

Threshold Concepts in Biochemistry

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Julian Pakay; Hendrika Duivenvoorden; Thomas Shafee; and Kaitlin Clarke

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About the Authors



Dr Julian Pakay is a teaching-focused academic in the Department of Biochemistry and Chemistry at La Trobe University with over 12 years' experience teaching Biochemistry and Molecular Biology at all tertiary levels from 1st year through to Masters. He has completed a graduate certificate in higher education and is a fellow of the Higher Education Academy.

His teaching is informed by both experience and a scholarly approach to identify deficiencies in student understanding. His main research interests are to developing strategies to improve teaching of quantitative literacy¹ and to improve employability skills through authentic learning^{2, 3}. This work earned the education award at the 44th FEBS congress in Krakow, 2019.⁴ His research has informed curriculum development and led to authoring an open

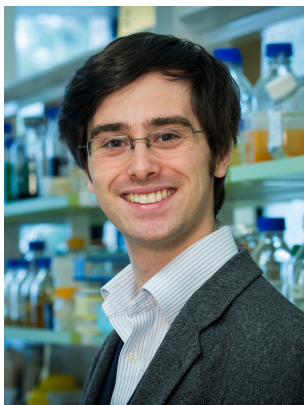
education textbook on quantitative literacy in biomedical science, *Foundations of Biomedical Science* (in preparation). He has successfully authored numerous research articles, conference papers and education articles. In 2018, he was awarded the La Trobe Pro-Vice Chancellor's Teaching Award for redeveloping the Biochemistry capstone curriculum to support students by focusing on current concepts, functioning knowledge and employability skills.



Dr Hendrika Duivenvoorden is an education-focused lecturer and course coordinator of the Master of Genome Analytics within the School of Biological Sciences at Monash University. Over the past nine years she has taught at three independent universities, with experience teaching both undergraduate and master's year levels. Hendrika has completed a Graduate Certificate in Higher Education, is a Higher Education Academy Fellow and is the co-founder of the 'Biology Researchers in Greater Higher education and Training' (BRIGHT) group at Monash, to empower emerging education-focused academics to plan and publish their education research. Hendrika has published 12 research articles (three as first author) and was an invited speaker at the 2021 Monash University Learning and Teaching conference.

Hendrika's passion lies in transforming curricula and resources to be more engaging, authentic, and interactive for students, informed by her field and current research. She also has an interest in electronic teaching resources and assessments, on which she has presented at the Monash Science Faculty Annual Science Symposium in 2020. In 2021 Hendrika was awarded the Faculty of Science Dean's Citation for Outstanding Contributions to Student Learning.

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4. Pakay, J., J. Young, and F. Carroll. 2019. "Improving quantitative literacy in incoming biomedical science students." In 44th FEBS Congress. Krakow, Poland.



Dr Thomas Shafee is a data scientist and bioinformatician with experience in extracting actionable insights from large and complex datasets, and communicating complex concepts to a broad range of audiences. After completing a PhD in evolutionary biochemistry at University of Cambridge and Medimmune, he transitioned to postdoctoral work in bioinformatics and data science at Hexima and La Trobe University. There he developed a wide array of skills in data analysis, particularly network and multidimensional scaling analyses as well as a range of statistical techniques.

He also has a keen interest in visualising data in a human-intelligible way in order to make abstract information more intuitive. This links into his wide experience in science communication to a range of stakeholders (other academics, industry partners, students, clinicians, policymakers, and the broader public). As well as direct data analysis and visualisation, he is able to advise on research data management and ethics best practices – whether securely handing sensitive data or efficiently disseminating open data.

Having a versatile skillset has enabled him to provide advice and expertise to national and international organisations ranging from pharmacology (MIMS Australia) to psychology (H-GAPS), Antarctic research (SCAR), and knowledge dissemination initiatives (Wikimedia Foundation).

Kaitlin Clarke is a researcher and casual teaching academic, completing a PhD in the Department of Biochemistry and Chemistry at La Trobe University. Kaitlin holds a bachelor's and master's degree by Research in Biochemistry. Her high achievement throughout undergraduate studies were recognised by academic achievement awards including the La Trobe University Vice Chancellors Award and the ASM Nancy Millis Student Award in microbiology. She prepared and delivered a talk presenting her postgraduate research at the international FAOBMB conference in 2021. As a specialist subject support tutor and demonstrator, Kaitlin has been involved in curriculum development and extensive experience in guiding undergraduate students grappling with difficult concepts. Additionally, she has several years' experience in laboratory demonstration/tutoring including Anatomy at The University of Newcastle and Biochemistry at La Trobe University helping students to bridge the gap between theory and practical application. She brings this experience and importantly a “near-peer” sensibility to the project. Kaitlin is a capable author and recently submitted a 10,000-word review first author paper to *Frontiers in Immunology* which will be published this year.

Acknowledgements

We acknowledge and thank the Open Educational Resources Collective, a Council of Australian University Librarians (CAUL) initiative, for their generous grant which made this book possible.

CAUL has been at the forefront of sector-wide initiatives that focus on developing the capacity and capability to publish open textbooks and other open educational resources in Australia and New Zealand.

Chapter 1: Studying biology at the molecular level

1.1 Introduction

This e-book is designed to be a relatively succinct resource for learning the key threshold concepts in biochemistry and molecular biology. Since these key concepts underpin everything in biology, this resource is designed for any students beginning a tertiary course related to biology, not just those who are focused on biochemistry or molecular biology. It is also useful for students who may be coming back to study after a hiatus and need to refresh their understanding of concepts or for anyone who wants a reasonably quick primer on biology at the molecular level. This e-book is not designed to be a replacement for traditional textbooks but rather this text focuses on the key threshold concepts agreed by experts in this discipline to be particularly important for establishing a solid foundation. These concepts are by their nature ones which many students tend to find initially difficult and problematic but, as the name ‘threshold concepts’ implies, once understood will provide a gateway for further understanding the discipline.

Overview of the contents

Chapter 2 makes a case for all biologists to have a fundamental literacy of life at the molecular level, and we provide some advice (based on hard-won experience!) on how to effectively study this discipline. We provide an overview of what biochemistry and molecular biology are and how they came to be. The story of their development provides a way to gain an overview of the discipline. The ‘black boxes’ in understanding of the early pioneers mirror the black boxes that all students must draw around unfamiliar concepts and their relationships as they learn them for the first time.

In Chapter 3, we define life itself at the molecular level, concentrating on what all life on Earth has in common, from the smallest microorganisms through to complex multicellular organisms like us.

Subsequently, each chapter tackles an overarching **threshold concept**. In turn we focus on:

Chapter 4: The flow of genetic information in living systems

Chapter 5: How matter and energy are transformed in living systems

Chapter 6: How cells control and regulate their internal environments

Chapter 7: How structure is related to function at the molecular level

Chapter 8: The importance of the theory of evolution to understanding biology at the molecular level

Navigating the material



The overarching threshold concepts are broken down into smaller threshold concepts. You will recognise these by the **doorway** icon alongside them. Learning these and replacing prior misconceptions will open the door to greater overall understanding.



Work through the material actively – take notes and check your understanding at every opportunity. Wherever you see the **knowledge check** icon there will be some limited choice or short-answer questions to test your vocabulary or fundamental understanding.



Wherever you see the **discussion question** icon, there will be some questions probing your deeper understanding of the topic. These will require more thought and ask you to apply your knowledge to a complex problem. These can make good questions to discuss collaboratively with peers, tutors, study groups and so on.

We hope that once you understand these threshold concepts in biochemistry and molecular biology you will be inspired to continue learning more!

Australia and New Zealand in focus



Wherever you see the **Australia and New Zealand in focus** icon we will highlight the contributions made to this discipline by researchers from Australia and New Zealand or how Australia and New Zealand contribute a unique perspective to the discipline.

You should not only feel proud and inspired by these historical contributions but also by the current research occurring in Australia and New Zealand. Our biochemistry and molecular biology communities rank amongst the world's best in terms of the number and quality of publications per head of population and our research facilities are world-class. We sincerely hope this specific focus allows you to recognise the opportunities this discipline can provide to you and the importance to our society as a whole.

1.2 Information for instructors

Biochemistry and molecular biology represent one of the fastest-growing fields of scientific research and technical innovation, and the resulting biotechnology is increasingly applied to other fields of study. So, an understanding of biochemistry and molecular biology (we use these terms largely interchangeably here) is critically important for students in all biological disciplines. However, at the same time, the content is inherently complex, highly abstract, and often deeply rooted in the pure sciences – mathematics, chemistry, and physics. This makes it difficult to learn and to teach.

Due to the rapid pace of change and accumulation of knowledge in biochemistry it is conceded that it is impossible to cover content exhaustively. Therefore, decisions must be made regarding which information is crucial to the study of biochemistry and which information can be omitted. In response to this, several studies have been undertaken to identify the key foundational concepts and skills required by majors in the discipline. Our experience in teaching biochemistry is consistent with others in that many students fail to grasp these concepts early. This presents a major barrier to learning, progressing in the discipline, and attaining the requisite skills. Almost always, the barriers to learning are misconceptions related to understanding key threshold concepts.

Threshold concepts have been identified in numerous disciplines and while often they are relatively few, mastering them causes a transformative shift in a student's understanding and appreciation of that field and empowers students to connect prior and new knowledge in more sophisticated ways. Threshold concepts are partly defined by their transformative nature but also by their 'troublesome' nature. In 2014, a study¹ funded by the American National Science Foundation identified the key threshold concepts critical for biochemistry in five major areas:

1. The central importance of the theory of evolution to all biological sciences
2. Matter and energy transformation
3. Homeostasis, control and regulation
4. Biological information
5. Macromolecular structure and function

A key barrier to understanding these concepts is their abstract nature. This resource attempts to lower this barrier through careful explanation of domain-specific language using vocabulary students already understand, by using visualisations and metaphors of complex concepts and by applying knowledge checks in which students can check their understanding. Students will also be able to apply their newly acquired conceptual understanding to novel problems with access to worked solutions.

This e-book is designed as a succinct and focused resource, specifically aimed at helping students grasp key threshold concepts in biochemistry – a cognitively demanding task. By focusing on the specific information required to understand the threshold concept and minimising extraneous detail, our intention is that cognitive load will be reduced. This will free students to focus on mastering the foundational concepts necessary in this discipline.

1. Loertscher, J., Green, D., Lewis, J., Lin, S., & Minderhout, V. (2014). Identification of threshold concepts for biochemistry. *CBE Life Sciences Education*, 13(3), 516–528.

1.3 Target audience

This resource is primarily aimed at novice students beginning their studies of biochemistry or molecular biology. However, given the fundamental importance of the threshold concepts covered to the mastery of the discipline and their inherently abstruse nature, this resource will be useful for reinforcement and remediation for students entering more advanced courses, where it will be assumed knowledge. This is particularly relevant for those students who failed to grasp these concepts early on or to help rebuild knowledge for those students who have misconceptions. Furthermore, it is worth noting that many science courses no longer require prerequisite knowledge in biology and this resource can help bridge the gap into tertiary study.

It will also be useful for students in other biological disciplines (e.g. genetics, microbiology or biomedical science) that are underpinned by basic biochemistry and molecular biology. In addition, it will make a useful primer for students engaged in other biological disciplines with a tangential biochemical/molecular focus (e.g. zoology or botany) where a basic literacy in the foundational knowledge of the threshold concepts of biochemistry or molecular biology is increasingly desired.

The open nature of this resource also allows it to be freely used by instructors in all of the disciplines above as directed reading or for the problems and case studies within, which can be selectively used to supplement other course materials.

We have included content that focuses on biochemistry and molecular biology through a prism of Australian and New Zealand research. The discipline in Australia and New Zealand is represented by outstanding Nobel laureates including Howard Florey (Australia), Maurice Wilkins (New Zealand), Peter Doherty (Australia) and Elizabeth Blackburn (Australia's first female Nobel laureate). We hope that students feel inspired by this and also by current research. This e-book promotes the discipline from a uniquely local perspective and allows students to recognise opportunities for themselves and the importance of the field to society. However, if you are instructing students from outside Australia or New Zealand, feel free to substitute our local content with something more relevant for your students.

1.4 Why study biology at the molecular level?

There is a molecular basis to life. That is, all biological function depends on events that occur at the molecular level. We now understand that these events are coordinated by (often) complex molecular machinery even if we do not fully understand every aspect of their function. These complex molecular machines are comprised of large molecules or clusters of molecules that include proteins, nucleic acids such as deoxyribonucleic acid (DNA), carbohydrates and lipids.

For example, we now understand the molecular basis of inheritance – that DNA, ribonucleic acid (RNA) and the genetic code transmit hereditary information from parents to offspring. We understand how functional gene products (e.g. proteins) work. That is, we are able to relate the complex and varied structure of proteins to their functional roles. We have developed techniques to map the interactions of these molecules, quantitate their abundance, determine their localisation within the cell and study the processes they mediate.

This **molecular revolution** began in earnest in the 1950s following key scientific breakthroughs that linked our understanding of inheritance to the internal molecular machinery of cells. It has transformed *every* aspect of biology. Ecologists can now track organisms using their DNA (see *Monitoring the platypus with environmental DNA*), evolutionary relationships can be accurately mapped using molecular data, taxonomy is resolved by molecular data, understanding the molecular basis of disease has revolutionised diagnosis and treatments, and molecular understanding has improved agriculture and land management.

If one visits most modern biology laboratories regardless of their discipline, whether it is biomedicine, agricultural biology, zoology, microbiology or botany, they tend to appear alike. This is because there is an incredible similarity and overlap in the technical approaches used, regardless of the biological system being studied. Any differences you do notice will likely be due to the underlying biological system under investigation. For example, a microbiology laboratory may use agar plates upon which to culture microorganisms or a plant biology laboratory may use a greenhouse to grow the plants under study. However, even laboratories that study disparate systems may use common approaches such as culturing vinegar flies (*Drosophila*) or zebra fish (both animals are common genetic models for various useful reasons). The actual molecular techniques used are likely to be very similar. It is not uncommon for researchers in biology who are working at the molecular level to never see the organism under investigation! All the experiments are undertaken using material extracted from the organism in question. It is also possible that a biologist working on a specific problem does not even see biological material at all! This is because due to the staggering amount of data available, some investigations can now even be undertaken solely *in silico*; that is, by analysing online databases.

You will find biochemists and molecular biologists working in all biological disciplines. Even if they describe themselves as a zoologist, botanist or ecologist, they will often have functional literacy, if not proficiency, in biochemical and molecular techniques.



Figure 1.1 A typical biology laboratory: Almost all modern biology laboratories look alike (at least at the cursory level) as much of the research is focused on understanding biology at the molecular level. The researcher here could be working on almost any aspect of biology from conservation ecology through to human disease. Image: 'Woman In White Long Sleeved Laboratory Gown Standing' by Polina Tankilevitch from Pexels used under Pexel license.

Monitoring the platypus with environmental DNA



All organisms shed DNA into the environment as they lose cells, hair, skin, scales, faeces etc. With recent improvements in technology, it is now possible to collect and sequence this DNA. These trace amounts of DNA, known as environmental DNA (eDNA), can be used to determine which species are present in a particular environment without having to directly observe or capture them. As every organism has a unique DNA sequence, their shed DNA provides a signature that can be used to trace them. For example, from a small sample of water it is possible to obtain a genetic profile of all the organisms present in a waterway.

Australia and New Zealand have been at the forefront in using eDNA to manage both endangered and also invasive species. The iconic Australian animal the platypus is at risk due to habitat loss and drought. eDNA is allowing this notoriously elusive species to be monitored. By understanding its range, environmental scientists are able to make better informed decisions regarding land and waterway management. Since the collection of eDNA is a simple task – a water sample is all that is required – monitoring can be assisted by members of the public. In Australia, one of the world's largest citizen science projects, *The Great Australian Platypus Search*, was launched in 2021, to map platypus populations across the state of Victoria.



Figure 1.2 A wild platypus from a river in Victoria, Australia: By using techniques to monitor environmental DNA (eDNA) it is possible to determine the distribution of species like the platypus without having to directly observe or capture them. Image: 'Wild Platypus' by Klaus from Wikimedia Commons used under CC BY-SA 2.0.

What exactly is analysed in eDNA?

Typically, a gene that is highly conserved and found in all species is used for eDNA analysis. For example, for analysis of the microbiome (all the microbes in an environment) the 16S ribosomal RNA gene is used for identifying species present (all life has ribosomes – see Chapter 3.) For animals, the mitochondrial cytochrome c oxidase I (COI) gene is often used. This gene is useful as it is found in mitochondrial DNA, and mitochondria are found in all eukaryotes. Each cell has many copies of this DNA, so there are more copies of mitochondrial DNA than nuclear DNA. Importantly, there are regions in the sequence of this gene that show enough variability for it to provide a unique signature, allowing it to be used to distinguish individual species.

Chapter 2: How to study biology at the molecular level

2.1 What is molecular biology?

Biochemistry and molecular biology are used interchangeably in this book, and they are increasingly recognised as a unified discipline, rather than as two distinct disciplines or subdisciplines. Probably this is due to widespread adoption of the techniques of both disciplines by all biologists. However, historically molecular biology emerged from a convergence between biochemistry and genetics. **Biochemistry** is centred in chemistry and principally deals with understanding the function of proteins. **Genetics** is principally concerned with the function of genes and inheritance. **Molecular biology** emerged later, once the molecular mechanisms (biochemistry) of gene function were determined, and focuses on the relationship between genes and functional gene products (which are in most cases proteins). So molecular biology could be viewed as the nexus between biochemistry and genetics.

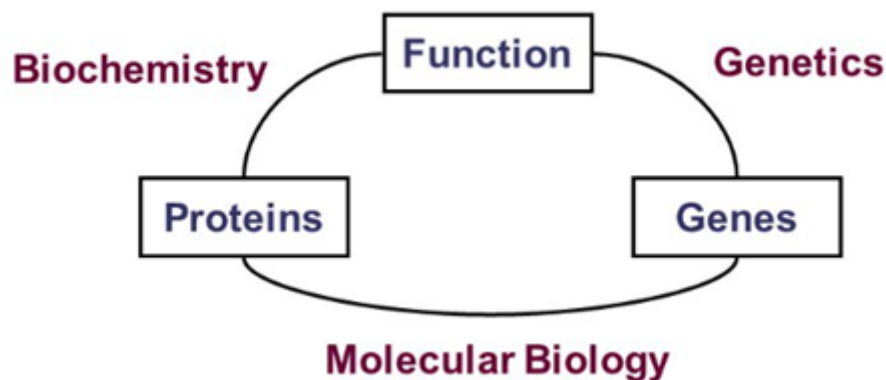


Figure 2.1 The relationship between biochemistry, genetics, and molecular biology: Molecular biology was born out of discoveries which linked genes and inheritance to with functional gene products (proteins).

