sea level, air is 784 times less dense than water, and a volume of air at sea level has 0.13% of the density of the same volume of water.

The importance of these factors in relation to life can be illustrated by looking at two animals that live in water as well as in the air or on land, such as the black-throated loon (*Gavia arctica*) and the ringed seal (*Pusa hispida*), as shown in Figure A1.1.10.

Figure A1.1.10 The black-throated loon, *Gavia arctica*, (left) and the ringed seal, *Pusa hispida*, (right)

Top tip!

When referring to an organism, either the common name, e.g. black-throated loon, or the scientific name, for this example *Gavia arctica*, is acceptable.

The black-throated loon (*Gavia arctica)* is a diving bird species, catching its prey (mostly fish) underwater. It breeds in the vicinity of deep freshwater lakes throughout northern Europe, the west coast of Alaska, and Asia. From August, it migrates south to areas around the Black Sea and the Mediterranean Sea, and to north-east Atlantic coasts and the eastern and western Pacific Ocean. It returns to its breeding grounds in early April when sea ice in those areas has melted.

Ringed seals (*Pusa hispida)* live in the Arctic and sub-arctic regions of the North Pole. They live on packs of ice, but also spend much of their time in the sea, under the ice. They are quite small seals, usually less than 1.5 m in length, and have a distinct pattern of dark spots surrounded by light grey rings on its fur – explaining its common name.

Specific heat capacity and the temperature of water

A relatively large amount of energy is required to raise the temperature of water, because a lot of energy is needed to break the large number of hydrogen bonds that restrict the movement of water molecules. This property of water is its specific heat capacity. Consequently, aquatic environments (rivers, ponds, lakes and seas) are very slow to change temperature when the surrounding air temperature changes. Aquatic environments have relatively more stable temperatures than terrestrial (land) environments. As organisms, and the cells from which they are made, are largely composed of water, water's ability to absorb and lose heat without undergoing a large temperature change also provides thermal cushioning within the organisms themselves, protecting cells and organisms from large fluctuations in temperature.

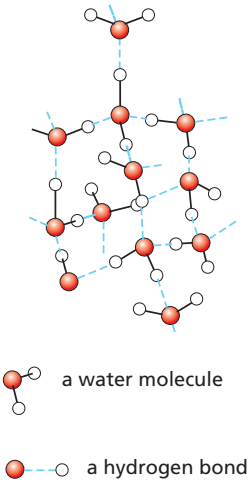


Figure A1.1.11 In ice the water molecules are hydrogen bonded in an open tetrahedral lattice, which makes ice less dense than liquid water

The relatively stable sea temperatures enable seals to live and feed throughout the year. The specific heat capacity of air is lower than water, so air temperature tends to fluctuate more. In winter, the very low air temperatures cause surface water to freeze. One of the interesting properties of water is that, unlike many other substances, it floats when it freezes because the density of ice is lower than that of liquid water. This is due to the behaviour of hydrogen bonds and how they make water molecules interact (Figure A1.1.11). Water has its lowest density at 4 °C. The ice forms a platform on which seals can live. Ringed seals have claws to dig through ice to produce holes so that they can emerge from their aquatic habitat to breathe. This enables them to live under and on the ice throughout the year.

Thermal conductivity

Top tip!

Restricting convection is as important as restricting conduction in maintaining the body temperature of birds.

Water has a higher thermal conductivity than air, with water conducting heat 28 times better than air. By trapping air in its feathers, the black-throated loon forms an effective insulating layer between its skin and the outside air. Feathers also restrict convection currents by trapping a thin layer of air that is not able to move easily, which also helps to maintain the body temperature of the bird. In contrast, the seal relies on thick blubber to insulate its body. Layers of ice also have insulating properties because ice's thermal conductivity is low, like the thermal conductivity of air, which stops heat being transferred into the surroundings, even when the temperature is very low. The ice traps thermal energy in the water beneath the ice, increasing sea temperatures.

Buoyancy

The black-throated loon can swim large distances underwater. However, bird anatomy is adapted for life in the air and on land, with hollow bones to decrease weight and air trapped between feathers to provide insulation. These adaptations can be problematic in water, as buoyancy needs to be overcome to catch underwater prey species. The loon has solid bones to increase its weight and to compress air from its lungs and feathers to decrease buoyancy and enable successful diving.

Fat is stored in animals as adipose tissue, usually under the skin (subcutaneous fat). Aquatic diving mammals, such as seals, have a great deal of subcutaneous fat, which is known as blubber. Blubber acts as a buoyancy aid, as well as providing thermal insulation.

4 **Explain** the properties of water using examples of two animals that live in water as well as in the air or on land, such as the black-throated loon (*Gavia arctica*) and the ringed seal (*Pusa hispida*).

Viscosity

Viscosity is the resistance to flow. Water is more viscous than air (see Table A1.1.1). Bird plumage is adapted to hold and deflect air to make lift easier to achieve flight. When the black-throated loon flies, the light feathers can move through the air easily and with minimum friction.

Interactions between water molecules at the surface of water form surface tension (page X). Below the surface, however, water molecules slide past each other very easily. This property is described as low viscosity. The hydrodynamic shapes of the loon and seal enable both animals to move through the water easily. Both animals need to produce resistance against the water to achieve movement. The seal uses its flippers (modified arms) to propel itself through the water. The black-throated loon has webbed feet that provide a large surface area to push against water. The feet are located laterally and towards the back end of the body to allow maximum propulsion; it also avoids the formation of turbulent eddies in the water and therefore reduces drag.

ATL A1.1C

Find out about another two animals that live in water – one should be a mammal that also lives on land, and the other should be a bird. Research the adaptations that help them to survive in these environments, using the same factors that are covered in this section: buoyancy, viscosity, thermal conductivity and specific heat capacity.

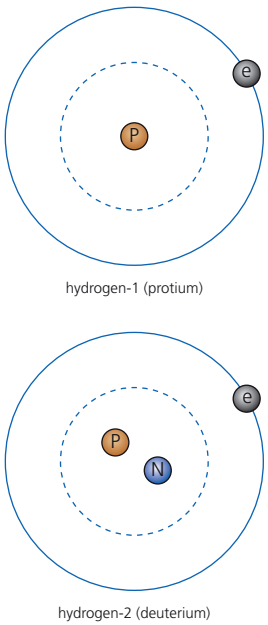


Figure A1.1.12 Isotopes of hydrogen

Extraplanetary origin of water on Earth

The Earth formed approximately 4.5 billion years ago, in an environment too hot for water to condense into liquid. This means that the Earth's water must have an extraplanetary origin. As the distance from the Sun increases, water vapour can condense directly into water ice. It is in these regions that water could first have formed, thereby providing the origin of Earth's water.

Researchers examining the composition of asteroids, and the meteorites that form by breaking off from them, have hypothesised that asteroids are most likely to be the source of Earth's water. Such asteroids still contain ice and organic material (amino acids), and so could have delivered water and organic molecules to Earth, which are both critical for the possible evolution of life (see page X for further discussion of the origin of life). A group of meteorites, known as **carbonaceous chondrites** – some of the oldest meteorites in the solar system – can be up to 28% water and have a water composition similar to ocean water. The water molecules are incorporated in the crystal structures of minerals. The composition of water, and its possible origin, can be assessed using isotopes of hydrogen (Figure A1.1.12) and the relative proportions in which they appear: deuterium (hydrogen-2) has a nucleus with one proton and one neutron, while protium (hydrogen-1) has just one proton in its nucleus. With hydrogen isotopes that closely match Earth's seawater, the water in these meteorites could have been the source of the Earth's oceans.

HL ONLY

Top tip!

There are many hypotheses for how water first arrived on the Earth, including being carried on icy comets and the creation of water beneath the planet's surface itself. For this syllabus we are only considering the asteroid hypothesis.

Two 4.5-billion-year-old meteorites containing liquid water, found on Earth, support this hypothesis. When meteorites heat up, such as during an impact with our planet, they release their water as gas, which is then trapped by the Earth's gravitational attraction.

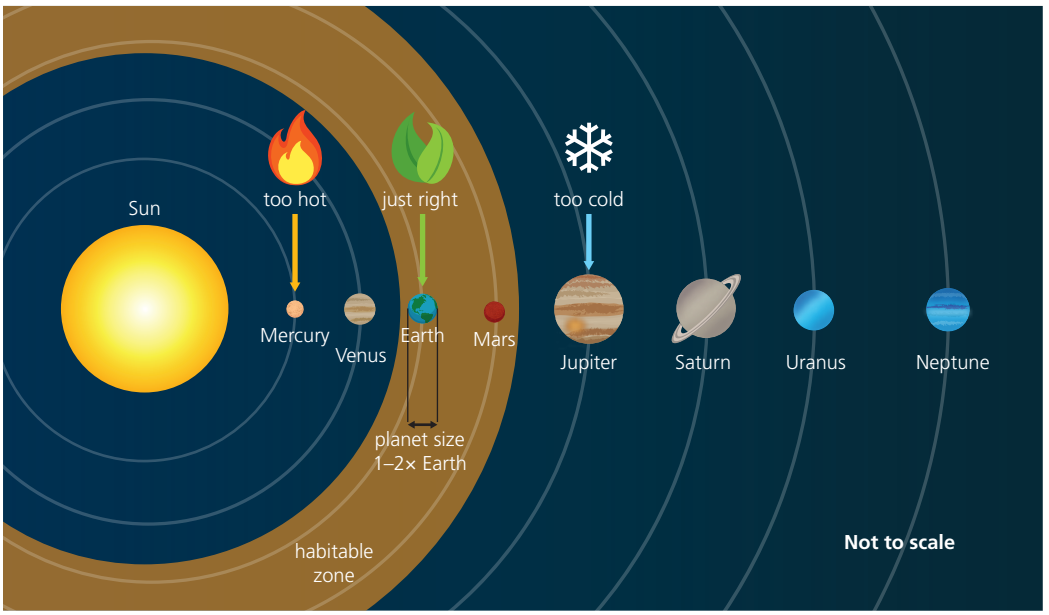
The Earth's current deuterium to protium ratio also matches ancient eucrite achondrites, one type of meteorite which originates from a large asteroid known as Vesta, located in the outer asteroid belt (the asteroid belt is located between the orbits of Mars and Jupiter). Carbonaceous chondrites and eucrite achondrites are therefore hypothesised to have delivered water to Earth.

Once present on Earth, the temperatures were cool enough to allow water vapour to condense into liquid water. Gravity enabled the water to be retained on the Earth's surface, rather than being dispersed into space.

The relationship between the search for extraterrestrial life and the presence of water

◆ **Goldilocks zone**: also known as the 'habitable zone'; the area around a star where it is not too hot or too cold for liquid water to exist on the surface of surrounding planets.

Given life's dependence on water, any planet where life as we know it is to exist must also have water present. Planets where water can exist must be at the right distance from their nearest star – too close and the water boils and evaporates, too far away and the water is frozen. The distance from the star where liquid water, and therefore life, can exist is called the **Goldilocks zone**, from the nineteenth-century British fairy tale *Goldilocks and the three bears*.



■ **Figure A1.1.13** The 'Goldilocks zone' for our solar system; Mars is also in the habitable zone along with Earth but it is too small to keep an atmosphere, which is needed to sustain life

5 **State** what is meant by the 'Goldilocks zone'.

ATL A1.1D

Scientists at NASA are planning to send astronauts to Mars (www.nasa.gov/topics/moon-to-mars). Carrying water from Earth to Mars is impractical – it is far too heavy to carry all the water required for a mission in a rocket. The plan would be to collect water from Mars itself: to do that, scientists would need to know if and where water is located.

What evidence is there for water on Mars, both now and in the past? What would the presence of water tell us about the possibility of finding life on Mars? What type of organisms could we expect to find on Mars?

To establish whether a distant planet may contain water, scientists use a technique called 'transit spectroscopy'. As a planet passes in front of its nearest star ('transits'), light passes through the planet's atmosphere; this light is analysed to see which wavelengths are being absorbed or deflected. This analysis shows which elements and molecules, such as water, are present in the atmosphere. In this way, planets outside of our solar system (exoplanets) may be said to have a 'water signature'. Scientists are looking for exoplanets that have a water signature, that are the right distance from their nearest star and are also the right size for life to exist. Discoveries such as the planet Kepler-186f, which is Earth-size and located in a Goldilocks zone, are likely contenders for life existing in other solar systems.

ATL A1.1E

Working in small groups, make a presentation about the conditions needed for life and how scientists are looking for such exoplanets. What techniques are there for finding water on other planets? What other exoplanets have been found? Could these planets ever be a potential home for humanity? The presentations should clearly explain the science behind 'Goldilocks planets' and how scientists go about looking for them.

LINKING QUESTIONS

1. How do the various intermolecular forces of attraction affect biological systems?
2. Which biological processes only happen at or near surfaces?

A1.2 Nucleic acids

Guiding questions

- How does the structure of nucleic acids allow hereditary information to be stored?
- How does the structure of DNA facilitate accurate replication?

SYLLABUS CONTENT

This chapter covers the following syllabus content:

- A1.2.1 DNA as the genetic material of all living organisms
- A1.2.2 Components of a nucleotide
- A1.2.3 Sugar–phosphate bonding and the sugar–phosphate 'backbone' of DNA and RNA
- A1.2.4 Bases in each nucleic acid that form the basis of a code
- A1.2.5 RNA as a polymer formed by condensation of nucleotide monomers
- A1.2.6 DNA as a double helix made of two antiparallel strands of nucleotides with two strands linked by hydrogen bonding between complementary base pairs
- A1.2.7 Differences between DNA and RNA
- A1.2.8 Role of complementary base pairing in allowing genetic information to be replicated and expressed
- A1.2.9 Diversity of possible DNA base sequences and the limitless capacity of DNA for storing information
- A1.2.10 Conservation of the genetic code across all life forms as evidence of universal common ancestry
- A1.2.11 Directionality of RNA and DNA (HL only)
- A1.2.12 Purine-to-pyrimidine bonding as a component of DNA helix stability (HL only)
- A1.2.13 Structure of a nucleosome (HL only)
- A1.2.14 Evidence from the Hershey–Chase experiment for DNA as the genetic material (HL only)
- A.1.2.15 Chargaff's data on the relative amounts of pyrimidine and purine bases across diverse life forms (HL only)

◆ **Nucleic acid**: polynucleotide chain of one of two types, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

◆ **Genetic code**: the order of bases in DNA (of a chromosome) that determines the sequence of amino acids in a protein.

◆ **Deoxyribonucleic acid (DNA)**: a form of nucleic acid consisting of two complementary chains of deoxyribonucleotide subunits, and containing the bases adenine, thymine, guanine and cytosine.

◆ **Ribonucleic acid (RNA)**: a form of nucleic acid containing the pentose sugar ribose, and the organic bases adenine, guanine, uracil and cytosine.

Link

Viruses and their life cycles are covered in more detail in Chapter A2.3 (HL only), page XX–XX.

DNA as the genetic material of all living organisms

DNA is a **nucleic acid**. Nucleic acids are the information molecules of cells (and also of viruses – see below, and page XX) found throughout the living world. The code containing the information in nucleic acids, known as the **genetic code**, is universal. This means that it makes sense in all organisms. It is not specific to a few organisms or to just one group, but to all groups and species.

There are two types of nucleic acid found in living cells: **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**. DNA is the genetic material and occurs in the chromosomes of the nucleus (and also certain cell organelles, chloroplasts and mitochondria – see pages XX–XX). While some RNA also occurs in the nucleus, most is found in the cytoplasm – particularly in the ribosomes.

Some viruses use RNA as their genetic material, such as SARS-CoV-2, the virus that causes COVID-19. Other diseases caused by RNA viruses include the common cold, influenza, Dengue fever, hepatitis C, rabies, Ebola, polio, mumps and measles. Viruses depend on the cells of living organisms to survive and replicate, and so are not considered to be living; thus it is true to say that DNA is the genetic material of all living organisms.

Both DNA and RNA have roles in the day-to-day control of cells and organisms, as we shall see shortly. First, we will look into the structure of nucleotides and the way they are built up (synthesized) to form the unique DNA double helix.

Concept: Unity

All living organisms and viruses contain nucleic acids. This universality of the genetic code indicates the inter-connectivity of life on Earth and explains how viruses can take over and use the biological machinery of cells.

Top tip!

The presence of genetic material in a structure does not necessarily indicate life. Viruses, which are usually considered to be non-living, contain genetic material. In addition, DNA is chemically stable so can persist in dead organic matter and some fossils.

ATL A1.2A

DNA has long been known as a major chemical in the nucleus. DNA is associated with proteins in the nucleus and, during the early 1900s, proteins were considered better candidates as molecules able to transmit large amounts of hereditary information from generation to generation rather than DNA.

Find out about the early work on DNA by Friedrich Miescher and Phoebus Levene. What did they conclude about the structure and role of proteins and DNA in the nucleus, and to what extent were their hypotheses falsified by subsequent work? Use this site to find out more: **www.dnaftb.org/15/animation.html**

Inquiry 1: Exploring and designing

Exploring; designing

DNA can be extracted from any tissue. Using the following statements about DNA, plan a method to extract DNA from animal or plant tissue:

- Cell walls can be broken up by heating and mashing.
- DNA is soluble in water.
- DNA is not soluble in ethanol.
- DNA is found in the nuclei of cells.
- Nuclei have a membrane around them made of lipids (fats).
- Cell membranes are made of lipids (fats).
- Detergents (such as washing up liquid) dissolve fats.
- Salty water causes DNA to clump together to make larger molecules in solution.
- Precipitates form more easily in cold liquids.

Write out a set of instructions for DNA extraction and explain why you need to take each step.

This site has a methodology you can follow: **https://learn.genetics.utah.edu/content/labs/extraction/howto**

◆ **Nucleotide**: phosphate ester of a nucleoside – an organic base combined with pentose sugar and phosphate (Pi).

◆ **Cytosine**: a pyrimidine nitrogenous base found in nucleic acids (DNA and RNA) that pairs with guanine.

◆ **Guanine**: a purine nitrogenous base found in nucleic acids (DNA and RNA) that pairs with cytosine.

◆ **Adenine**: a purine nitrogenous base, found in the coenzymes ATP and NADP and in nucleic acids (DNA and RNA), that pairs with thymine.

◆ **Thymine**: a pyrimidine nitrogenous base found in DNA that pairs with adenine.

◆ **Pentose**: a 5-carbon monosaccharide sugar.

◆ **Condensation**: formation of larger molecules involving the removal of water from smaller component molecules.

Components of a nucleotide

A **nucleotide** consists of three substances combined through covalent chemical bonding. These are:

- a **nitrogenous base** – the four bases of DNA are **cytosine** (C), **guanine** (G), **adenine** (A) and **thymine** (T)
- a **pentose** sugar – deoxyribose occurs in DNA and ribose in RNA
- a **phosphate** group (phosphate diester).

These components are combined by an enzyme-controlled **condensation** reaction to form a nucleotide. Condensation reactions occur when two molecules combine, producing water as a by-product. Enzymes are biological catalysts that speed up and control biological reactions. Since any one of the four bases can be incorporated, four different types of nucleotide can be found in DNA.

How these components are combined is shown in Figure A1.2.1, together with the diagrammatic way the components are represented to illustrate their spatial arrangement. Simple shapes are used rather than complex structural formulas, and these shapes are all that are required here. (You need to be able to draw simple diagrams, using these symbols.)

Top tip!

In diagrams of nucleotides, use circles, pentagons and rectangles to represent phosphates, pentose sugars and bases. The positions of the components relative to each other need to be accurately represented.

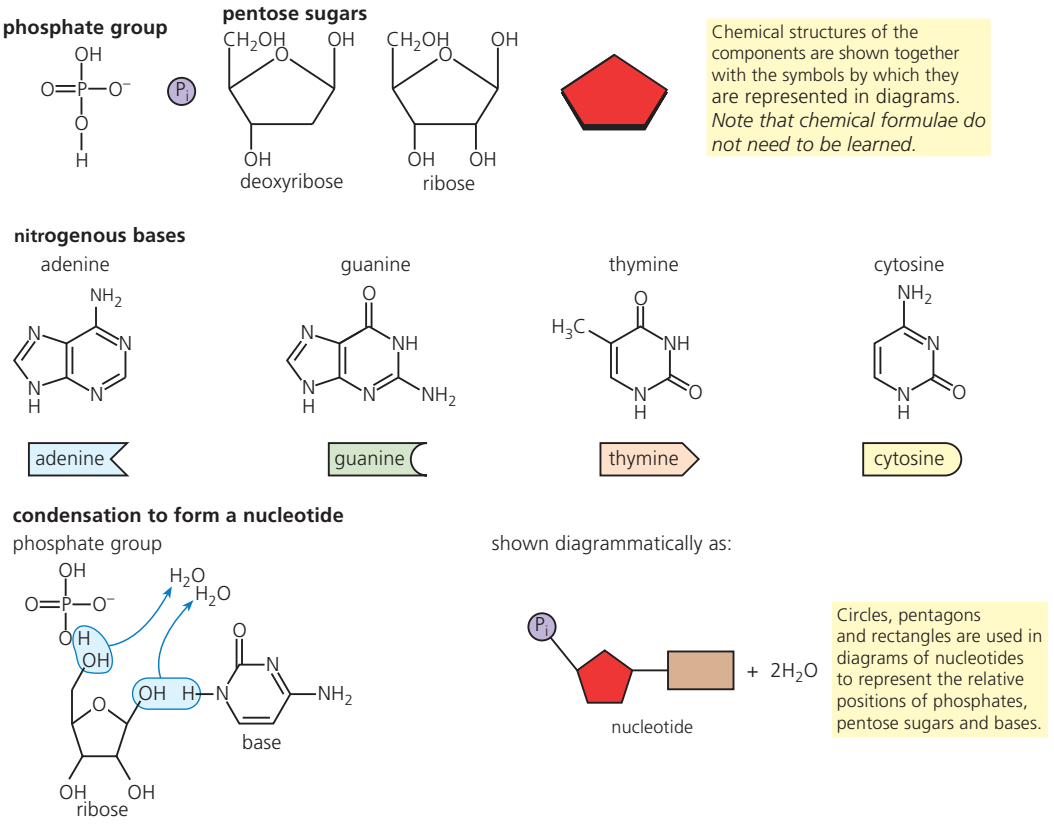


Figure A1.2.1 The components of nucleotides

1 **Distinguish** between a nitrogenous base and a base found in inorganic chemistry.

◆ **Purine**: One of two types of chemical compound used to make nucleotides, the building blocks of DNA and RNA. Examples are adenine and guanine.

◆ **Pyrimidine**: One of two types of chemical compound used to make nucleotides. Examples are cytosine, thymine and uracil. Cytosine and thymine are used to make DNA; cytosine and uracil are used to make RNA.

◆ **Polynucleotide**: a long, unbranched chain of nucleotides, as found in DNA and RNA.

The bases can be divided into two groups: the **purines** (adenine and guanine) and the **pyrimidines** (cytosine and thymine), based on their molecular structure (see Figure A1.2.1).

The sugar–phosphate 'backbone' of DNA and RNA

Sugar–phosphate bonding

Nucleotides may chemically combine, one nucleotide at a time, by condensation reactions to form large molecules (with high values of molar mass) called nucleic acids or **polynucleotides** (Figure A1.2.2). So, nucleic acids are very long, thread-like (linear) macromolecules with alternating sugar and phosphate molecules forming the 'backbone'. This part of the nucleic acid molecule is uniform and unvarying. Sugar–phosphate bonding creates a continuous chain of covalently bonded atoms in each strand of DNA (and also RNA) nucleotides, which forms a strong backbone to the molecule.

Nucleotides become chemically combined together, phosphate to pentose sugar, by covalent bonds, with a sequence of bases attached to the sugar residues. Up to 5 million nucleotides condense together in this way, forming a polynucleotide (nucleic acid).

