

PREFACE

Cell biology sits awkwardly in a competitive syllabus. It is taught early, so it tends to be learned as vocabulary — a list of organelles, a list of transporters, a list of checkpoint proteins — and it is examined late, where the questions ask not what a structure is called but what happens when it fails, what the experiment showed, and which of four plausible statements cannot be true. Most of the marks lost in this unit are lost in the gap between those two experiences.

This book was written to close that gap. Our aim throughout has been to explain mechanisms rather than catalogue them, and to say plainly how each thing came to be known. A student who understands why a potassium channel admits the larger K^+ ion and turns away the smaller Na^+ , why a microtubule collapses the moment it loses its GTP cap, or why a lysosomal enzyme must be stamped with mannose-6-phosphate before it can find its way home, will answer a question they have never seen before. A student who has memorised the same facts as a list will not.

The fifteen chapters follow a deliberate arc. We begin at the boundary — the cell itself, the cell theory and the walls that surround bacteria, plants and fungi — and then build the plasma membrane from its molecules upward before asking how anything crosses it, passively and then actively. From the surface we move inward: the endomembrane system, mitochondria and plastids, and the sorting machinery that delivers each protein to its correct address. The nucleus, genome organization and chromatin follow, and the book closes with the processes by which a cell divides, distributes its chromosomes, dies on schedule, and — in the microbial case — grows, adapts and acquires resistance.

Two things have guided the writing. The first is the previous-year question paper: we have let it decide where to go deep and where a paragraph is enough, and where a concept is repeatedly tested through a diagram or a classical experiment we have presented it that way rather than in prose alone. The second is the figure. Cell biology is a visual subject, and a mechanism understood from a good diagram is retained long after the paragraph describing it has gone.

We have also tried to be honest about what is settled and what is not. Where a model is contested, or where a familiar textbook statement has been overtaken, we say so rather than passing on a tidy answer that the current literature no longer supports. A student who knows which parts of a subject are still moving is better prepared, not worse.

The book assumes no prior specialisation. It begins where a graduate in any branch of the life sciences begins and reaches the level at which a research paper can be read with understanding, so it should serve equally for CSIR-NET, GATE, ICMR, IIT JAM, CUET-PG and university examinations.

We are grateful to our students, whose questions shaped almost every explanation in these pages and whose confusions were far more instructive than their successes; to our colleagues at IFAS for their careful reading; and to our families for their patience. Errors that remain are ours, and we will be glad to hear of them.

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ACKNOWLEDGEMENTS

No book of this size is ever finished by its authors alone. It is the slow work of many hands — colleagues, mentors, reviewers, designers and, most of all, students — and to every one of them we owe a clear debt. The pages that follow are here because of their patient effort across many months.

OUR STUDENTS

Our first and most heartfelt thanks belong to the students of the Institute for Advanced Studies (IFAS). Their questions, their stubborn curiosity and their seriousness about the life sciences gave this volume both its reason and its shape. Every chapter has been tested in classrooms, sharpened by a query from the back row, and rewritten until the explanation actually worked for the student sitting in front of us. The book exists because they exist.

READING EVERY CHAPTER

We are deeply grateful to Mr. Digambar Jagtap, and Mr. DadaSaheb Sondge for the generous gift of their time, for reading every chapter with a careful eye, and for offering suggestions that were unfailingly thoughtful. Their scholarly attention has shaped this book in ways the careful reader will sense throughout — wherever the prose moves cleanly, wherever a difficult idea lands without struggle, their influence is at work.

SECTION BY SECTION

Special thanks go to Mr. Pranshu Dwivedi and Mr. Vishal Bhujbal, who worked through the manuscript section by section and figure by figure. Their fine-grained comments improved both the clarity of the explanations and the pedagogical rhythm of the chapters, and helped steer the book steadily toward the readers it was written for.