2.2 What makes biochemistry and molecular biology so difficult?

Biochemistry and molecular biology have a reputation for being difficult to understand. This is not altogether undeserved! When you begin these disciplines, there is a lot of new information, many new terms to learn and some difficult concepts. One aspect that makes biochemistry and molecular biology difficult is that they draw on knowledge from other disciplines – most heavily from biology, which provides the relevance; but also chemistry, which provides the molecular understanding; and to a certain extent mathematics and physics (see Figure 2.2). As a student you will need to be prepared to integrate knowledge from these other disciplines. If you were to specialise in biochemistry or molecular biology, it is likely you would take classes at some point in all of these disciplines.

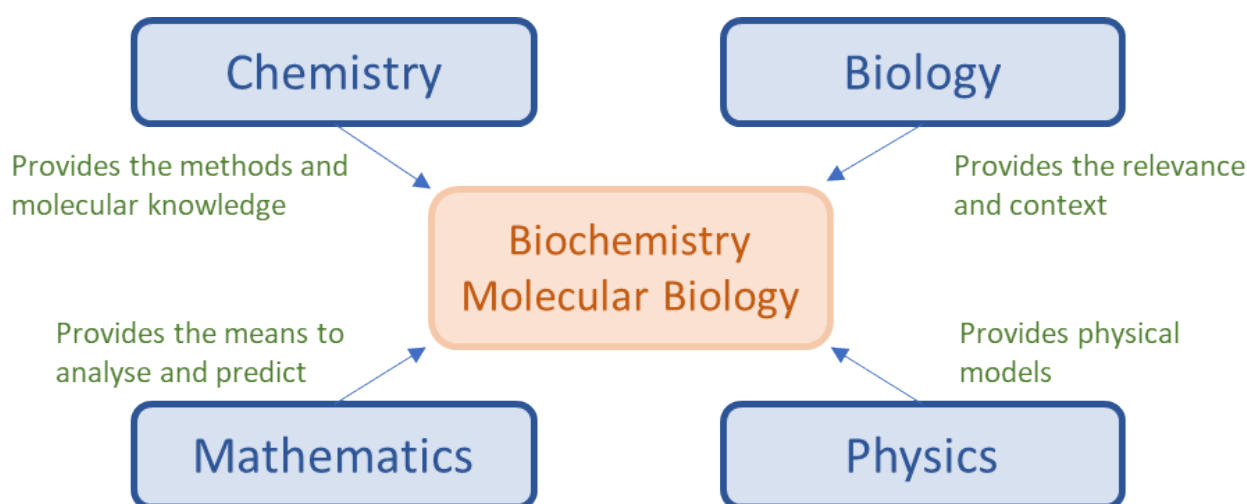


Figure 2.2: While biochemistry and molecular biology are disciplines themselves, the underlying concepts rely heavily on other disciplines.

2.3 How much chemistry will I need?

This is a very common question from students beginning a biological discipline. You *will* need to understand some chemistry! This text assumes some familiarity with basic concepts. For example, you should understand atomic structure, elements, periodicity and molecular structure. You will need to know about covalent and non-covalent bonding, polarity (electronegativity), ionisation, acids and bases, and pH. A knowledge of chemical reactions is important, so stoichiometry, oxidation-reduction chemistry and catalysis should be familiar concepts to you. A basic understanding of organic chemistry and the common functional groups is also very useful.

If these terms are unfamiliar, or you realise you need to refresh these concepts, it is worth spending some time reading an introductory chemistry text.



Knowledge check: How is your chemistry?

This short diagnostic will test your knowledge of chemistry and provides links to further resources to help you get up to speed if necessary.



An interactive H5P element has been excluded from this version of the text. You can view it online here:

<https://oercollective.caul.edu.au/threshold-concepts-in-biochemistry/?p=141#h5p-7>

Help – this is all too abstract!

Biology at the molecular level is complex. It involves multiple processes that interact and impinge on each other. Life represents an emergent property of these processes acting together (see Chapter 3). So by definition it is impossible to understand these processes fully by looking at them in isolation. Things only start to make sense after you learn how they work together.

This is a problem when you first begin learning and it is normal for things to initially seem a bit abstract! That is, it is normal to learn about a process but not be completely sure about how it fits into biology in general. For example, consider how information flows through a biological system (see Figure 4.6). Genetic information is stored as discrete hereditary units called genes in DNA (a nucleic acid) and the information is transcribed to RNA (also a nucleic acid) before being translated into protein. Proteins are the functional products of genes.

It might thus seem reasonable to start your learning with DNA, since it represents the start of that process. The problem with this approach is that it is impossible to really understand how this transfer of information works without understanding proteins (the functional products of genes) in the first place! It is the proteins that facilitate the whole process.

In Figure 2.3 the proteins that catalyse this transfer of information are written in pink. DNA polymerase, a protein, makes new copies of DNA during cell division, RNA polymerase, another protein, makes an RNA transcript using the DNA as a template and ribosomes (a molecular machine made from many proteins and also RNA molecules) translate the information in the RNA into proteins.

Okay, so since proteins are important, then the answer might be to start with understanding what proteins are and how they work. But the problem now is that proteins are encoded by genes and therefore understanding proteins fully requires understanding how DNA works!

Whatever you start with will require drawing a 'black box' around something else, and things will only start to make sense once you understand enough concepts (see Figure 2.3). This example of the interplay between DNA and protein, which plagues most beginner biology students, also mirrors the situation the pioneers of molecular biology faced. These researchers made significant inroads into understanding processes such as inheritance or biological catalysis often with very limited knowledge of the biological molecules responsible.

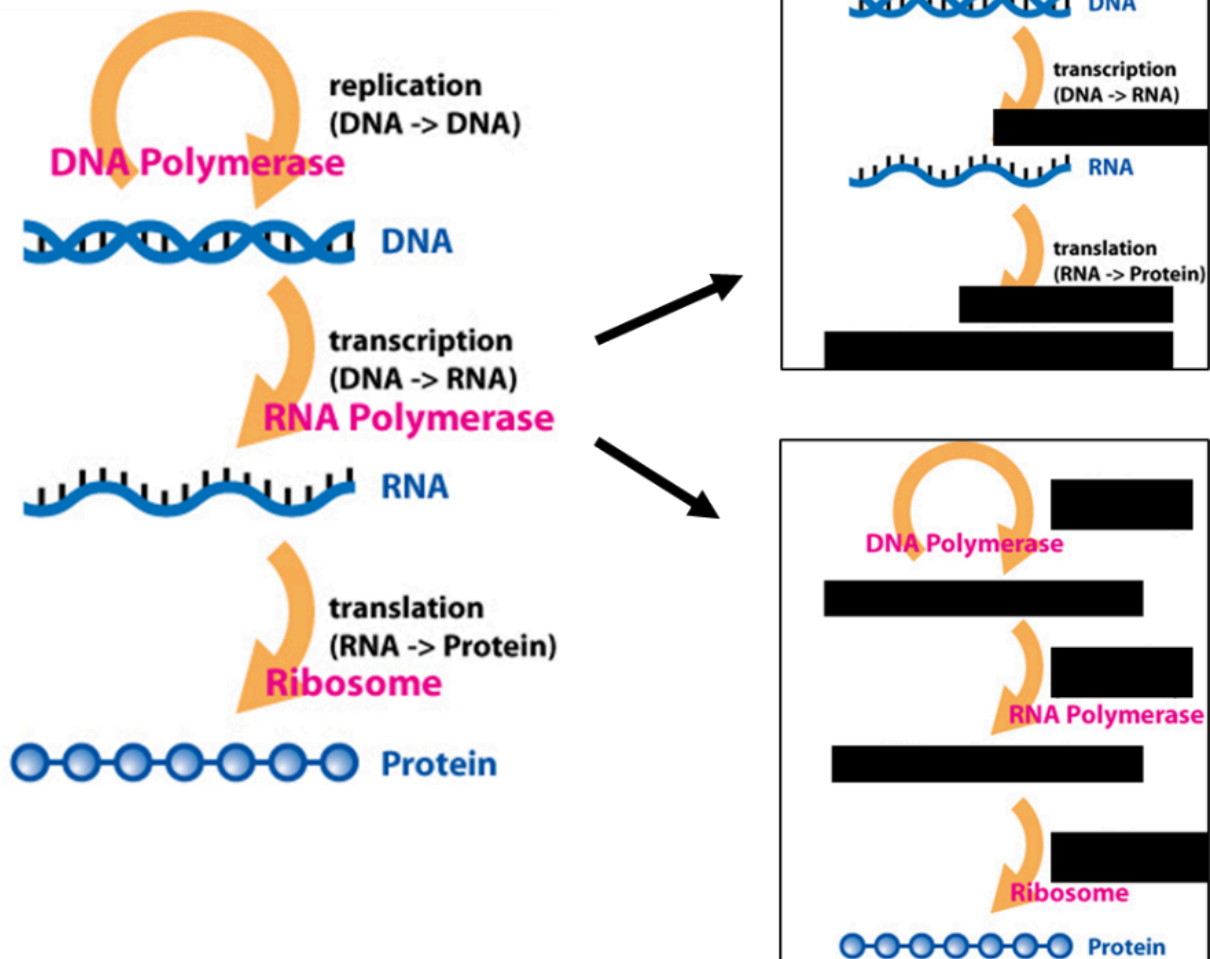


Figure 2.3: Understanding the flow of biological information, from DNA to RNA to protein, requires an understanding of both nucleic acids and proteins. Whichever you learn about first, requires drawing a “black box” around the other. It is only when you learn about both DNA and proteins does the process become clear.
Image: Adapted from Central Dogma of Molecular Biochemistry with Enzymes by Daniel Horspool from Wikimedia Commons used under CC BY-SA 3.0.

Can it be less abstract?

One way to make things less abstract is to try to get an overview first. This will initially seem overwhelming, and you have to accept that lots of detail will be missing, but once you have a sense of the bigger picture, then as you learn things in more detail, they will fit easier in place. A quick way to get an overview is to follow the timeline of the major discoveries in biology which led to our current understanding of molecular biology. The major concepts will be revealed to you sequentially, it will be easier to remember new terms if you know their origins, and you will learn about DNA and protein in terms of their function.

2.4 Advice for studying biology at the molecular level

Yes, biochemistry and molecular biology at times can be challenging, but it is not all bad news! There are a few things to note and a few approaches you can follow that should make things easier than they initially appear or pay dividends in the long run.

All extant life is related. This is a very good thing!

All extant life (living organisms which exist now on Earth) has a common ancestor. The evidence for this is presented in Chapter 3. This means that even with all of the amazing diversity of life around us, because all living things are related, they work in fundamentally the same way at the molecular level. For example, all life uses DNA to store genetic information and codes it essentially in the same way, and all life uses the same or similar basic molecular building blocks and organises them in the same way. This means that once you understand a few basic principles, you will be able to apply this knowledge to all living things. Imagine how difficult it would be if this were not the case, and we had to learn everything from scratch when we moved on to a new organism!

Finding the patterns

Biology (and in fact all science) is concerned with discovering relationships between variables. That is, it is about discovering trends and patterns. The most useful discoveries in biology are those that are explanations for general phenomena. Similarly, when you are learning a discipline, it is most useful to learn the principles that have the broadest applicability.

When you first learn biology at the molecular level it might seem that there is a bewildering amount of information to learn. There is a lot! But you will also realise that many processes work in a similar way or even use the same or very similar components.

In his essay ‘Evolution and Tinkering’, published in *Science* in 1977, the French biologist and Nobel laureate, François Jacob described evolution as a ‘dogged tinkerer’. What he meant by this is that evolution should not be compared to engineering, where a new function is designed and created from scratch. Rather, the process is similar, in Jacob’s words, to a ‘tinkerer [who] picks up an object which happens to be in his stock and gives it an unexpected function. Out of an old car wheel, he will make a fan; from a broken table, a parasol.’

We often mention **adaption** when discussing evolution. This is where changes to a population or species occur due to selective pressure of the environment (see **Growth, reproduction and evolution** in Chapter 3). However, there is another important process called **exaptation**. This is where a trait can shift in function during evolution. That is, like how the tinkerer works, the trait is given an unexpected new function.

This might be difficult to understand at this stage but will become clearer after you read chapters 3 and 4. At the moment just understand that because evolution will reuse and repurpose biological components for new functions, you will see that similar mechanisms occur in different cellular processes. The same molecule or mechanism will be seen in different pathways, and different complex signalling or biosynthetic pathways will contain the same modular components.

For example, the molecule adenosine is a component of DNA, RNA, adenosine triphosphate (ATP), coenzyme A, nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD) and cyclic adenosine monophosphate (cAMP). These participate in many different cellular processes but once you first learn about adenosine you will much more easily see how it fits into all these different places.

Another example is G-protein coupled receptors, a large superfamily of cell surface receptors that have incredibly diverse functions (photoreception, taste, pain and smell; they also mediate cell recognition, metabolism and growth) despite having a very similar structure and common mechanism of action. All G-protein coupled receptors share two properties – they have seven transmembrane domains, and they interact with specialised proteins (called G proteins) to influence intracellular pathways after binding extracellular signals (see Figure 2.4).

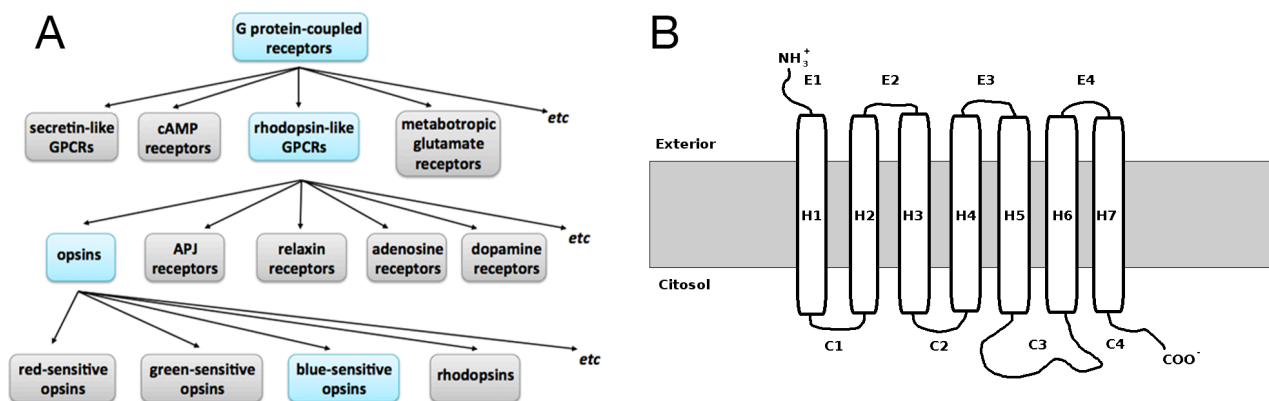


Figure 2.4 A: The G-protein coupled receptors are a diverse family of proteins which have myriad biological functions. To illustrate this the diversity of the opsin sub-family are shown with the short-wave light sensitive opsin proteins highlighted in blue. Image: 'Hierarchical relationship of G-protein' from www.edi.ac.uk used under CC BY 4.0.

Figure 2.4 B: Despite the diversity of functions this entire family of proteins share a common canonical structure containing seven transmembrane domains and a common mode of action elicited through a coupled g-protein. Image: 'GPCR' by Retama from Wikimedia Commons used under CC BY-SA 3.0.

Getting to grips with the language

Part of the challenge you will face initially with biochemistry is learning new terms. The American author and educator Neil Postman said¹:

Biology is not plants and animals. It is language about plants and animals ...

This quote highlights the importance of language when learning about biology. It is not just about learning new words but also how to use words you already know in a biological or scientific context. Without understanding language and naming conventions to a level where you use them in your speech and writing, your learning will be limited. This is not trivial – in science classes the number of new terms can be comparable to foreign language classes!

In addition, there are two ways in which language is confusing in biochemistry:

1. Postman, N. (1980). Language Education in a Knowledge Context: A Review of General Semantics, 37(1), 25–37.

1. Words are often borrowed from everyday English and repurposed. This can make it difficult as you will need to re-learn a word you know but in its scientific context.

For example, when we speak about *tissues* in everyday language, the brand Kleenex may come to mind. However, in cell biology, *tissues* refer to a level of organisation in multicellular organisms consisting of a group of either functionally or structurally similar cells plus their surrounding intercellular material.

Similarly, words like *transcription* or *translation* have a meaning in everyday written and spoken language. However, in molecular biology they specifically refer to the transfer of genetic information in living organisms.

With this kind of terminology, the key is to ensure that you understand the *processes* they are associated with. Understanding them in context is the key to not confusing their counterpart meaning in everyday English.

2. Many words are long and complex and have origins from other languages, principally Greek and Latin. These, just because of their unfamiliarity and length, will be difficult to read and intimidating to say aloud.

Becoming fluent in the language of biology

When learning anything complex it is a good idea to break it down into smaller parts to make it more accessible.

Scientific words often have three parts – a prefix, a root and a suffix, as shown in the following table.

Prefix	Root	Suffix
Appears at the beginning of a word. Many are used frequently, so you will eventually learn their meaning.	The root of a word often has a standalone meaning. These often have a Greek or Latin origin.	Appears at the end of a word to provide additional meaning.
<i>hypo</i> – below <i>hyper</i> – above <i>cyclo</i> – ring <i>poly</i> – many <i>endo</i> – within <i>exo</i> – outside of	<i>iso</i> – equal <i>allo</i> – other <i>chloro</i> – green <i>cyte</i> – cell	<i>-phillic</i> – love, affection <i>-phobic</i> – hate, fear <i>-lysis</i> – breakdown <i>-some</i> – body <i>-oma</i> – abnormal, cancer

You will be surprised how often the same prefix, root or suffix will appear in different words. Once you learn a few, things will start to get easier. For example, the Greek word *therm* means ‘heat’. You will find this word in a large family of scientific terms, including exothermic, thermogenesis, thermoregulation, isothermal, ectotherm, thermodynamics, hypothermia and many others.

Knowing the word origin (etymology) of a term can also help to identify its likely meaning and make it easier to understand in biochemistry.