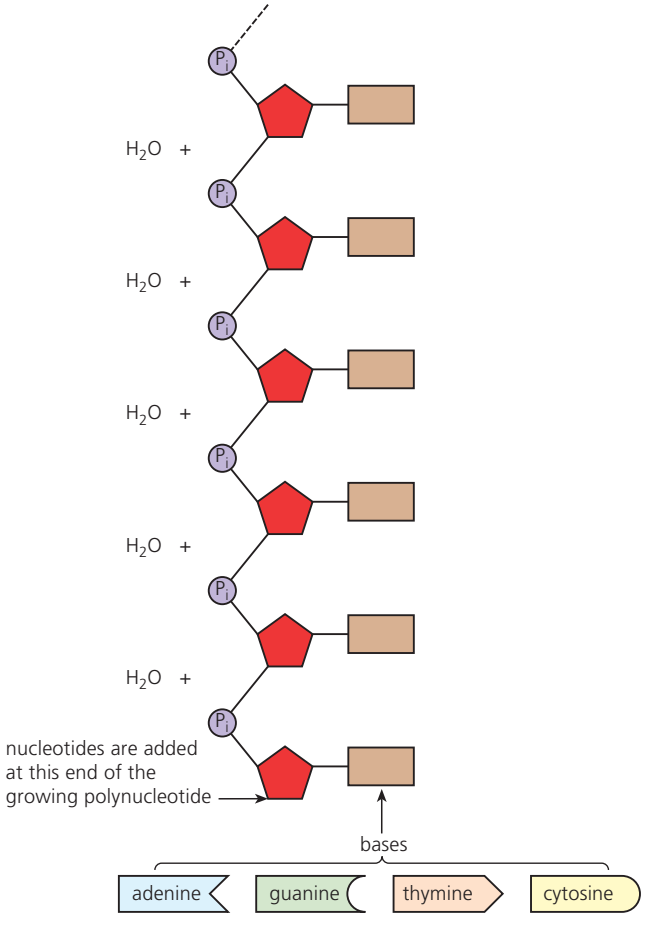


Figure A1.2.2 How nucleotides make up nucleic acid

Along the strand, each base is attached to a pentose sugar molecule. The bases project sideways (Figure A1.2.2). Since the bases vary, they represent a unique sequence that carries the coded information held by the nucleic acid.

Bases in each nucleic acid form the basis of a code

Information in DNA lies in the sequence of the nitrogenous bases – cytosine, guanine, adenine and thymine – forming the genetic code. This sequence dictates the order in which specific amino acids are assembled and combined to synthesize a protein. The code lies in the sequence in one of the DNA strands, the coding strand. The other strand is complementary to it (see Figure A1.2.6, page XX). The coding strand is always read in the same direction, by enzymes.

The code is a three-letter or triplet code, meaning that each sequence of three bases stands for one of the 20 amino acids, and is called a **codon**. With a four-letter alphabet (C, G, A, T), there are 64 possible different triplet combinations ($4 \times 4 \times 4$).

Nucleic acids code for the production of proteins in cells. Proteins make up about two-thirds of the total dry mass of a cell. They differ from carbohydrates and lipids in that they contain the element nitrogen and sometimes the element sulfur, as well as carbon, hydrogen and oxygen.

Link

For more on proteins see Chapter B1.2, page XX–XX, and Chapter D1.2, page XX–XX. The codons for the 20 amino acids found in proteins are in Chapter D1.2, page XX.

2 **Distinguish** between a nitrogenous base, a nucleotide and a nucleic acid.

RNA polymers

◆ **Codon**: three consecutive bases in DNA (or RNA) which specify an amino acid.

◆ **Polymer**: large molecules made up of repeating subunits (monomers).

◆ **Uracil**: a pyrimidine nitrogenous base found in RNA (not DNA); it pairs with adenine.

RNA molecules are relatively short in length, compared with DNA. In fact, RNA molecules tend to be from a hundred to thousands of nucleotides long, depending on their particular role.

The RNA molecule is a **polymer**. In messenger RNA (mRNA) it is a single strand of polynucleotide in which the sugar monomer is ribose (see page XX). The bases found in RNA (Figure A1.2.3) are cytosine, guanine, adenine and **uracil** (which replaces thymine of DNA).

The carbon atoms in organic molecules such as ribose can be numbered (Figure A1.2.4). The numbering runs from right to left, clockwise. This enables the bonds between adjacent sugars and their phosphate neighbours to be identified, along with the direction in which the polynucleotide is orientated.

Top tip!

Make sure you can draw and recognize diagrams of the structure of single nucleotides and RNA polymers.

◆ **Messenger RNA (mRNA)**: single-stranded ribonucleic acid, formed by the process of transcription of the genetic code in the nucleus, that then moves to ribosomes in the cytoplasm.

◆ **Transfer RNA (tRNA)**: short lengths of RNA that combine with specific amino acids prior to protein synthesis.

◆ **Ribosomal RNA (rRNA)**: molecule that forms part of the protein-synthesizing organelle known as a ribosome.

◆ **Phosphodiester bond**: the linkage between the 3' carbon atom of one sugar molecule and the 5' carbon atom of another (deoxyribose in DNA and ribose in RNA) in nucleic acids.

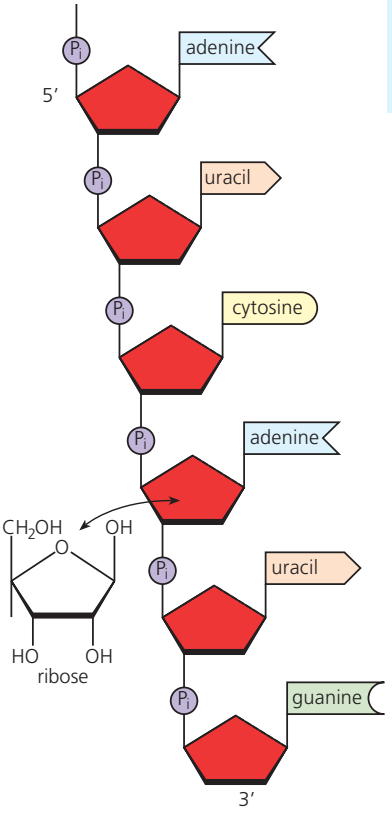


Figure A1.2.3 An RNA polymer

3 **Draw** a labelled diagram of:
- **a** an RNA single nucleotide
- **b** an RNA polymer.

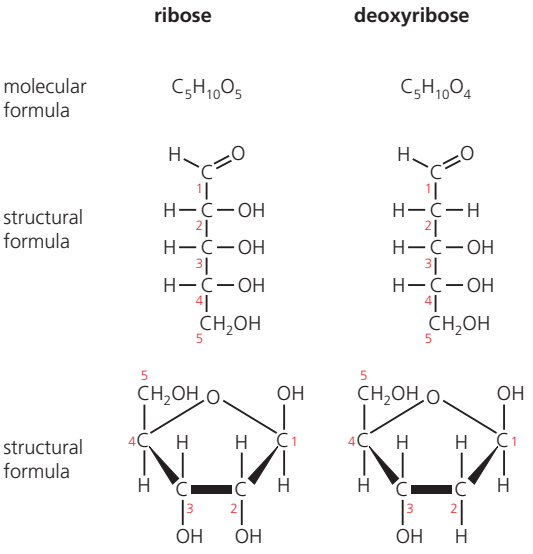


Figure A1.2.4 The numbering of carbon atoms in ribose and deoxyribose (in their straight chain (linear) and cyclic forms)

It is the convention to refer to the first carbon as 1' carbon, the second as 2' carbon, and so on.

There are three functional types of RNA: **messenger RNA (mRNA)**, **transfer RNA (tRNA)** and **ribosomal RNA (rRNA)**. mRNA is formed in the nucleus and is transported out through nuclear pores to the ribosomes in the cytoplasm. tRNA and rRNA are also made in the nucleus and occur in the cytoplasm.

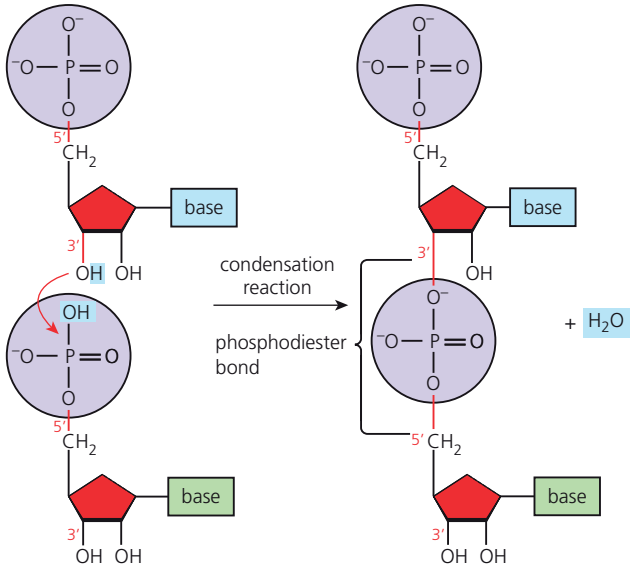


Figure A1.2.5 RNA is formed by the condensation of nucleotide monomers

RNA is formed by the condensation of many nucleotide monomers. These condensation reactions link the pentose sugar and phosphate groups of adjacent nucleotides, so forming the new strands (Figure A1.2.5). The bond formed between adjacent nucleotides is called a 3'–5' **phosphodiester bond**.

◆ **Double helix**: two interlocking helixes joined by hydrogen bonds between the pairs of purine–pyrimidine bases (A pairs with T and G with C). The helical structure makes a 360° twist after each 10 nucleotides, i.e. every 3.4 nm.

4 **Explain** what is meant by *antiparallel strands*.

The DNA double helix

The DNA molecule consists of two antiparallel polynucleotide strands, paired together, and held by hydrogen bonds. The two strands take the shape of a **double helix** (Figure A1.2.6).

The two strands are termed 'antiparallel' because one runs from a 5' carbon to a 3' carbon, and the other from a 3' carbon to a 5' carbon.

Top tip!

Note: when drawing DNA's structure, two antiparallel strands should be drawn, but the helical shape is not required. Adenine (A) should be shown paired with thymine (T), and guanine (G) paired with cytosine (C). For completion, Figure A1.2.6 shows the numbers of hydrogen bonds between adjacent bases – this detail does not need to be included when drawing the structure of DNA in exams. Only a small section of the DNA need be included to illustrate the way in which the nucleotides are arranged.

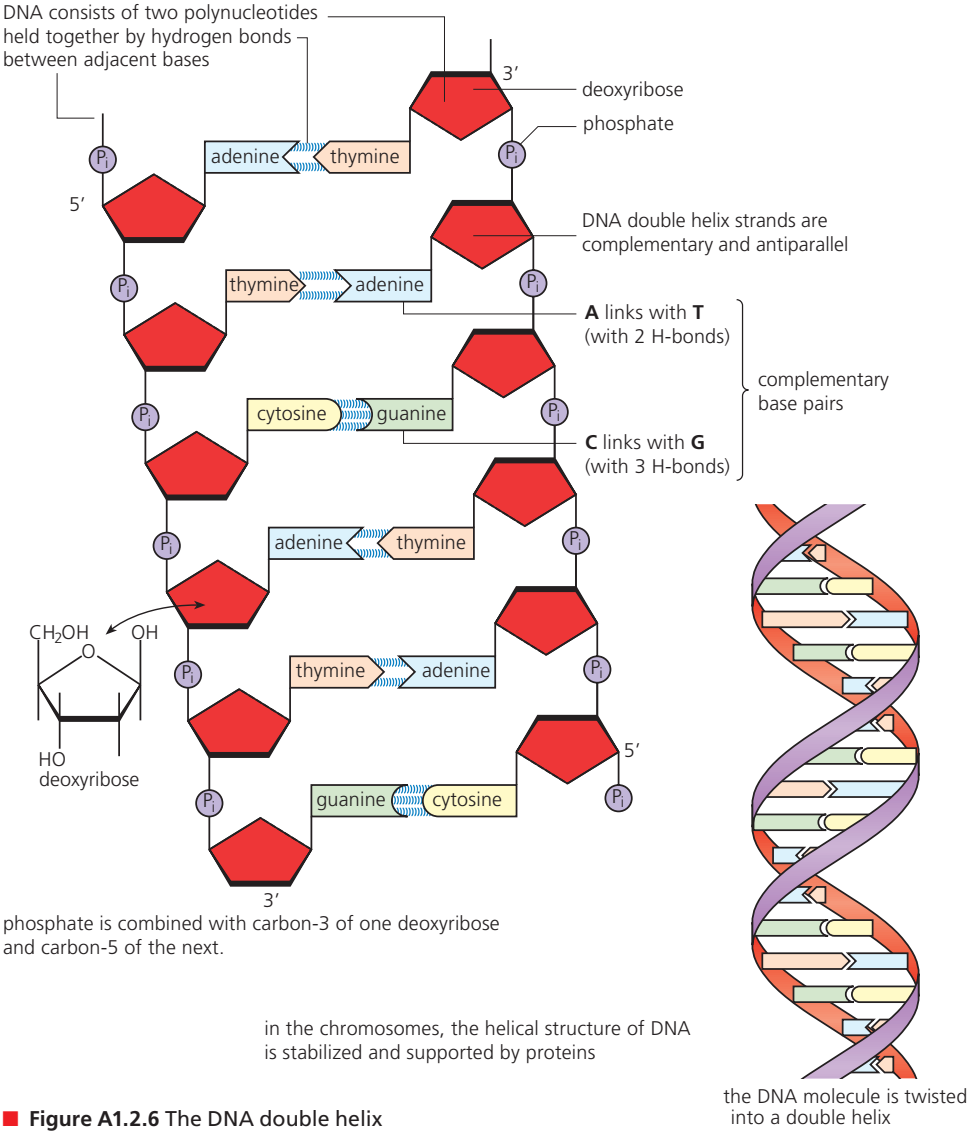


Figure A1.2.6 The DNA double helix

Nature of science: Models

Creating the model of DNA

A model is a simplified description of a biological system, a concept or biological process. Models are used to describe and explain phenomena that cannot be experienced directly or to simplify complex systems such as ecosystems. The first model of the structure of DNA was developed in 1953 by Francis Crick and James Watson (see Figure A1.2.11 on page X). Bringing together experimental results from other scientists, Crick and Watson used evidence to deduce the likely structure of the DNA molecule. Models are useful because they allow biologists to mimic the real world, make calculations and test predictions. Biological models include mathematical models that can be simulated on computer software. Models, like theories, may be modified as new experimental data are discovered.

Common mistake

A common mistake when drawing DNA is to link nitrogenous bases to phosphates rather than the pentose sugars. Another common error is to link the phosphate groups to the oxygen in the sugar ring, rather than to C4 via C5. Pay careful attention to the detail contained in the DNA molecule, and how the different parts of the nucleotide connect together, and make sure you can accurately recreate this from memory. Practise drawing molecular diagrams.

TOK

What are the implications of having, or not having, knowledge?

The 'object' shown in Figure A1.2.7 is an X-ray diffraction image of a DNA fibre, taken by Raymond Gosling in May 1952 at King's College London. Calculations by James Watson and Francis Crick from the photograph gave crucial dimensions for the double helix model they built.

The publication in 1953 in *Nature* by Watson and Crick of DNA's structure and a mechanism for replication of DNA marked a paradigm shift from classical genetics to molecular genetics.

The initial implications of this new scientific knowledge to Watson, Crick and other biologists (the knowers) were that scientists could now develop new molecular knowledge about the genetic code and protein synthesis based on new forms of experiments.

At a later stage, knowledge of DNA structure and complementary base pairing led to recombinant DNA research, genetic engineering, human molecular genetics, monoclonal antibodies and, most recently, CRISPR (gene editing). This new DNA-based knowledge has led to more effective medical treatments, new drugs and better disease diagnosis (for patients). However, there are new ethical implications arising from potential CRISPR editing of the inherited germline ('designer babies') for non-therapeutic and enhancement reasons.

Figure A1.2.7 X-ray diffraction image of DNA

Genetic fingerprinting is perhaps the most well-known DNA technology to the general public (another group of knowers) and is a 'tool' used to track down relatives, establish paternity and identify dead bodies. However, it has also led to the conviction of many criminals and to the freeing from prison of many individuals who were wrongly convicted.

Possible social implications of these new advances in genetic knowledge are that genetic fingerprinting may act as a deterrent to certain kinds of crime and lead to greater satisfaction with the criminal justice system. There will also be economic costs associated with the adoption of the forensic use of DNA technology, and another implication might be the unauthorised disclosure or misuse of the data, e.g., typing by insurance companies.

Hence, the development and application of scientific knowledge has led to ethical decisions that can be justified through utilitarian reasoning: the idea that whether actions are ethical or not depends on their effects. Utilitarianism 'maximises utility', and favours technology that produces the largest amount of 'good' or 'happiness'.

However, some social scientists fear that this type of research into the human genetic code will encourage some people to argue that social problems, such as violence and drug abuse, are explainable in terms of human genes, and therefore deterministic abnormalities. They are worried that social interventions such as counselling, affirmative action and educational opportunities will lose funding.

ATL A1.2B

Crick and Watson brought together the experimental results of many other scientists, and from this evidence they deduced the likely structure of the DNA molecule. Rosalind Franklin (Figure A1.2.8) is often called 'the forgotten woman of DNA'. What role did she play in developing the first model of DNA? Why has she not been recognized in the same way as Watson and Crick in this pivotal scientific breakthrough?

Figure A1.2.8 Rosalind Franklin produced the key X-ray diffraction pattern of DNA at King's College London

- **Gene**: heritable factor that consists of a length of DNA that codes for a protein.
- **Chromosome**: length of DNA that carries specific genes in a linear sequence.
- **Locus**: the particular position of a gene on homologous chromosomes.
- **Allele**: different versions of the same gene.

Link

The concept of the gene pool is covered in more detail in Chapter D4.1, page X–XX.

Genes

Within the DNA molecule, there are sections that code for proteins – these sections are called **genes**. A gene is a heritable factor that influences a specific character. By 'character' we mean some feature of an organism, such as 'height' in the garden pea plant or 'blood group' in humans. 'Heritable' means genes are factors that pass from parent to offspring during reproduction.

Chromosomes

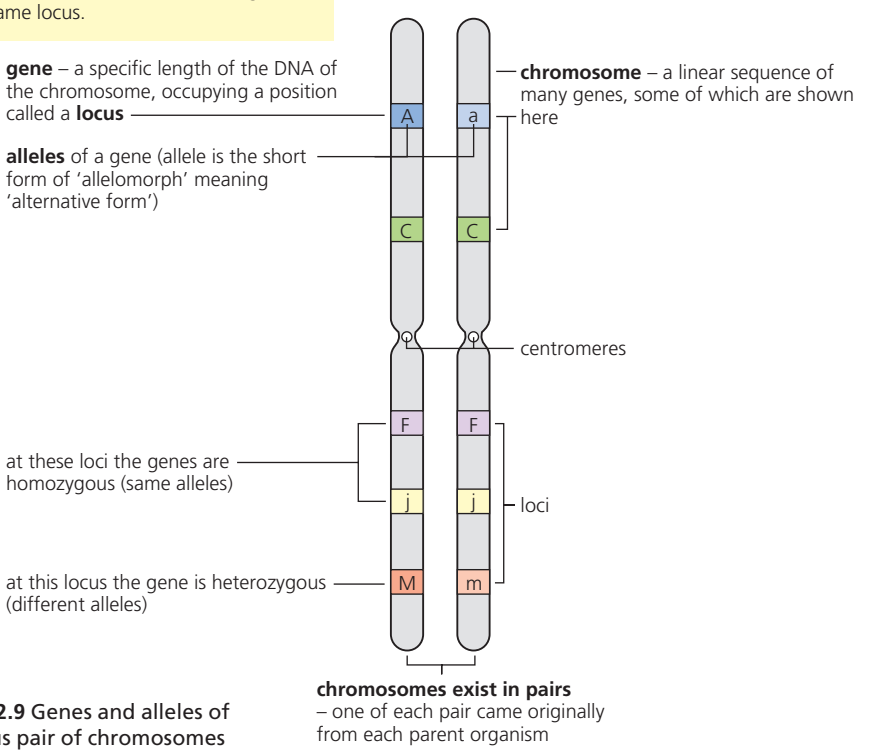
Genes are located on **chromosomes**. Each gene occupies a specific position on a chromosome; therefore each chromosome is a linear series of genes. Furthermore, the gene for a particular characteristic is always found at the same position or **locus** (plural, loci) on a particular chromosome. For example, the gene controlling height in the garden pea plant is always present in the exact same position on one particular chromosome of that plant. However, that gene for height may code for 'tall' or it may code for 'dwarf', as we shall see shortly. In other words, there are different forms of genes. In fact, each gene has two or more forms and these are called **alleles**. The word 'allele' just means 'alternative form'. For a given gene, many alleles may exist in the **gene pool** of the species.

◆ **Homologous chromosomes**: pairs of chromosomes, one from each parent, that carry the same sequence of genes (but not necessarily the same alleles of those genes).

Now, the chromosomes of eukaryotic cells occur in pairs called **homologous chromosomes**. ('Homologous' means 'similar in structure'.) One of each pair came originally from one parent, and the other one of the pair came from the other parent. So, for example, humans have 46 chromosomes, 23 coming originally from each parent in the process of sexual reproduction. Homologous chromosomes resemble each other in structure and they contain the same sequence of genes.

In Figure A1.2.9, we see some of the genes and their alleles in place on a homologous pair of chromosomes.

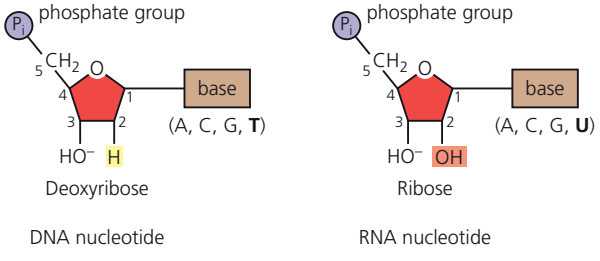
The **loci** are the positions along the chromosomes where genes occur, so alleles of the same gene occupy the same locus.



■ **Figure A1.2.9** Genes and alleles of a homologous pair of chromosomes

Top tip!

You should be able to sketch the distinction between ribose and deoxyribose.



■ **Figure A1.2.10** DNA and RNA nucleotides

Differences between DNA and RNA

Both DNA and RNA are made of nucleotides although they have different structures. One main difference is that DNA has the pentose sugar deoxyribose while RNA contains ribose (Figure A1.2.10).

5 **Construct** a table to **distinguish** between DNA and RNA.

Top tip!

When distinguishing between DNA and RNA, you need to refer to the number of strands present, the types of nitrogenous bases and the type of pentose sugar.

◆ **Complementary base pairing**: this describes how the nitrogenous bases of nucleic acids align with each other in a specific way, i.e. adenine pairs with thymine (or uracil in RNA) and cytosine with guanine; complementary bases are held together by hydrogen bonds.

Role of complementary base pairing

The pairing of bases is between adenine (A) and thymine (T), and between cytosine (C) and guanine (G), simply because these are the only combinations that fit together along the helix. This pairing, known as **complementary base pairing**, also makes possible the very precise way that DNA is copied in a process called replication. From the model of DNA in Figure A1.2.6, we can also see that when A pairs with T, they are held together by two hydrogen bonds; when C pairs with G, they are held by three hydrogen bonds. Only these pairs can form hydrogen bonds. Due to base pairing and the formation of specific hydrogen bonds, the sequence of bases in one strand of the helix determines the sequence of bases in the other. Complementary base pairing allows genetic information to be replicated and expressed.

Link

The process of DNA replication is covered in more detail in Chapter D1.1, page X–XX.

Top tip!