CONCEPTS AND STRUCTURE

We thank Mr. Lalji Kanoujia and Dr. Rakesh Kumawat for their patient work of sorting and compiling the central concepts of the book and matching each one to its proper chapter and section. Their careful curation turned a sprawling stack of notes into a navigable reference, and it is largely owing to their effort that the overall structure of the volume holds together as a coherent whole.

PROOFREADING AND INDEX

Sincere thanks also to Mr. Lokesh and Mr. Mohit for their attentive proofreading and for verifying the index with diligence. The care they brought to small things — a missing comma, a stray citation, a cross-reference pointing at the wrong page — is precisely what separates a reference book that readers trust from one that quietly slips out of their hands.

COVER, DIAGRAMS AND PRODUCTION

This book is also the outcome of a collaborative effort that would not have been possible without the outstanding work of the IFAS Publication Team. Special thanks to Mr. Vikendra Metha for translating our ideas into a beautiful cover and for rendering the diagrams throughout the text with craftsmanship and imagination. His team also deserves our sincere thanks for the formatting, typesetting and production work that has given this volume its finished appearance — the book you are holding owes its readable surface to their care.

Finally, our humble greetings to everyone who contributed and remains, in these pages, unmentioned. A reference book of this scale grows through countless small acts — a query resolved late at night, a page re-read for a missing word, a diagram redrawn for the third time. To each of those unnamed hands, our sincere thanks. The pages that follow are as much yours as they are ours.

With sincere thanks,

Dr. Kailash Choudhary
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CHAPTER

1

The Cell and the Cell Wall

Every organism alive today is built of cells, and every one of those cells came from a cell before it. That single sentence took two centuries to earn. It began with a man looking at a sliver of cork through a home-made microscope and seeing rows of empty boxes, and it ended with the realisation that those boxes were the walls of something that had once been alive. This chapter follows that history, asks what a cell is and how the first one might have arisen, and then turns to the structure that Hooke actually saw — the wall. It is the wall that decides a bacterium's shape, resists the pressure that would otherwise burst it, gives wood its strength, and provides the target for the most successful class of antibiotics ever made.

LEARNING OBJECTIVES

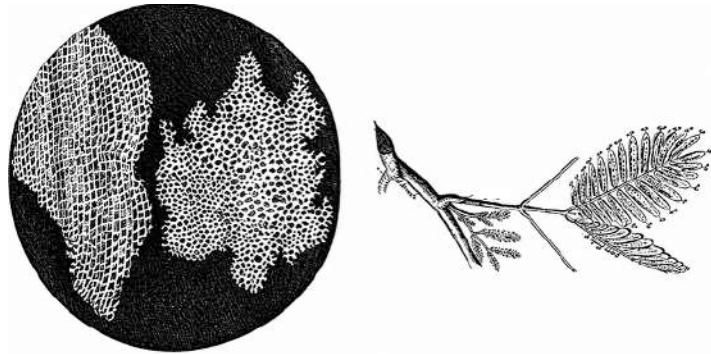
- Recount the discovery of the cell and state the three classical tenets of the cell theory.
- Give the modern interpretation of the cell theory
- Outline the stages by which the first cell is thought to have arisen
- Compare prokaryotic and eukaryotic cells in size, organization, genome and division.
- Explain the three-domain classification of life and the evidence from 16S rRNA on which it rests.
- Describe the bacterial cell wall and the difference between Gram-positive and Gram-negative envelopes.
- Draw the peptidoglycan subunit and identify its unusual amino acids
- Describe lipopolysaccharide, the outer membrane and the periplasm
- Explain how the wall protects against osmotic lysis
- Describe the archaeal envelope and why it resists both lysozyme and penicillin.
- Describe the plant cell wall and the roles of the primary and secondary wall.
- Explain how plasmodesmata connect neighbouring cells, and distinguish symplast from apoplast.
- Describe the layered composition of the fungal cell wall.

1.1 The Discovery of the Cell and the Cell Theory

The cell was not discovered by reasoning; it was discovered by looking. In the seventeenth century a new instrument, the compound microscope, made visible a world that no one had suspected, and the history of biology divides neatly into the periods before and after that instrument existed.