For example, the word **eukaryote** is a noun describing a cell containing a nucleus. It is derived from the prefix **eu-** (from Greek, meaning good, well or true), the root **kary** (from the Greek word *karyon*, which means kernel or nut but now indicates the cell nucleus) and the suffix **-ote** (a singular form of the Greek-derived word *osis*, which means process or state).

Resources

There are many useful websites that can help you with understanding the etymology of words.

- **Online Etymology Dictionary**
- **The Free Dictionary** also contains a thesaurus, medical dictionary and many other features. You can listen to a pronunciation of almost all words in the dictionary.

When learning new words, writing them in a sentence of your own construction can help integrate them into your vocabulary faster. Also say them aloud. Try not to be intimidated by their length – most of the time they will be phonetic (sound the way they are spelled). So just break them down into their component parts and at first say them slowly until you get used to pronouncing them! When you can confidently say them in your head, reading will be much easier, and your learning will rapidly accelerate.

Concepts rule!

The reason for learning anything in the first place is to be able to apply that knowledge to novel situations. This means that it is always much more important to learn **concepts** rather than isolated facts. Biochemistry and molecular biology as disciplines are no different. This does not mean that facts are unimportant! It is just that understanding ideas and concepts will help you learn facts faster. **Learn to understand rather than just simply memorise.**

An excellent example of the importance of concepts versus facts is the development of the periodic table of the elements. The Russian chemist Dmitri Mendeleev set out to order the known chemical elements in the 1860s (at the time only 63 elements were known). He noticed that there was a correlation between atomic weight and the chemical properties of elements. This led to ordering elements by increasing atomic weight but also into horizontal rows called periods, where each period number

indicates the number of electron orbitals for the elements in that row. As elements were discovered they were added logically to the gaps in the table.

Group Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1 H																	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	* 71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	* 103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Nh	114 Fl	115 Mc	116 Lv	117 Ts	118 Og
			* 57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb		
			* 89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No		

Figure 2.5 The periodic table of the elements represents the advantage of learning concepts over facts: By understanding the significance of the organisation of the rows (groups) and periods it is possible to predict the properties of an element from its position in the table. Image: 'Hierarchical relationship of G-protein' by Offnfopt from Wikimedia Commons used under CC BY-SA 4.0.

For students of chemistry (and biochemistry!) where knowledge of the elements is important, it is far better to understand the main concepts underlying the periodic table than to memorise the chemical properties of individual elements.

Conceptual learning is important but there is a hierarchy of concepts too. Some are more important than others in the sense that by learning them they will allow you to connect many different concepts. We call these **threshold concepts**, and mastering them is the purpose of this e-book.

Mastering the threshold concepts

A threshold concept refers to one of the core concepts in a subject whose understanding is key to transforming the way you understand the subject as a whole. To understand what we mean by a threshold concept, consider this example from the unrelated discipline of history.

When you begin learning history you may focus on names and dates and accept an initial historical account you read to be accurate. However, as you study in more depth, you begin to understand that historical accounts can be biased, and there can in fact be different competing historical accounts. Often there is no clear definition of why something happened or even a definitive account of what actually happened! The **threshold concept** in this case is that there is an **interpretive** aspect to history. This is transformative as once you understand it, you will never read history in the same way again.

So, threshold concepts are **transformative** (create a significant shift in your understanding), proba-

bly **irreversible** (once learnt are difficult to unlearn), **integrative** (allow you to make links between different topics) and potentially **troublesome** (difficult to initially grasp and often counterintuitive).

The focus of this resource is to help you understand the key threshold concepts in biochemistry and molecular biology. These have been identified by research² funded by the American National Science Foundation. Key threshold concepts critical for biochemistry were identified in five major areas:

1. The central importance of the theory of evolution to all biological sciences
2. Matter and energy transformation
3. Homeostasis, control and regulation
4. Biological information
5. Macromolecular structure and function

Applying active learning

Despite the importance of concepts some things just need to be committed to memory. However, memorisation is far more effective if it is done by active learning rather than passive learning. If you simply read text or watch lectures, the information will wash over you. However, if you write things down (preferably by hand), create diagrams and flowcharts and constantly check your knowledge by answering questions, you will be far more likely to successfully commit important information to memory. Give yourself plenty of time to study, and leave enough time between knowledge checks to ensure you are not just testing your short-term memory.

2. Loertscher, J., Green, D., Lewis, J., Lin, S., & Minderhout, V. (2014). Identification of threshold concepts for biochemistry. *CBE Life Sciences Education*, 13(3), 516–528.

Chapter 3: The universality of life

3.1 How can we define life?

Previously, we defined biochemistry and molecular biology as the study of chemical processes within and relating to living organisms. But how do we define life itself? This might seem like a simple question, but there is no single, widely accepted definition of life. Even biologists (who study life) don't agree on what 'life' is! When we think about life, most often our definitions are operational. That is, they allow us to differentiate non-living matter from living matter but don't give a definition for life.

If you search for a succinct definition of life you might come across NASA's contribution:

Life is a self-sustaining chemical system capable of Darwinian evolution.

This compact definition was formulated for astrobiologists. These scientists are searching for extraterrestrial life and do not want to be restricted to a definition that applies only to the example of life on Earth. However, the NASA definition is controversial. It is broad enough to encompass viruses, which most biologists would not include as living organisms, and it may exclude organisms not capable of reproduction such as mules (which are sterile, even though we would agree that a mule is alive).

Given that life is so difficult to define, rather than attempting to provide a succinct yet all-encompassing answer, it is more useful to describe life or living things by their common characteristics. These include:

- Organisation
- Metabolism
- Homeostasis
- Growth, reproduction and evolution

Organisation

Living systems are organised, meaning simply that they are arranged in a particular manner. When we use the term **organised** in biology it includes two fundamental ideas. One is that in living systems, things are arranged in a hierarchical manner; the other idea is that living systems tend to be less random than their surrounding environment.



All living systems exhibit a hierarchical level of organisation, have metabolic processes, maintain homeostasis and are capable of growth and reproduction as well as the capacity to evolve.

The hierarchy of organisation

Living systems exhibit a **hierarchical level of organisation** where each level of the hierarchy exhibits greater complexity than the previous level and is comprised of components from the previous level.

Understanding this hierarchy is the key to understanding how life works mechanistically; that is, how all the components fit together.

We will go into more depth with the common components of life, but it is worth getting an overview of them now. Figure 3.1 demonstrates this hierarchical organisation of life, starting with amino acids and proteins. We could certainly show a similar hierarchy using DNA, lipids (fats) or sugars as the example.

We can think of the hierarchy of organisation of life beginning with atoms forming molecules. The most common elements found in living organisms are carbon, hydrogen, oxygen and nitrogen (two other relatively common elements are phosphorous and sulphur). Atoms from these elements make up about 96% of any life form. For example, hydrogen and oxygen atoms combine to form water, which is a major component of all living things.

Molecules can combine to form more complex molecules such as amino acids. Some of these molecules can combine further to form macromolecules such as proteins. These macromolecules can combine further to form complexes such as chromosomes, or large protein complexes which themselves provide the basic architecture of cells.

Cells are common to all living things and are considered the fundamental unit of life. That is, a cell is the smallest unit that we consider to be alive. A living organism can be a single cell, or it can be multicellular. In multicellular organisms, the organisation can be incredibly complex, with many different types of specialised cells. These cells can cluster to form tissues which in turn form organs and organ systems, which make up the organism. The hierarchy continues as communities of organisms interact with their physical environment to create ecosystems. All the ecosystems on Earth together form the biosphere.



Living systems exhibit a hierarchical level of organisation where each level exhibits greater complexity than the previous level and is comprised of components from the previous level.

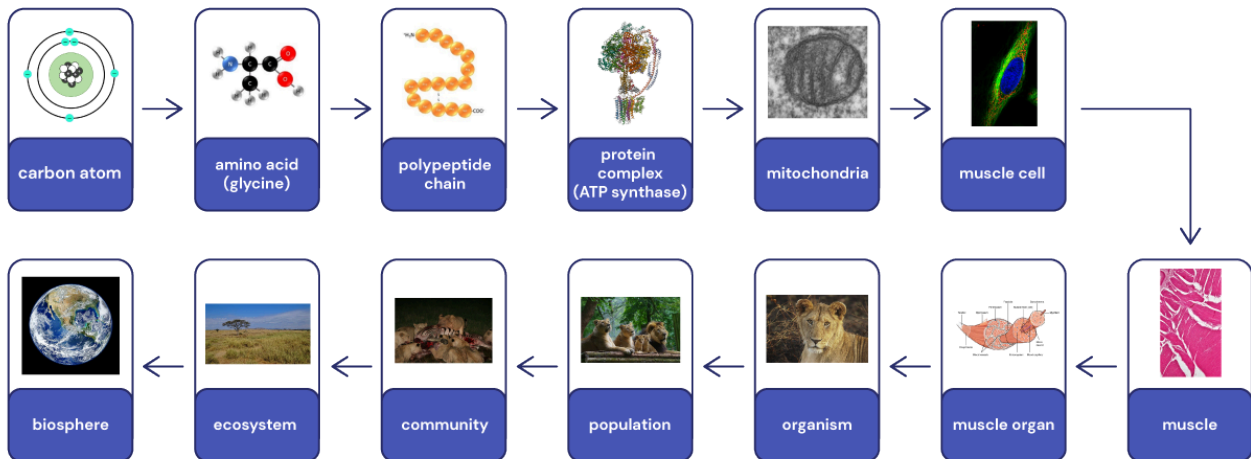


Figure 3.1 The hierarchical organisation of life: The simplest component of living things is the atom. Atoms combine to form molecules. Molecules can combine to form macromolecules. Multiple macromolecules help form cells. Cells function together as tissues, and tissues can combine to form organs. Organs work together to form organ systems and organ systems combine to make a living organism. Groups of the same organism living together in an area is called a population, and interacting populations form a community. Communities interact not only with each other but also with the physical environment and are known as ecosystems. All the ecosystems together make up the biosphere, which is the full extent of life on Earth.

How does this organisation come about?

How organisations arise is not a trivial question. By understanding it you will understand a key concept in biochemistry – that a constant input of energy is needed in order to maintain life.

The second law of thermodynamics states that:

the entropy of isolated systems left to spontaneous evolution cannot decrease as they always arrive at a state of thermodynamic equilibrium where the entropy is highest at the given internal energy.

Okay, so what does this mean?

First, we need to define what is meant by **entropy**. Entropy is a measurable physical property that is associated with disorder, randomness and uncertainty. So the second law of thermodynamics is stating that isolated systems will tend to become more random and disordered over time.

A good analogy is a neatly stacked pile of bricks. Over time you might expect some bricks to fall and the stack to eventually collapse. Another good analogy might be your bedroom which, if like the authors', tends to become messy and disorganised over time! The reverse – reforming a neat stack of bricks or tidying your bedroom – won't happen spontaneously. You must put some energy (work) into the system.



Figure 3.2 A neatly stacked pile of bricks collapsing over time is another good analogy for the second law of thermodynamics: Over you time you would expect the pile of bricks to eventually collapse, that is move from order to disorder. That is, you would expect the entropy of this system to increase. The only way to decrease the entropy would be to re-stack the bricks, that is put energy into the system. Image: 'Fallen Bricks' by Tomas Castelazo from Wikimedia Commons used under CC BY-SA 4.0.

But living systems exhibit complex organisation, which represents a decrease in entropy. Does that mean that life violates the second law of thermodynamics?

The answer is no because living systems are not isolated. Energy enters the system (on Earth, primarily from the Sun) and is used to decrease entropy locally. This local decrease in entropy is compensated by an increase in entropy somewhere else. For our stack of bricks, if a worker were to restack a random (disordered) pile of bricks (decreasing local entropy), they would release heat from their muscles and small molecules such as carbon dioxide and water broken down from larger molecules in food by their metabolism (increasing the entropy of the universe).

The complex organisation that living systems exhibit means they are in a state of thermal disequi-

librium – they can only maintain order via a constant input of energy. Note that this is possibly the characteristic of living systems that would be most useful for astrobiologists trying to find extraterrestrial life. That is, since we have no idea of the form extraterrestrial life might take, it might be better to just look for a local region of decreased entropy (less randomness, increased organisation) relative to the surroundings!



Living systems are in a state of thermal disequilibrium and need a constant input of energy.

The concept that energy is required for maintaining the hierarchical organisation exhibited by life will help us understand the next important characteristic of all life – metabolism.

Metabolism

As mentioned above, organisms need to maintain a non-equilibrium state with respect to energy. They do this by importing chemicals to produce energy that can be used to drive various cellular processes. **Metabolism** is the term used to describe all the chemical reactions involved in these cellular processes. These chemical reactions are essential to life as they provide the energy and the basic building blocks that enable organisms to maintain their complex organisation, grow and reproduce.

The fact that chemicals need to be imported into the cell means that the cell needs to be defined by a perimeter. A distinct boundary between the organism and the rest of the world is required. The basic barrier for all organisms is a **cell membrane**, and it appears to be a homologous structure across all organisms. That is, cell membranes were present in the common ancestor to all life and have been inherited ever since.

Organisms may confine their metabolic reactions to within cells, but they must also exchange resources with their environment. Organisms are able to import (capture), in a controlled manner, energy and matter (nutrients) from their environment and to export (excrete) waste products to their environment. It is the cell membrane that modulates the transport of chemicals into and out of cells and is thus critical to metabolism.

How can metabolism create order?

In the previous section we saw that life represents a local decrease in entropy that must be balanced by an overall increase in entropy of the universe. What is the nature of that entropy increase?

Remember that systems tend toward disorder rather than order. This means that chemical reactions that *create* order, such as the activity of complex macromolecules and structures within cells, are still ‘unfavourable’ in that they lead to an overall decrease in entropy.

So, imported energy is used to drive thermodynamically unfavourable chemical reactions, creating disequilibrium with the environment. As we saw, this is represented by, say, the release of small molecules such as carbon dioxide and water from breaking down larger food molecules and the release of heat from these chemical reactions.

Types of metabolism

We can broadly categorise metabolism into two processes: **catabolism** (obtaining energy from nutrients) and **anabolism** (production of new cell components, usually through processes that require the power obtained from nutrient catabolism). The key to understanding metabolism is that these processes are linked; that is, catabolic processes are used to drive anabolic processes.

A chemical reaction will not proceed spontaneously unless the products of the reaction have lower energy than the reactants, so that some energy is available from and released by the process. What we mean here by energy is 'free energy'. In simple terms free energy is a measure of the capacity of the system to do work.

This can be more easily understood if we look at a familiar chemical reaction like the combustion of petrol. Octane (a molecule in petrol) contains more energy than the water and carbon dioxide molecules that are released after burning the petrol. This means that the reaction has the capacity to do work, such as drive an internal combustion engine. Combustion is an example of an **exergonic** reaction, in which the reactants have more energy than the products.

Conversely, when the free energy of the products is greater than the reactants, the reaction is known as **endergonic**. Endergonic reactions can only proceed when there is an input of energy. Given that many of the reactions in a cell are endergonic, such as the production of macromolecule polymers such as proteins from amino acids, how can these occur?

The answer is by coupling exergonic and endergonic reactions, so the free energy provided by the first (exergonic) reaction can be used to fuel the second (endergonic) process. The metabolism of glucose to produce ATP to fuel polypeptide synthesis is a good example.

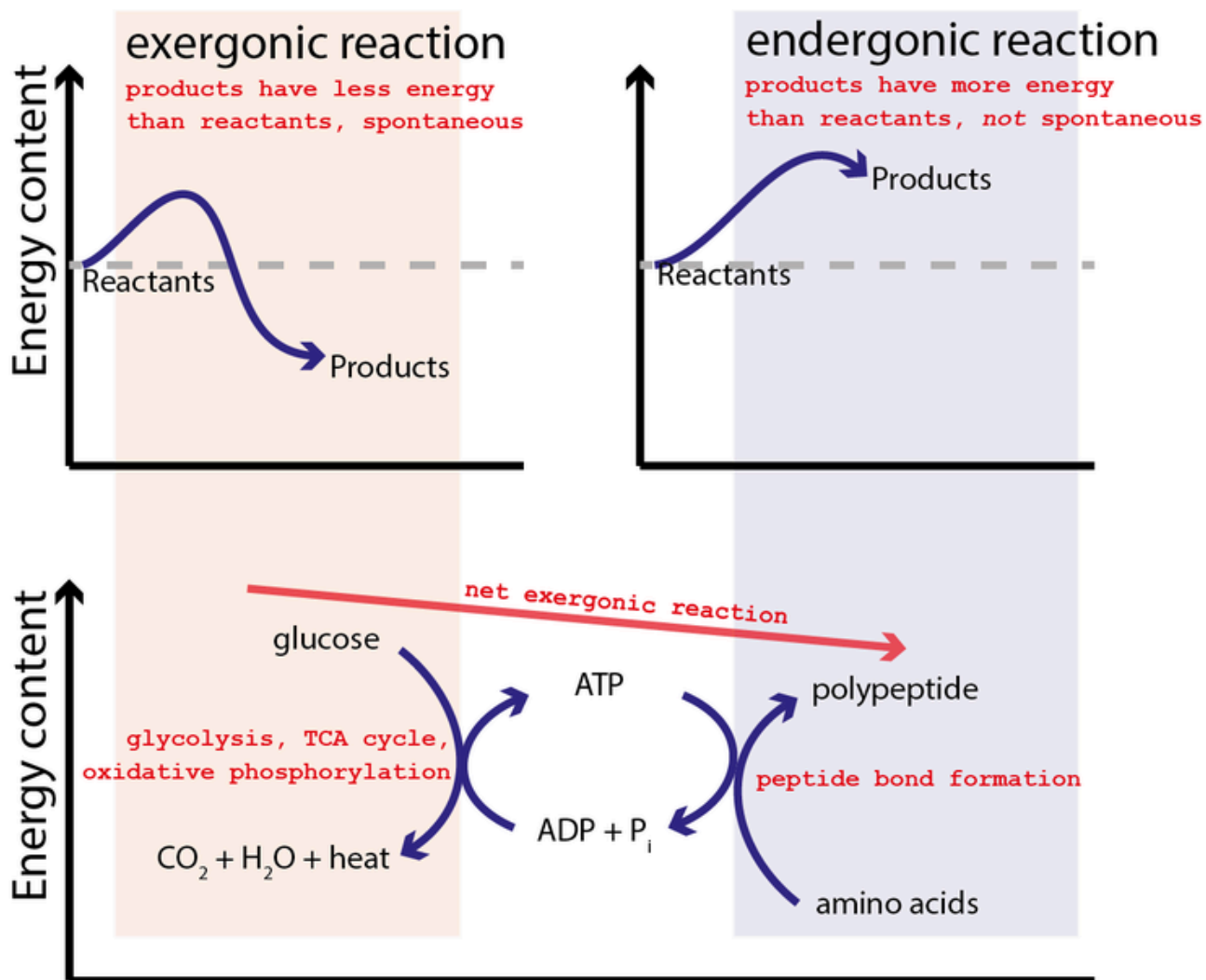


Figure 3.3 A reaction will not proceed spontaneously unless the products of the reaction have lower energy than the reactants: This is called an exergonic reaction. A reaction where the products have higher energy than the reactants (endergonic reaction) can only proceed when there is an input of energy. In the graph below we have an exergonic reaction (the metabolism of glucose to produce ATP) coupled to an endergonic reaction (synthesis of a polypeptide from amino acids) via the hydrolysis of ATP. ATP is used as a universal energy currency in cells to drive unfavourable, endergonic reactions. Image: 'Coupled Reactions' by Petrucci et.al from Libretext Chemistry used under CC BY-NC-SA 4.0.