Complementarity is based on hydrogen bonding. The bases pair up in the way they do due to the hydrogen bonds that form between the pairs of bases.

ATL A1.2C

Watson and Crick developed their model of DNA in 1953. The first model they developed was not successful, however, as the various components did not fit together correctly. They developed their final and correct model once all the parts of nucleotides were accurately represented. By building a model, they were able to understand how DNA can replicate (copy) itself, thereby passing on genetic information from generation to generation.

Can you think of a way of representing DNA using material you have in the lab or in your home? How can you represent the different components and how they are connected together?

6 In base pairing, organic bases are held together (A–T, C–G) by hydrogen bonds. **State** which parts of these organic molecules form the hydrogen bonds.

Diversity of possible DNA base sequences

Although the genetic code is comprised of only four bases – A, C, T and G – the order in which they can be combined is immeasurable. The diversity of possible DNA base sequences means that DNA has a limitless capacity for storing information, with diversity by any length of DNA molecule and any base sequence possible.

An indication of the storage capacity of DNA is the number of genes that can be contained within it. Species vary in the number of genes they have – some have many more than others. Table A1.2.1 lists the numbers of genes present in a range of common organisms. Notice that the list includes one bacterium, as well as certain plants and animals, and that the water flea has more genes than a human, but the fruit fly has fewer.

Table A1.2.1 Estimated approximate numbers of protein-coding genes

Species (animals and protists)	Number of genes	Species (plants, fungi, prokaryotes)	Number of genes
Daphnia (water flea)	31 000	*Oryza sativa* (rice)	41 500
Homo sapiens (human)	20 000	*Vitis vinifera* (grape)	30 450
Canis familiaris (domestic dog)	19 000	*Arabidopsis thaliana* (rockcress)	27 000
Drosophila melanogaster (fruit fly)	14 000	*Saccharomyces cerevisiae* (yeast)	6 000
Plasmodium (malarial parasite)	5 000	*Escherichia coli* (bacterium)	4 300

Concept: Diversity

The four-letter genetic code (A, C, T and G, coding for A, C, U and G in RNA) allows a huge variety of different proteins to be coded for. The human body contains thousands of different proteins, each with a specific function. The evolution of DNA over millions of years (see Chapter A4.1, page X) has led to the vast diversity of life seen on Earth, from microscopic bacteria to large organisms such as the blue whale and redwood trees.

Another indication of the data-storing capability of DNA is the number of base pairs that DNA contains (Table A1.2.2).

Table A1.2.2 A comparison of genome size

Species	Total number of base pairs (bp)
T2 phage (a virus specific to a bacterium)	3 569 (3.5 kb)
Escherichia coli (bacterium)	4 600 000 (4.6 Mb)
Drosophila melanogaster (fruit fly)	123 000 000 (123 Mb)
Oryza sativa (rice)	430 000 000 (430 Mb)
Homo sapiens (human)	3 200 000 000 (3.2 Gb)
Paris japonica (canopy plant)	150 000 000 000 (150 Gb)

Genome size refers to the amount of DNA contained in a genome (which is the genetic code in one complete set of chromosomes), where 1 Mb = 1 000 000 bp and 1 Gb = 1000 Mb. Table A1.2.2 expresses the number of base pairs in terminology familiar from computer data storage (Mb and Gb), although here the units are different: gigabases (Gb) and megabases (Mb) rather than gigabytes and megabytes.

In a human cell, the DNA held in the nucleus measures about 2 m in total length. This length contains 3.2 Gb of 'data' – a phenomenal quantity of genetic code. Within this DNA, it is estimated that humans have between 20 000 and 25 000 protein-coding genes. These figures are an indication of how DNA offers an enormous capacity for storing data with great economy.

Conservation of the genetic code

We now know that the 64 codons in the genetic code of DNA have the same meaning and code for the same amino acids in nearly all organisms. This supports the idea of a common origin of life on Earth; that the very first DNA has sustained an unbroken chain of life from the first cells on Earth to all cells in organisms alive today. Only the most minor variations in the genetic code have arisen in the evolution and expansion of life since it originated 3.5 billion years ago.

◆ **Genome**: the whole of the genetic information of an organism or cell.

◆ **Mutation**: a change in the amount or the chemical structure (i.e. base sequence) of DNA of a chromosome.

Over many generations, changes in the sequence of bases in the **genome**, and therefore in the mRNA and order of amino acids that they assemble, can occur due to **mutations**. Many sequences, both in areas which code for proteins (so-called 'coding sequences') and those that do not (known as **non-coding sequences**), persist unchanged, however, or with only minor modifications over many generations: these are known as **conserved sequences**. It is possible that highly conserved sequences have a functional value, although the reasons for non-coding sequences are unclear. Even if the base sequences in coding areas of DNA change, the sequences of amino acids they code for may not, because each amino acid has several different mRNA codes, and so mutations in a coding sequence do not necessarily affect the amino acid sequence of its protein product (these are called synonymous mutations).

TOK

Highly repetitive DNA sequences were once described as 'junk DNA', the label 'junk' showing a degree of confidence that those sequences had no role. To what extent do you think the labels and categories used in the pursuit of knowledge affect the knowledge that we obtain?

The most highly conserved genes are those that can be found in all organisms. These include proteins required for transcription and translation, and those found in ribosomes. The fact that such genes exist indicate that all life is interlinked, with universal ancestry for all life on Earth. Histone proteins, which help to package DNA within nuclei (see Theme A2, page XX), are also highly conserved in terms of sequence and structure, again suggesting universal ancestry for all species.

Common mistake

A common mistake is to equate 'genome', or genomic size (which is the size of the genome), to the total number of genes in an organism, rather than the correct definition – the total amount of DNA.

Directionality of RNA and DNA

We can identify direction or polarity in the DNA double helix. The phosphate groups along each strand are bridges between carbon-3 of one sugar molecule and carbon-5 of the next, and one chain runs from 5' to 3' while the other runs from 3' to 5' (see Figure A1.2.6). (Remember, the carbon atoms of organic molecules can be numbered, page x.) That is, the two chains of DNA are antiparallel, as illustrated in Figure A1.2.6. The existence of direction in DNA strands becomes important in DNA replication (when DNA is copied), when the genetic code is transcribed into mRNA (a process called **transcription**), and when the message encoded in the mRNA is read to form proteins (a process called **translation**).

Link

Translation and transcription are discussed further in Chapter D1.2, page XX.

Information in the DNA lies in the sequence of the bases: cytosine (C), guanine (G), adenine (A) and thymine (T). This sequence dictates the order in which specific amino acids are assembled and combined together. The code lies in the sequence in one of the strands, the coding strand; the other strand is complementary to it. It is the coding strand that becomes the template for transcription. The coding strand is always read in the same direction (in the 3' to 5' direction). A single-stranded molecule of RNA is formed by complementary base pairing (the RNA strand is synthesized in the 5' to 3' direction). The mRNA are translated in the 5' to 3' direction into amino acids by a ribosome to produce a polypeptide chain. The details of these processes will be explored in subsequent chapters (see pages XX–XX).

Common mistake

DNA has a role beyond coding for proteins. Although the genes within chromosomes code for polypeptides, some regions of DNA do not code for proteins but have other important functions. Some regions of DNA regulate the expression of genes, and other sections code for the RNA that attaches to amino acids and also play a role in the formation of proteins at ribosomes (tRNA), for example.

Purine to pyrimidine bonding

When Watson and Crick assembled the first model of DNA in 1953 (see page X), they used cardboard cut-outs to represent the different bases and other nucleotide subunits. Their first attempts used molecular shapes for thymine and guanine that were incorrect, and they arranged the different atoms of different elements from which the bases were made in the wrong configuration. This meant that the DNA model did not fit together correctly, as the lengths of the base pairings were incorrect. Following suggestions from the American scientist Jerry Donohue, in which the correct shapes for the bases were proposed, Watson made new cardboard cut-outs of the two bases, and found that the

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complementary bases now fitted together perfectly (i.e., A with T and C with G), with each pair held together by hydrogen bonds (Figure A1.2.6). The structure also matched Chargaff's rules (see page X, later in this section).

Tool 1: Experimental techniques

Physical molecular modelling

Physical model making helped Watson and Crick to establish the structure of DNA in a number of ways:

- it allowed them to combine what was known about the chemical content of DNA with information from X-ray diffraction studies
- by building scale models of the components of DNA, they were able to attempt to fit them together in a way that agreed with the data from other sources, such as Chargaff's rules
- they made several arrangements of the scale model until they found the best one that fitted all the data.

Figure A1.2.11 Watson and Crick with their demonstration model of DNA

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Crick and Watson had a distinctive method of working, including reinterpreting already-published data and developing others' studies, leading to the building of models (Figure A1.2.11).

To what extent were their achievements the product of both cooperation and competition?

In researching this, remember to consult a variety of relevant sources of information.

Watson and Crick discovered that the base pairings, A to T and C to G, are of equal length. This means that whatever the base sequence, the DNA helix has the same three-dimensional structure. The hydrogen bonding between complementary bases also confers stability (Figure A1.2.12), making DNA the ideal molecule for the storage of information in cells.

Nucleosome structure

In cells with a true nucleus (eukaryotes – see page XX), DNA occurs in the chromosomes in the nucleus, along with protein. More than 50% of a chromosome contains protein. While some of the proteins of the chromosome are enzymes involved in copying and repair reactions of DNA, the bulk of chromosome protein has a support and packaging role for DNA.

Why is packaging necessary?

Take the case of human DNA. In the nucleus, the total length of the DNA of the chromosomes is over 2 m. We know this is shared out between 46 chromosomes, and that each chromosome contains one very long DNA molecule. Chromosomes are different lengths, depending on the number of genes they contain, but we can estimate that within a typical chromosome of 5 μm length (where 1 μm = 1/1000 mm), there is a DNA molecule approximately 5 cm long. This means that about 50 000 μm of DNA is packed into 5 μm of chromosome.

This phenomenal packaging is achieved by coiling the DNA double helix and looping it around protein beads called **nucleosomes**, as illustrated in Figure A1.2.13.

The packaging proteins of the nucleosome, called **histones**, are a basic (positively charged) protein containing a high concentration of amino acid residues with additional basic groups ($-NH_2$), such as lysine and arginine (see also page XX, Theme B). In nucleosomes, eight histone molecules combine to make a single bead. Around each bead, the DNA double helix is wrapped in a double loop.

◆ **Nucleosome**: a sequence of DNA wound around eight histone protein cores – a repeating unit of eukaryotic chromatin.

◆ **Histone**: basic proteins (rich in the amino acids arginine and lysine) that form the scaffolding of chromosomes.

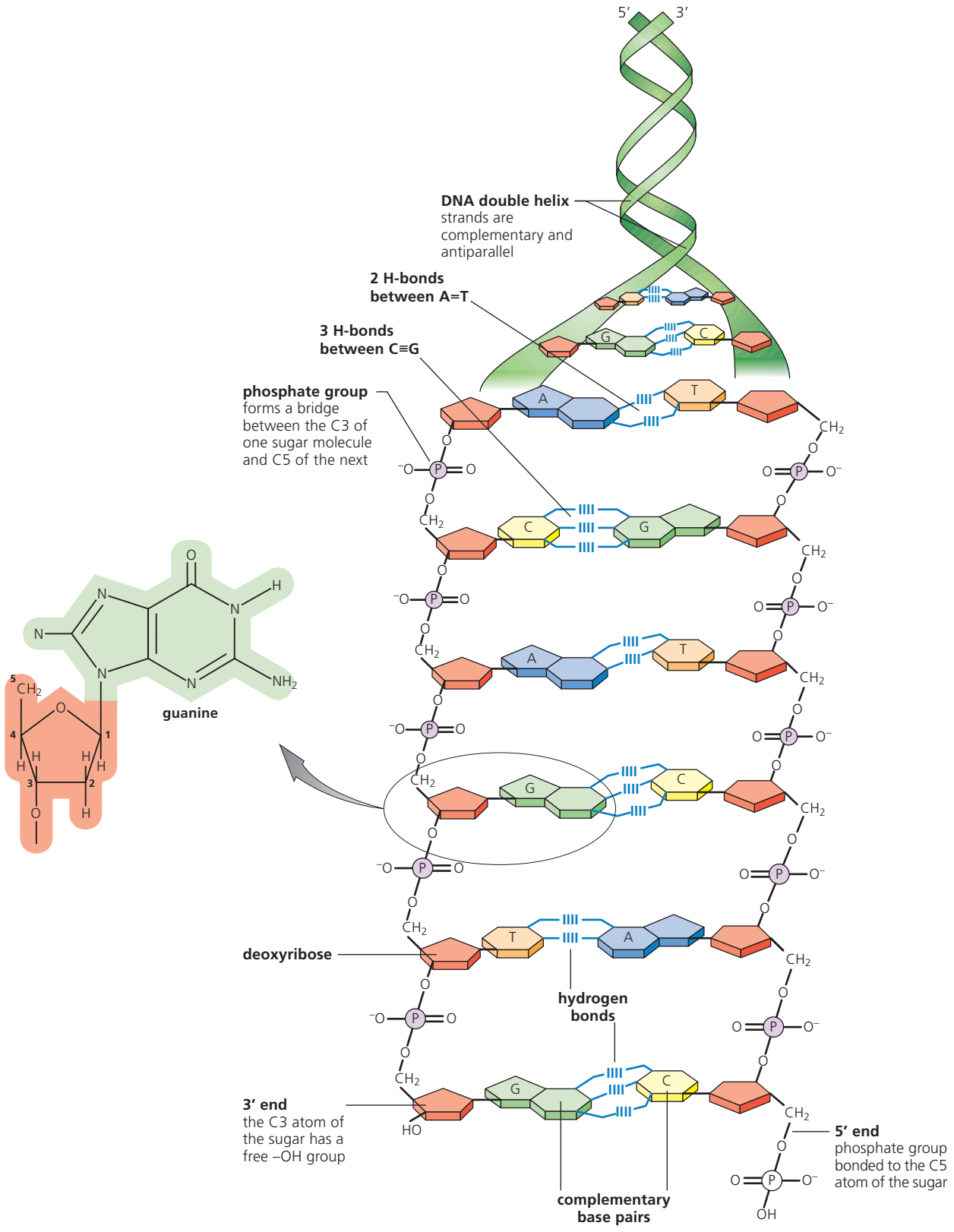
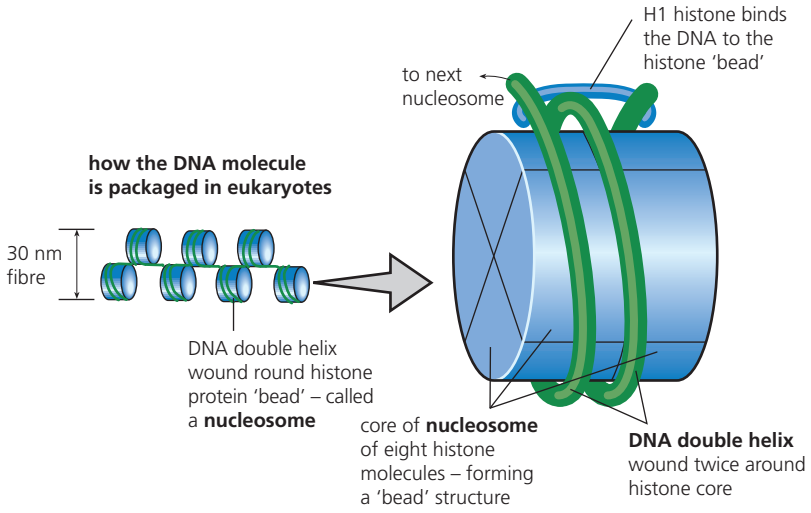
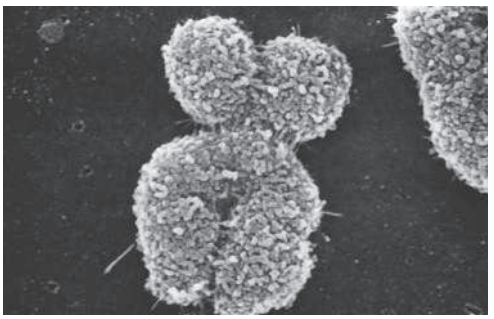


Figure A1.2.12 Direction, base pairing and hydrogen bonding between purine and pyrimidine bases in the DNA double helix



Electron micrograph of metaphase chromosome (× 40 000) **– at this stage the chromosome is at maximum condensed state**



the packaging of DNA in the chromosome

Figure A1.2.13 The nucleosome and supercoiling of DNA

◆ **Non-histone chromosomal protein**: proteins that remain in the chromatin once histone proteins have been removed; they play a key role in the regulation of gene expression.

At times of cell division, when the nucleus divides, the whole beaded thread is coiled up, forming the chromatin fibre. The chromatin fibre is again coiled, and the coils are looped around a 'scaffold' protein fibre made of a **non-histone chromosomal protein**. This whole structure is folded (supercoiled) into the much-condensed chromosome (Figure A1.2.13).

Clearly, the nucleosomes are the key structures in the safe storage of these phenomenal lengths of DNA that are packed in the nuclei. However, nucleosomes also allow access to selected lengths of the DNA (particular genes) during transcription – a process we will discuss shortly.

7 **Explain** a main advantage of chromosomes being 'supercoiled' during the process of cell division.

Tool 1: Experimental techniques

Digital molecular modelling

A molecular visualization of DNA was created for the 50th anniversary of the discovery of the double helix. The dynamics and molecular shapes were based on X-ray crystallographic models and other data. You can observe an animation of the packaging of the DNA molecule in nucleosomes at:

www.lindenbiomedical.com/animation

We can conclude that the much smaller genomes of prokaryotes (organisms without a true nucleus, i.e. bacteria) do not require this packaging, as protein is absent from the circular chromosomes of bacteria. Here, the DNA is described as 'naked'.

Common mistake

A group of bacteria called eubacteria have DNA that is not associated with histone proteins – this is termed 'naked' DNA. Some students incorrectly use this term to describe DNA that is not enclosed in a nuclear membrane, as is the case in all bacteria. The term 'naked DNA' should be reserved for DNA that is not associated with histone proteins.

Use of molecular visualization software

Molecular visualization software can be used to study the association between the proteins and DNA within a nucleosome. This page shows DNA wrapped around a nucleosome:
www.wehi.edu.au/wehi-tv/nucleosomes

Search for your own image of a nucleosome using the Protein Data Bank (PDB):

Access the PDB: **www.rcsb.org/pdb/home/home.do**

1. At the top of the page, search for 'nucleosomes' – this will take you to a list of images. Select one – this will take you to a page that has an image of the nucleosome and information about it. At the top of the page, select '3D view'. This will show you an image that you can use your mouse to drag, rotate and zoom in and out of the structure. Alternatively, you can 'Select Orientation' from the menu on the right.
2. Rotate the nucleosome so that you can see the two copies of each histone protein, with DNA wrapped around each. Each protein has a tail that extends out from the core. DNA is wrapped nearly twice around the octamer core.
3. You can alter the image by selecting a different style of molecular visualization. The default is 'Mol* (Javascript)' but other options can be accessed on the menu at the bottom right of the screen ('Select a different viewer'). If 'JSmol' is selected, the structure can be seen using a variety of different styles and colours.
4. Access the JSmol viewer. Select 'colour by amino acid'. What role do the positively charged amino acids play in the association of the protein core with the negatively charged DNA?

This site also has a molecular visualization of a nucleosome:
www.mcb.ucdavis.edu/courses/jsmol/Nucleosomejs.htm

It is possibly to modify the image using the selection underneath the visualization. For example, 'Show protein as cartoons'. The original view can be restored by clicking on 'Restore original view'.

Count how many times the DNA is wound around the histones (to make this easier, you may want to 'Hide protein'). Count the number of histone proteins (H2A, H2B, H4 and H3). Note the tails coming from the histone core. The N-terminal tail that projects from the histone core for each protein is used in regulating gene expression through chemical modification.

Another site allows you to download free software to view the three-dimensional structure of molecules: **https://pymol.org/2/**

This programme enables you to upload PDB files and allows you to zoom in and rotate molecules.

The Hershey and Chase DNA experiment

Since about 50% of a chromosome consists of protein, it is not surprising that scientists once speculated that the protein of chromosomes might be the information substance of the cell. For example, there is more chemical 'variety' within a protein than in nucleic acid. However, this idea proved incorrect. We now know that the DNA of the chromosomes holds the information that codes for the sequence of amino acids from which the proteins of the cell cytoplasm are synthesized.

How was this established?

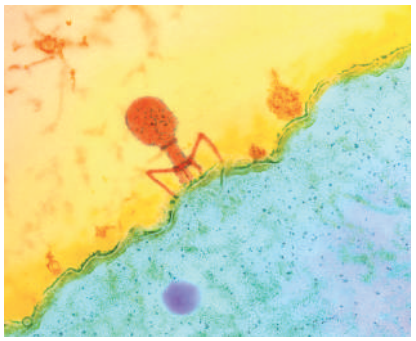
◆ **Bacteriophage**: a virus that parasitizes bacteria (also known as a phage).

The evidence for the unique importance of DNA was proved by an experiment carried out by two experimental scientists, Martha Chase and Alfred Hershey, with a bacteriophage virus. A **bacteriophage** (or **phage**) is a virus that parasitizes a bacterium. A virus particle consists of a protein coat (capsid) surrounding a nucleic acid core. Once a virus has gained entry to a host cell, it may take over the cell's metabolism, switching it to the production of new viruses. Eventually, the remains of the host cell break down (lysis) and the new virus particles escape – now able to repeat the infection in new host cells. The life cycle of a bacteriophage, a virus with a complex 'head' and 'tail' structure, is shown in Figure A1.2.14.

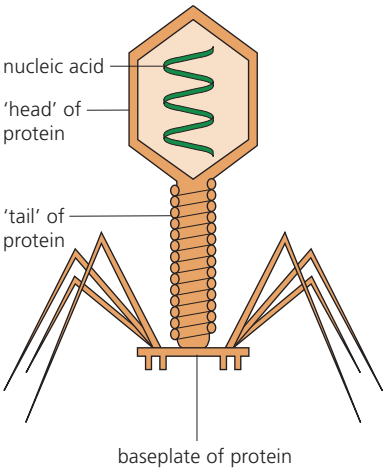
Link

The life cycle of a virus is covered more fully in Chapter A2.3, page X–X.

electron micrograph of bacteriophage infecting a bacterium

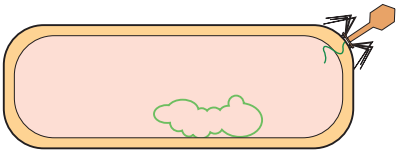


structure of the phage



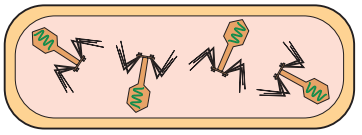
steps to replication of the phage

1 The phage attaches to the bacterial wall and then injects the virus DNA.



2 Virus DNA takes over the host's synthesis machinery.

3 New viruses are assembled and then escape to repeat the infection cycle.

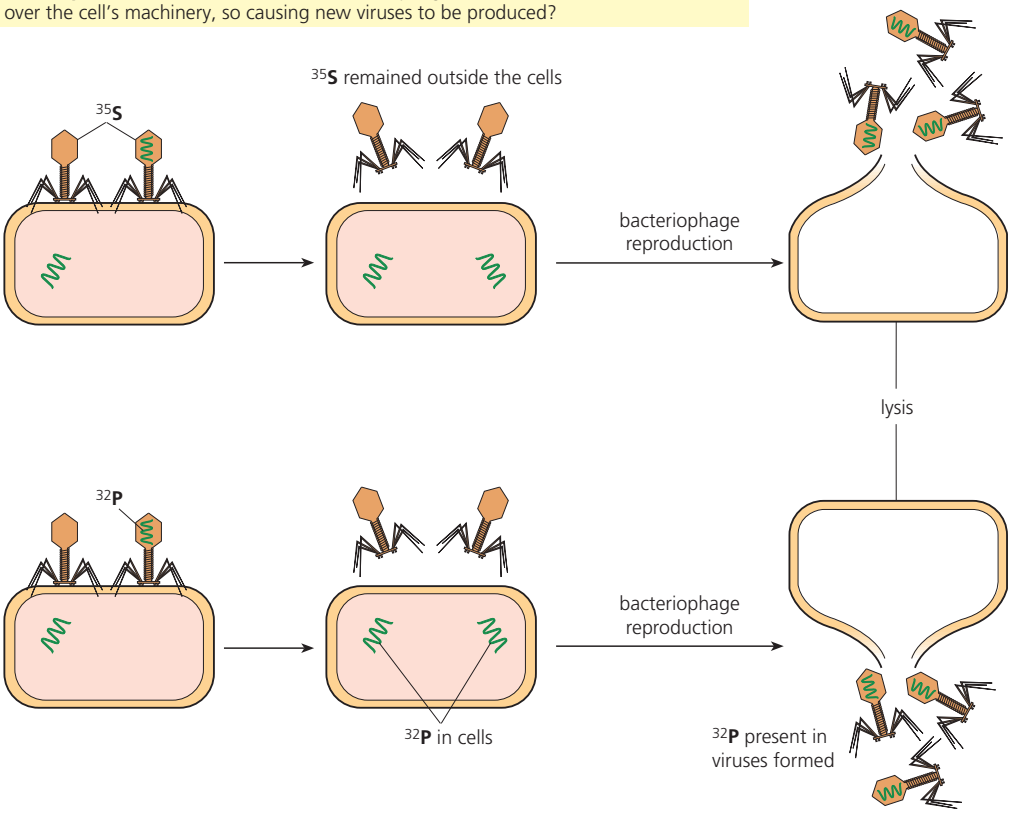


■ **Figure A1.2.14** The life cycle of a bacteriophage

In 1952, Chase and Hershey used a bacteriophage that parasitizes the bacterium *Escherichia coli* to answer the question of whether genetic information lies in the protein coat (capsid) or the DNA (core) (Figure A1.2.15).