In 1665 Robert Hooke turned a primitive compound microscope on exceedingly thin slices of bottle cork and published what he saw in *Micrographia*. The cork was divided into a multitude of tiny pores, which he named **cells** — from the Latin *cella*, a small room, and *cellulae*, the hexagonal compartments of a honeycomb (Figure 1). It is worth being clear about what Hooke had and had not found. The magnification available to him was low, and what he took for the fundamental units were in fact only the empty walls of dead plant tissue. He could see no nucleus, no organelles, nothing within, and he concluded that the *cellulae* were not living things at all.

Figure 1.1 Hooke's drawing of cork as it appeared in *Micrographia* (1665). The regular compartments he named cells are the empty walls left behind by dead plant tissue — the first structure of the kind ever illustrated, and, as it happens, the subject of the rest of this chapter.



Better lenses came in 1673 with Anton van Leeuwenhoek, whose single-lens instruments magnified almost three hundred times. Where Hooke had seen only compartments, Leeuwenhoek saw movement. He described a multitude of microscopic organisms in water — his *animalcules*, among them protozoa and bacteria — and went on to examine red blood cells and spermatozoa. The living cell, rather than the dead wall, had been seen for the first time.

Animal cells were described long after plant cells, and for a mundane reason: animal tissue is fragile and tears rather than sectioning cleanly, so thin slices were hard to prepare. Biologists were convinced that life had a fundamental unit but could not agree what it was. The connection was finally made by Henri Dutrochet, who declared that the cell is the fundamental element of organization and, importantly, that it is not merely a structural unit but a physiological one. Meanwhile in 1804 Karl Rudolphi and J. H. F. Link were awarded a prize by the Royal Society of Science at Göttingen for solving the problem of the nature of cells — they were the first to prove that cells possess independent walls of their own.

Figure 1.2 Theodor Schwann (1810–1882) and Matthias Jakob Schleiden (1804–1881), whose parallel conclusions about plants and animals became the first two tenets of the cell theory.



Theodor Schwann (1810–1882)



Matthias Jakob Schleiden (1804–1881)

Cells are the smallest building blocks of life, and they are small indeed — most measure between 1 and 100 micrometres. They vary enormously in shape and size because they do enormously varied jobs. One property, however, is universal and turned out to be the key to the whole subject: a cell can make more of itself by dividing. If a cell exists, it came from another cell, and it can give rise to new ones.

In 1838 the German botanist Matthias Jakob Schleiden concluded that every part of a plant is either made of cells or derived from cells. In 1839 the German physiologist Theodor Schwann extended the same claim to animals — a bolder step than it now sounds, because far more was known then about plant structure than about animal structure. Their two statements became the first two tenets of the cell theory:

- All living organisms are composed of one or more cells; the cell is the structural unit of life.
- The cell is the most basic functional unit of life.

The third tenet came from Rudolf Virchow in 1855, who concluded that all cells arise from pre-existing cells — in Latin, *Omnis cellula e cellula*. With that addition the classical cell theory was complete:

- All cells arise only from pre-existing cells.

KEY POINT

Hooke (1665) named cells but saw only dead cell walls; Leeuwenhoek (1673) first saw living cells. Schleiden (1838, plants) and Schwann (1839, animals) supplied the first two tenets, Virchow (1855) the third — *Omnis cellula e cellula*.

1.2 Modern Interpretation, and the Exceptions

The classical statement has been extended rather than replaced. The generally accepted modern cell theory holds that:

- All known living things are made up of one or more cells.
- All living cells arise from pre-existing cells by division.
- The cell is the fundamental unit of structure and function in all living organisms.
- The activity of an organism depends on the total activity of its independent cells.
- Energy flow — metabolism and biochemistry — occurs within cells.
- Cells contain DNA, found specifically in the chromosome, and RNA, found in the nucleus and the cytoplasm.
- All cells are basically the same in chemical composition in organisms of similar species.