Living systems couple exergonic reactions and endergonic reactions to allow unfavourable endergonic reactions to occur. Exergonic reactions release free energy that fuels endergonic reactions.

Homeostasis

Homeostasis (from the Greek words for same and steady) in essence means a maintenance of stability. In biological systems, all living organisms actively use processes to maintain the internal conditions necessary for survival. So, while living systems may appear to be constant – in other words, in a steady state – they require continual work (energy input) to maintain, and they are never actually at equilibrium.

The processes that control the internal conditions of an organism are driven by **negative feedback loops**. For example, many organisms need to maintain a relatively constant internal temperature. Any change in external conditions that leads to a change in body temperature triggers a process to counteract that change – in humans that process might be shivering if externally it is colder than internally or sweating if it hotter. These kinds of homeostatic processes are involved in maintaining all of the key conditions in organisms: pH, nutrient levels, electrolyte concentrations and so on.

Though we can understand homeostasis at the broad physiological level when we think of something like body temperature in humans, ultimately there is a cellular and molecular basis for homeostasis. This differs from process to process but there is some commonality. All homeostatic control mechanisms can be likened to a simple machine and have at least three interdependent components: a sensor, a control centre and an effector (see Figure 3.4).

For example, the levels of a physiological variable like blood glucose need to be maintained within an optimal range despite food intake and physiological status. If glucose levels rise above this optimal range, receptors in the body are activated (sensors), and these receptors in turn stimulate the pancreas (control centre) to release insulin (effector). Insulin stimulates the liver to take up blood glucose and store it as glycogen (a polymer of glucose monomers), thereby reducing blood glucose levels to within the optimal range and maintaining homeostasis.

One of the major goals of biochemistry is to understand the molecular mechanisms that underlie homeostasis by mechanistically characterising these components; that is, determining how they fit together and work. Being able to understand and identify negative feedback loops is key to understanding the majority of cellular processes.

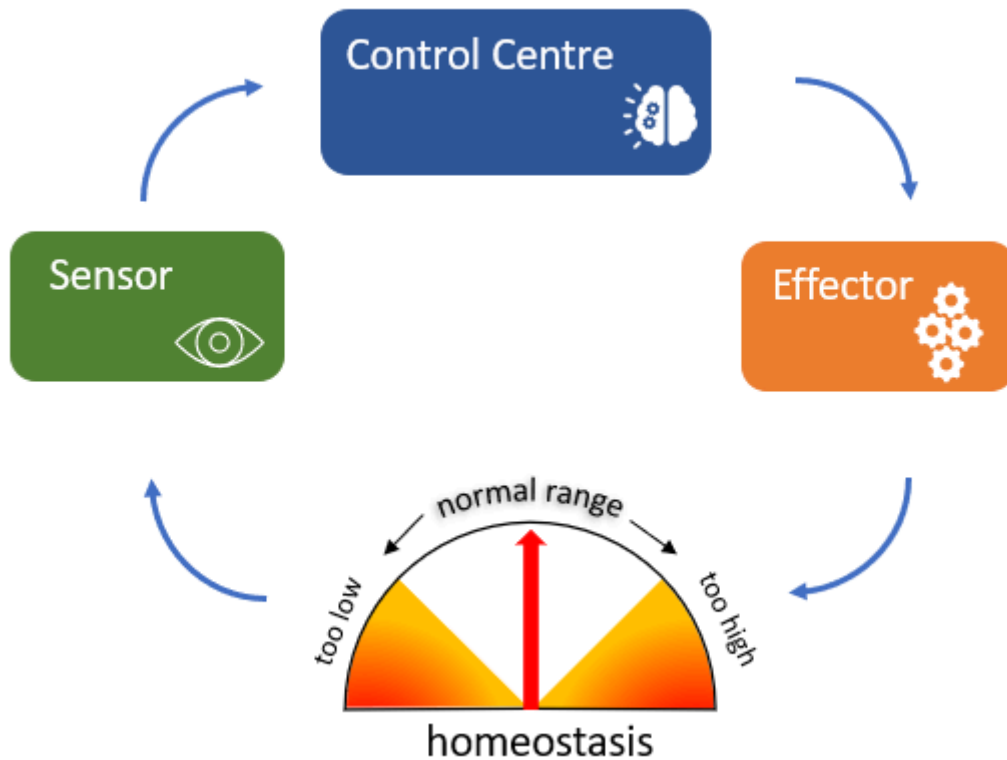


Figure 3.4 The general mechanism for maintaining homeostasis: A variable is monitored by a sensor and if it deviates from a normal or optimal range, which is determined by the control centre then a response is initiated via the control centre to an effector which creates the response required to maintain homeostasis.



All living things exhibit 'homeostasis', which is the ability to maintain a steady internal environment regardless of their external environment. Homeostasis is maintained by negative feedback loops.

Growth, reproduction and evolution

The final key characteristics shared by all life are growth, reproduction and evolution. You might wonder why these are grouped as such, but you will see that they are very much intertwined.

Growth by itself cannot be a defining characteristic of life as many non-living things grow, such as mountains or crystals. These non-living things generally grow by accretion – material is accumulated on their outside over time. Living things, on the other hand, grow from internal

processes. This growth (unlike simple accretion of matter) is usually characterised by some transformative change, which we call **development**.

The twin characteristics of growth in living organisms are an increase in mass and an increase in the number of individuals (**reproduction**). In multicellular organisms, growth occurs by cell division, which may occur continually through the life of the organism (e.g. in many plants) or only up to a certain age (e.g. in most animals). Unicellular organisms such as bacteria also grow by cell division, so cell division is their means of reproduction (growth).

In the majority of higher ordered multicellular organisms, growth (increase in mass) and reproduction (increase in number) are mutually exclusive events and cell division is the means of tissue growth and maintenance. To slightly complicate things, cell division and cell growth are not always the same thing in all organisms. It is possible that cells divide repeatedly without exhibiting any growth. This is seen in very early embryo development of a number of organisms where the fertilised egg cell simply partitions itself into many cells without increasing mass.

Unicellular organisms and individual cells within multicellular organisms cannot simply divide ad infinitum (forever), otherwise they would become smaller with each division! The individual cells must increase in size and alter their composition to prepare for further cell division. So even individual cells demonstrate growth and development. The processes governing the growth and development of individual cells is known as the **cell cycle**. This is a repeating series of processes where cells acquire materials, synthesise molecules from them and partition these materials into two daughter cells.

So, if all living things grow in terms of increasing mass and by development, and all living systems are capable of reproduction, how is this linked to evolution?

What is evolution?

Evolution is described in a number of different ways by different people. Some definitions are much more expansive than others and often incorporate the term **adaptation**. Charles Darwin, in his book *On the Origin of Species*, did not use the term **evolution** but instead described the process of species development as ‘descent with modification’. This is a useful definition and we can reword it to:

Evolution is the change in the characteristics of a species over several generations.

The fact that this definition mentions ‘generations’ links the changes in characteristics of species to reproduction and therefore implies that these are **heritable** changes. For a change to be heritable (able to be passed from parent to offspring) it must happen to genetic material (i.e. DNA).

This may appear initially contradictory. You might imagine that for reproduction to occur successfully, DNA must be copied faithfully and passed onto the offspring (see Chapter 4). This is largely true but at the same time, evolution would not be possible without changes to DNA. The answer to this apparent contradiction is that change does occur to DNA during reproduction, but it is limited.

There are two ways in which DNA changes: by **mutation** and, in the case of species that reproduce sexually, by **recombination**. Mutations are changes to DNA that affect its sequence. Recombination occurs during meiosis, when maternal and paternal genes are regrouped into different combinations

during the formation of gametes (sex cells). For the purposes of understanding evolution, we will only focus on mutation here.

How does DNA mutate?

For a cell to divide, first it must accurately and completely duplicate the genetic information encoded in its DNA. This is a complex problem because of the great length of DNA molecules. The cellular machinery that copies DNA is not perfect. Although it has a repair/correction function when it makes an error, a very small number of mistakes still slip through and remain coded. Plus, DNA can get damaged from things in the environment such as radiation or some chemicals.

The DNA in any cell in a multicellular organism can accumulate mutations but **the only mutations that contribute to evolution are those that occur in germ cells** (the cells that give rise to gametes – egg and sperm cells) as *these* cells contain the DNA that gets passed to the next generation. This brings up another important point – with evolution, individuals themselves do not change but instead there are changes to traits across generations.

Mutation is random!

It is important to emphasise this point as it is a source of confusion. Mutation does not occur in order to improve an organism (new traits do not arise in response to need). Mutation merely represents errors in genetic replication. Most of the changes (mutations) either do not cause any change to the characteristics of the species (they are neutral) or are in fact problematic (detrimental or deleterious). Very, very few mutations will be beneficial or advantageous in any way.



Variation among individuals is a fundamental requirement for evolutionary change. The processes that produce variation, mutation and recombination are essentially random.

So, evolution is not possible without mutation, and mutation is not possible without a good possibility of some adverse consequences.

From this we can deduce that the heritable mutation rate must be very low. If it were high, it would put an impossible ‘genetic burden’ onto the next generation. They would be stuck with too many deleterious mutations to be able to live.

Now that we have the capability to easily sequence an entire genome (all of the DNA in an organism) we know how often mutations occur in reproduction. For example, we can see that for humans, at birth, children have about 70 new genetic mutations compared to their parents. This number makes sense given the size of the human genome ($\approx 6.3 \times 10^9$ base pairs) and the known rate of mutation ($\approx 1.1 \times 10^{-8}$ per site per generation). If you multiply these numbers, you get an estimate of the number of mutations per generation.

$$\begin{aligned}
 &6.3 \times 10^9 \text{ (size of genome in base pairs)} \\
 &\times 1.1 \times 10^{-8} \text{ (rate of mutation per base pair)} \\
 &\approx 70 \text{ mutations per generation}
 \end{aligned}$$

Fortunately for us, these mutations appear randomly in our DNA sequence and most of our DNA (> 98%) does not directly affect our characteristics. Therefore most of these mutations won't be problematic for us.

How does evolution occur?

Given the low rate of mutation and the fact that most mutations won't really do anything (neutral mutations), how do species evolve? The answer is that mutation is not enough, and other processes embed mutations within a population or species.

When a gene is mutated, it becomes slightly different. We refer to the variants of a particular gene as **alleles**. When we talk about changes to the characteristics of a population or species, what we are really discussing are the changes to the frequencies of alleles (gene variants) in the population or species. The major processes that affect the frequencies of alleles are **natural selection**, **genetic drift** and **gene flow**.

Some alleles give an organism a slight advantage in a particular environmental context. This advantage will manifest as increased success at reproduction (often described in genetic terms as fitness). This process is known as **natural selection**. In natural selection, the environment exerts a pressure on a population such that individuals with certain alleles will survive and reproduce more successfully. For example, individuals with a certain trait, say insects with more similar colouration to their habitat, might be less likely to be predated on by birds than insects of the same species with colouration that contrasts their habitat. If the predatory bird is present in the environment, a selective pressure will be exerted with respect to colouration.

Genetic drift occurs because the alleles that make it into the next generation in a population are a random sample of the alleles in a population in the current generation. Just by chance, not every allele will make it through; some will be over-represented while others will decline in frequency.

Gene flow can be thought of as a variation of genetic drift. When individuals migrate from one area to another, they bring with them different allele frequencies from the existing population. If there is subsequent limited mating with the existing population, coupled with different selective pressures in the new environment, substantial change can occur, including the evolution of new species.

The effects of genetic drift and gene flow can also manifest strongly when a population experiences a bottleneck in numbers (a rapid decrease for a period).



Evolution is a two-step process: the first step (which is random) involves the generation of variant by mutation, whereas the second step (natural selection, genetic drift, gene flow) determines which randomly generated variants persist into the next generation.

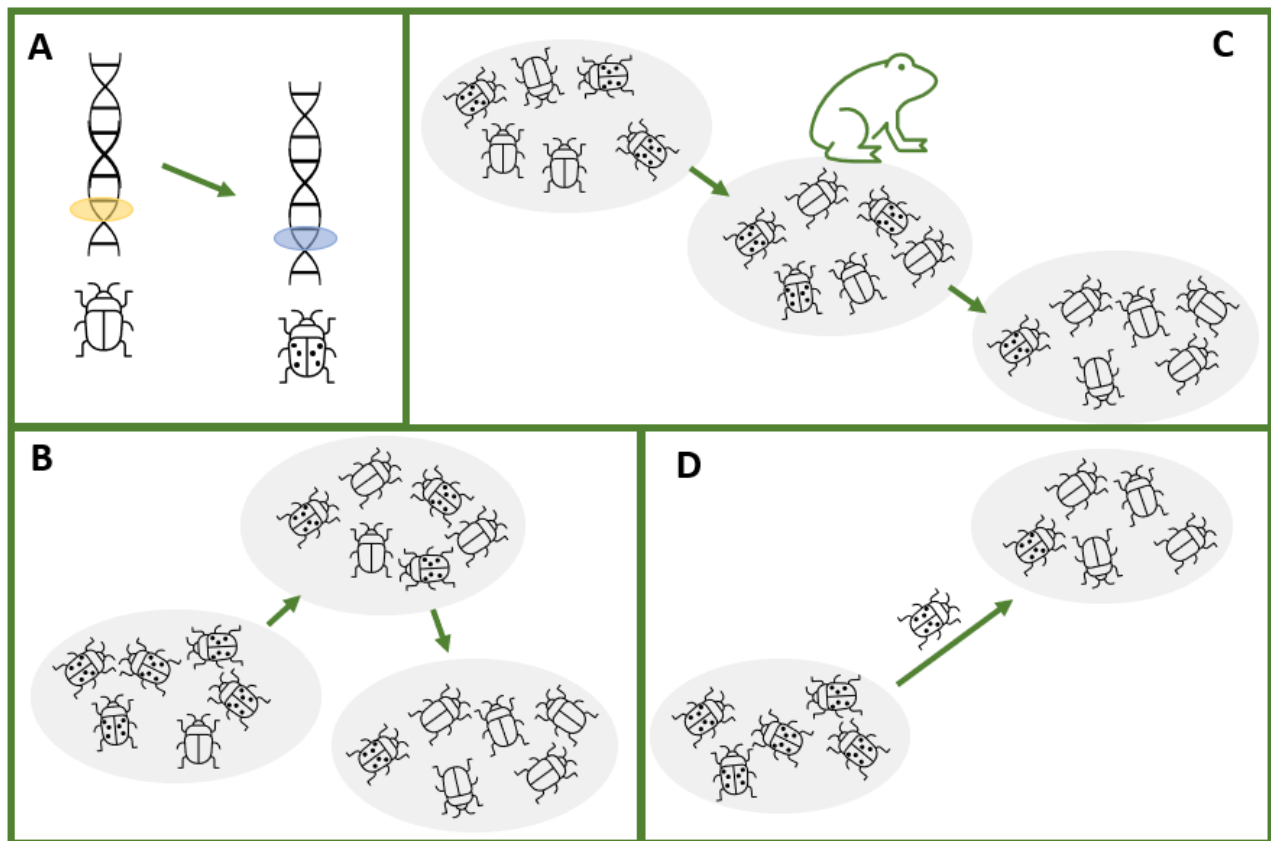


Figure 3.5 The major mechanisms underpinning evolution: A. Mutations (changes to DNA sequence) are a random process that can lead to variant alleles and changes to characteristics. B. Genetic drift occurs to the random distribution of alleles which occur over time. C. Natural selection is occurs where an allele provides a reproductive advantage in a particular environment. This causes a change in the distribution of the allele in response to a differentially selective environmental pressure (in this case predation). D. Gene flow cause a change in the distribution of alleles in a population due to migration.

How did life on Earth arise?

The origin of life on Earth represents a conundrum, which is that on Earth today, all life comes from pre-existing life. But in principle life must have at some point arose abiotically (from non-living matter). We have evidence for life on Earth from around 3.5 billion years ago. The Earth is 4.6 billion years old, so it took perhaps a billion years for whatever chemical evolution took place to result in biological life.

Earlier ideas that microbial life ‘spontaneously generated’ from nutrient broth have been shown to be incorrect by careful experiment. So far, researchers have been unable to create a living and self-replicating cell from organic molecules, although a number of experiments have demonstrated that given the right conditions, the organic building blocks of life, such as amino acids, can form from inorganic precursors.

The problem is that all life contains complex macromolecules that are polymers (DNA is a polymer of nucleotides and proteins are a polymer of amino acids) but in extant cells, polymer formation is catalysed by enzymes which are proteins and therefore polymers themselves! There is some evidence

that under the right conditions polymers such as RNA can spontaneously form from monomer precursors.

Even if polymers can spontaneously form, this leaves another problem. How could these polymers be self-replicating? The polymer would need to be both the hereditary molecule and make itself by being its own catalyst.

The RNA world hypothesis

This is problematic for DNA as there are no known natural DNA molecules that display catalytic activity. RNA, on the other hand, might be a better candidate. There are two differences that distinguish DNA from RNA. RNA contains the sugar ribose, while DNA contains the slightly different sugar deoxyribose (DNA's sugar component lacks one oxygen atom compared to RNA's), and RNA has the base uracil while DNA has thymine. These small differences in structure allow RNA to take on a much wider range of conformational structures than DNA.