Two batches of the bacteriophage were produced, one with radioactive phosphorus atoms (^{32}P) built into the DNA core (so here the DNA was labelled) and one with radioactive sulfur atoms (^{35}S) built into the protein coat (here the protein was labelled). Note that sulfur occurs in protein, but there is no sulfur in DNA. Likewise, phosphorus occurs in DNA, but there is no phosphorus in protein. So, we can be sure the radioactive labels were specific.

Is it the **protein coat** or the **DNA** of a bacteriophage that enters the host cell and takes over the cell's machinery, so causing new viruses to be produced?



■ **Figure A1.2.15** The Hershey–Chase experiment

Only the DNA part of the virus got into the host cell (and radioactively labelled DNA was present in the new viruses formed). It was the virus DNA that controlled the formation of new viruses in the host, so Hershey and Chase concluded that **DNA carries the genetic message**.

Two identical cultures of *E. coli* were infected, one with the ^{32}P-labelled virus and one with the ^{35}S-labelled virus. Subsequently, radioactively labelled viruses were obtained only from the bacteria infected with virus labelled with ^{32}P. In fact, the ^{35}S label did not enter the host cell at all. Chase and Hershey's experiment clearly demonstrated that it is the DNA part of the virus which enters the host cell and carries the genetic information for the production of new viruses.

8 **Deduce** what would have been the outcome of the Hershey–Chase experiment (Figure A1.2.15) if protein had been the carrier of genetic information.

ATL A1.2D

Find out more about Alfred Hershey and his work with Martha Chase using this site: **www.dnaftb.org/18/animation.html** Select 'animation'; and click on the icon 'jump to' to move to the fourth section where Alfred Hershey discusses his research on bacteriophage genetics.

Nature of science: Global impact of science

The Hershey–Chase experiment illustrates how technological developments can open up new possibilities for experiments. When radioisotopes were made available to scientists as research tools, the Hershey–Chase experiment became possible.

After the nuclear explosions over Japan at the end of the Second World War, the US government publicised the peacetime benefits of nuclear knowledge through nuclear power and the medical uses of radioisotopes. The availability of reactor-produced radioisotopes equipped virus researchers (and biochemists more generally) with a valuable new research tool. Isotopes are especially suitable for studying the dynamics of chemical transformation over time, through metabolic pathways or life cycles.

Chargaff's data on the relative amounts of pyrimidine and purine bases

The discovery of the principle of base pairing by Watson and Crick was the result of their interpretation of the work of Erwin Chargaff. In 1935, Chargaff had analysed the composition of DNA from a range of organisms and found rather remarkable patterns. Apparently, the significance of these patterns was not immediately obvious to Chargaff, though.

His discoveries were:

- the numbers of purine bases (adenine and guanine) always equalled the number of pyrimidine bases (cytosine and thymine)
- the number of adenine bases equalled the number of thymine bases, and the number of guanine bases equalled the number of cytosine bases.

What does this mean?

The organic bases found in DNA are of two distinct types with contrasting shapes:

- cytosine and thymine are pyrimidines or single-ring bases
- adenine and guanine are purines or double-ring bases.

Only a purine will fit with a pyrimidine between the sugar–phosphate backbones, when base pairing occurs (Figure A1.2.6, page X). So, in DNA adenine must pair with thymine, and cytosine must pair with guanine.

Nature of science: Falsification

The problem of induction

Biology is not a body of unchanging facts, but a process of generating new biological knowledge, theories and laws, using the scientific method. There is no single agreed scientific method, but a number of variations, all of which can be used to generate new scientific knowledge. The scientific method you will be familiar with from your practical work (investigations) is known as the Baconian or **inductive** scientific method.

The inductive scientific method begins with observations and the collection of raw data. The data are analysed and a hypothesis is generated. An investigation is then designed to test the validity of the hypothesis. A general theory may then be generated from the specific data and the hypothesis.

Biologists use both inductive and deductive reasoning to study biological problems.

Inductive reasoning (Figure A1.2.16) is sometimes termed the 'bottom up' approach. When inductive reasoning is used, specific observations and measurements may show a general pattern. This may then lead to a hypothesis that can be further explored and may also lead to the drawing of some general conclusions.

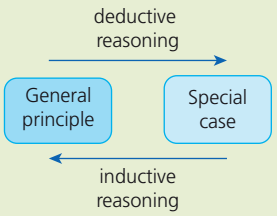


Figure A1.2.16 Deductive versus inductive reasoning

In this case, one might construct an inductive argument along the following lines:

Organisms A, B and C all have characteristic X.

Therefore, all items in the same class as A, B and C probably also have X.

For example:

- This sawfly stung me. It is a hymenopteran.
- This wasp stung me. It is a hymenopteran.
- This fire ant stung me. It is a hymenopteran.

There is a pattern here: it might seem that all hymenopterans (a large order of insects) have stingers.

One potential issue here is the **problem of induction**. Using data from many specific observations discovered in the past to create general observations about what will always happen in the future, is to assume; namely that the future will be just the way it was when you gathered your data. But you may be as yet unaware of something in your past observations which means the generalisation is not true. For example, many hymenopterans (stingless bees and ants, male honeybees (drones), etc.) do not have stingers. Perhaps your previous observations only *happen* to be of those hymenopterans that did have a stinger, you just never saw all the others that did not! (You might not discover this unless you test every single hymenopteran species for stinging capability, and this is simply impractical.)

Inductive reasoning involves forming generalisations from specific examples. Biology uses inductive reasoning – generalisations based on empirical evidence – as the basis of its justification for knowledge. A reliable scientific conclusion will be based on a large number of repeated investigations.

However, inductive reasoning can never give certainty. We also cannot be sure that the generalizations made in the past will continue to hold in the future. The impossibility of reaching certainty through induction is known as the 'problem of induction'. Inductive generalisations (biological theories) may, therefore, be shown to be wrong by new data and should only be thought of as 'tentative'. A single counter-example falsifies an inductive conclusion. (However, some theories, such as cell theory or the 'theory of gravity', are so well confirmed that there is little room for rationally doubting them.)

As a counter to the view that scientists are simply looking for further data to confirm their hypotheses (which are always only tentative anyway), the philosopher Karl Popper rejected the idea that science creates new knowledge by inductive steps. He suggested that scientists may work intuitively and creatively to generate a hypothesis before collecting data. This guides the scientist to plan and carry out investigations to collect data to test the hypothesis. The data will then either support the hypothesis or falsify (i.e. disprove) the hypothesis, but the falsifying data are actually more helpful in developing knowledge. Popper suggested that **falsification** is an important part of the scientific process because a hypothesis which is confirmed after one or many experiments, may yet for some unknown reason be falsified later. However, if a hypothesis is shown to be false, then genuine knowledge is gained: the hypothesis is *not* true.

The Russian-American biochemist Phoebus Levene (1869–1940), who discovered ribose sugar in 1909 and deoxyribose sugar in 1929, suggested (incorrectly, with hindsight) the structure of nucleic acid as a repeating tetramer. He called the phosphate–sugar–base unit a nucleotide (Figure A1.2.17).

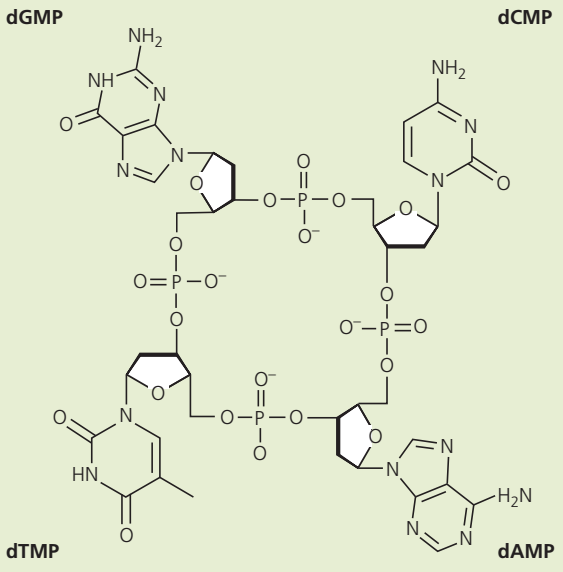


Figure A1.2.17 Possible structural formula of a tetranucleotide, later shown to be incorrect, proposed by Levene around 1910

Levene did not recognize that the compositions of nucleic acids were organism-specific, and he did not recognize that in organisms the four nucleotides are not present in equal amounts. This was due to the inaccuracy of the analytical techniques available at that time, which did not allow a reliable determination of the relative amounts of nucleotides in nucleic acids.

It was only in the second half of the 1940s that Erwin Chargaff established the organism-specificity of nucleic acids and the special relationships among the amounts of nucleotides in any organism. The tetranucleotide hypothesis became obsolete after the structure of DNA was determined, since it was realized that a structure in which a four-member unit is being repeated could not carry the genetic information that must be involved in heredity.

The tetranucleotide hypothesis, and Chargaff's falsification, is an example of how the problem of induction can be addressed by the certainty of falsification. In this case, Chargaff's data falsified the tetranucleotide hypothesis that there was a repeating sequence of the four bases in DNA.

◆ **Falsification**: a process used by scientists in which a hypothesis is tested by trying to show that it is false. Where a hypothesis cannot be shown to be false after repeated experiments conducted by different groups of scientists, it is considered a strong hypothesis.

LINKING QUESTIONS

1 What makes RNA more likely to have been the first genetic material, rather than DNA?
2 How can polymerization result in emergent properties?

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A2.1 Origins of cells

Guiding questions

- What plausible hypothesis could account for the origin of life?
- What intermediate stages could there have been between non-living matter and the first living cells?

SYLLABUS CONTENT

This chapter covers the following syllabus content:

- A2.1.1 Conditions on early Earth and the pre-biotic formation of carbon compounds (HL only)
- A2.1.2 Cells as the smallest units of self-sustaining life (HL only)
- A2.1.3 Challenge of explaining the spontaneous origin of cells (HL only)
- A2.1.4 Evidence for the origin of carbon compounds (HL only)
- A2.1.5 Spontaneous formation of vesicles by coalescence of fatty acids into spherical bilayers (HL only)
- A2.1.6 RNA as a presumed first genetic material (HL only)
- A2.1.7 Evidence for a last universal common ancestor (HL only)
- A2.1.8 Approaches used to estimate dates of the first living cells and the last universal common ancestor (HL only)
- A2.1.9 Evidence for the evolution of the last universal common ancestor in the vicinity of hydrothermal vents (HL only)

Conditions on early Earth and the pre-biotic formation of carbon compounds

◆ **Greenhouse gas**: a gas that contributes to the greenhouse effect by absorbing infrared radiation.

◆ **Greenhouse effect**: process in which greenhouse gases trap outgoing long-wave radiation from the Earth, causing the planet to be warmer than it would otherwise be.

Around 4.3 billion years ago, much of the surface of the Earth was molten rock. This time is known as the Hadean eon (from Greek mythology where 'Hades' is the God of the Underworld, and the term is associated with 'Hell', indicating the conditions on Earth at the time). As Earth cooled, gases released by volcanic activity formed the atmosphere. The atmosphere included ammonia (NH_3), nitrogen, methane, water and significantly higher levels of carbon dioxide compared to today's atmosphere (see Figure A2.1.1). Both methane and carbon dioxide are **greenhouse gases** – this means that they absorb and react with infrared radiation emitted from the surface of the planet, causing the surface of the Earth to heat up, resulting in higher temperatures (a process known as the **greenhouse effect**).

1 **Compare and contrast** the atmospheric conditions of the early Earth with the atmosphere of today.

The atmosphere today has relatively high levels of oxygen, essential for sustaining life. Also important for the preservation of life on the Earth's surface is the presence of ozone in the stratosphere (Figure A2.1.2). Ozone (O_3) is formed naturally through the interaction of solar ultraviolet (UV) radiation with molecular oxygen (O_2). On the early Earth there was a lack of free oxygen and, therefore, ozone in the atmosphere, resulting in ultraviolet light penetration and high levels of UV light at the surface of the planet. UV radiation 3.7 billion years ago was 100 times more intense than today. UV causes damage to DNA and causes it to mutate – lower levels of UV allow life to exist on the surface of the Earth today.

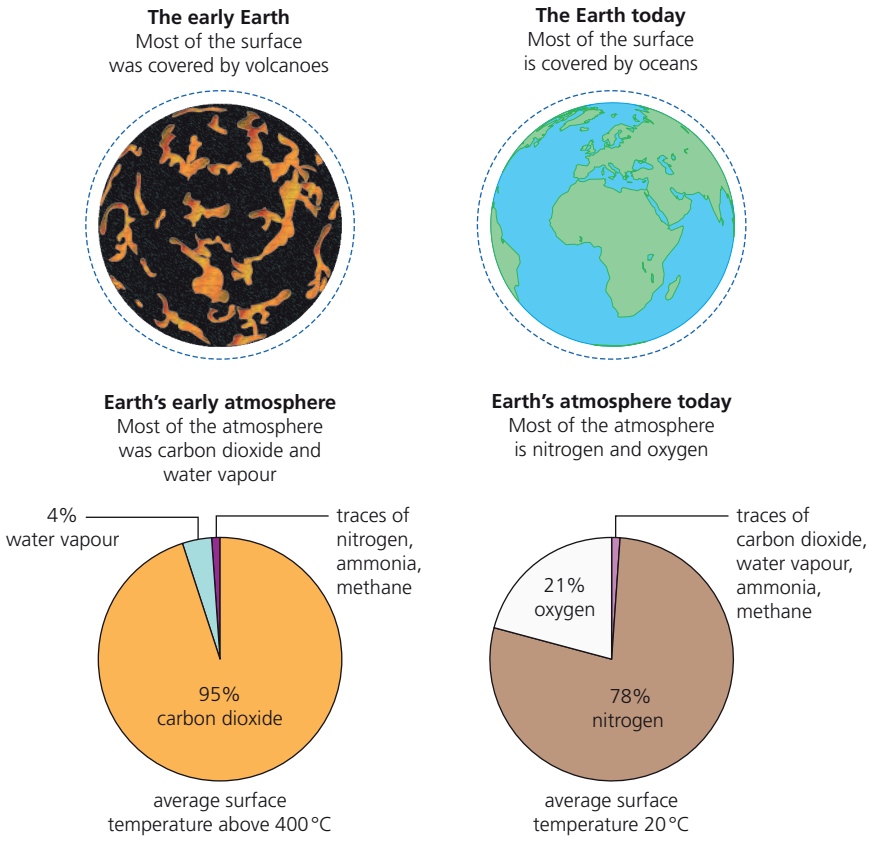


Figure A2.1.1 The atmosphere of the early Earth and the atmosphere today

The conditions on the early Earth may have caused a variety of carbon compounds to form spontaneously by chemical processes that do not occur now. If energy was added to the gases that made up Earth's early atmosphere, the building blocks of life (for example, amino acids, peptides, ribose, nucleobases, fatty acids, nucleotides and oligonucleotides) could have been created. The idea that high energy or UV radiation led to the formation of the first biological molecules is called the 'primordial (or pre-biotic) soup' hypothesis and was originally proposed independently by Alexander Oparin in 1924 and JBS Haldane in 1929.

However, high-energy UV light is destructive of the chemistry of early life. When a molecule is destroyed, it is broken into smaller, very reactive pieces that undergo additional reactions, eventually recombining to form larger high-energy molecules. For example, pyruvic acid, a molecule that is central to key metabolic pathways in cells, forms larger molecules when dissolved in water and illuminated with UV light.

2 **List** the gases which cause the greenhouse effect.

Research has shown that the bases of nucleotides are extremely efficient at reducing the harmful effects of UV radiation, thereby protecting the pentose sugar and phosphate components of nucleic acids. In the presence of strong UV light, RNA was shown to be more likely to form chains than other molecules. It is therefore possible that the high UV levels on primordial Earth, rather than being an obstacle to the origin of life, may have acted as a selection factor that drove the process forwards.

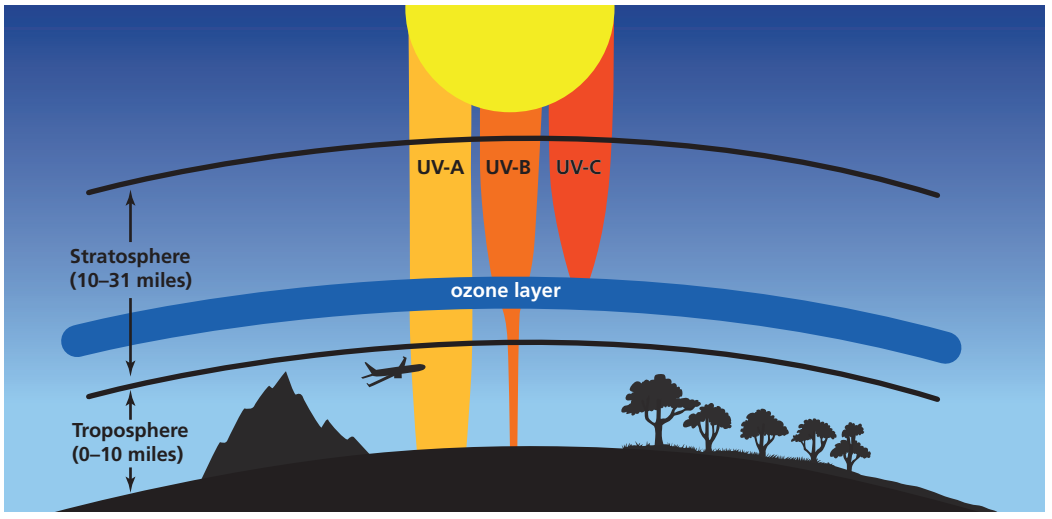


Figure A2.1.2 Ozone absorbs the most energetic frequencies of ultraviolet radiation, known as UV-C and UV-B, which are frequencies that harm living organisms

Cells as the smallest units of self-sustaining life

Link
Plasma membranes are covered in detail in Chapter B2.1, page X–X.

Cells are the fundamental self-sustaining units of life that reproduce via cell division. All present-day cells are believed to have evolved from a common ancestral cell that existed around 4 billion years ago.

All cells are enclosed by a plasma membrane that separates the inside of the cell from its environment. All cells contain DNA to store genetic information and use it as a template for the synthesis of RNA molecules and proteins.

The identification of cellular life processes, e.g., respiration, nutrition, reproduction and so on (see also page XX), does not define life and it does not explain how life appeared. The three principles below can be used to define life:

Link
Natural selection is covered in Chapters A4.1, page X, and D4.1, page X.

- Living organisms must be able to evolve through natural selection. This requires them to be able to reproduce and have a hereditary system that must show genetic variation.
- Life forms are contained and separate from, but in communication with, their surrounding environment, like a cell.
- Life forms are chemical and physical 'machines' that receive and respond to information.

There is also a need to take a systems approach and not just to view life as simply a large set of biochemical reactions at the molecular level. Systems biologists view living organisms as complex systems that process information about themselves and their environment.

Link
For more on Darwinian evolution, see Chapter A4.1, page XX.

NASA's working definition of life is: 'life is a self-sustaining chemical system capable of Darwinian evolution'. This definition of 'life' would include viruses, but many of the problems in attempting to define life are because there is only one example – life on Earth – where all the organisms have the same basic biochemistry. It is therefore difficult to distinguish which properties of life on Earth are unique and which are needed in a general sense to qualify as 'life'. The most promising place for life in our solar system is probably Mars since it may have sub-surface pockets of water.

However, some scientists have proposed that life could evolve on other planets in the universe using a liquid other than water, e.g., ammonia. If these hypothetical organisms existed, they might be described as 'weird life' because they would have to be fundamentally different from terrestrial life.

Link
Viruses are covered in detail in Chapter A2.3, page X–X.

Viruses are regarded as non-living. Viruses are non-cellular and therefore lack organelles to carry out metabolism independently, e.g., releasing energy in the form of ATP (see page XX) and protein synthesis. They can only replicate inside living cells using their cellular components.

However, viruses do have some features of living organisms. They do not respond to stimuli from their surroundings and do not exhibit homeostasis (the keeping of internal conditions within narrow limits, such as temperature), but they do respond to external stimuli, such as evading immunity, adapting to drug treatment (with antivirals) or changing host range by mutation (especially RNA viruses).

Viruses contain hereditary material in the form of nucleic acids (DNA or RNA) with genes that code for specific structures, e.g. capsid coat proteins, and reverse transcriptase and integrase (for retroviruses).

The genomes of viruses are prone to rapid mutation and are one factor in the evolution of viruses by natural selection. Many viruses can engage in a form of recombination known as genetic shift.

The spontaneous origin of cells

Cells are consistent across all biological systems and are highly complex structures that can currently only be produced by the division of pre-existing cells. However, the first cells must have had non-biotic (non-biological) origins, and one of the most important questions in biology is how did these first cells evolve? Answers may be found by looking at the features of cells and then considering how they first evolved and appeared.

All cells have three common features:

1. a stable, partially permeable membrane that surrounds cell components
2. genetic material that can be passed on when new cells are formed, and which controls the function and behaviour of cells
3. metabolic processes that allow energy generation, enabling growth, self-maintenance and reproduction.

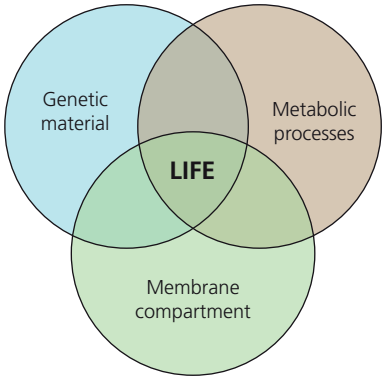


Figure A2.1.3 Three factors essential for sustaining life

In biology, the term 'evolution' specifically means the processes that have transformed life on Earth from its earliest beginnings to the diversity of forms we know about today, living and extinct. It is an organizing principle of modern biology. It helps us make sense of the ways living things are related to each other, for example.