Four kinds of infectious agent sit outside this scheme, and each fails it for a different reason.

Viruses are acellular. They possess a nucleic acid genome, DNA or RNA, and an outer protein coat, but they carry no machinery for replicating that genome. They reproduce only inside a host cell, using the host's replication machinery. Because every living organism is expected to replicate its own genome and reproduce itself, a virus cannot be accommodated by the cell theory.

Viroids go further. Discovered by Theodor Diener in 1971, a viroid consists of nothing but a short circular strand of RNA capable of self-replication, with no protein coat whatever to protect it. The first one found causes potato spindle tuber disease. Like viruses, viroids commandeer the host machinery to copy their RNA, and they damage the plant by RNA-interference-mediated gene silencing.

Virusoids, or satellite RNAs, are essentially viroids that have been packaged inside the coat protein of a helper virus. Their genomes are tiny — single-stranded circular RNAs of only 220 to 388 nucleotides — and they encode no protein at all, serving only to replicate themselves and to silence host genes. The first virusoid was found in *Nicotiana velutina* infected with velvet tobacco mottle virus.

Prions abandon nucleic acid altogether. A prion is an infectious protein that induces the normal cellular form of the same protein to misfold, propagating the abnormal conformation through the brain. It has no associated nucleic acid and no envelope. Prion diseases affect humans and animals and are sometimes transmitted through infected meat; the commonest human form is Creutzfeldt–Jakob disease.

DO YOU KNOW?

A viroid genome is smaller than a single average gene — a few hundred bases of circular RNA with no protein-coding capacity at all. It causes disease not by making a toxin but by folding into a structure that the plant's own RNA-silencing machinery mistakes for a target, so the plant effectively silences its own genes.

1.3 The Origin of the First Cell

Evidence places the origin of life at least 3.8 billion years ago, nearly 750 million years after the formation of the Earth about 4.5 billion years ago. The route from chemistry to a cell is conventionally divided into five stages.

Synthesis of simple organic molecules. In the 1920s Oparin and Haldane proposed that the primitive atmosphere, lacking oxygen but rich in H_2 , NH_3 and H_2O , would allow simple organic molecules to form under the influence of lightning, ultraviolet radiation and volcanic activity. Methane was among the earliest organic compounds, and from such beginnings came amino acids and other monomers. Stanley Miller demonstrated the principle experimentally in the 1950s by passing electric sparks through a mixture of these gases and recovering amino acids.

Formation of macromolecules. Monomers polymerised into macromolecules when dripped onto hot surfaces; Sidney Fox called the abiotically produced polypeptides *protenoids*, and showed that they form microspheres. Clay minerals may have acted as surfaces on which such polymerisation reactions were concentrated and templated.

Self-replicating molecules. The decisive property is the ability to copy oneself. Of the biomolecules present in cells today, only the nucleic acids can do this, and RNA can do it while also acting as a catalyst. This dual capacity is the basis of the RNA **world** hypothesis — the proposal that RNA once carried out both the informational and the catalytic work of the cell by itself. The discovery of ribozymes, and the fact that the peptidyl transferase centre of the modern ribosome is built of RNA rather than protein, remain the strongest evidence for it.

Formation of the first cell. The first cell is envisaged as self-replicating RNA enclosed in a phospholipid membrane, a structure that would already have permitted RNA-directed protein synthesis. In time the chemically more stable DNA displaced RNA as the store of genetic information, and RNA was left with the intermediary roles it still holds.

Evolution of metabolism. The earliest organisms absorbed nutrients from their surroundings. To secure a reliable energy supply, cells evolved metabolic pathways — first anaerobic glycolysis, which drew down the available glucose, and then photosynthesis. The earliest photosynthetic bacteria are thought to have used H_2S as an electron donor before the transition to H_2O , a change that released oxygen into the atmosphere. That oxygenation transformed the planet, made aerobic respiration possible and opened the way to the energy-expensive complexity of the eukaryotic cell.