Lending weight to the idea that RNA may have been the ancestral polymer of life was the discovery that RNA, because it could take on many different conformational structures, could also be catalytic. This discovery led to a Nobel prize in 1989 for US scientists Thomas Cech and Sidney Altman, who demonstrated that some RNA molecules can catalyse their own cleavage.

Catalytic RNA molecules are known as **ribozymes**. Ribozymes play universal and central roles in cellular information processing. The ribosome is a large complex of RNA and proteins that reads the genetic information in a strand of RNA to synthesise proteins. The key catalytic activity of the ribosome, the formation of peptide bonds to link two amino acids, is catalysed by its RNA component. The ribosome is actually a ribozyme!

With the possibility of catalytic RNA molecules, a single molecule or family of similar molecules could potentially store genetic information and replicate themselves, with no proteins needed initially. The discovery of ribozymes led to the formulation of the **RNA world hypothesis**. In this hypothesis, populations of catalytic RNA molecules undergo a molecular evolution conceptually identical to biological evolution by natural selection. RNA molecules would make copies of each other, making mistakes and generating variants. The variants that were most successful at replicating themselves would increase in frequency in the population of catalytic RNA molecules.

At some point in the lineage leading to our last universal common ancestor, DNA became the preferred long-term storage molecule for genetic information. This is probably because DNA molecules are more chemically stable than RNA (deoxyribose is more chemically inert than ribose). Also, having two complementary strands means that each strand of DNA can serve as a template for replication of its partner strand, providing some innate redundancy. These and possibly other traits gave cells with a DNA hereditary system a selective advantage so that now all cellular life on Earth uses DNA to store and transmit genetic information.

However, this is still just a hypothesis, and a different idea with many adherents is that metabolism in the form of self-sustaining networks of metabolic reactions may have come first. In this hypothesis, initially simple pathways might have produced molecules that acted as catalysts for the formation of more complex molecules. Eventually, the metabolic networks might have been able to build large molecules such as proteins and nucleic acids.

Regardless of the theory put forward for the initial self-replicating system, there are still many other questions. For example, a basic property of all cells is the ability to compartmentalise and maintain

an internal environment distinct from the external environment. This is facilitated by encapsulation by a lipid membrane. How this first encapsulation took place and what lipids may have comprised the ancestral membranes remains a major question.

The oldest fossils



Australia can claim the oldest fossils on Earth. These are found in Archean rocks from near Marble Bar in Western Australia. These fossils, known as stromatolites (Greek for layered rock), are layered sedimentary formations created by microorganisms and dated as approximately 3.45 billion years old. This is remarkable given that the oldest rocks on Earth date only fractionally older at 3.8 billion years. Initially, the idea that these fossils represented ancient life was controversial but recent isotopic analysis revealed that they were characteristic of biological matter.

During the time these fossils were formed the Earth's atmosphere did not contain very much oxygen. Oxygen started appearing around 3 billion years ago and became more abundant around 2.5 to 2.3 billion years ago. It is speculated that these very early microorganisms may have used simplified photosynthesis that produced methane rather than oxygen. Eventually, species evolved like cyanobacteria (confusingly, sometimes called blue-green algae) which through photosynthesis fundamentally changed the Earth's atmosphere by producing oxygen.

Australia is also one of the few places where you can find living versions of these stromatolites, in the shallow waters of Shark Bay, Western Australia. These living stromatolites are formed by photosynthetic cyanobacteria, and they provide us with a glimpse of what life may have been like for much of Earth's biological history. These were the dominant fossil-forming organism for much of the Archean (4 billion to 2.5 billion years ago) and Proterozoic (2.5 billion to 541 million years ago) eras. Living stromatolites are made by a complex community of microbes and suggest the possibility of a similar complex community producing the ancient fossil stromatolites. If this is the case, it would mean that life first evolved well before 3.5 billion years ago!



Figure 3.6: Living stromatolites near Shark Bay, Western Australia, one the few places on Earth where these can be found. These calcareous deposits are deposited by gradual accretion from microbial communities, predominantly photosynthetic cyanobacteria. They give us a glimpse of what life might have looked like for much of Earth's biological history. Image: 'Shark Bay stromatolites' by Alicejmel from Wikimedia Commons used under CC BY-SA 4.0.

3.2 The cellular Basis of life

As mentioned above there is a cellular basis to all life. All living things are either single cells themselves or multicellular (made up of cells).

Why are cells the fundamental unit of life?

All the processes that characterise life – metabolism, homeostasis, growth and reproduction – can only exist within the confines of cells. So, defining a perimeter and confining all processes within it is a fundamental aspect of the organisation that allows life to exist. We call this aspect of organisation **compartmentalisation**. All cells define their perimeter by a cell membrane. Without an intact cell membrane, the cell has no organisation and cannot live. Many cells, including our own, exhibit further internal compartmentalisation and confine specialised processes and functions to areas defined by internal membranes.

Things common to all cells (suggesting a common ancestry)

Although all life is either cells or made up of cells, the cells themselves show incredible diversity in size, shape and function. However, before focusing on the differences between cells it is worth thinking about what they have in common. There are four components that all cells share:

1. **All cells have a plasma membrane.** This is an outer covering that separates the cell's interior from its surrounding environment. The membrane is primarily composed of phospholipids. It not only defines the perimeter of the cell and contains the cell's components, it also provides a way for the cell to interact with its environment in a controlled manner. The plasma membrane allows the selective passage of molecules into or out of the cell.
2. **All cells have a cytoplasm.** The cytoplasm is a gel-like fluid inside the cell (comprised of water, salts and typically a surprisingly high concentration of macromolecules) that provides a medium for chemical reactions. Diagrams of cells often give the wrong impression of cytoplasm as a simple bag of water. In reality, the cytoplasm is crowded with macromolecules. The cytoplasm of the bacteria *Escherichia coli* contains 300–400 mg/ml of macromolecules (see Figure 3.7). This makes the cytoplasm viscous and affects the properties of all of the components in a cell. For instance, the high concentration of macromolecules reduces the volume of water available for other molecules, effectively increasing their concentration.

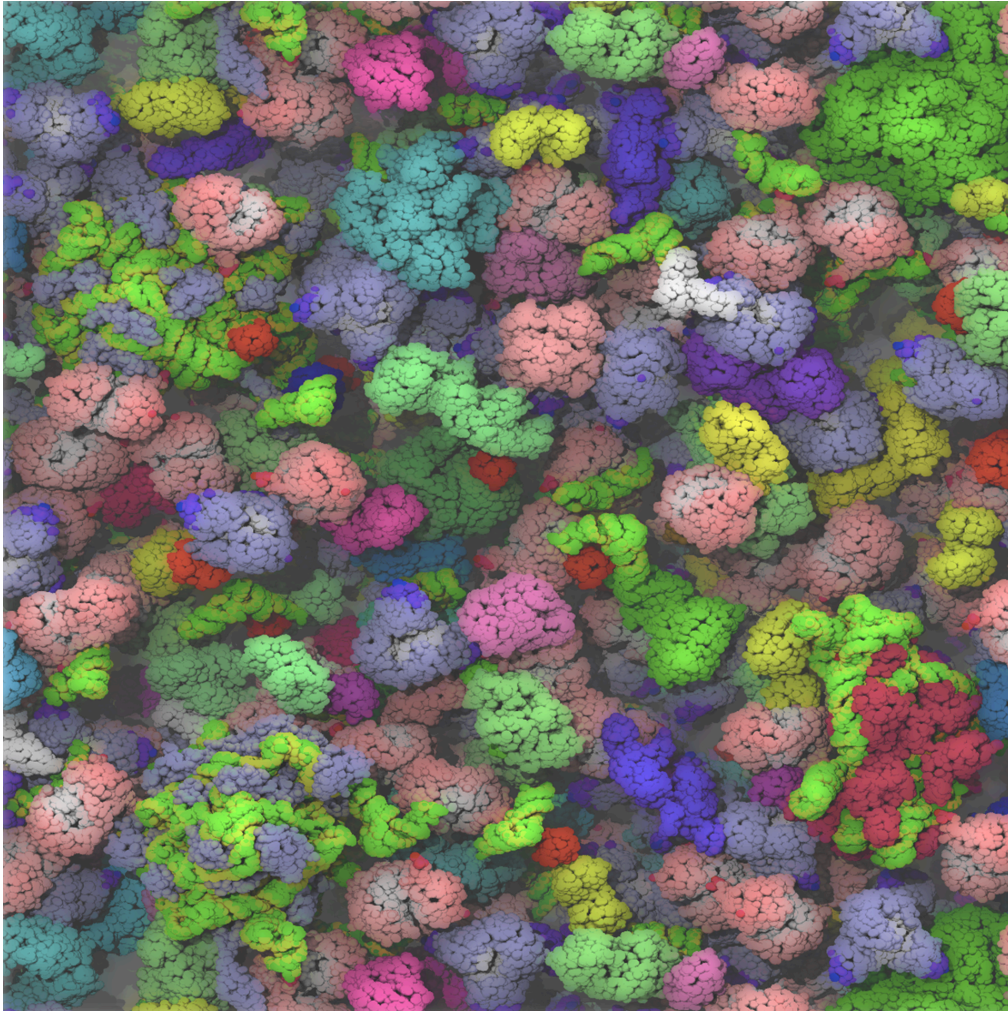


Figure 3.7 A computer generated image modelling the cytoplasm of the gram-negative bacterium, *Escherichia coli*: This image, which would be similar for the cytoplasm of any cell, shows the cytoplasm to be very densely packed with macromolecules, and a highly crowded environment. This accurate depiction differs from many text-book representations of this environment. (McGuffee, S. R. and A. H. Elcock (2010, March). Diffusion, crowding & protein stability in a dynamic molecular model of the bacterial cytoplasm. *PLoS Computational Biology* 6 (3), e1000694+.) Image: The cytoplasm model by McGuffee SR, Elcock AH from Diffusion, Crowding & Protein Stability in a Dynamic Molecular Model of the Bacterial Cytoplasm used under CC BY 4.0.

3. **All cells use DNA as their genetic material.** In fact, all cells store their hereditary information in the same form of double-stranded DNA using the same four types of nucleotide monomers (adenine, thymine, cytosine and guanine or A, T, C and G). All cells use the same mechanism to replicate their DNA, when they grow and reproduce, of semi-conservative replication where each strand acts as a template for the new strand. All cells transcribe portions of their DNA into RNA, which acts as an intermediary species for the production of proteins (some RNA is functional in its own right; e.g. ribosomal RNA). And all cells use the same four types of nucleotide monomers (adenine, uracil, cytosine and guanine) to make the RNA polymer. Furthermore, the genetic code (the set of rules defining how the four-letter code of DNA is translated into the 20-letter code of amino acids making up proteins) is nearly universal with only a few exceptions reported (see Chapter 4).
4. **All cells contain ribosomes, which are the sites of protein synthesis.** Often the ribosome is described as an organelle, but it is not enclosed by a membrane and better thought of as a macromolecular machine. Ribosomes are composed of RNA (approximately 60%)

and protein (approximately 40%). While ribosomes can differ between species, the core of the ribosome and how it functions is the same in all living systems.

These shared characteristics of all cells, particularly the use of the same genetic material, a near universal genetic code and the ubiquitous use of ribosomes for protein synthesis, point to all life on Earth sharing a common ancestor. This last universal common ancestor (LUCA) of all cells is thought to have lived approximately 4 billion years ago.



All cells have a plasma membrane and cytoplasm, use DNA to store genetic information and almost universally use the same genetic code, and use ribosomes to synthesise proteins.

The evolutionary relationship between organisms is known as **phylogeny**. Since all life uses the same hereditary DNA molecule and common processes (e.g. protein synthesis), it is possible to use differences in the DNA sequences of common genes to establish how closely related species are to one another. Since cells all use ribosomes, and all for the same purpose of protein synthesis, the structure of ribosomes changes very slowly over evolutionary time. This makes them a useful way to measure evolutionary relationships over long periods. Figure 3.8 shows a phylogenetic model (a phylogenetic tree) based on comparing the sequences of ribosomal RNA (rRNA) from many species. This tree places all life in three distinct domains – bacteria, archaea and eukarya – with our last universal common ancestor at the bottom. The lineage of eukaryotes is more complicated as they are almost certainly the result of two or more cells merging in a process called **endosymbiosis** (see Chapter 4).

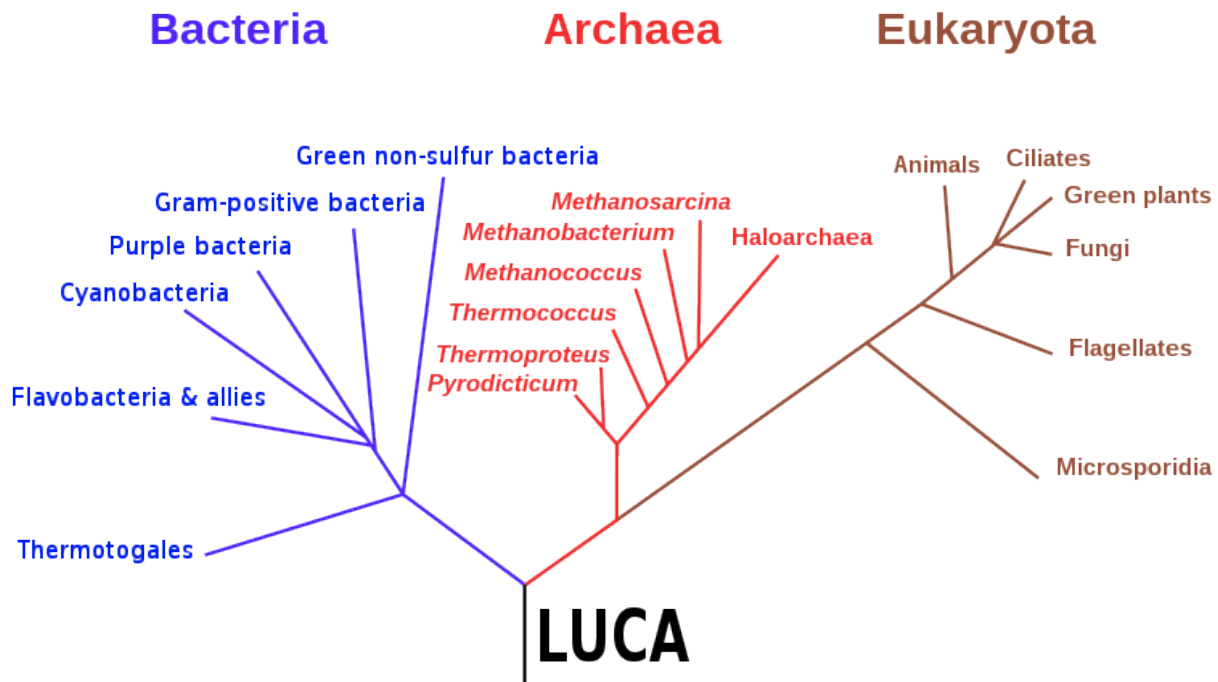


Figure 3.8 The three-domain model of life based on comparing ribosomal RNA sequences between species: This model was proposed by Carl Woese, Otto Kandler, and Mark Wheelis in 1990. There is growing evidence that eukaryotes may have originated within a subset of archaea. In any event, it is accepted today that there are three distinct domains of organisms in nature. Image: 'Phylogenetic tree of life 1990 LUCA' by Chiswick Chap from Wikimedia Commons used under CC BY-SA 4.0.

Despite this lineage as described, cells are classified into two broad groups: **prokaryotes** and **eukaryotes**. Bacteria and archaea are all prokaryotes. Typically, prokaryotes are all unicellular organisms (though some species are capable of forming colonies). Eukaryote cells are in the domain eukarya. Eukaryotic cells are much larger than prokaryotic cells (typically 10 to 100 times bigger) and can be either unicellular or multicellular.

However, the major difference between eukaryotes and prokaryotes is that eukaryotic cells have internal compartmentalisation and prokaryotes do not. The internal compartmentalisation of eukaryotes consists of internal membrane structures that enclose particular cell constituents and processes. These structures are known as **organelles**.

The most obvious difference in this respect is the presence of a **nucleus** in eukaryotes and the absence of one in prokaryotes. The nucleus is a membrane-bound organelle that functions as the site of DNA storage of DNA. Prokaryote literally means 'before the nucleus' (*pro* means before and *karyon* means nut or kernel).

Eukaryotic cells have several other membrane-bound organelles located within their cytoplasm that are not found in prokaryotic cells. The major ones are the **mitochondria** (an important site for energy production); rough and smooth **endoplasmic reticulum** (an interconnected network of membrane-enclosed tubules that transport synthesised proteins); **Golgi complex** (for protein secretion); and in the case of plant cells, **chloroplasts** (which conduct photosynthesis).

Compartmentalisation in eukaryotes has some functional implications. Due to compartmentalisation, cell division in eukaryotes is a much more complicated and regulated process (mitosis) while prokaryotes can divide by simple binary fission. The presence of a nucleus in eukaryotes means that

transcription (which occurs in the nucleus) is decoupled from translation (which occurs in the cytoplasm). In prokaryotes these two processes occur simultaneously.



Eukaryotes have internal membranes (compartmentalisation) including a nucleus while prokaryotes do not. This has functional implications for many processes, including cell division and gene expression.



Discussion questions

1. What happens to an organism that reaches a state of thermodynamic equilibrium?
2. Many chemical reactions in the cell are energetically unfavourable, such as synthesising nucleic acid polymers from nucleotide monomers. How do unfavourable chemical reactions occur in the cell?
3. Exobiologists are researchers who seek life outside the Earth. Therefore, they need a definition of life that determines what they will look for. In 1994, NASA scientists involved in their exobiology program proposed the following definition: 'Life is a self-sustaining chemical system capable of Darwinian evolution'.
 - a. This appears to be a simple definition. Which of the characteristics common to all life does it support?
 - b. Can you think of any entity generally agreed upon to be living that is excluded by this definition?
4. Do you think that NASA's definition is practical for exobiologists seeking life outside the Earth? In 2013, Azua-Bustos and Vega argued that regardless of the types of life on Earth or that might be found elsewhere in the universe, all life should share the attribute of decreasing internal entropy at the expense of free energy obtained from their surroundings. How could this be practically measured?
5. A human baby acquires, on average, approximately 70 random mutations to its genomic DNA compared to its parents. Why do the vast majority of these mutations have no phenotypic effect?
6. Homeostatic mechanisms are controlled by negative feedback loops. What are positive feedback loops? Can you think of one example of a positive feedback loop that would be deleterious to an organism and one that might be beneficial to an organism?
7. Explain why the following statement is incorrect.