The evolution of life in geological time has involved major steps – none more so than the origin of the first cells. Unless these first cells arrived here from somewhere else in the universe, they must have arisen from non-living materials, starting from the components of the Earth's atmosphere at the time. We can only speculate about these very first steps.

Concept: Diversity

The common features of all cells have allowed a large diversity of forms to evolve over a period of some 4 billion years.

Following on from the discussion above, the formation of living cells from non-living materials would have required the following steps:

- the synthesis of simple organic molecules, such as sugars and amino acids
- the assembly of these molecules into polymers (page X)
- the development of self-replicating molecules, such as the nucleic acids
- the retention of these molecules within membranous sacs, so that an internal chemistry developed, different from the surrounding environment.

As well as the features shown in Figure A2.1.3, and the processes listed above, these elements must have spontaneously self-assembled to form the first cells.

3 **List** the three common features shared by all cells.

4 **Outline** the steps that would be needed for the formation of living cells from non-living materials.

Nature of science: Hypotheses

The origin and the evolution of the earliest cells are among the most intriguing topics being debated in the scientific community. Traditionally, two approaches have been used to understand how life on the Earth originated. The bottom-up approach, favoured by chemists, for example Miller's experiment (see Figure A2.1.4, page X), attempts to reconstruct the conditions of primitive Earth. The top-down approach is favoured by biologists, who study modern organisms to find the relics of their ancestors to reconstruct ancient metabolic pathways and molecular processes.

However, knowledge about evolutionary history is not restricted to perfectly replicating a point in the geological past or finding fossils. Biologists interested in chemical evolution or the emergence of the first protocell can carry out experiments to test the mechanisms upon which a theory rests – and can do so in laboratory conditions that match what scientists do know about conditions that likely existed somewhere on the pre-biotic Earth. However, one of the problems of testing hypotheses in this way is that the exact conditions on pre-biotic Earth cannot be replicated. This approach can be illustrated for the three main competing theories for the origin of life.

The three competing theories for the origin of life

1 Protocell-first

◆ **Protocell**: pre-cellular or cell-like entity, e.g. a lipid droplet with a few molecules inside.

A cell-like compartment that had a basic metabolism but lacked a fully developed genetic system; it arose spontaneously with the ability to grow and then divide into daughters that tended to resemble the mother cell. These **protocells** evolved adaptively until they eventually acquired a genetic system (likely RNA, then later DNA).

Sample prediction: cell-like units capable of growing and dividing without genetic molecules could be engineered or, better still, be seen to arise spontaneously in the laboratory under controlled conditions. The exact nature of the earliest cells would be difficult to prove, however, because the first protocells did not fossilize.

2 Gene-first

A genetic molecule (thought to be RNA) or a small set of genetic molecules arose spontaneously, capable of replication. The replicators evolved adaptively by natural selection, forming genetic variants that could assemble a cell membrane and start metabolising.

Sample predictions: a context could be found in which complex RNA molecules form spontaneously; spontaneously arising RNAs can show collective self-replication and open-ended evolution.

3 Metabolism-first

A self-sustaining system of simple reactions, capable of feeding on nutrients and energy arose spontaneously, perhaps adsorbed on to a mineral surface. The chemical mixture evolved adaptively, eventually evolving cells (perhaps via selection for dispersal) and genetic systems (perhaps via selection for catalysis of metabolic reactions).

Sample predictions: chemical mixtures given a flux of nutrients/energy in the laboratory should sometimes demonstrate autocatalysis and show evidence of adaptive evolution; autocatalytic reaction systems can produce lipids and genetic polymers.

Many scientists favour the metabolism-first theory because rapid growth, replication and division, which are essential processes for protocells' evolution, all require significant amounts of energy.

Top tip!

Claims in science, including hypotheses and theories, must be testable. In some cases, scientists struggle with hypotheses that are difficult to test. In this case, the exact conditions on pre-biotic Earth cannot be replicated and the first protocells did not fossilize.

TOK

Knowledge that is beyond the capability of science is perhaps knowledge that we do not have the technology to discover at this current moment in time. Although it is possible to investigate the origin of the first cells, this is not something that can be verified as it is not possible to check whether the hypotheses are true. The validity of hypotheses regarding the origin of life can only be tested by accumulating evidence that supports a particular theory. What knowledge, if any, is likely to always remain beyond the capabilities of science to investigate or verify?

Evidence for the origin of carbon compounds

The molecules that make up living things are built mainly from carbon, hydrogen and oxygen, with some nitrogen, phosphorus and sulfur; a small number of other elements are also present (metals and their ions are very important in living organisms). Today, living things make these molecules by the action of enzymes in their cells, but for life to originate from non-living material, the first step was the non-living synthesis of simple organic molecules.

Apparatus like this has been used with various gases to investigate the organic molecules that may be synthesized.

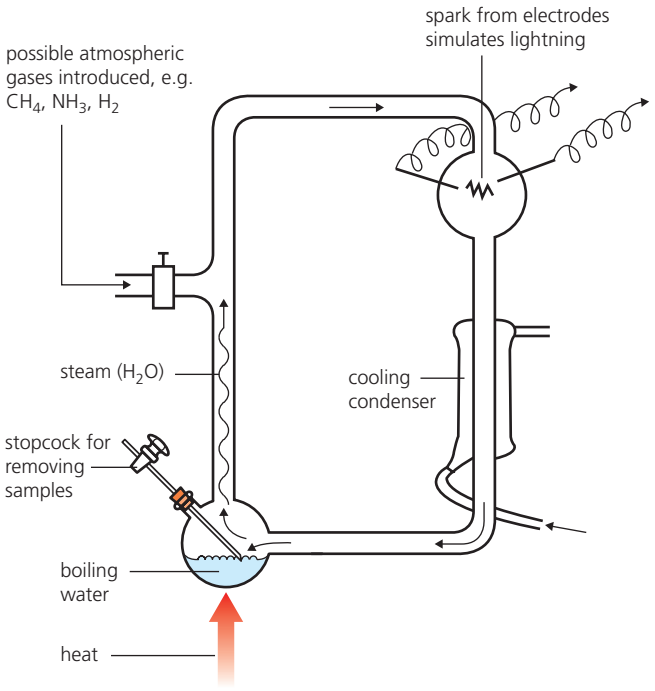


Figure A2.1.4 Apparatus for simulating early chemical evolution

SL Miller and HC Urey (1953) investigated how simple organic molecules might have arisen from the chemicals present on Earth before there was life. They used a reaction vessel in which specific environmental conditions could be reproduced (Figure A2.1.4). For example, strong electric sparks (simulating lightning) were passed through mixtures of methane, ammonia, hydrogen and water vapour for a period of time. They discovered that amino acids (some known components of cell proteins) were formed naturally, as well as other compounds.

This approach confirmed that organic molecules can be synthesized outside cells, in the absence of oxygen.
The experiment has subsequently been repeated, sometimes using different gaseous mixtures and other sources of energy (ultraviolet radiation, in particular), in similar apparatus.
The products have included amino acids, fatty acids and sugars such as glucose. In addition, nucleotide bases have been formed and, in some cases, simple polymers of all these molecules have been found. So, we can see how it is possible that a wide range of organic compounds could have formed on the pre-biotic Earth, including some of the building blocks of the cells of organisms.

TOK

To what extent can you argue that Miller and Urey's experimental response to a seemingly insoluble issue was a uniquely scientific response?

ATL A2.1A

Carry out further research into Stanley Miller and Harold Urey's work on synthesizing organic molecules in a pre-biotic world: **www.dnaftb.org/26/animation.html**

What first led Urey and Miller to study the origin of life on Earth? What research came before them, and what work followed their discoveries?

Assembly of the polymers of living organisms

For polymers to be assembled in the absence of cells and enzymes would have required a concentration of biologically important molecules such as monosaccharides (simple sugars – the building blocks for polysaccharides), amino acids (building blocks for proteins) and fatty acids (for lipid synthesis). They would need to come together in 'pockets' where further chemical reactions between them were possible. Clays have been shown to be important in the **polymerization** of monomers – they promote phosphodiester bond formation (see page X) by binding and concentrating nucleotides. Microscopic layers of clay may have played a similar role in the formation of the first polyribonucleotides. This might have happened in water close to lava flows from volcanoes or at the vents of submarine volcanoes where the environment is hot, the pressure is high and the gases being vented are often rich in sulfur compounds (e.g. H_2S) and other compounds. There is some evidence for the latter (see page XX).

◆ **Polymerization**: process by which relatively small molecules, called monomers, combine chemically to produce a larger molecule called a polymer.

Evaluating the Miller–Urey experiment

Inquiry 3: Concluding and evaluating

Evaluating

An **evaluation** is an important procedure towards the end of a scientific investigation. Did you demonstrate your hypothesis? What were the limitations and strengths of the investigation? How could you improve the experiment? What else could you measure or change?

When commenting on limitations, consider the procedures, the equipment, the use of equipment, the quality of the data (for example, their **accuracy** and **precision**) and the relevance of the data. To what extent may the limitations have affected the results? Propose realistic improvements that address the limitations.

◆ **Evaluation**: make an appraisal by weighing up the strengths and limitations.

◆ **Accuracy**: how close to the true value a result is.

◆ **Precision**: describes the reproducibility of repeated measurements of the same quantity and how close they are to each other.

The conditions of the Miller–Urey experiment were believed at the time (1953) to simulate the atmosphere of early Earth, which was assumed to be reducing (hydrogen rich) and rich in methane. However, current thinking is that methane was in low abundance in the early atmosphere (except perhaps for brief periods), with carbon largely in the form of carbon dioxide (oxygen rich). Also, the Miller–Urey experiment used electrical discharges rather than UV light to simulate high-energy input into the early Earth system. However, organic molecules such as amino acids and bases are generated when carbon dioxide, nitrogen and water are subjected to ionizing (nuclear) radiation and ultraviolet light, as well as electrical discharges.

Despite the success of the experiments of Miller and Urey, efforts to reproduce the conditions of pre-biotic chemistry have not until recently succeeded in generating nucleotides. Nucleotides have now been chemically synthesized via a new approach involving four simple organic molecules – cyanamide, cyanoacetylene, glycolaldehyde and glyceraldehyde – that are readily produced under reasonable pre-biotic conditions.

Spontaneous formation of vesicles

All cells are made from membranes that separate genetic material and chemical reactants in metabolic processes from the external environment. It is likely that the earliest cells, or protocells, were formed from basic membranes.

But how did these protocells form?

Fatty acids are likely to have formed the components of protocell membranes because they are **amphipathic**, which means that they have a polar end that is attracted to water and a non-polar end that is repelled by it. Scientists have shown that if a few lipid molecules are in water, they form a monolayer on the surface of water and, with more lipid present, bilayers form. Lengths of these bilayers are likely to have formed **microspheres** (Figure A2.1.5) or very small **vesicles**. Vesicles therefore form spontaneously (i.e. without an external cause or stimulus) by coalescence of fatty acids into spherical bilayers.

Perhaps simple microspheres, surrounding a portion of a pre-biotic 'soup' of polymers and monomers, were the forerunners of cells. These may have formed membrane systems with a distinctive internal chemistry, as they developed a chemical environment different from their surroundings.

◆ **Amphipathic**: a molecule that has two different affinities – a polar end that is attracted to water and a non-polar end that is repelled by it.

◆ **Microsphere**: a microscopic hollow sphere made from a lipid bilayer.

◆ **Vesicle**: membrane-bound sac.

Link

Phospholipids and cell membranes are covered in detail in Chapters B1.1, page X–X, and B2.1, page X–X.

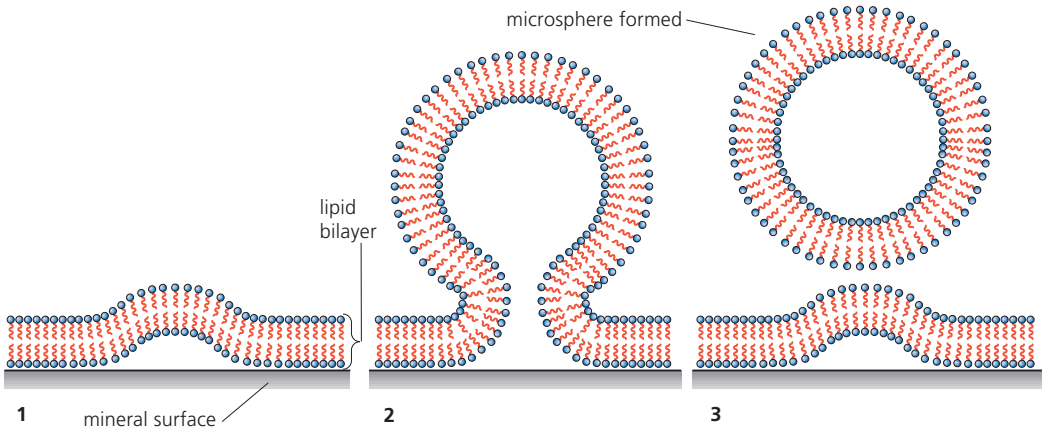


Figure A2.1.5 Steps in the formation of microspheres

Cell membranes are made from modified lipids, called phospholipids, which have structures with hydrophobic tails and hydrophilic heads. It is likely that fatty acids rather than phospholipids formed the first membranes as they are chemically simpler than phospholipids.

The evolution of cell membranes, from primitive to more modern, may therefore have followed the following pathway:

- Protocells formed from fatty acids. Fatty acids are extremely stable compounds and may have accumulated to significant levels on the early Earth.
- Condensation of fatty acids with glycerol to form triglycerides, a highly stabilizing membrane component.
- Phosphorylation (the addition of a phosphate to the triglyceride) forms the simplest phospholipid.

RNA as a presumed first genetic material

For the evolution of life from a mixture of polymers and their monomers, two special situations need to emerge:

- a 'self-replication' system
- an ability to catalyse chemical change.

Today in living cells these essential situations are achieved by DNA, the home of the genetic code, and enzymes, which are typically large, globular proteins (see Chapter C1.1, page XX). However, neither of these have been synthesized in any experiments that repeat Miller and Urey's demonstration of how biologically important molecules might have been synthesized in the pre-biotic world.

So what may have filled the roles of DNA and enzymes in the origin of life?

A likely answer came as a by-product of a genetic engineering experiment, investigating the enzymes needed to join short lengths of RNA. It was discovered that RNA, as well as being information molecules, may also function as enzymes. Perhaps short lengths of RNA combined the roles of information molecules and enzymes in the evolution of life itself.

In Chapter A1.2, we explored the structure of RNA and its similarities and differences to DNA. We have also seen how hydrogen bonds between adjacent bases confer stability in DNA molecules. Messenger RNA (mRNA) is clearly a simpler molecule than DNA (it is single stranded rather than double stranded), and hydrogen bonds can occur between nucleotides in the same chain, causing RNA to fold up in a unique way, determined by its nucleotide sequence. This folding can confer enzymatic properties on the RNA – a property that would have been needed in the earliest forms of life. This is known as the **RNA world** hypothesis.

◆ **RNA world**: hypothesis that proposes that the earliest life forms (protocells) may have used RNA alone for the storage of genetic material.

Concept: Unity

As nucleic acid is in all living organisms, this suggests a means by which the first cells arose.

The RNA world

◆ **Central dogma**: the idea that the transfer of genetic information from DNA of the chromosome to mRNA to protein (amino acid sequence) is irreversible.

The **central dogma** of molecular biology is that DNA makes RNA, which makes protein (Figure A2.1.6). Nucleic acids are required for protein synthesis, but proteins are required to synthesize nucleic acids. This makes it difficult to see how this interdependent system could have evolved by natural selection.

Top tip!

The idea that information always flows in this direction (DNA to RNA to protein) in cells was called the central dogma of cell biology, implying it was always the case. However, in retroviruses such as HIV (see Chapters A2.3 and C3.2), the information in RNA in the cytoplasm is translated into DNA within a host cell and then becomes attached to the DNA of a chromosome in the host's nucleus.

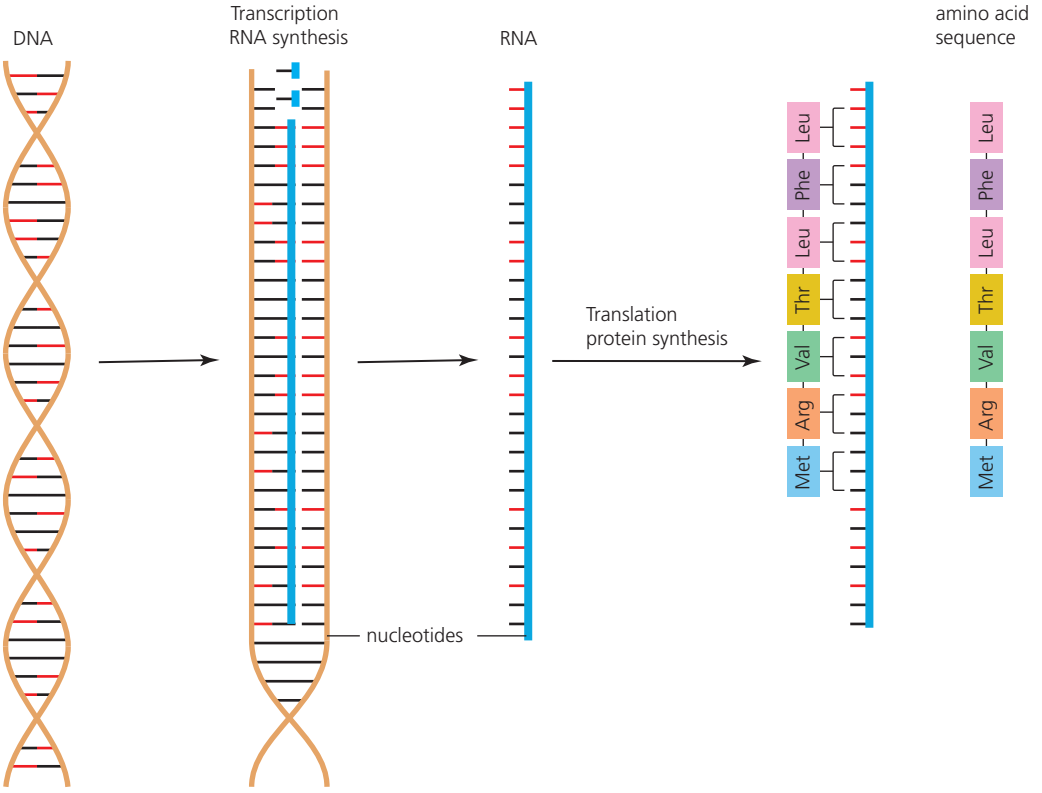


Figure A2.1.6 The central dogma showing flow of information from DNA to mRNA to proteins

ATL A2.1B

Crick's original formulation of the central dogma was: 'Once information has got into a protein it cannot get out again.'

What did he mean by this statement and how does it allow for reverse transcription, retroviruses and other biological phenomena?

Read about Crick's ideas here: **www.ncbi.nlm.nih.gov/pmc/articles/PMC5602739**

One view is that an RNA world existed in protocells before modern cells containing DNA and proteins. According to the RNA world hypothesis, RNA is now an intermediate between genes and proteins, but it stored genetic information and catalyzed chemical reactions in protocells. Only later in evolutionary time did DNA take over as the genetic material and proteins become the major catalysts and structural components of modern cells.

However, there still appear to be relics of the RNA world in modern cells. RNA primers (short nucleic acid sequences) are used in eukaryotic DNA replication, and ribosomal RNA appears to be involved in the catalysis of peptide bond formation in the ribosome. RNA molecules that have catalytic properties are known as **ribozymes**. These molecules have a unique folded three-dimensional shape that acts as an active site. In 1989, Thomas Cech and Sidney Altman were awarded the Nobel Prize in Chemistry for the discovery of catalytic RNA.

The unique potential of RNA molecules to act both as carriers of genetic information and as catalysts is thought to have enabled them to have a central role in the origin of life. Although self-replicating systems of RNA molecules have not been found in nature, scientists are attempting to synthesize them in the laboratory.

Evidence that RNA arose before DNA in evolution can be found in the chemical differences between them in their pentose sugars. Ribose present in RNA is readily formed from methanal (H_2CO), which is one of the principal products of the Miller–Urey experiment (page XX). In modern cells, deoxyribose is produced from ribose in a reaction catalyzed by a protein-based enzyme.

◆ **Ribozyme**: RNA molecule capable of acting as an enzyme.

Top tip!

Catalysis, self-replication of molecules, self-assembly and the emergence of compartmentalization were necessary requirements for the evolution of the first cells.

The other differences between RNA and DNA, the stable double helix of DNA and the use of the base thymine rather than uracil, further increase DNA's chemical stability by making the molecule easier to repair by enzymes.

The basic chemical reaction of the ribosome – joining together amino acids from an RNA template – is ultimately catalysed by RNA. This is perhaps even stronger evidence for the RNA world than the more generalized ability of RNA to act as a catalyst of various reactions, as it is consistent with the idea that protein synthesis could have first been developed in a pre-protein world, using an RNA enzyme (the early ribosome) to make the proteins from an RNA template.

The RNA world hypothesis has been an important paradigm shift in the scientific study of life's origins. Although this concept does not fully explain how life originated, it has helped to guide scientific thinking and has served to focus experimental efforts.

TOK

The concept that RNA can have both informational and functional roles, and that ribozymes can act as catalysts for chemical reactions between other RNA molecules, represented a paradigm shift in how scientists viewed the evolution of early life. The RNA world hypothesis provided a means by which the first nucleic acids could have developed and the role they played in the first cells.

What role do paradigm shifts play in the progression of scientific knowledge?

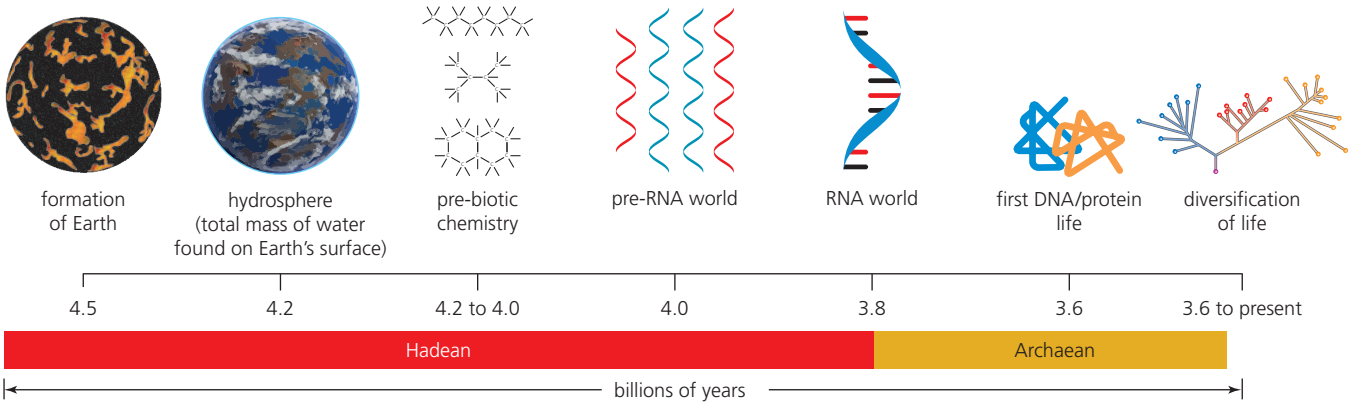


Figure A2.1.7 Geological timeline for the early Earth and the appearance of life; the sequence of events shows the formation of Earth through the Hadean eon and into the Archaean eon, and the corresponding events in the origin of life according to the RNA world hypothesis

5 **Describe** two properties of RNA which may have contributed to the origin of life.