KEY POINT

The proposed order is: simple organic molecules (Oparin–Haldane, demonstrated by Miller) → macromolecules (Fox's protenoid microspheres) → self-replicating RNA (the RNA world) → RNA enclosed in a phospholipid membrane → DNA replaces RNA as the genetic material → glycolysis, then photosynthesis and the oxygenation of the atmosphere.

1.4 Prokaryotic and Eukaryotic Cells Compared

Every living organism — bacterium, blue-green alga, plant or animal — has a cellular structure and is built of one cell or of many. Those consisting of a single cell are **unicellular**: bacteria, blue-green algae, some algae and the protozoa. Those built of many are **multicellular**: the fungi, most plants and the animals. Cells fall into two great classes, **prokaryotes** and **eukaryotes**. The terms were introduced by Chatton in 1937 and brought into general use by Hans Ris in the 1960s.

It is thought that every organism now living derives from a single primordial cell that existed more than three billion years ago; the family resemblances between all living things are too strong to be explained any other way. One landmark on that road occurred about 1.5 billion years ago, with the transition from small cells of relatively simple internal structure to the larger and radically more complex cells found in plants and animals.

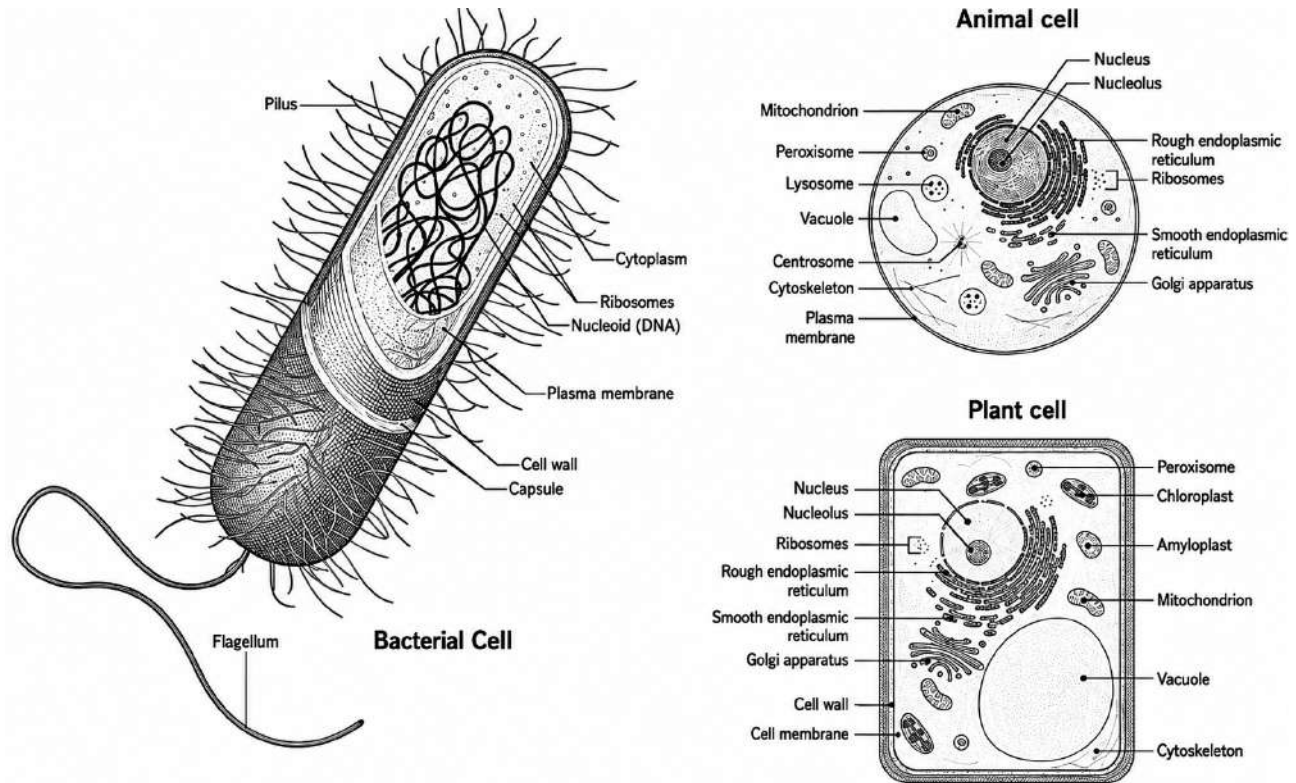


Figure 1.3 A prokaryotic cell (left) compared with animal and plant cells. The prokaryote has no nucleus and no membrane-bounded organelles; its genome lies in the nucleoid, a region of the cytoplasm that is not membrane-separated from it. The eukaryotic cells are compartmentalised, with the DNA enclosed in a double-membraned nucleus.

Prokaryotes include all the bacteria, and are divided into two great groups, the archaeobacteria and the eubacteria. They are relatively small cells bounded by a plasma membrane and, characteristically, by a cell wall whose composition differs from organism to organism. They have no nucleus — although they certainly have DNA, usually circular, sometimes linear — and no membrane-bounded organelles, though they do contain ribosomes. Under the microscope the protoplasm shows a chromosomal region of fibrous appearance, the **nucleoid**, and the cytoplasm around it. Bacteria and Archaea are the two prokaryotic domains.

Prokaryotic cells are structurally simple but biochemically extravagant. They are spherical or rod-shaped, commonly a few micrometres across, usually with a tough protective wall beneath which the plasma membrane encloses a single cytoplasmic compartment of DNA, RNA, protein and small molecules. In the electron microscope the interior looks like a matrix of varying texture with no obvious organization. They divide simply, by **binary fission**, and they divide fast: under optimal conditions a single cell dividing every twenty minutes gives rise to five billion cells — roughly the human population — in under eleven hours. Where food is plentiful, survival of the fittest tends to mean survival of whoever divides fastest.