'As animals evolve, they adapt to their environment. If they encounter a problem such as

increased toxic chemicals in their environment, they evolve new mechanisms to mediate the toxicity by changing their DNA. Changing their DNA ensures that their offspring will be resistant too.'

Chapter 4: Information flow through organisms

4.1 What is a gene?

On planet Earth there was a living being, and in the living being there was a cell, and in the cell there was a nucleus, and in the nucleus there were chromosomes, and the chromosomes were made up of DNA, and DNA was made up of an alphabet that produced life ...

A **gene** is a section of DNA made up of sequences of the four nucleotide monomers adenine (A), thymine (T), cytosine (C) and guanine (G). It is the instruction to make a single product, such as a protein. Genes might seem like they are a jumble of monomers, but amazingly just those four ‘letters’ – A, T, C and G – encode all living things in DNA. This same short alphabet in combination is used by you (a human), the mosquito biting your leg, the bacteria on the mosquito’s leg and the virus inside the mosquito. And can you believe that the tiny mosquito has almost as many genes as you do?

4.2 Genotype vs phenotype

The genotype of an organism is the genes it has. You can't see them, and they are inherited from the parents of the organism (if sexual reproduction occurs). The phenotype is what results when the genes are expressed; for example, you have the genes (genotype) for attached earlobes, and the phenotype is you physically having attached earlobes. Some phenotypes can be seen with the naked eye, like hair and eye colour, while others are obscured, like blood type. Phenotypes are determined by genotype but can also be influenced by the environment (this is called epigenetics). For example, you might have inherited a predisposition for high cholesterol from your parents, but your diet can affect your cholesterol levels as well.

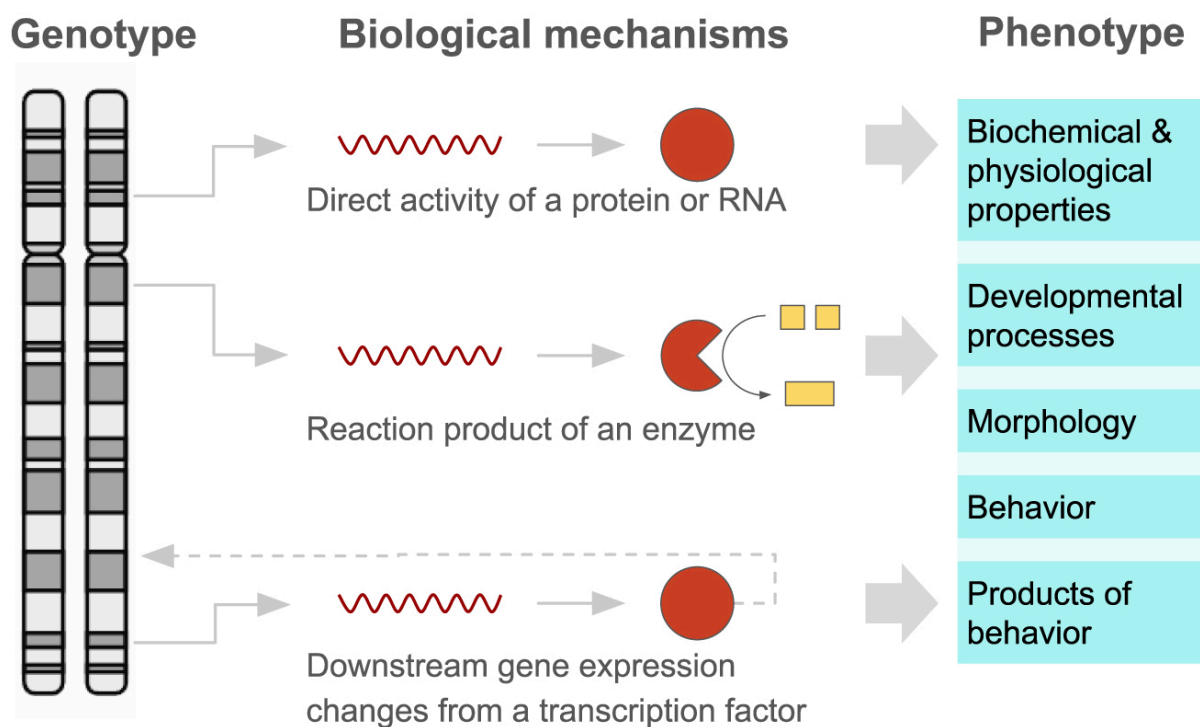


Figure 4.1 Genotype to phenotype: The sum of an organism's genetic material along with its environment determine its phenotype. This is mediated via various biological mechanisms: either the direct activities of gene products or their downstream effects. Illustration by Thomas Shafee.



Phenotypes are determined by genotype but can also be influenced by the environment.

4.3 The flow of biological information

Information flows from genes to proteins but not in reverse.

The gene expression factory

You can think of a cell as a factory. In the middle of the factory is the head office (nucleus) with the instructions to make everything in the factory, including the machinery (proteins), workers (proteins), and even some other rooms (organelles). When something new needs to be made, the big manual of instructions (DNA) in the head office is sorted through and the specific instructions (one or more genes) are found. The instructions are not allowed to leave the head office, so must be photocopied (**transcribed**) into a single copy (**messenger RNA** or mRNA). This single copy leaves the head office, heads out to the factory floor (cytoplasm) and is read by a machine (ribosome). The ribosome translates the photocopy into a different language entirely – a chain (of amino acids) – and when this **translation** is finished the chain can contort into a new shape and becomes a functioning piece of machinery or worker that might be used in the factory or in a neighbouring factory (**exported**) or back in the head office (nucleus).

This analogy explains **gene expression**, when we go from the original gene to a functional ‘product’. Watch this video for a detailed visualisation of the process from DNA to protein.

From DNA to protein – 3D



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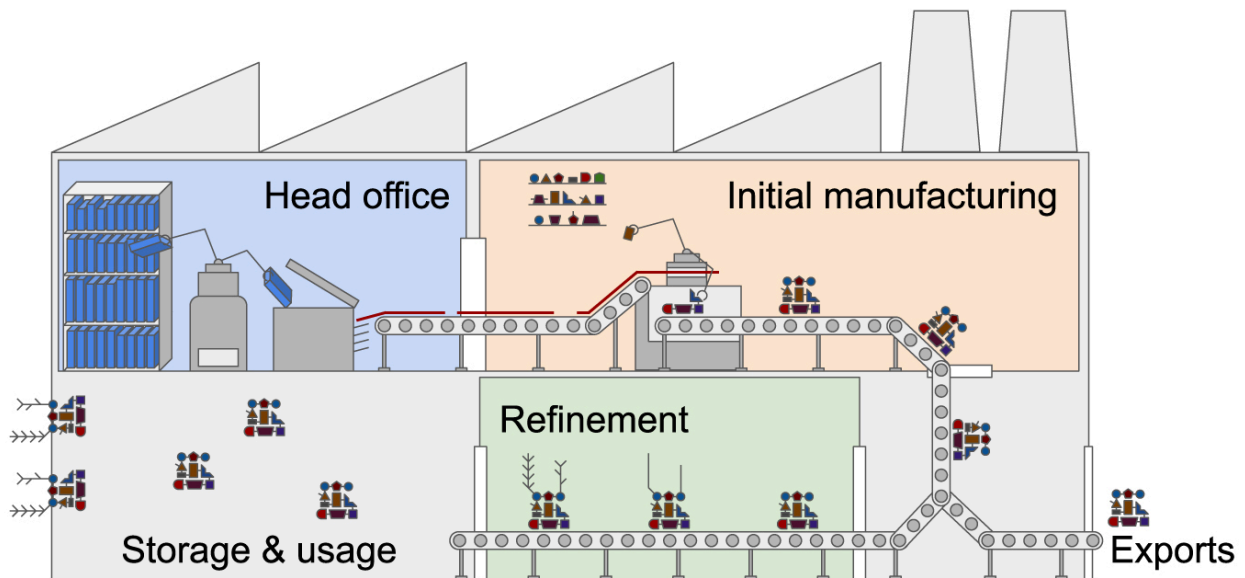


Figure 4.2 The cell as a factory: In some ways, a cell is like an automated factory. Instructions (DNA) stored in the head office (nucleus) are photocopied (transcription), with the instruction copy (mRNA) sent to initial manufacturing (endoplasmic reticulum) to be used as the blueprints for building (translation) a specified product (protein). Sometimes this product undergoes further refinement and processing (Golgi), with final products being stored, used in the factory itself or its walls, or exported outside. Illustration by Thomas Shafee.

DNA vs RNA

There are two main differences between deoxyribonucleic acid (DNA) and ribonucleic acid (RNA):

1. DNA is normally double stranded and RNA is normally single stranded (in humans anyway; other organisms are different).
2. DNA is made up of adenine (A), thymine (T), cytosine (C) and guanine (G) bases. Each base is attached to a phosphate and a sugar molecule, which together make a nucleotide. RNA is made up of A, C and G nucleotides and instead of thymine, it has uracil (U).

In DNA, A always pairs with T, and C always pairs with G (the straight letters stick together and the curvy letters stick together) to create a **base pair**. In RNA, A always pairs with U. These pairings are how complementary strands 'stick together' and how by looking at only one strand you can replicate the DNA (if you want to pass it onto a daughter cell) or transcribe it into RNA.

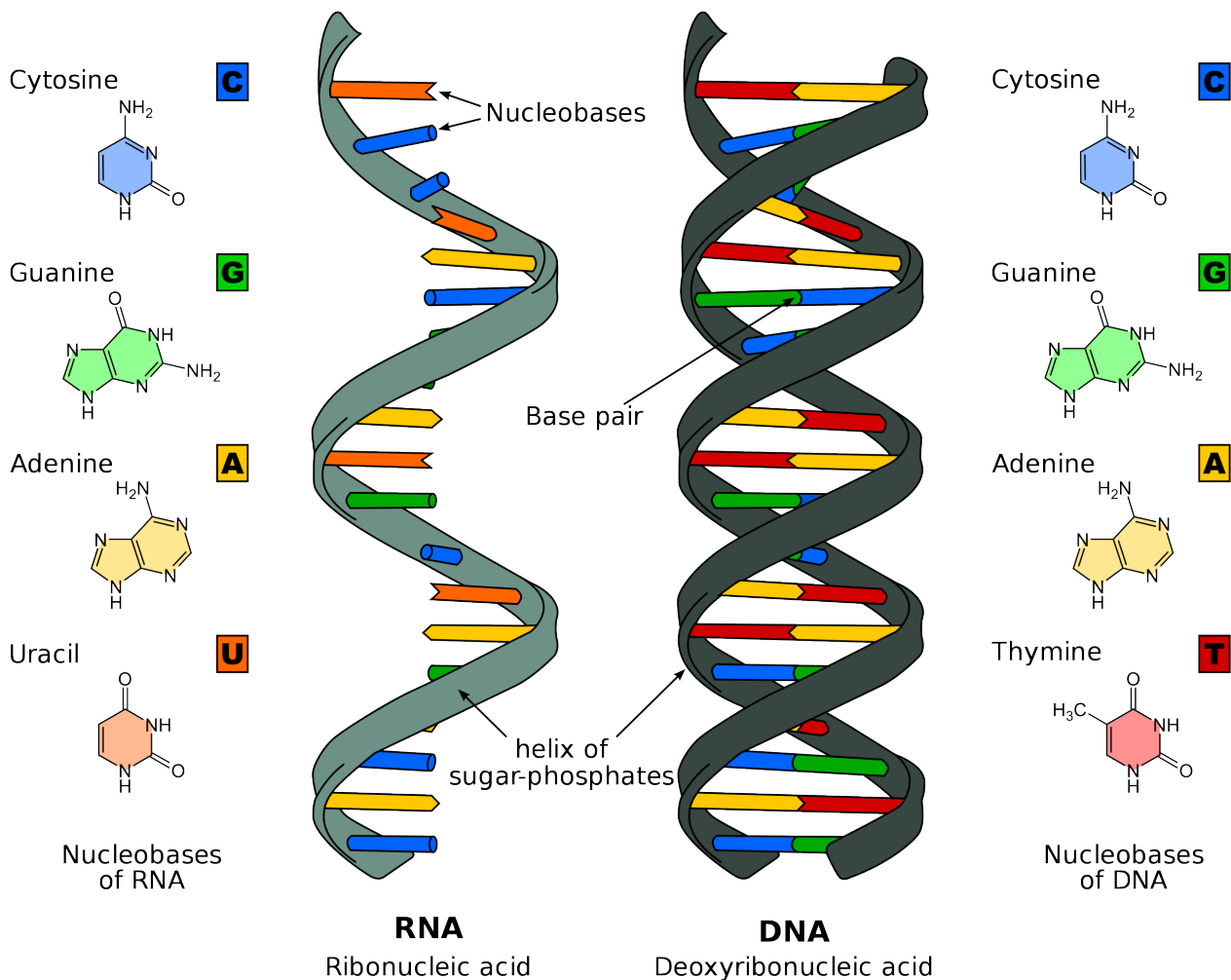


Figure 4.3 The nucleotides of RNA and DNA: RNA and DNA are commonly shown as ribbons, where the sugar-phosphate backbone forms a helix and the nucleobases point inwards. RNA and DNA share three of their bases: adenine, cytosine and guanine. The fourth base is uracil for RNA and thymine for DNA, which differ by a single methyl group. In the double helix of DNA, the base-pairing of these nucleotides (A-T and C-G) hold together the familiar double helix structure. Image: Difference DNA RNA-EN by sponk from Wikimedia Commons used under CC BY-SA 3.0.

Transcription

There is different machinery involved in the process from DNA to protein. During transcription, RNA polymerase finds the gene that needs to be transcribed and unwinds ('unzips') the double DNA strand at this location (Figure 4.4). The RNA polymerase reads the template strand of the DNA and brings in the necessary nucleotides to build a complementary mRNA strand (with uracil instead of thymine) (Figure 4.5A). When it has finished transcribing the gene (following cues from within the DNA code), the RNA is released and the RNA polymerase 'zips' the double-stranded DNA back together. The mRNA requires some processing before it can leave the nucleus: introns (segments of DNA or RNA that do not code for proteins) are removed through splicing, the poly-A tail (a long sequence of 'AAAAAA') is added to the 3' end (three prime end) to increase the stability of the molecule and a methylated 'cap' is added to the 5' end (five prime end) to help initiate protein synthesis and protect the mRNA from being degraded. In the opposite of what you might think from the names, introns are removed and exons (segments of DNA or RNA containing coding information) stay in, because intron is derived from 'intragenic' (inside a gene) and exons are 'expressed'.

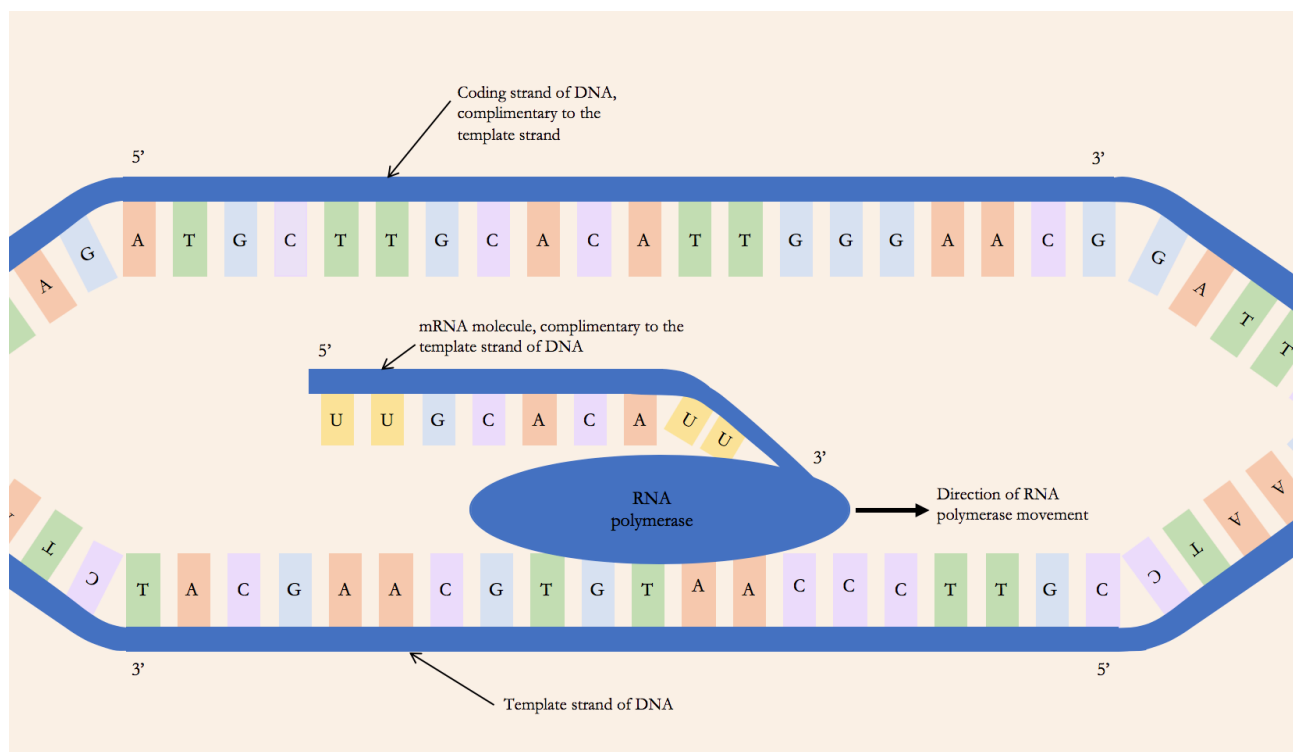


Figure 4.4 Transcription by RNA polymerase: RNA polymerase transcribing the template strand of DNA to produce an mRNA molecule, complementary to the template strand of DNA. Image: RNA polymerase transcribing template strand DNA by Kep17 from Wikimedia Commons used under CC BY-SA 4.0.

Perhaps confusingly, the ‘template’ strand of the DNA is called the non-coding strand, and the non-template strand is called the coding strand. This is because the DNA non-template strand will have the same sequence as the mRNA strand transcribed from this gene, except T’s will be U’s. In Figure 4.4 you can see that the mRNA molecule has the same sequence (apart from U/T) as the top coding strand, and these are both complementary to the template strand.

‘Transcription’ outside of biology means to convert audio or speech into written text in the same language. Therefore, as DNA and RNA use the same ‘language’ of base pairs, this process is called transcription.