Top tip!

RNA can be replicated and also has some catalytic activity so may have acted initially as both the genetic material and the enzymes of the earliest cells.

Evidence for a last universal common ancestor

◆ **Common ancestor**: the most recent species from which two or more different species have evolved.

Fossil remains and other evidence (such as anatomical and biochemical similarities) provide evidence about how species are related. The skeletons of the apes, for example, show that the gibbon, gorilla, chimpanzee, orang-utan and human are related and share a **common ancestor** (an ancestor species they all share), and suggest how evolution by natural selection has allowed them to adapt to different environments and lifestyles.

DNA can be used to determine similarities and differences between species. Species with very similar genes will be closely related, whereas those with very different DNA will be only distantly related. As we have already discussed, all life on Earth is related to each other and, ultimately, we all share a common ancestor, which is believed to have existed some 4 billion years ago. This organism is therefore the evolutionary link between the abiotic phase of Earth's history and the biotic phase. This organism is known as **LUCA**, which stands for '**L**ast **U**niversal **C**ommon **A**ncestor'. If all life on Earth is represented as a tree, LUCA is the organism at the base of the tree (Figure A2.1.8). There is an unbroken line of descent from us to LUCA. All organisms share the same biochemistry, the same bases in DNA and the same shared amino acids. The shared genetic code of (most) organisms – the code for translating RNA to protein – is very good evidence for common ancestry, since multiple codes would be possible if different lineages somehow independently evolved protein synthesis.

Concept: Unity

All living organisms can be related to a universal common ancestor, from which the tree of life arose.

These evolutionary relationships have been established by comparing the sequence of bases (nucleotides) in the ribosomal RNA (rRNA) present in species of each group.

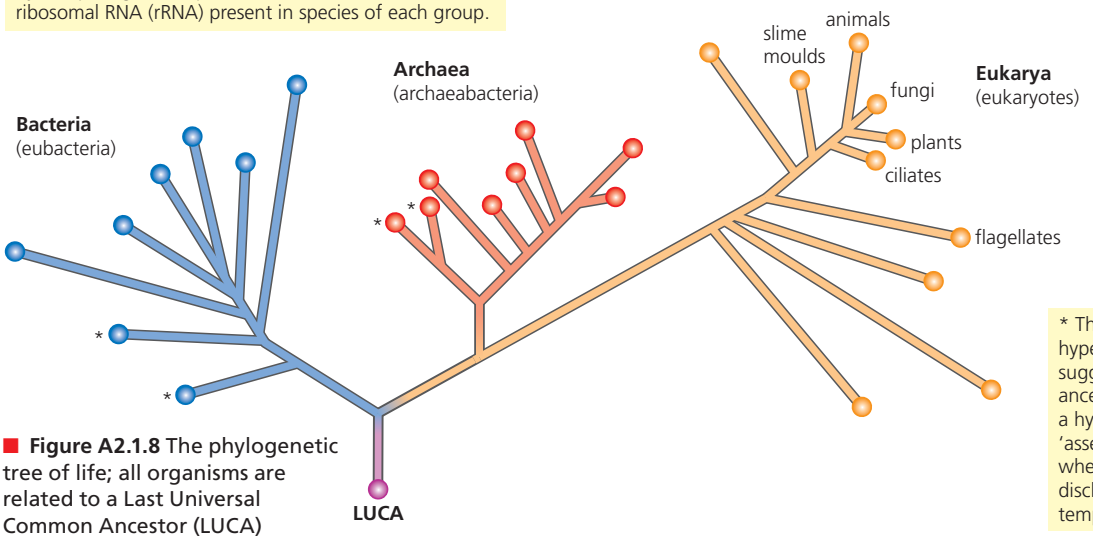


Figure A2.1.8 The phylogenetic tree of life; all organisms are related to a Last Universal Common Ancestor (LUCA)

* The shortest branches lead to hyperthermophilic species, which suggests that the universal ancestor of all living things was a hyperthermophile (possibly 'assembled' at deep ocean vents where volcanic gases are discharged into water at high temperature and pressure).

Common mistake

LUCA should not be referred to as a 'protocell'. The term protocell refers to a pre-cellular or cell-like entity (e.g. a lipid droplet with a few molecules inside; something much less elaborate than, for example, the ancestral prokaryote).

We have already discussed how the genetic code is universal (see Theme A1.2, page XX). The genetic code of all life on Earth contains a record of the origin and evolution of DNA through time, and the proteins for which it forms the 'blueprint'. Scientists have analysed DNA from modern bacteria (the eubacteria) and from two archaea (a separate group of extremophile prokaryotes, which can live in extreme environments such as deep ocean vents, in high-temperature habitats such as geysers, in salt pans, in acidic conditions and in polar environments) and searched for genes shared by them as an indicator of genes that were inherited from a common ancestor. Researchers searched DNA databanks, analysing the genomes of 2000 modern microbes sequenced over two decades: 355 gene families were discovered that were widespread among the bacteria from six million total genes, which means they were likely to be genes passed down from LUCA. Care was taken by researchers to eliminate genes that could have been transferred between bacteria laterally (through processes such as conjugation – the process by which one bacterium transfers genetic material to another through direct contact – see page XX) rather than by descent. Genomic analysis was then used to predict the likely structure and function of LUCA (given that DNA codes for proteins, and that proteins determine the structure and function of organisms). This analysis is discussed below (page XX).

It is likely that other forms of life evolved at the same time as LUCA but then became extinct by competing for common resources, making LUCA the surviving organism from which all species evolved. It is also likely that descendants of LUCA also competed with species that subsequently became extinct, shaping the tree of life that we see today.

ATL A2.1C

Figure A2.1.8 shows the three-domain 'model' of life. Recent research has suggested that a 'two-domain' model may better reflect the evolution of life on Earth. This is illustrated in this journal paper: **www.nature.com/articles/nmicrobiol2016116**

Find out about the two-domain tree of life. How does it relate to what you know about the origin of the first cells, for example the endosymbiont theory, page XX, Chapter A2.2?

This 2017 article: *Looking for LUCA, the Last Universal Common Ancestor* is a good starting point: **https://astrobiology.nasa.gov/news/looking-for-luca-the-last-universal-common-ancestor**

TOK

What is the role of imagination and intuition in the creation of hypotheses in the natural sciences?

Science is creative in a similar way to art, music or literature. Scientists must use their imagination to formulate a hypothesis – that is, a testable scientific explanation.

Although imagination, faith and intuition (guiding a scientist in one particular direction) may be used in developing hypotheses and theories about the origin of cells, the validity of scientific arguments must eventually be tested by experimentation or, if that is not possible, simulation.

Biologists may often disagree about the choice of methodology and the value and importance of specific data, or about the appropriateness of particular assumptions and simplifications that are made – and therefore disagree about what conclusions are justified. However, they tend to agree about the principles of logical reasoning that connect evidence (data) and assumptions with conclusions.

What differentiates the natural sciences from other knowledge is subjecting hypotheses to empirical testing by observing whether predictions derived from a hypothesis are confirmed from relevant observations and, if possible, experiments. According to Karl Popper, a hypothesis is scientific if there is possibility of falsification.

The central hypothesis regarding the origin of the cell is that organic molecules self-assembled within a vesicle to form the first protocell (Figure A2.1.9) approximately 4 billion years ago.

Biologists have focused on four critical processes: the formation of organic molecules such as amino acids and nucleic acids (especially RNA), the polymerization of these molecules, the formation of membranes, and the development of metabolic pathways for energy transfer.

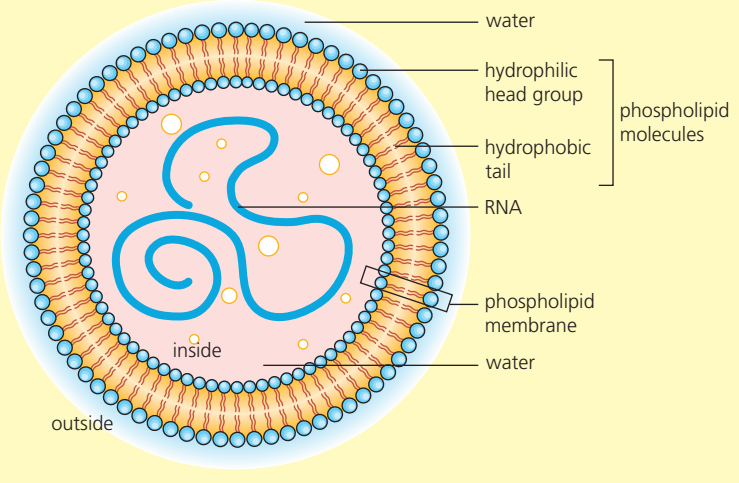


Figure A2.1.9 A possible protocell consisting of self-replicating RNA and proteins within a lipid vesicle

Competing theories exist for how each of these processes evolved, and in what sequence. Although there is a scientific consensus that organic molecules came first via chemical evolution, views differ between scientists over whether metabolism, polymerized molecules (nucleic acids and proteins) or membranes then evolved.

For example, the 'iron–sulfur world' model was initially proposed as a hypothesis without experimental testing. The hypothesis postulated that the first steps in polymerization and protocell formation would take place in a hot and high-pressure (several kilometres under the sea) iron–sulfur hydrothermal environment under the sea.

The hypothesis gained support after the discovery of sub-ocean hydrothermal vents, but experimental designs to test it have been hampered by the challenges of simulating high pressures and temperatures in the laboratory.

We still do not know how life originated on the Earth, and we will possibly never know, since scientists are studying a historical problem for which critical evidence and data may have completely disappeared. The lack of sources forces scientists to imagine and then reconstruct and test the events that could have happened.

Estimating the dates of the first living cells and LUCA

Life has been evolving on Earth over an immense length of time. LUCA may have existed some 4 billion years ago, some 560 million years after the creation of the Earth. But how have scientists determined the age of the first life?

We learn something about the history of life from the evidence found in fossils. Fossilization is an extremely rare, chance event. Predators, scavengers and bacterial action normally break down dead plant and animal structures before they can be fossilized. Of the relatively few fossils formed, most remain buried or, if they do become exposed, are overlooked or accidentally destroyed. Bacteria are known to have been fossilized in rock.

Nevertheless, numerous fossils have been found and more continue to be discovered all the time. If the fossil, or the rock that surrounds it, can be accurately dated (using radiometric dating techniques), we have good evidence of the history of life. Radiometric dating measures the amounts of naturally occurring radioactive substances such carbon-14 (in relation to the amount of carbon-12), or the ratio of potassium-40 to argon-40.

Older rocks will contain more ancient groups of organisms. It can be expected that the oldest rocks on the planet (providing they allowed for fossilization to take place and biological remains were not destroyed by geological forces, for example in metamorphic rock where great pressure and heat create extreme conditions where fossils cannot survive) will contain evidence of the oldest life on Earth.

Genomic analysis also offers techniques to establish the age of ancient organisms. Changes occur in DNA over time. These gene mutations provide the key to estimating dates of the first living cells and the last universal common ancestor. By estimating the average time for mutations to take place, and then extrapolating this back through time, the dates when organisms shared a common ancestor can be estimated. Similarly, the amino acid composition of proteins can be used in a similar way, because change to the genetic code leads to alteration of protein composition and structure.

Biochemical changes, like those discussed above, may occur at a constant rate and, if so, may be used as a 'molecular clock'. If the rate of change can be reliably estimated, it does record the time that has passed between the separation of evolutionary lines. By examining the changes in specific genes common to first life and species alive today, the molecular clock can be used to estimate when these genes converged in a common ancestor, and the rate of biochemical change used to estimate the time over which these changes would have occurred, giving a date for the earliest life on Earth.

Top tip!

Fossils in a rock layer (stratum) that has been accurately dated give us clues to the community of organisms living at a particular time in the past (although this is an incomplete picture). The fossil record may also suggest the sequence in which groups of species evolved and the timing of the appearance of the major groups.

Link

The molecular clock is discussed further in Chapter A3.2, page X.

Evidence for the evolution of LUCA

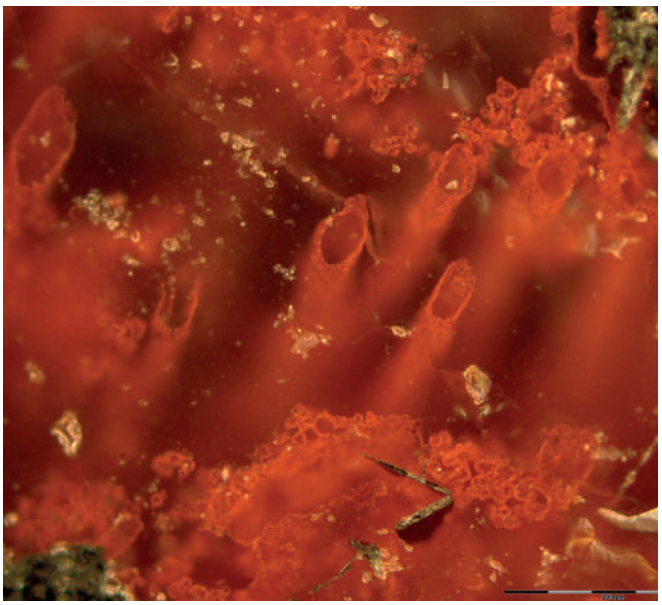
Given that LUCA probably evolved in deep-sea hydrothermal vents, rocks formed by ancient seafloor hydrothermal vent precipitates are the most likely to contain fossils of first life.

Scientists have found fossilized evidence of bacteria from ancient seafloor hydrothermal vent precipitates from the Nuvvuagittuq Greenstone Belt in Quebec, Canada. Rock was cut into hundredth-of-a-millimetre slices and examined under a light microscope. These sedimentary rocks contained structures similar to those produced by modern bacteria found at hydrothermal vents. The fossils have been dated to at least 3.77 billion years old, but could be up to 4.28 billion years old, making them the oldest fossil remains to be found to date, indicating that these are some of the first cells to have existed on the Earth.

◆ **Chemosynthesis**: the synthesis of glucose by bacteria using energy derived from reactions involving inorganic chemicals, such as hydrogen gas, hydrogen sulfide (H_2S), ammonia (NH_3) or iron(II) and iron(III) ions.

◆ **Extremophile**: an organism that lives in conditions of extreme temperature, acidity, alkalinity, salinity, pressure or chemical concentration.

The fossil structures are very small, around half the width of human hair. They are tubes made of haematite, the mineral form of iron(III) oxide (Figure A2.1.10). They are like filamentous microbes from modern hydrothermal vent precipitates. The Nuvvuagittuq rocks also contain carbonate and carbonaceous material, which provides supporting evidence of oxidation and biological activity. Iron-oxidizing microbial communities, which take iron atoms out of the water and remove electrons from it for energy transfer within metabolism, can currently be found associated with widespread hydrothermal vents at the ocean floor. It is likely that the bacteria found in the Nuvvuagittuq rock formation had a similar biochemistry. While different to the metabolism indicated for LUCA, the environment found at deep-sea hydrothermal vents provides many opportunities for diverse forms of energy generation using **chemosynthetic** pathways.



■ **Figure A2.1.10** Haematite tubes found in Nuvvuagittuq – the remains of ancient bacteria, at least 3.77 billion years old

LUCA would have contained conserved genes that are present in all cells. Genetic sequences for conserved genes typically involve proteins associated with ribosomes, where the genetic code is translated into proteins. Because protein synthesis is the most energy-intensive activity of a cell (around 75% of a cell's ATP is used for protein synthesis) these conserved genes tell us that LUCA released and used energy. These universal conserved sequences from genomic analysis do not tell scientists the metabolic nature of this first ancestor, and so other techniques are needed.

The 355 protein families determined from the genetic analysis discussed above (page XX) are not distributed throughout all organisms, and so can be used to suggest the likely physiology of a possible LUCA. For example, LUCA contained a gene for making a protein called 'reverse gyrase' (an enzyme that helps maintain DNA's structure and stability), which is found today in **extremophiles** existing in high-temperature environments including hydrothermal vents.

The properties and functions of these proteins indicate that LUCA had the following characteristics:

- anaerobic (survived without oxygen)
- CO_2-fixing (converted carbon dioxide into glucose)
- H_2-dependent (used molecular hydrogen as an energy source, rather than sunlight)
- N_2-fixing (converted nitrogen into ammonia, for subsequent synthesis of amino acids)
- thermophilic (survived in areas of very high temperature – up to 122 °C).

The analysis indicated that modern-day microbes with similar physiologies include *Clostridia* (an anaerobic bacteria found in soil and the intestines of humans and other animals) and methanogens (anaerobic bacteria that produce methane as a waste product).

◆ **Autotrophic**: synthesizing glucose from simple inorganic substances using an external source of energy.

The genes identified by scientists as being passed down from LUCA were those of an **autotrophic** (i.e. it can synthesize glucose) extremophile organism that probably lived in hydrothermal vents: areas where seawater and magma meet on the ocean floor (Figure A2.1.11). LUCA inhabited a geochemically active environment rich in hydrogen, carbon dioxide and iron. As discussed above, similar prokaryotic organisms still live in these environments, among the toxic plumes of sulfides and metals. Given the genetic analysis and recent fossil evidence, many researchers believe this is where life first began.

6 **Explain** why LUCA (the Last Universal Common Ancestor) is thought to be the evolutionary link between the abiotic phase of Earth's history and the biotic phase.

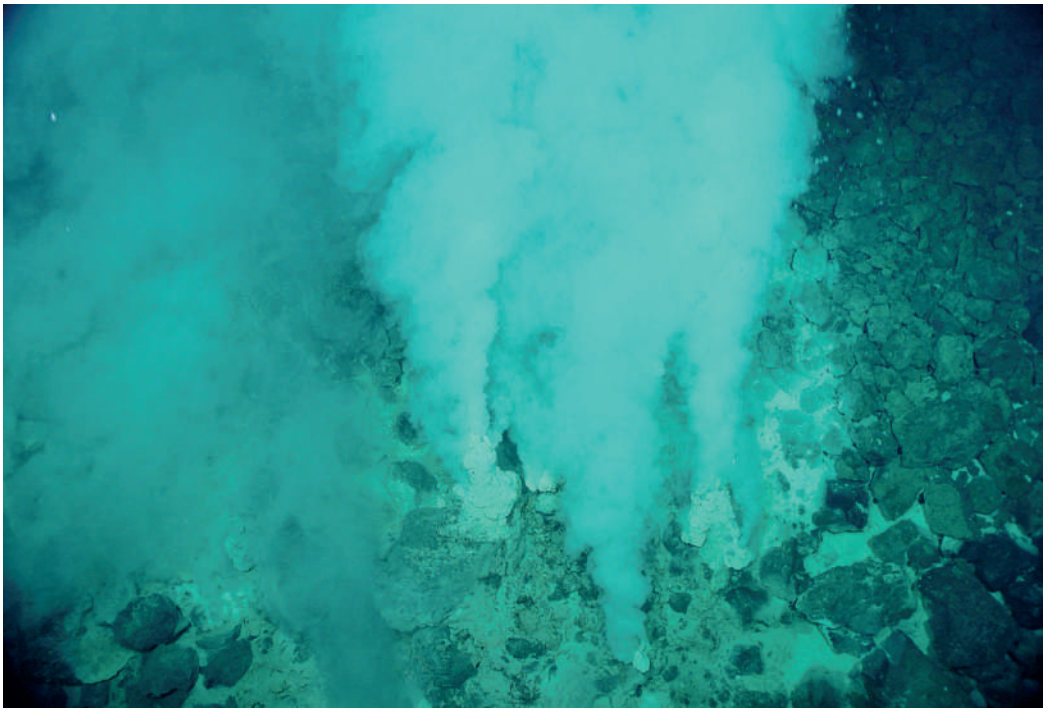


Figure A2.1.11 A hydrothermal vent

ATL A2.1D

Tardigrades are an example of an extremophile organism that can survive in the vacuum of space. Research this organism and find out about the range of conditions it can survive in. What physiological adaptations does it have to survive in such extreme environments? Start your reading here: **https://serc.carleton.edu/microbelife/topics/tardigrade/index.html**

LINKING QUESTIONS

1 For what reasons is heredity an essential feature of living things?
2 What is needed for structures to be able to evolve by natural selection?

A2.2 Cell structure

Guiding questions

- What are the features common to all cells and the features that differ?
- How is microscopy used to investigate cell structure?

SYLLABUS CONTENT

This chapter covers the following syllabus content:

- A2.2.1 Cells as the basic structural unit of all living organisms
- A2.2.2 Microscopy skills
- A2.2.3 Developments in microscopy
- A2.2.4 Structures common to cells in all living organisms
- A2.2.5 Prokaryote cell structure
- A2.2.6 Eukaryote cell structure
- A2.2.7 Processes of life in unicellular organisms
- A2.2.8 Differences in eukaryotic cell structure between animals, fungi and plants
- A2.2.9 Atypical cell structure in eukaryotes
- A2.2.10 Cell types and cell structures viewed in light and electron micrographs
- A2.2.11 Drawing and annotation based on electron micrographs
- A2.2.12 Origin of eukaryotic cells by endosymbiosis (HL only)
- A2.2.13 Cell differentiation as the process for developing specialized tissues in multicellular organisms (HL only)
- A2.2.14 Evolution of multicellularity (HL only)

Introduction to cells

Common mistake

Students sometimes use the terms 'cell' and 'tissue' as if they are synonymous (i.e. are the same thing) – this is not the case. Cells are the basic structural unit of all living organisms, whereas tissues are a collection of cells of similar structure and function.

The cell is the basic structural unit of all living organisms – it is the smallest part of an organism that we can say is alive. It is cells that carry out the essential processes of life. We think of them as self-contained units of structure and function.

Cells are extremely small – most are only visible as distinct structures when we use a microscope (although a few types of cell are just large enough to be seen by the naked eye).

Observations of cells were first reported over 300 years ago, following the early development of microscopes. You may have already used a light microscope to view living cells, such as the single-celled organism *Amoeba*, shown in Figure A2.2.1.

Concept: Unity

All living organisms are made from cells. Some organisms are made of single cells (such as protists and bacteria) and others are multicellular (animals, plants and most fungi). In multicellular organisms, cells are the building blocks for tissues.

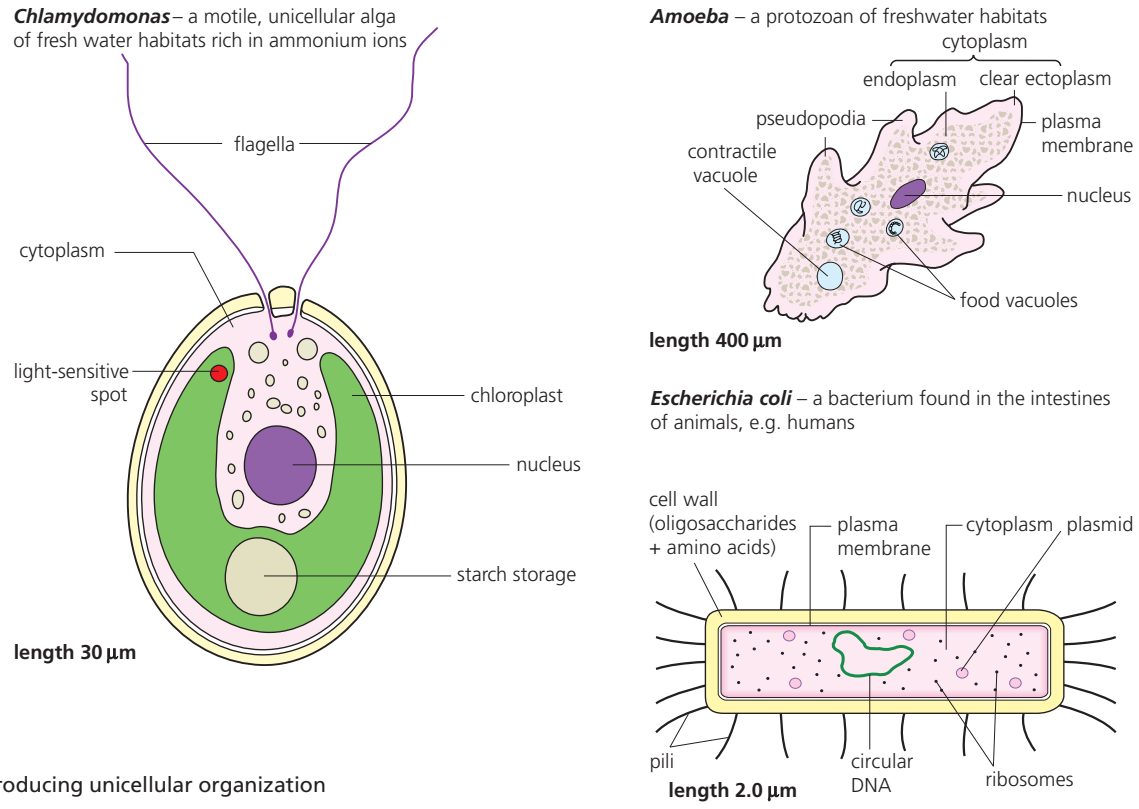


Figure A2.2.1 Introducing unicellular organization

- **Unicellular**: consisting of a single cell (e.g. prokaryotes, protists and some fungi).
- **Protists**: eukaryotes consisting of single-celled organisms.
- **Multicellular**: consisting of many cells (e.g. animals, plants and most fungi).