That speed is also what makes bacteria so adaptable. A population maintained in a large vessel in the laboratory will evolve within a few weeks, by spontaneous mutation and natural selection, the ability to use sugars it could not use before. In nature they occupy an enormous variety of ecological niches and show a corresponding richness of biochemistry.

There are species able to use virtually any organic molecule as food — sugars, amino acids, fats, hydrocarbons, polypeptides, polysaccharides — and some that obtain their carbon from CO_2 and their nitrogen from N_2 . For all their simplicity, bacteria have existed longer than any other organism and remain the most abundant cells on Earth.

Eukaryotes arose from cells sometimes called proto-eukaryotes, which over time acquired a mitochondrial symbiont and later developed a nucleus. As atmospheric oxygen rose, anaerobic life met a crisis. Some lineages died out.

Others adapted: some acquired the capacity to respire aerobically; some retreated into niches where oxygen was absent and thrive there still; some became predators or parasites of aerobic cells; and some took the cleverest route of all, entering an intimate symbiosis with an aerobic cell.

Multicellularity probably began when daughter cells stayed together in small colonies after dividing rather than separating, and came into its own in eukaryotes, whose cells could differentiate and organise into tissues with specialised functions.

The defining properties of eukaryotic cells follow from one idea, **compartmentalization**. Most of the DNA is enclosed by a double membrane in the nucleus (*karyon* in Greek), separated from the rest of the cell's contents; the endoplasmic reticulum, Golgi apparatus, lysosomes and the other organelles occupy the cytoplasm in which most metabolism occurs.

Prominent among them are two organelles each bounded by its own double membrane, chemically distinct from the nuclear membranes: mitochondria, an almost universal feature of eukaryotic cells, and chloroplasts, confined to photosynthetic eukaryotes — plants, but not animals or fungi. Both almost certainly have a symbiotic origin.

One consequence of that architecture is the diversity of cell type it permits. Animals have evolved between one and two hundred distinct cell types in a multicellular body, against ten to twenty in plants, fungi and protocista. The major differences between the two classes of cell are collected in Table 1.1.