Translation

Now that the ‘photocopy’ of the gene has been made, the mRNA can leave the nucleus through a little pore and find a ribosome. The ribosome reads the RNA in **codons** (three nucleotides in a row) and recruits **transfer RNA** (tRNA) with a complementary anti-codon to the RNA sequence. The tRNAs have a specific anti-codon at their ‘feet’, and carry a specific amino acid on their ‘head’. Within the ribosome, when the tRNA anti-codon matches up with the codon in the mRNA the amino acid is released from the head of the tRNA and joins the growing amino acid chain. This process continues until all the mRNA codons have been matched with tRNA anti-codons and a ‘stop’ codon is reached; then the amino acid chain is complete. This amino acid chain may then undergo further processing, fold into a certain shape or join with other amino acid subunits to make a functional protein. Note that some genes do not undergo translation, they are destined to remain as RNA such as ribosomal or transfer RNAs (see Figure 4.5 B, C).

As the RNA and amino acids are in different ‘languages’, this process is called translation.

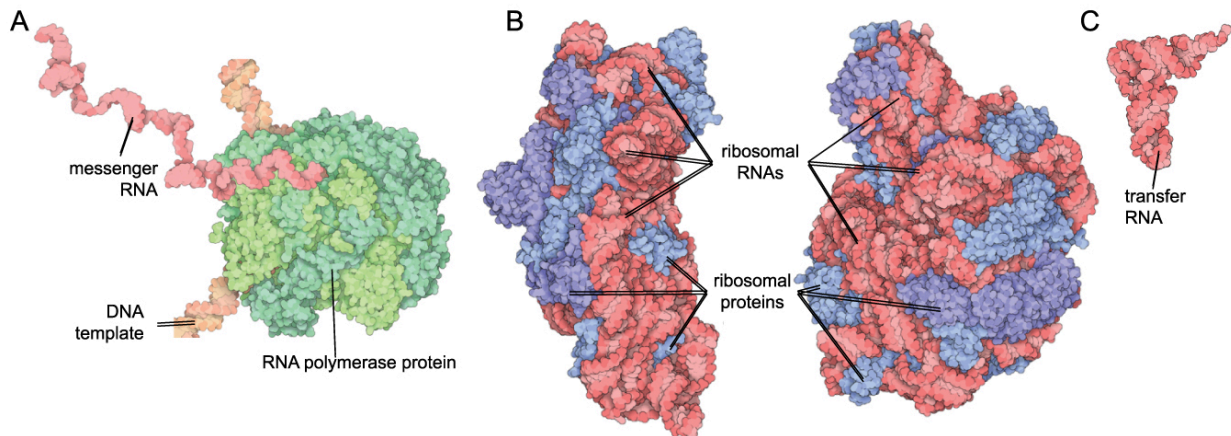


Figure 4.5 Different examples of RNA: A: Messenger RNA (mRNA) in pink is the best known type of RNA, produced as a copy of a stretch of DNA to take information out of the nucleus. B: Ribosomes contain multiple small ribosomal RNAs (rRNA) in red and multiple proteins in blue in order to translate mRNAs into their encoded proteins. C: Transfer RNA (tRNA) is a key intermediate in protein production, binding to each codon in the mRNA messenger and carrying the relevant amino acid to the ribosome. Images: Adapted from 2014: Tour of the Protein Data Bank from PDB-101 by Goodsell used under CC BY 4.0.



Transcription is copying (with some modification) DNA into RNA.
Translation is decoding RNA into protein.

The central dogma of molecular biology

The **central dogma** of molecular biology is an explanation of the flow of genetic information in a biological system. It was first stated by Francis Crick, who together with James Watson and Maurice Wilkins was awarded a Nobel prize in 1962 for explaining the helical structure of DNA. The dogma is often stated as:

DNA is transcribed to RNA which is translated to protein.

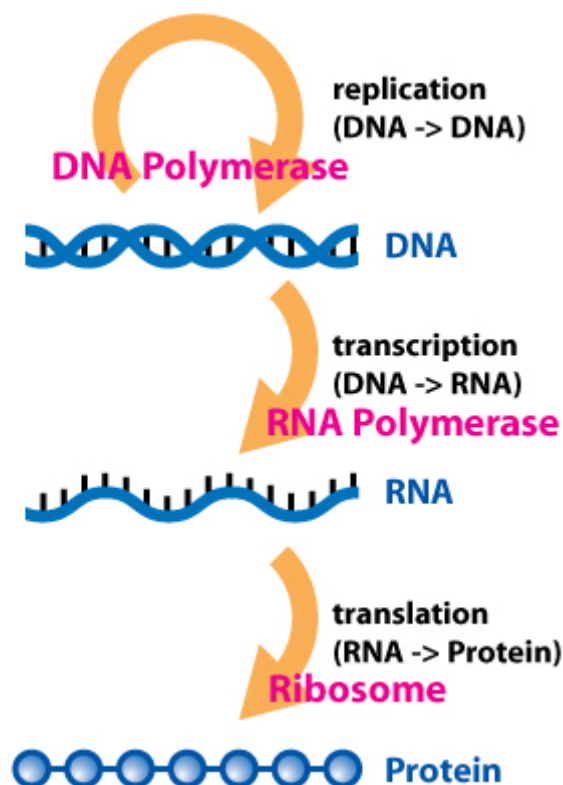


Figure 4.6 Overview of the central dogma:
 Depicting the one-way flow of biological information from DNA to RNA to protein and identifying the key enzymes and machinery involved at each step. Image: 'Central Dogma of Molecular Biochemistry with Enzymes' by Daniel Horspool from Wikimedia Commons used under CC BY-SA 3.0.

Decoding: understanding the triplet code

As we've explained, the four little letters of the DNA alphabet encode all life. Ribosomes read the mRNA in groups of three nucleotides called codons, and the specific order of the nucleotides in the codon determine which amino acid joins the chain. The amino acid codon table (Figure 4.7) shows how to determine which amino acid is encoded for by the codon in the mRNA. The next video illustrates how to use this table.

How to read a codon chart



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Second Base									
First Base									Third Base
	UUU	phe	UCU	ser	UAU	tyr	UGU	cys	
	UUC		UCC		UAC		UGC		
	UUA	leu	UCA		UAA	STOP	UGA	STOP	
	UUG		UCG		UAG		UGG	trp	
	CUU	leu	CCU	pro	CAU	his	CGU	arg	
	CUC		CCC		CAC		CGC		
	CUA	leu	CCA		CAA	gln	CGA		
	CUG		CCG		CAG		CGG		
	AUU	ile	ACU	thr	AAU	asn	AGU	ser	
	AUC		ACC		AAC		AGC		
	AUA		ACA		AAA	lys	AGA	arg	
	AUG	met START	ACG		AAG		AGG		
	GUU	val	GCU	ala	GAU	asp	GGU	gly	
	GUC		GCC		GAC		GGC		
	GUA		GCA		GAA	glu	GGA		
	GUG		GCG		GAG		GGG		

Figure 4.7 Amino acid codon table: mRNA nucleotides are read in groups of three nucleotides, called codons. This table reveals which amino acids are encoded by the nucleotide codons. The order of the first, second and third base is important for determining the amino acid it encodes. Image: 'Amino Acid Codon Table' by Scott Henry Maxwell from Wikimedia Commons used under CC BY-SA 4.0.

There are four important codons highlighted in the table to keep in mind:

- AUG is known as the **start codon**. Ribosomes know to look for it as it signals where to start translating the mRNA and also establishes the reading frame. AUG encodes for methionine, which is almost always the first amino acid in the chain but can also be inserted in other locations along the chain.
- UAA, UAG and UGA are known as **stop codons**. They do not encode for an amino acid and instead at this position the amino acid strand will be released from the ribosome and translation is complete.

Codon redundancy

Studying the amino acid codon table (Figure 4.7) we can see that there is some redundancy – for example, UCU, UCC, UCA and UCG all encode for the amino acid serine, while CGU, CGC, CGA, CGG, AGA and AGG encode for arginine. This makes sense. There are only 20 amino acids, but 64 possible combinations of the four nucleotides ($4 \times 4 \times 4$). It's remarkable that all life uses this same coding system to build proteins from the genes we inherit.

This redundancy is also useful if there is a mutation at the third base of a codon (e.g. a C mutated to an A in UCC) – the amino acid encoded would not be different; we would still get serine added to the amino acid chain. You might think a single amino acid change isn't too much of an issue, but it could have detrimental effects. A single change could alter the shape of the final protein, alter the active site of an enzyme or change the hydrophobic/hydrophilic nature of the protein so that it no longer sits in the plasma membrane. This might not sound problematic, but if the enzyme has an important job or the plasma membrane protein pumps a molecule that your cell can't live without then a small amino acid change could be fatal. Imagine if you had another accelerator pedal in your car instead of a brake. It looks like a trivial change (you have the same number of pedals), but it's likely to be seriously detrimental. It is important to keep in mind that a lot of mutations in DNA code are not 'solved' by this codon redundancy though.

Limitations to information flow

As we've discussed, DNA is transcribed into RNA and RNA is translated into protein (figures 4.8 and 4.9). If you were given a string of DNA letters, you would be able to transcribe this yourself into RNA (knowing what bases/nucleotides complement each other) and then, using the amino acid coding table, decode the order of amino acids that would result if this mRNA was translated. Note that this flow of information only goes one way. Protein cannot be turned back into RNA or DNA.

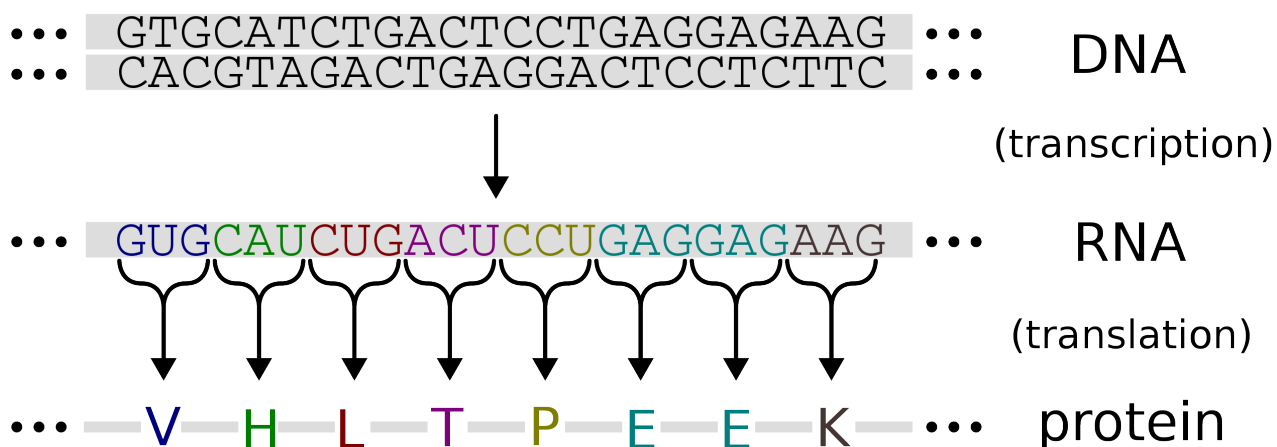


Figure 4.8 DNA to RNA to protein showing the triplet code: Double-stranded DNA is transcribed to single-stranded RNA. The RNA is read in codons (illustrated in different colours) which translates to amino acids to build a protein. This illustration is of the first few amino acids for the alpha subunit of haemoglobin (without the start codon). Image: 'Genetic code' by Madprime (Madeline Price Ball) from Wikimedia Commons.



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Protein cannot revert to RNA or DNA; biological information only flows in one direction.

4.4 Genomes

The genome is all the genetic material contained within a cell and contains the information needed for an organism to grow, mature and function. However, your genome includes more than just protein-coding genes.

How many genes are there?

The Human Genome Project, completed in 2003, identified that humans have roughly 20,000 protein-coding genes. Researchers were expecting many more protein-coding genes, but it has been revealed that about 1–2% of our DNA encodes proteins. The rest was thought to be ‘junk DNA’ that we don’t need, but it turns out there is a lot of important information hidden in this non-coding DNA. The non-coding portion of the genome includes regulatory information (what should be expressed when) and RNA components such as transfer RNA and ribosomal RNA. Some of it probably is ‘junk’, however, that has remained in our genome throughout our evolution. Or perhaps we just haven’t discovered its purpose yet.

How is DNA organised?

In 2022, it was revealed that each of your cells has 3,054,815,472 nucleotides (A, T, C and Gs) in its DNA. That equates to about 2 metres of DNA per cell! How does that fit into a nucleus that is only approximately 0.01 mm wide? A bit like how a long slinky can collapse to much smaller than its extended length, the DNA ladder rolls up, wraps around histones (little balls of protein) and supercoils its way into chromosomes. The next video shows how the supercoil works, along with other processes discussed above.

DNA animation



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Typically, humans have 23 pairs of **chromosomes**: 44 autosomes and two sex chromosomes. Chromosomes are labelled in order of size, with chromosome 1 being the largest and chromosome 22 the smallest (the X and the Y sex chromosomes are always put as a pair at the end even though their

size is different – see Figure 4.10). You inherit 23 chromosomes from your biological mother and 23 chromosomes from your biological father. Biological females have two X chromosomes (one from each parent), while biological males have one X (from their mother) and one Y chromosome (from their father).

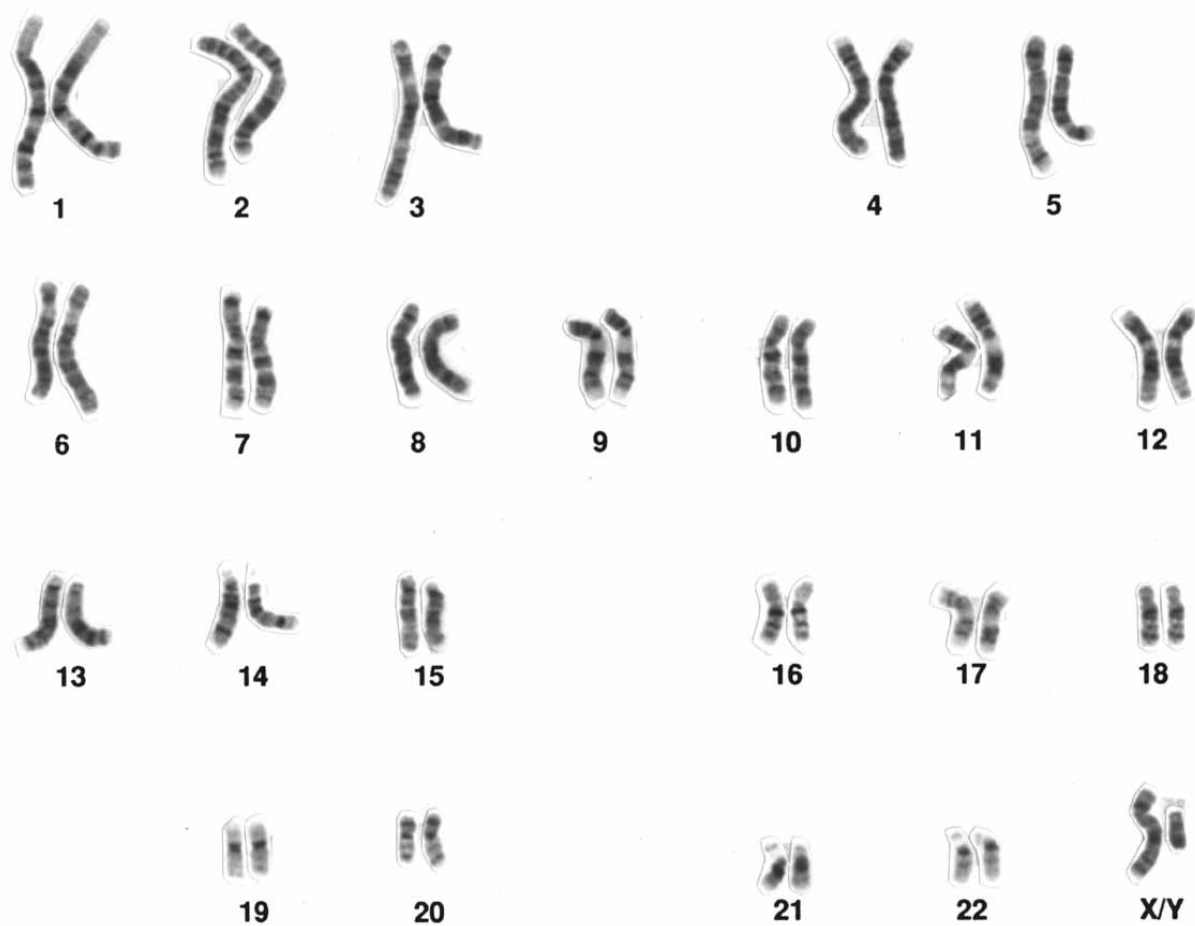


Figure 4.10 Karyogram of human cell with X and Y chromosomes after G-banding: To generate this karyogram (a diagram of the complete set of chromosomes), a cell was arrested in metaphase of mitosis and the chromosomes were stained with Giemsa stain, producing 'G-banding' in which each chromosome has a unique pattern. The 22 pairs of autosomes and an X and Y chromosome from a human cell are shown. The chromosomes are displayed in homologous pairs in descending size, with the largest autosome first, the smallest autosome (22) second last and then the sex chromosomes (X and/or Y) which are always shown last. Image: 'Human male karyotype' by National Human Genome Research Institute from Wikimedia Commons.

As we have seen, DNA exists tightly coiled into chromosomes, but it will uncoil for two reasons: gene expression (transcription explained above) and **DNA replication**. DNA replication occurs early in mitosis or meiosis, when the cell divides to form daughter cells. It involves DNA polymerase copying the entire chromosome from end to end. It is like transcription, except that the whole chromosome is copied, not just a single gene, and it is completed by DNA polymerase so that the resulting copied chromosome is made up of DNA, not RNA. When all 46 chromosomes have been copied, the cell is ready to divide. Here is a great cartoon video comparing mitosis and meiosis.

Mitosis vs meiosis: Side by side comparison



One or more interactive elements has been excluded from this version of the text. You can view them online here: <https://oercollective.caul.edu.au/threshold-concepts-in-biochemistry/?p=56#oembed-2>

The ends of chromosomes



At both ends of each of your chromosomes, there are little ‘caps’ called telomeres. Telomeres protect chromosomes during cell division, preventing degradation of the DNA. Interestingly, telomeres shorten as we age. When they get too short, the cell becomes inactive and no longer divides (this is called senescence and is why we age).