Unicellular and multicellular organisms

Some organisms are made of a single cell and are known as **unicellular**. Examples of unicellular organisms are introduced in Figure A2.2.1. There are vast numbers of different unicellular organisms in the living world, many with very long evolutionary histories. One type of unicellular organism is in a kingdom called the Protoctista (such as *Chlamydomonas* and *Amoeba* in Figure A2.2.1 – organisms in this kingdom are referred to as **protists**) and another are the bacteria (*Escherichia coli*, Figure A2.2.1).

Other organisms are made of many cells and are known as **multicellular** organisms. Examples of multicellular organisms are mammals and flowering plants.

Top tip!

Much of the biology in this book is about multicellular organisms and the processes that go on in these organisms. But remember, single-celled organisms carry out all the essential functions of life too, within a single cell.

Cell theory

All organisms are composed of one or more cells. **Cell theory** includes the idea that cells are the unit of structure and function in living organisms. The cell theory states that:

- cells can only arise from pre-existing cells
- living organisms are composed of cells, which are the smallest unit of life
- organisms consisting of only one cell carry out all functions of life in that cell; cells perform life functions at some point in their existence.

Although most organisms conform to cell theory, there are exceptions (see page XX).

Many biologists contributed to the development of the cell theory. This concept evolved gradually in western Europe during the nineteenth century because of the steadily accelerating pace of developments in microscopy and biochemistry.

Table A2.2.1 Units of length used in microscopy

1 metre (m) = 1000 millimetres (mm)
1 mm (10^{-3} m) = 1000 micrometres (μm) (or microns)
1 μm (10^{-6} m) = 1000 nanometres (nm)

Cell size

Since cells are so small, we need appropriate units to measure them. The **metre** (symbol **m**) is the standard unit of length used in science (it is an internationally agreed unit, or **SI unit**).

Look at Table A2.2.1, showing the subdivisions of the metre that are used to measure cells and their contents.

These units are listed in descending order of size. You will see that each subdivision is one thousandth of the unit above it. The smallest units are probably quite new to you; they may take some getting used to.

So, the dimensions of cells are expressed in the unit called a **micrometre** or micron (**μm**). Notice, this unit is one thousandth (10^{-3}) of a millimetre. This gives us a clear idea about how small cells are when compared to the millimetre, which you can see on a standard ruler.

Bacteria are really small, typically 0.1–2 μm in size, whereas the cells of plants and animals are often in the range of 50–150 μm or larger. In fact, the lengths of the unicellular organisms shown in Figure A2.2.1 are approximately:

Escherichia coli 2 μm

Chlamydomonas 30 μm

Amoeba 400 μm (but its shape and, therefore, length varies greatly).

Cell size determines the rate of diffusion of substances across the plasma membrane; by being small, this rate is maximized (see Chapter B2.3, page XX).

Table A2.2.2 shows the average sizes of cells and their components in decreasing size. The organelles and other structures that are found in cells are discussed in detail later in this chapter (pages XX–XX).

Table A2.2.2 The size of cells and their components

Cell and component	Diameter	Cell component	Diameter
plant cell	40 μm (average)	lysosome	0.2–0.5 μm
animal cell	20 μm (average)	centriole	0.15 μm
nucleus	10–20 μm	microtubule	24 nm
chloroplast	5–10 μm	ribosome	20 nm
bacterium	1 μm	microfilament	7 nm
mitochondrion	0.5–1.5 μm	DNA molecule	2 nm

1 **Calculate** how many cells of 100 μm diameter will fit side by side along a millimetre.

Nature of science: Theories

Collecting and analysing biological observations can lead to important conclusions based on **inductive reasoning**. Induction involves formulating generalisations from many related specific observations (see page XX).

The claim of cell theory that 'all organisms will consist of one or more cells' is derived from inductive reasoning. This was based on microscopic observations by many biologists in a wide range of organisms. In inductive reasoning, biologists begin with specific observations and measurements and then detect patterns or common features. They may then formulate a hypothesis that can be tested, and finally develop a theory.

A related process known as **deductive reasoning** is used to test the theories, such as cell theory, produced by induction. Deductive reasoning proceeds from the more general to the more specific. This ultimately leads biologists to test the hypotheses with specific data that either support or falsify the theory.

The philosopher Karl Popper rejected the use of inductive reasoning in science, claiming that for induction to be true, every example of its inference must be true. Biologist have found that there are a small number of cells and organisms that are exceptions to the cell theory (page XX). However, cell theory remains an important and unifying concept in biology, necessary for inductive science.

Induction and deduction are important types of logical reasoning and both contribute to the construction of scientific knowledge (Figure A2.2.2). Inductive reasoning is a form of logic directly opposite to that of deductive reasoning. Inductive reasoning is covered in Chapter A1.2, page XX.

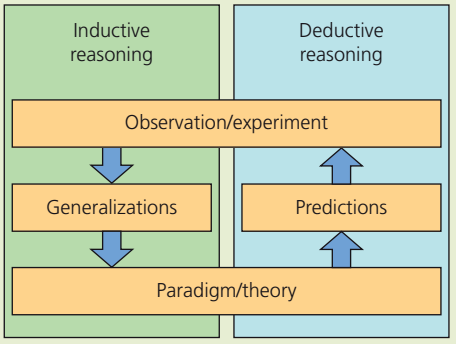


Figure A2.2.2 Inductive and deductive reasoning in the scientific method

Top tip!

You should have experience of making temporary mounts of cells and tissues, staining, measuring sizes using an eyepiece graticule, focusing with coarse and fine adjustments, calculating actual size and magnification, producing a scale bar and taking photographs. Adaptors are available that allow cameras or smartphones to be attached to the eyepiece lens to enable photos to be taken. See more on this in the Tools section on page XX.

Microscopy skills

Examining cells and recording structure and size

We use microscopes to magnify the cells of biological specimens in order to view them. Figure A2.2.3 shows two types of light microscope.

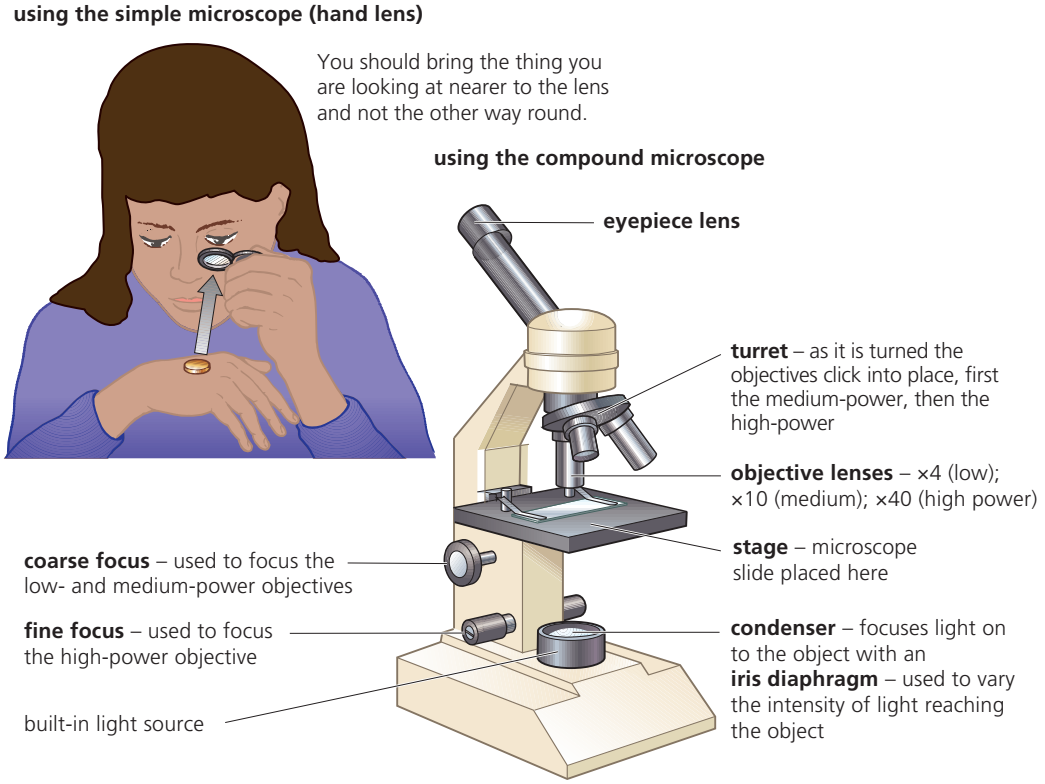


Figure A2.2.3 Light microscopy

In the simple microscope (**hand lens**), the instrument can be held very close to the eye and is today mostly used to observe external structure. Some of the earliest hand lenses detailed observations of living cells.

Light (compound) microscopes have been instrumental in understanding cell structure and function, and revealing organisms that are not visible with the naked eye. The **objective lens** forms an image (in the microscope tube) that is then further magnified by the **eyepiece lens**, producing a greatly enlarged image. Initially, coarse focus enables you to locate and see the specimen under low power. Fine focus can be used to resolve the image under higher powers.

Tool 1: Experimental techniques

The light microscope

1. Select a low-power lens. Make sure the lens clicks into position.
2. Examine the prepared slide without the microscope and note the position, colour and rough size of specimen.
3. Place the slide on the stage, coverslip uppermost, viewing it from the side. Position it with stage adjustment controls so that the specimen is lit up.
4. Focus using first the coarse and then the fine focusing controls. Use **both hands** to alter the focusing controls; this helps keep the controls working properly and to not go out of alignment.
   Note: The image will be reversed and upside down when seen by viewing the slide directly. When focusing, always move the stage down, away from the objective lens, to avoid moving the slide on to the objective lens (which could damage the lens and break the slide).
5. For higher magnifications, swing in the relevant objective lens carefully, checking there is space for it. Adjust the focus using the fine control only. If the object is in the centre of the field of view with the ×10 objective, it should remain in view with the ×40 objective.
6. When you have finished using the microscope:
   - turn the objective lens back to ×10 and then lower the stage
   - remove the last slide and return it to the correct section in the tray
   - clean the stage if necessary and check eyepiece lenses and objective lenses are clean
   - unplug the cable and store tidily, replacing the dust cover.

Common mistake

When using a compound light microscope:

- Never force any of the controls.
- Never touch any of the glass surfaces with anything other than a clean, dry lens tissue.
- Do not hold the microscope with one hand. When moving the microscope, hold the stand above the stage with one hand and rest the base of the stand on your other hand.
- Do not tilt the microscope. Always keep the microscope vertical (or the eyepiece may fall out).
- Do not touch the surface of lenses with your fingers.
- Do not allow any solvent to touch a lens.
- When focusing, move the stage down, away from the objective lens, to avoid moving the slide on to the objective lens.

Biological material to be examined by compound microscopy must be sufficiently transparent for light rays to pass through. When bulky tissues and parts of organs are to be examined, thin sections are cut. Thin sections are largely colourless.

Table A2.2.3 The skills of light microscopy

You need to master and be able to demonstrate these aspects of good practice
Knowledge of the parts of your microscope and care of the instrument – its light source, lenses and focusing mechanisms.
Use in low-power magnification first, using prepared slides and temporary mounts.
Switching to high-power magnification, maintaining focus and examining different parts of the image.
Types of microscope slides and the preparation of temporary mounts, both stained and unstained.

Tool 1: Experimental techniques

Preparation of a temporary mount

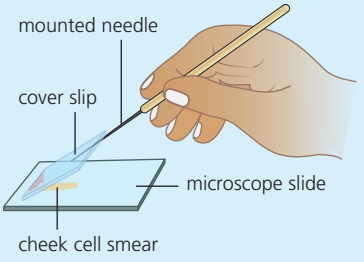
Cells can be mounted on slides so they can be viewed under a microscope. These can be disposed of once the cells have been seen and studied. Stains can be used to view cell features more clearly. Techniques describing how temporary mounts can be made are outlined here.

Living cells are not only very small but also transparent. In light microscopy it is common practice to add dyes or stains to introduce sufficient contrast and so differentiate structure. Dyes and stains that are taken up by cells are especially useful.

Observing the nucleus, cytoplasm and cell membrane in human cheek cells

Take a smear from the inside lining of your cheek using a fresh, unused cotton bud you remove from the pack. Touch the materials removed by the cotton bud on to the centre of a microscope slide and add a cover slip (see Figure A2.2.4). Dispose of the cotton bud safely and hygienically. Handle the microscope slide yourself and, at the end of the observation, immerse the slide in 1% sodium hypochlorite solution to sterilize the slide and cover slip. To observe the structure of human cheek cells, irrigate the slide with a drop of methylene blue stain (following the procedure shown in Figure A2.2.4) and examine some of the individual cells with medium- and high-power magnification.

Making a temporary mount



Irrigating a temporary mount

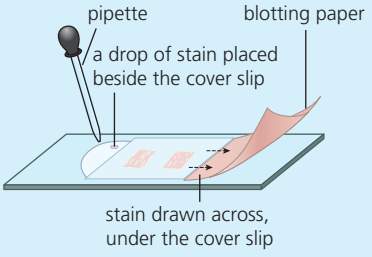


Figure A2.2.4 Preparing living cells for light microscopy

You could also observe chloroplasts in moss leaf cells, or the nucleus, cell wall and vacuole in an onion epidermis cell.

TOK

Living tissues prepared for examination under the microscope are typically cut into thin sections and stained. Both processes may alter the appearance of cells. Is our knowledge acquired with the aid of technology fundamentally different from that which we acquire from our unaided sense? If so, what may be done about this, in practical terms?

Tool 1: Experimental techniques

The eyepiece graticule

The size of a cell can be measured under the microscope. A transparent scale, called a **graticule**, is mounted in the eyepiece at the focal plane (there is a ledge for it to rest on). In this position, when the object under observation is in focus, so too is the scale. The size (for example, length or diameter) of the object may then be recorded in arbitrary units. Next, the graticule scale is calibrated using a **stage micrometer** – in effect, a tiny, transparent ruler, which is placed on the microscope stage in place of the slide and then observed. With the eyepiece and stage micrometer scales superimposed, the true dimensions of the object can be estimated in micrometres. Figure A2.2.5 shows how this is done.

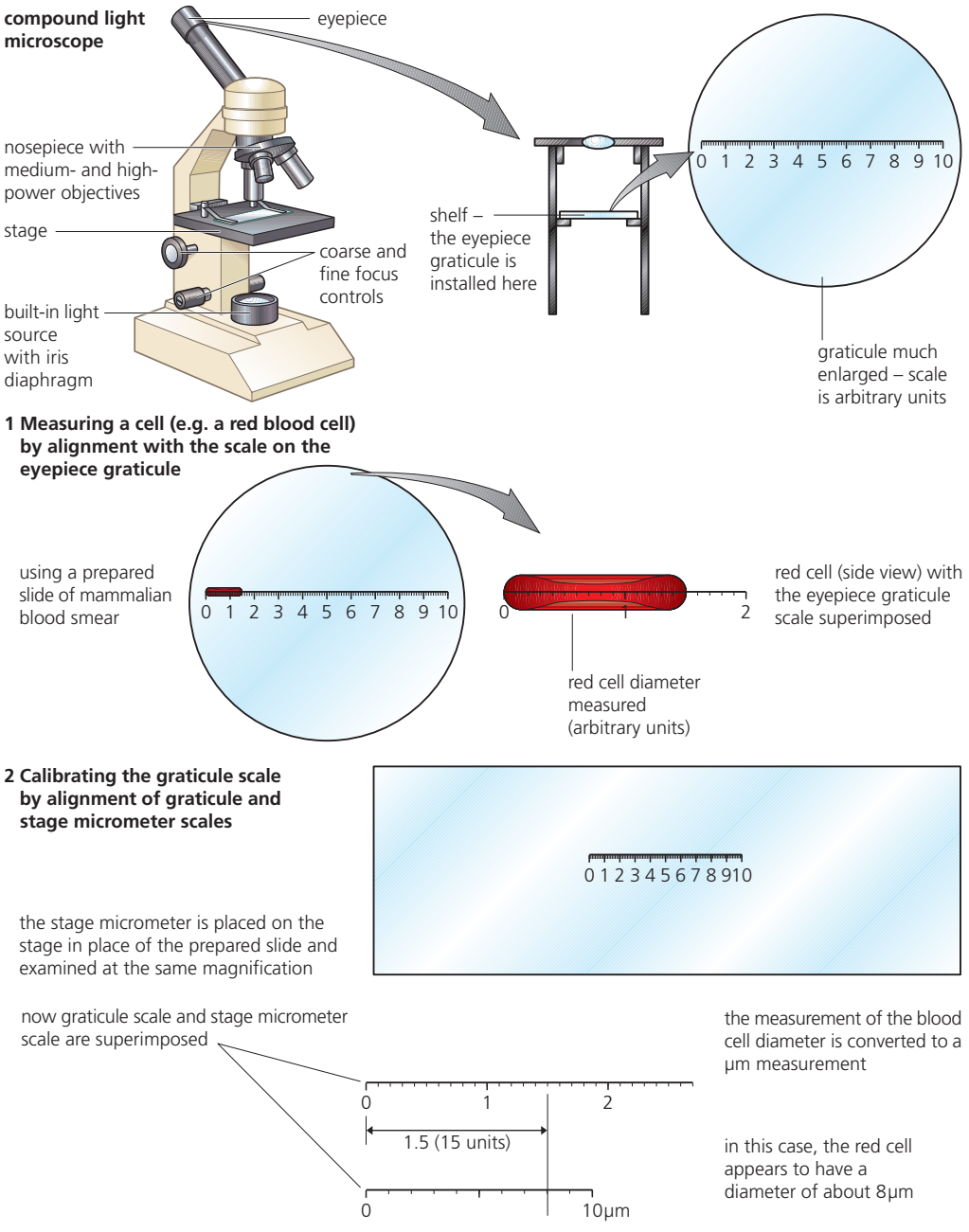
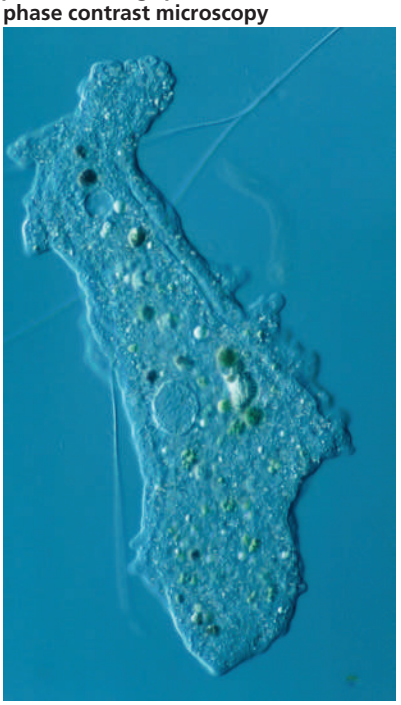


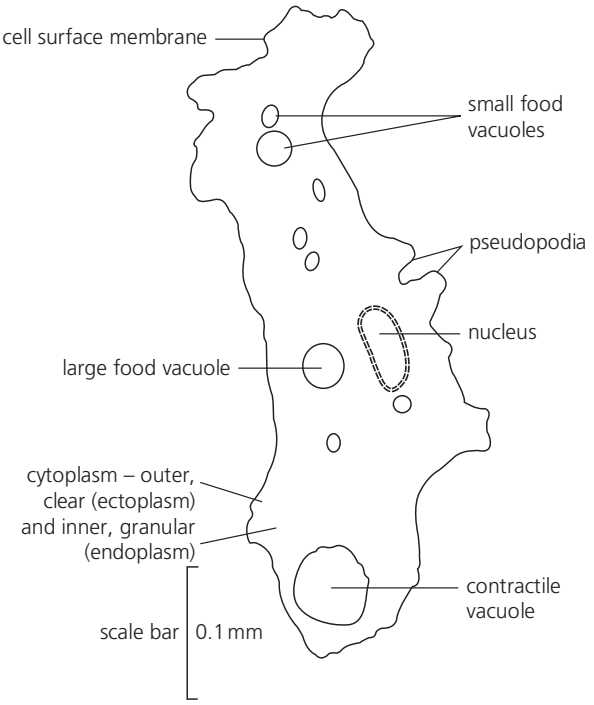
Figure A2.2.5 Measuring the size of cells

Once the size of a cell has been measured, a scale bar line may be added to a micrograph or drawing to record the actual size of the structure, as illustrated in Figure A2.2.6.

photomicrograph of *Amoeba proteus* (living specimen) – phase contrast microscopy



interpretive drawing



■ **Figure A2.2.6** Recording size by means of scale bars

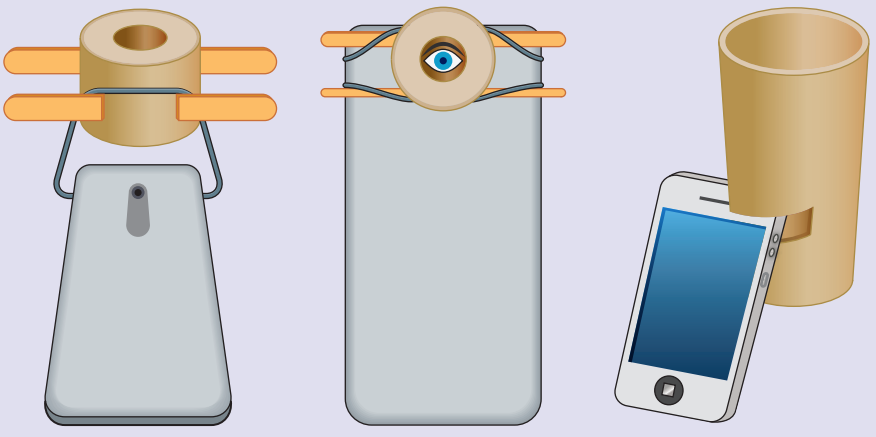
ATL A2.2A

Converting smartphones into 'CellCams'

Photographs of microscope images (photomicrographs) have traditionally been taken using expensive camera-mounted apparatus. However, smartphones can take high-quality images without the need for such specialized apparatus. It is difficult to take photographs using hand-held smartphones, because slight variations in the angle of the phone make the image invisible or partially obscured. Simple methods can be used to attach the smartphone to the eyepiece of the microscope, enabling images to be taken. Such adaptors enable you to create your own 'CellCam'.