Table 1.1 Major differences between prokaryotes and eukaryotes

Feature	Prokaryotes	Eukaryotes
Type of cell	Unicellular	Unicellular and multicellular
Cell size	0.2–2.0 μm in diameter	10–100 μm in diameter
Cell wall	Usually present; chemically complex (peptidoglycan)	When present, chemically simple (cellulose, chitin)
Nucleus	Absent; a nucleoid region instead	Present, with a nuclear envelope
Ribosomes	Present, smaller (70S)	Present, larger in the cytosol (80S); 70S in mitochondria and plastids
DNA arrangement	Generally circular	Linear
Compartmentalization	No membrane-bounded organelle or compartment	Single- and double-membrane compartments present
Mitochondria	Absent	Present
Endoplasmic reticulum	Absent	Present
Plasmids	Present	Very rarely found
Lysosomes and centrosomes	Absent	Present
Cell division	Binary fission	Mitosis
Flagella	Smaller, and structurally simple	Larger, with the 9+2 axoneme
Cytoskeleton	Homologues present (FtsZ, MreB)	Present and elaborate
Reproduction	Asexual	Asexual and sexual
Examples	Bacteria and Archaea	Plant and animal cells

2.9 Integral Membrane Proteins

The lipid bilayer is the structural unit of every biological membrane, but the proteins carry out essentially all of its active functions — transport, receptor activity and the rest. Most membrane proteins can be classified as **peripheral**, **integral** or **lipid-anchored**. Peripheral proteins are globular proteins that interact with the membrane mainly through electrostatic and hydrogen-bonding interactions with integral proteins. Integral proteins are strongly associated with the bilayer, with part of the protein embedded in it or extending all the way across. Lipid-anchored proteins, which Singer and Nicolson did not anticipate, associate with membranes through a variety of covalently attached lipid anchors. The classes are compared in Figure 2.20.

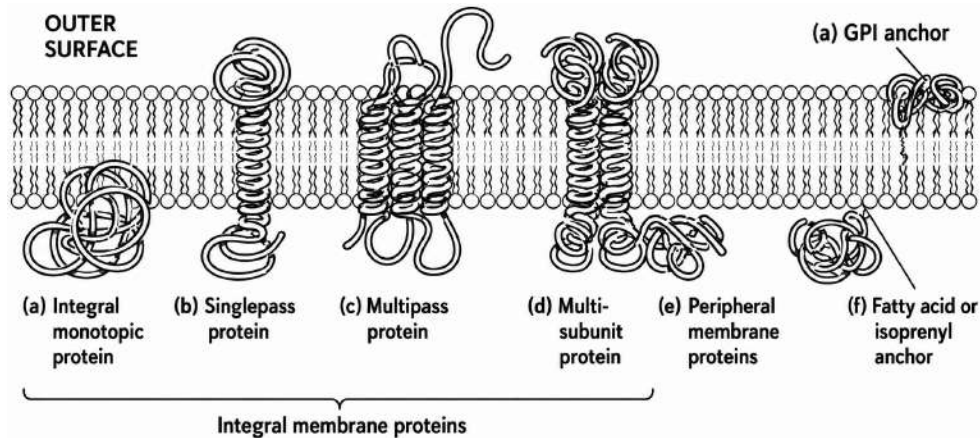


Figure 2.19 Classes of membrane protein: (a) integral monotopic, embedded in one leaflet only; (b) single-pass and (c) multipass transmembrane proteins; (d) a multisubunit complex; (e) a peripheral protein bound at the surface; (f) a protein anchored by a fatty acyl chain; (g) a GPI-anchored protein on the outer face.

Integral membrane proteins are diverse but fall into two general classes. A few are embedded in, and protrude from, only one side of the bilayer — the **integral monotopic** proteins. Most, however, are **transmembrane** proteins that span the membrane with hydrophilic regions protruding on both sides. These cross either once, as **single-pass** proteins, or several times, as **multipass** proteins; some multipass proteins are a single polypeptide, others are multisubunit assemblies.