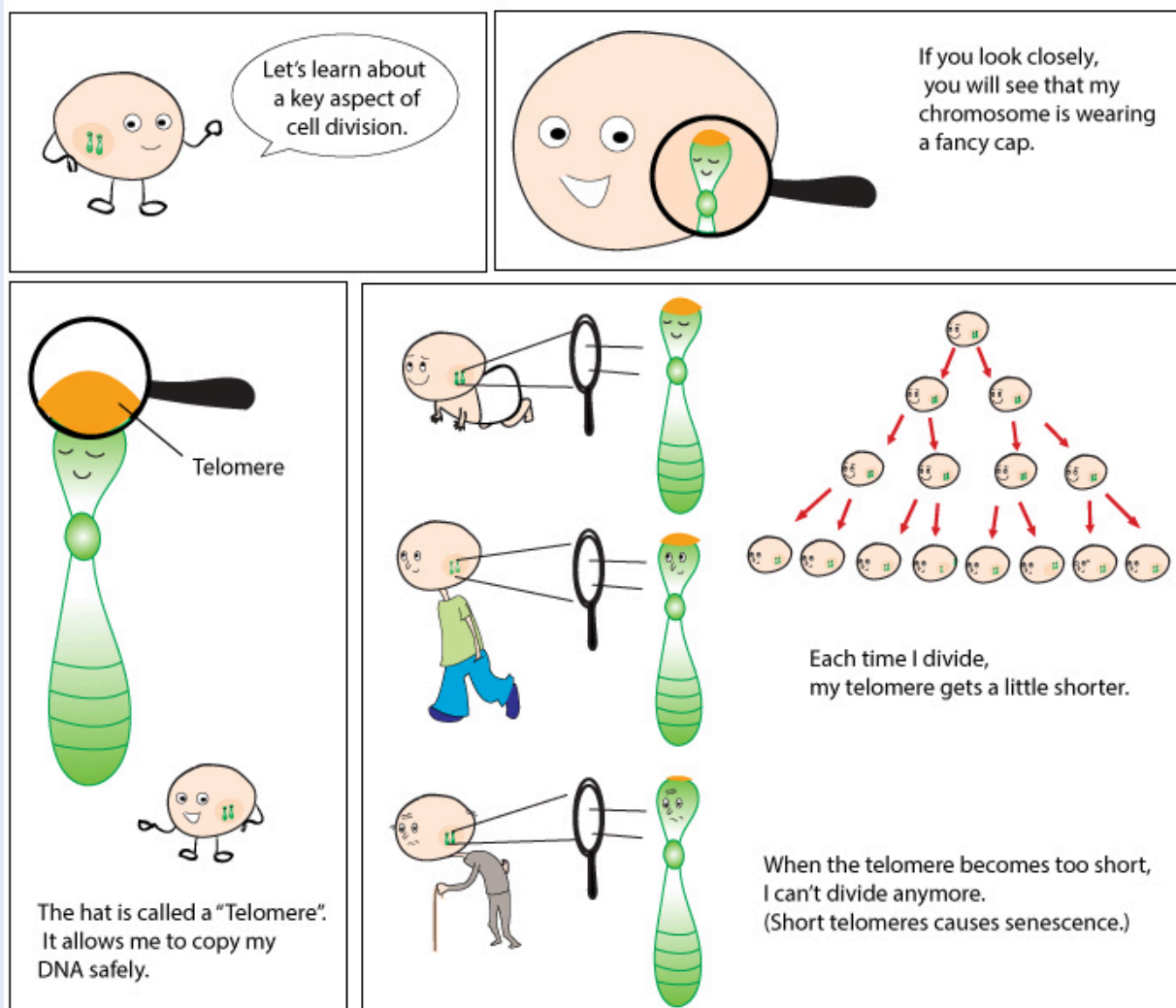
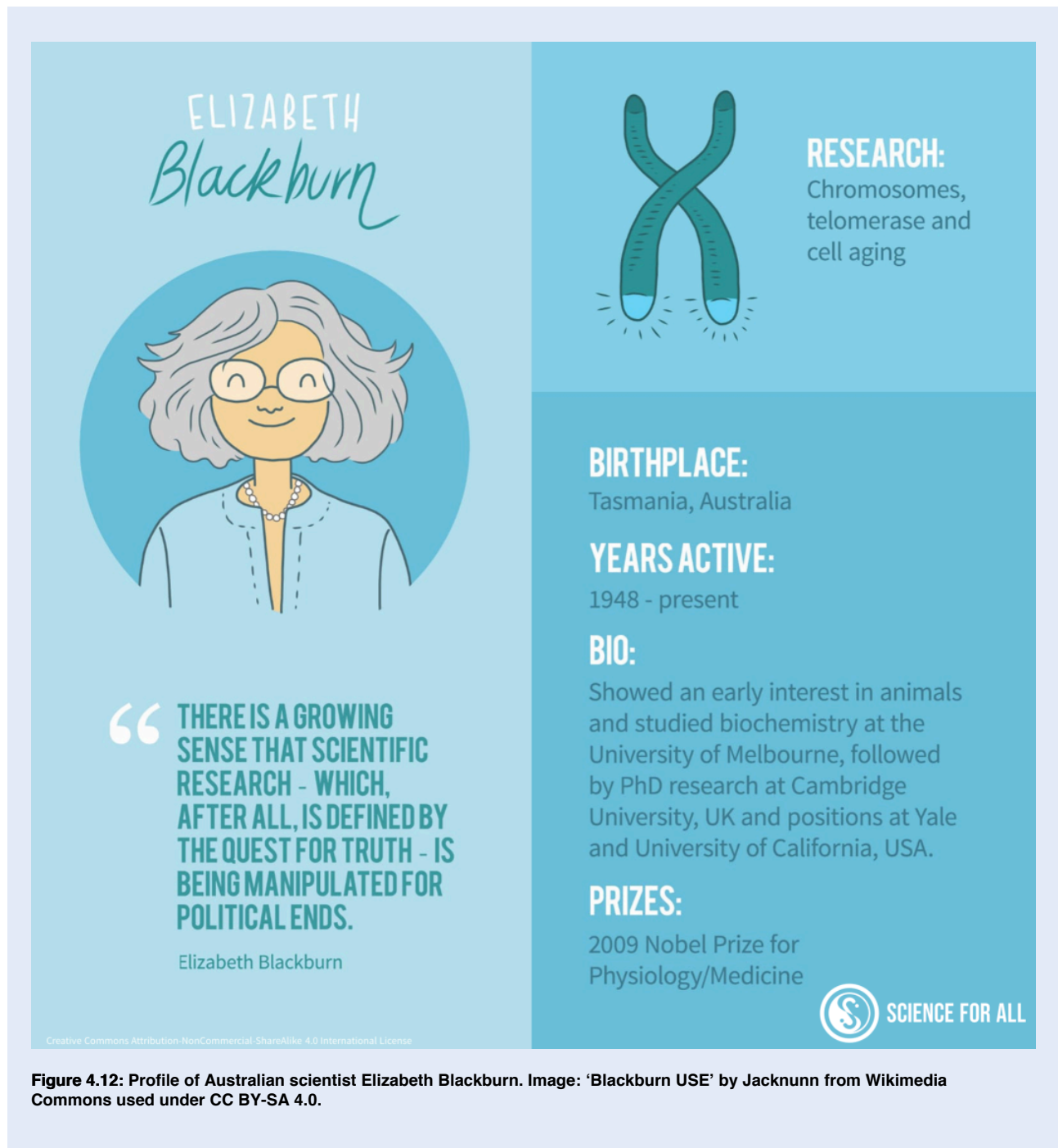


Figure 4.11 Telomeres shorten with age: Telomeres get a little bit shorter with each cell division, until they reach a stage when cells can no longer divide and instead become senescent; that is, they permanently stop dividing but do not die. Image: Telomere end replication problem by WassermanLab from Wikimedia Commons used under CC BY-SA 4.0.

Telomerase is the enzyme that replenishes telomeres, by adding DNA to the ends of chromosomes. In 1984, Australian scientist Elizabeth Blackburn along with Carol Greider discovered telomerase, for which they were awarded a Nobel prize in 2009 alongside Jack Szostak. Telomerase is not normally active in somatic (body) cells, but it is busy working in germ cells (those that make eggs and sperm), some stem cells and cancer cells. This is because these cells are constantly dividing, and if telomerase was not extending the chromosome's telomeres, the cells would undergo cell death (apoptosis) or enter senescence.



How and when do genes turn on?

Twenty-thousand protein-coding genes is a lot of information, and not all of it is relevant or needed all the time. Cells in your liver don't need to communicate visual information like the cells of your eye do. So how do cells know which nucleotides make up the genes that the cells need? And how do they know when to turn them 'on'? (As in, when to transcribe and translate the gene to make its associated protein.)

Gene regulation – how genes are turned on or off at certain times – is a complex process, but it is controlled (regulated) in a number of ways. The nucleotides before the start of the coding region of a

gene (called upstream) contain a number of regions that can aid in turning on or off gene expression. Just upstream of the transcription start site is the promoter region, where RNA polymerase binds to start transcription. Further upstream of this (up to thousands of nucleotides before the promoter region) are regulatory elements such as enhancer or silencer regions, which transcription factors bind to and influence if gene expression is 'enhanced' or 'silenced'. There may also be regulatory sequences after the open reading frame of the gene (Figure 4.11).

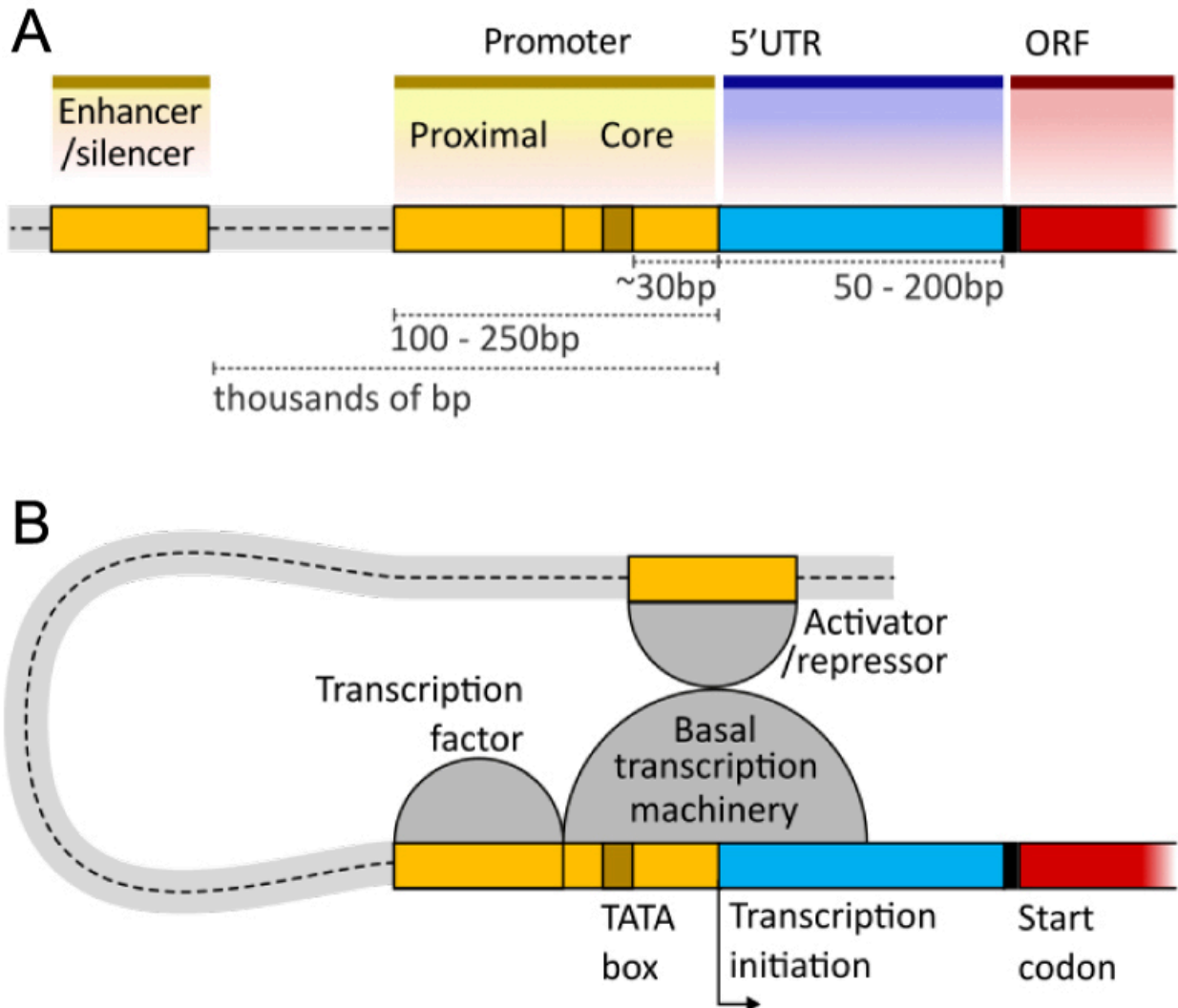


Figure 4.13 Regulatory regions in DNA: A: The structure of the start of a eukaryotic protein-coding gene. Regulatory sequence controls when and where expression occurs for the protein coding open reading frame (ORF, red). Promoter and enhancer regions (yellow) regulate the transcription of the gene into a pre-mRNA and can be right next to a gene or many base pairs (bp) or kilobases away upstream or downstream. The mRNA 5' and 3' untranslated regions (UTR, blue) regulate translation into the final protein product. B: The DNA loops on itself in order for the activator/repressor to interact with the basal transcription machinery. Image: Gene structure eukaryote 2 heavily adapted from image by Thomas Shafee & Rohan Lowe from Wikimedia Commons used under CC BY 4.0.

DNA methylation is another way gene expression can be controlled. If certain genes are not required to be expressed, they can be 'methylated' – the nucleotides have methyl groups attached so that the enzymes involved in transcription cannot access them to turn the gene 'on'. When the genes

are needed, they are unmethylated so that RNA polymerase has access to start transcription. DNA methylation is controlled by epigenetics – when gene expression is influenced by the environment.

Did you know mitochondria have their own DNA?

There is evidence that mitochondria, the organelles within the cell that make ATP (energy), were once a standalone organism, but a symbiotic relationship evolved and they ended up living inside eukaryotic cells (the endosymbiotic theory – see Figure 4.13). It is hypothesised that they were engulfed by a larger cell, the cell provided the mitochondria with food, and in return the mitochondria provided the cell with energy. This is why mitochondria have their own DNA, although some of their genes have integrated into the nuclear DNA. The same goes for chloroplasts in plant cells.

Mitochondrial DNA is inherited maternally (from your mother), as the mitochondria exist within the egg at fertilisation, but the mitochondria in the tail of the sperm is destroyed upon fertilisation.

The ENDOSYMBIOTIC THEORY

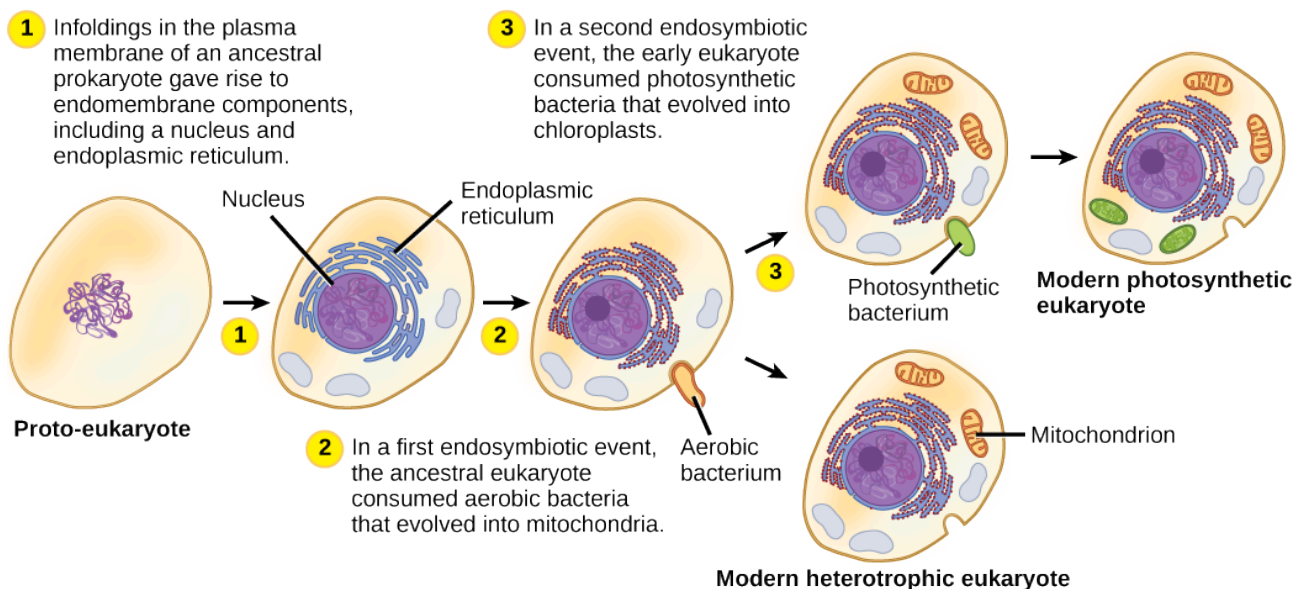


Figure 4.13: The endosymbiotic theory: The first eukaryote may have originated from an ancestral prokaryote that had undergone membrane proliferation, compartmentalisation of cellular function (into a nucleus, lysosomes and an endoplasmic reticulum) and the establishment of endosymbiotic relationships with an aerobic prokaryote, and in some cases a photosynthetic prokaryote, to form mitochondria and chloroplasts, respectively. Image: 'Endosymbiotic theory diagram' by CNX OpenStax from Wikimedia Commons used under CC BY 4.0.

4.5 Knowledge check



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Versioning History

This page provides a record of changes made to this textbook. Each set of edits is acknowledged with a 0.01 increase in the version number. The exported files for this book reflect the most recent version.

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Version	Date	Change	Details
1.00	April 2023	Published Chapter 1-4	

Review Statement

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Editing

This publication has been reviewed by an IPED accredited editor to improve the clarity, consistency, organization structure flow, and any grammatical errors.

Peer review

Full peer review to be conducted in the second half of 2023.