Take a toilet-paper cardboard tube (4.5 cm diameter and 4 cm in length) and add an adhesive foam weather-strip (1–2 cm wide) to the inside so that the cardboard tube fits securely around the eyepiece of the microscope. For a mid-mounted smartphone camera, attach the tube to the camera using ice-cream sticks secured either side of the tube using elastic bands (Figure A2.2.7a). For a corner-mounted camera, cut a slit in one edge of the tube and slot the camera inside (Figure A2.2.7b). Put the cardboard tube down over the eyepiece of the microscope. The CellCam can then be used to capture images or video of specimens.

Try making a CellCam using your smartphone and the methodology above and taking photos of specimens under the microscope. Remember to be careful when putting the cardboard tube over the eyepiece of the microscope.



a Mid-mounted camera **b** Corner-mounted camera

■ **Figure A2.2.7** Converting a smartphone into a 'CellCam': a) mid-mounted camera b) corner-mounted camera

Magnification of an image

◆ **Magnification**: how many more times larger an object appears.

Magnification is the number of times larger an image is than the specimen. The magnification obtained with a compound microscope depends on which of the lenses you use. For example, using a ×10 eyepiece and a ×10 objective lens (medium power), the image is magnified ×100 (10 × 10). When you switch to the ×40 objective (high power) with the same eyepiece lens, then the magnification becomes ×400 (10 × 40). These are the most usual orders of magnification you will use in your laboratory work.

2 **Calculate** what magnification occurs with a ×6 eyepiece and a ×10 objective.

There is actually **no limit to magnification**. For example, if a magnified image is photographed, then further enlargement can be made photographically. This is what may happen with photomicrographs shown in books and articles.

When an image from a light microscope is magnified photographically **the detail will be no greater**.

Tool 3: Mathematics

Calculating scales of magnification

Make a temporary mount of a plant cell using the techniques outlined on page XX. Pondweed is a good plant to use as the leaves are thin and easily removed from the plant. Draw one plant cell using the method shown on page XX. Use an eyepiece graticule to estimate the size (either the length or width) of a plant cell using the technique in Figure A2.2.5. Now calculate the magnification of the image you have drawn.

Magnification is given by the formula:

$$\text{magnification} = \frac{\text{size of image}}{\text{size of specimen}}$$

So, for a particular plant cell of 150 µm diameter, photographed with a microscope and then enlarged in a drawing or photographically, the magnification in a print showing the cell at 15 cm diameter (150 000 µm) is:

$$\frac{150\,000}{150} = \times 1000$$

If a further enlargement is made to show the same cell at 30 cm diameter (300 000 µm), then the magnification is

$$\frac{300\,000}{150} = \times 2000$$

3 Using the scale bar given in Figure A2.2.6, **calculate** the maximum observed length of the *Amoeba* cell.

4 **Calculate** the magnification of the image of *Escherichia coli* in Figure A2.2.1 on page XX.

Scale bars

To add a scale bar to your drawing of a biological specimen:

1. Use the stage micrometer and eyepiece graticule to work out the distance between two markings on the eyepiece graticule, i.e. the number of micrometres equivalent to one unit (or division) on the eyepiece graticule (Figure A2.2.5).
2. Remove the stage micrometer and place the specimen on the stage.
3. Measure the length of the specimen using the eyepiece graticule. The measurement will be in graticule units (which will depend on the magnification you are using).
4. Determine the length of the specimen in micrometres by multiplying the number of graticule units by the length represented by one unit. For example, if the length of the specimen is 20 graticule units, and the length of each unit represents 10 µm, the total length of the specimen will be 20 × 10 = 200 µm.

Top tip!

Scale bars can be used as a way of indicating the actual sizes in drawings and micrographs, and can be used to calculate magnification. Magnification is calculated by dividing the actual length of the scale bar by the length indicated on the scale bar.

Ideally your scale bar needs to be around 20% of the length of the specimen. If the specimen is 200 µm then the scale bar would be 20% of 200 = 40 µm. Now draw the specimen on a piece of paper or in your lab notebook and measure the length of your drawing. If the length of the drawing is 100 mm, then the scale bar needs to be drawn as 20% of this length = 20 mm. Draw a line next to your drawing 20 mm in length and mark on this the actual length it represents (40 µm).

The actual length represented by the scale bar should be a whole number, and so the measurement taken from the specimen may need to be rounded. For example, if the specimen is 52.5 µm, 20% of this number is 10.5 µm. This can be rounded down to give 10 µm – ratios can then be used to establish the length of the scale bar. 10/52.5 gives one ratio (the length of the scale bar to the actual length of the organism). If the length of the drawing is 96 mm, the second ratio is $x/96$ where x is the length of the scale bar. When the ratios are resolved:

$$10/52.5 = x/96$$

$$x = (10 \times 96)/52.5 = 18.3 \text{ mm}$$

The scale bar is then drawn to the length 18.3 mm, and the actual length that this represents recorded as 10 µm.

Top tip!

The size of cells, or components of cells, can be calculated given the amount of magnification and a scale drawing of the object. Simple equations can be used to calculate the magnification or actual size of the specimen.

- **I** = size of image (drawing of an object on paper)
- **A** = actual size of the object being measured
- **M** = magnification (the size of an object compared to its actual size, i.e. the number of times larger an image is than the specimen)

So, M = I/A; A = I/M and I = A × M.

A memory diagram can be used, showing how to calculate the magnification, actual size or image size of an object (Figure A2.2.8).

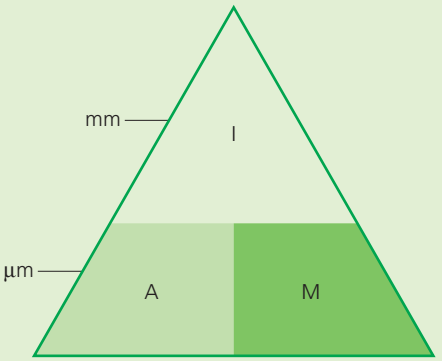


Figure A2.2.8 Memory diagram showing how to calculate the magnification, actual size or image size of an object

Remember the equation as AIM or IAM, and remember to convert units so that they are the same for both I and A.

Top tip!

Remember to convert all values to the same unit of measurement: if you do not do this, your results will be incorrect by a factor of 100, 1000 or even 1 000 000.

- Convert mm into µm by multiplying by 1000.
- Convert µm into mm by dividing by 1000.

5 A highly magnified electron micrograph of the bacterium *Escherichia coli* was accompanied by a scale bar of length 23 mm and labelled 1 µm. The following features were measured. Complete the following table. Express their actual size in appropriate units.

Feature	Measurement on scale bar (mm)	Actual size
thickness of the wall	1	
length of a flagellum	32	
width of the cell	24	
length of the cell	5	

Resolution of an image

◆ **Resolution**: the amount of detail that can be seen.

The **resolution** (resolving power) of a microscope is its ability to separate visually small objects that are very close together. If two separate objects cannot be resolved, they are seen as one object. Merely enlarging them does not separate them. Resolution is a property of lenses that is quite different from their magnification – and is more important.

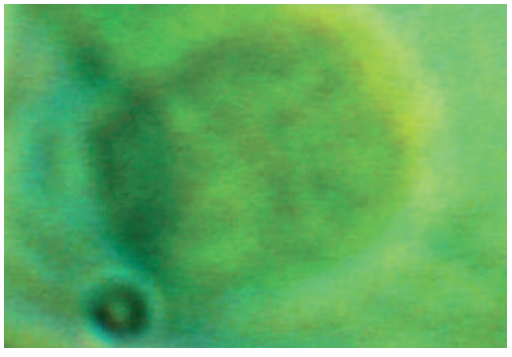
Resolution is determined by the wavelength of light. Light is composed of relatively long wavelengths, whereas shorter wavelengths give better resolution. For the light microscope, the limit of resolution is about 0.2 μm. As a result, two objects less than 0.2 μm apart may be seen as one object, which means that the image is blurred at low resolution. Figure A2.2.9 shows the importance of resolution over magnification. In this figure, an image of a chloroplast at the same magnification is shown at low (left-hand image) and high resolution (right-hand image). The high-resolution image has been taken using an electron microscope, and the low-resolution image with a light microscope. Details of the internal and external membrane structure cannot be seen under low resolution, however much the image is enlarged. This is because the membrane is too thin to be seen and resolved as separate structures using the light microscope.

6 Distinguish between resolution and magnification.

◆ **Quantitative data**: numerical measurements with units (and associated random uncertainty), which are often digitally recorded and processed.

◆ **Qualitative data**: descriptive information used to record the conditions in which data are recorded, or to describe the features or properties of an object.

a chloroplast enlarged (×6000) from a photomicrograph obtained by light microscopy



b chloroplast from a transmission electron micrograph

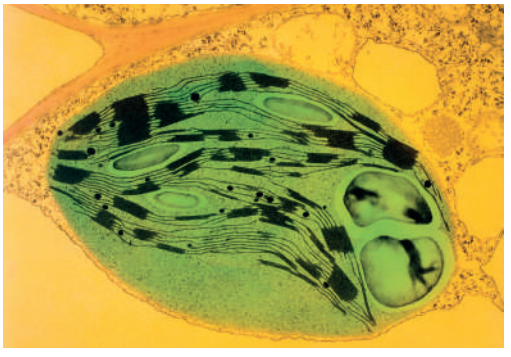


Figure A2.2.9 Magnification a) without and b) with resolution; the micrograph in b) has been colourized

Nature of science: Measurements

Using instruments is a form of quantitative observation

Biology – like all natural sciences – is an observation-based science, perhaps more so than chemistry or physics. Living organisms are very diverse and the first task of any biological investigation is the observation and recording of biological phenomena.

Observations in biology are varied and may include counting, drawing, photographing, video recording (of animal behaviour) or measuring changes in concentration or pH during biochemical reactions. **Quantitative observations** are ones that involve numerical data, for example the recording values for a dependent variable. **Qualitative observations** reinforce quantitative data: they record observations relevant to the study.

Observations and measurements are the basis for the development of new biological hypotheses. In biology, the observations often arise from questions of the form, 'How does variable X affect variable Y?' Knowledge acquired by the senses is known as **empirical data**.

Biologists often 'enhance' their senses by using technology with a variety of analytical instruments. This enables them to study phenomena that are beyond or outside the direct limits of our human senses.

For example, the electron microscope has been used to study the ultrastructure of cells and the technique of NMR (nuclear magnetic resonance spectroscopy) has been used to study the folding and unfolding of proteins. In this technique, molecules are studied by recording the interaction of high-frequency radio waves with the nuclei of molecules placed in a strong magnetic field. The effect of ultrasound has been studied on organisms such as bats, dolphins and porpoises which use it for 'echo location'. Humans are not able to directly perceive electromagnetic radiation or sound waves with these frequencies (energies).

Developments in microscopy

The technology used to view cells and their internal structure has developed through time, allowing greater detail to be discovered, which has revealed increasing knowledge of how cells function.

Electron microscopy – the discovery of cell ultrastructure

Microscopes were invented simultaneously in different parts of the world at a time when information travelled slowly. Modern-day advances in microscopy and communications have allowed for improvements in the ability to investigate and collaborate, enriching scientific endeavour.

◆ **Electron microscope (EM)**: microscope in which a beam of electrons replaces light so the powers of magnification and resolution are correspondingly much greater.

◆ **Ultrastructure**: fine structure of cells, determined by electron microscopy.

The **electron microscope (EM)** uses electrons to make a magnified image in much the same way as the optical microscope uses light. Electrons can show particle and wave properties, and need to travel through a vacuum. This means the biological material must be dead. However, because an electron beam has a much shorter wavelength, its resolving power is much greater. When the electron microscope is used with biological materials, the limit of resolution is about 5 nm. (The size of nanometres is given in Table A2.2.1, page XX.)

Only with the electron microscope can the detailed structures of cell organelles be observed. This is why the electron microscope is used to resolve the fine detail of the contents of cells, the organelles and cell membranes, collectively known as cell **ultrastructure**. It is difficult to exaggerate the importance of electron microscopy in providing our detailed knowledge of cells.

In the electron microscope, the electron beam is generated by an **electron gun**, and focusing is by **electromagnets**, rather than by glass lenses. We cannot see electrons, so the electron beam is focused on to a **fluorescent screen** for viewing, or on to a **photographic plate** for permanent recording (Figure A2.2.10).

In **transmission electron microscopy (TEM)**, the electron beam is passed through an extremely thin section of fixed, biological material. Membranes and other structures are stained with heavy metal ions, making them electron-opaque so they stand out as dark areas in the image.

In **scanning electron microscopy (SEM)**, a narrow electron beam is scanned back and forth across the surface of the specimen. Electrons that are reflected or emitted from this surface are detected and converted into a three-dimensional image (Figure A2.2.11).

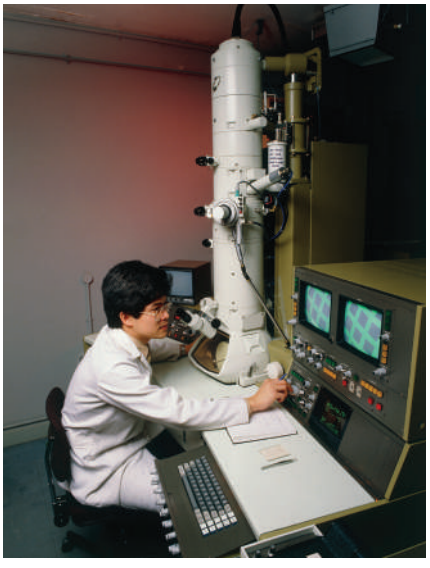


Figure A2.2.10 Using the transmission electron microscope

Electron microscopes have a greater resolving power than light microscopes. Their application to biology has established the presence and structure of all the cell organelles.

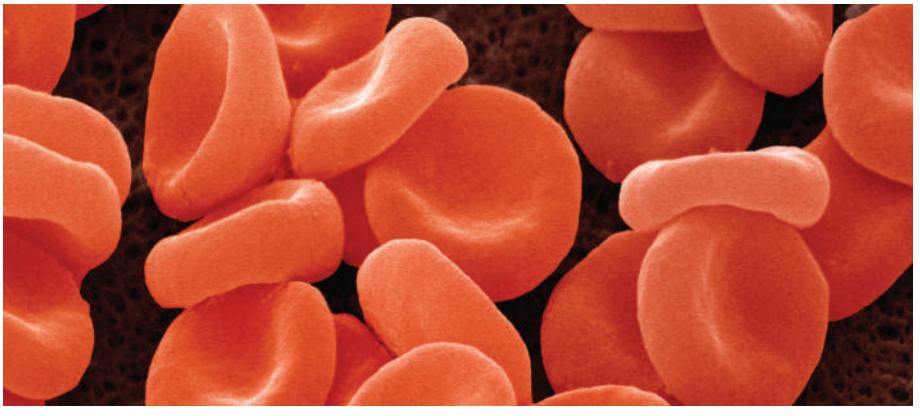


Figure A2.2.11 A scanning electron micrograph of red blood cells (5.7 µm in diameter); the image from an electron microscope is in black and white – this image has been coloured

7 **List** the features of cells that can be observed by electron microscopy that are not visible by light microscopy.

8 **State** two problems that arise in electron microscopy because of the nature of an electron in relation to the living cell.

TOK

The investigation of cell structures by observation of electron micrographs of very thin sections of tissue (after dehydration and staining) raises the issue of whether the structures observed are actually present (or are artefacts). The solution to this problem, described above, is an example of how scientific knowledge may require multiple observations assisted by technology.

The impact of electron microscopy on cell biology: the presence and structure of organelles

The nucleus is the largest substructure (organelle) of a cell and may be observed with a light microscope. However, most organelles cannot be viewed by light microscopy and none are large enough for internal details to be seen. It is by means of the electron microscope that we have learnt about the fine details of cell structure. Because electron microscopes have much greater resolving power than light microscopes, they can reveal structures that are not visible by light microscopy, such as organelles made from membranes.

ATL A2.2B

What do you think are the strengths and limitations of light microscopy compared to electron microscopy? Discuss in a group and draw up a comparison table.

Cryogenic electron microscopy

A more recent development in electron microscopy is a technique that involves flash-freezing solutions of proteins or other biomolecules and then exposing them to electrons to produce very high-resolution images of individual molecules. This technique is called **cryogenic electron microscopy**, because of the cryogenic temperatures used (Figure A2.2.12). The images are used to reconstruct the three-dimensional shape of the molecule, which in turn reveals its function. Cryogenic electron microscopy is used to reveal how proteins work, how they malfunction in disease and how to target them with drugs. The most recent cryogenic electron microscopes are able to locate individual atoms within a protein. In 2017, the Nobel Prize in chemistry was awarded to three scientists (Jacques Dubochet, Joachim Frank and Richard Henderson) for developing cryogenic electron microscopy.

Traditional electron microscopes place the specimen in a chamber that is kept under vacuum. This allows a pure beam of electrons to interact with the sample, without interference from particles that would be present in air. However, some materials, such as biological molecules, are not compatible with the high-vacuum conditions and intense electron beams used in traditional TEMs. Under the conditions of a vacuum, the water that surrounds the molecules evaporates, and the high-energy electrons destroy the molecules. Because cryogenic electron microscopes use frozen samples, less-intense electron beams can be used and the evaporation of water is no longer a problem.

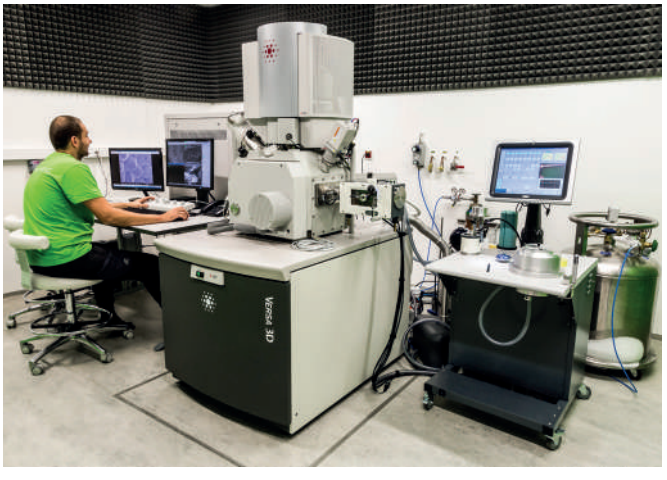


Figure A2.2.12 Cryogenic electron microscope

Prior to cryogenic electron microscopy, scientists relied on a technique called X-ray crystallography to view biomolecules, which involves crystallizing molecules, exposing them to X-rays and then reconstructing their shape from the patterns of diffracted X-rays. This technique was used to reveal the structure of DNA (see page XX).

Freeze fracture

In an **alternative method of preparation**, biological material is *instantly* frozen solid in liquid nitrogen. At atmospheric pressure this liquid is at −196 °C. At this temperature living materials do not change shape as the water present in them solidifies instantly.

This solidified tissue is then broken up in a vacuum, and the exposed surfaces are allowed to lose some of their ice; the surface is described as 'etched'.

◆ **Freeze etching**: preparation of specimens for electron microscope examination by freezing, fracturing along natural structural lines and preparing a replica.

Finally, a carbon replica (a form of 'mask') of this exposed surface is made and coated with heavy metal, such as gold, to strengthen it. The mask of the surface is then examined in the electron microscope. The resulting electron micrograph is described as being produced by **freeze etching**.

A comparison of a cell nucleus observed by both transmission electron microscopy and by freeze etching is shown in Figure A2.2.13.

Look at these images carefully.

The picture we get of nucleus structure is consistent; therefore, we can be confident that our views of cell structure obtained by electron microscopy are realistic.

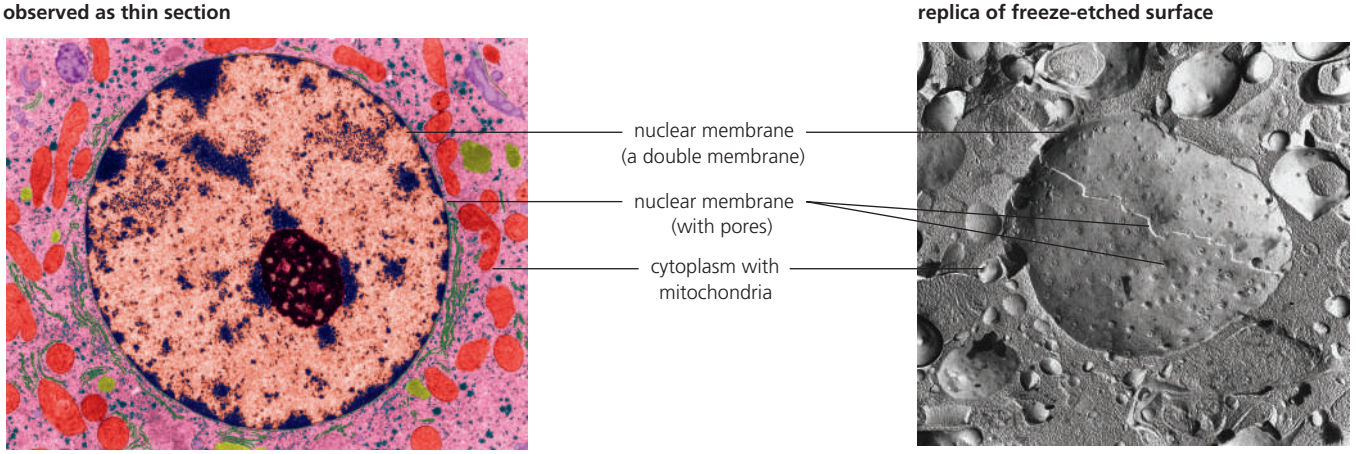


Figure A2.2.13 Electron micrographs from thin-sectioned and freeze-etched material showing the nucleus of a liver cell

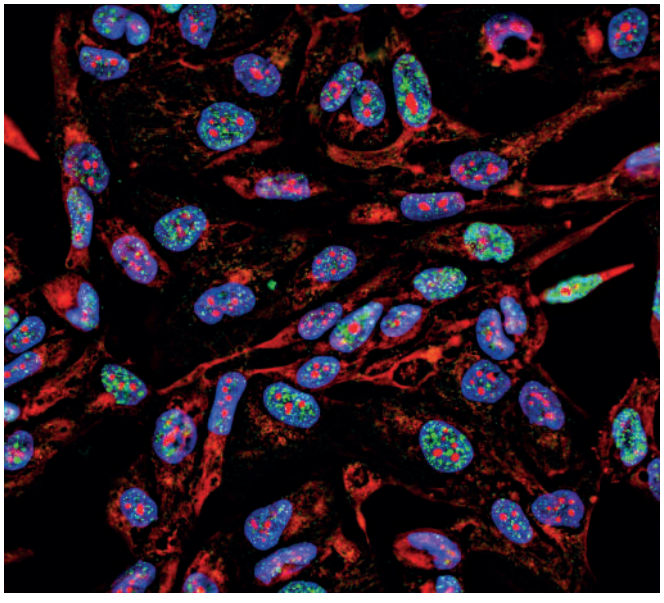


Figure A2.2.14 Fluorescent imaging: immunofluorescence of cancer cells with nuclei in blue, cytoplasm in red and damaged DNA in green

Fluorescence microscopy

Fluorescent dyes absorb light at one wavelength and emit it at another longer wavelength. Some such dyes bind specifically to target molecules in cells, e.g., DNA, and can reveal their cellular location when examined with a fluorescence microscope.

A fluorescence microscope is used to detect cells stained with fluorescent dyes. This is similar to an ordinary light microscope except that the illuminating light is passed through two sets of filters. The first set filters the light before it reaches the specimen, passing only those wavelengths that excite the specifically chosen fluorescent dye. The second filter blocks out this light and passes only those wavelengths emitted when the dye fluoresces. Dyed objects show up in bright colour on a dark background (Figure A2.2.14).

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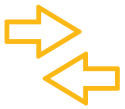

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