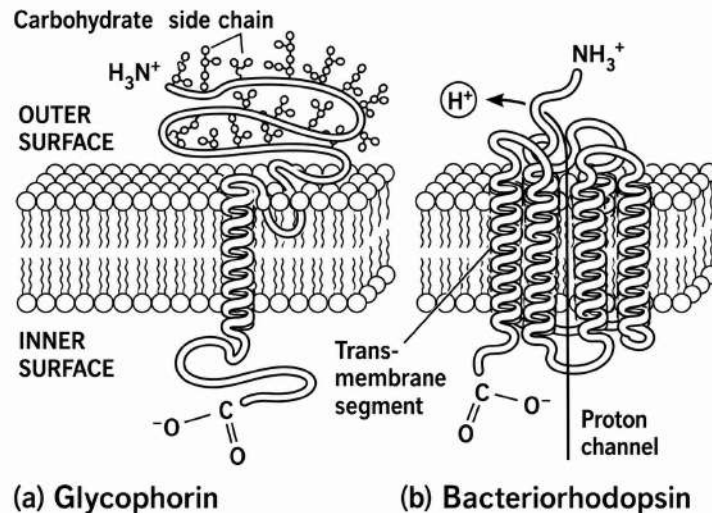
The hydrophobic core of a transmembrane segment is dominated by the **α -helix**, because that secondary structure satisfies the highly polar N–H and C=O groups of the peptide backbone by hydrogen-bonding them to each other, leaving no unpaired polarity to be buried in the hydrocarbon. A minimum of about 19 to 20 continuous hydrophobic amino acids is needed to span the hydrophobic core, and that number is the basis of the prediction methods of Section 2.12. Glycophorin and bacteriorhodopsin, drawn together in Figure 2.21, are the standard single-pass and multipass examples.

2.9.1 Glycophorin — a single-pass protein

Glycophorin has only a small hydrophobic core of 19 amino acids anchoring it in the bilayer, and that segment is an α -helix. Its N-terminus faces the extracellular side and its C-terminus the cytosol, and most of its mass lies outside the cell, exposed to the aqueous environment.

Glycophorin is heavily glycosylated on its N-terminal extracellular region, and those sugars are rich in **sialic acid**, giving the red cell a strongly hydrophilic, negatively charged coat. The oligosaccharides attached to this domain constitute the ABO and MN blood-group antigenic specificities of the red cell, and the same extracellular portion serves as the receptor for influenza virus. Glycophorin has a total molecular weight of about 31,000 and is roughly 40 per cent protein and 60 per cent carbohydrate — an unusual composition that makes the point that a membrane protein need not be mostly protein.

Figure 2.20 Two integral membrane proteins compared. (a) Glycophorin crosses the membrane once through a 19-residue α -helix, with a heavily glycosylated N-terminal domain outside the cell. (b) Bacteriorhodopsin crosses seven times as a compact bundle of α -helices, with very little of the protein exposed to water.



2.9.2 Bacteriorhodopsin — a seven-pass protein

Bacteriorhodopsin has a hydrophobic core that spans the bilayer seven times. It is not rod-like but globular, and it belongs to the class of proteins involved in transport and similar activities that require the protein to be embedded deep in the bilayer. Such proteins consist of several hydrophobic α -helical segments joined by hinge regions, so that the chain winds back and forth across the membrane in a zig-zag.

Bacteriorhodopsin clusters in the purple patches of *Halobacterium halobium* and is a light-driven **proton pump**. When the organism is deprived of the oxygen it needs for oxidative metabolism, it switches to capturing energy from sunlight and uses that energy to pump protons out of the cell. The resulting proton gradient is potential energy, later used to make ATP. In the purple patches the protein is arranged in hexagonal arrays, the polypeptide crossing the membrane seven times in seven α -helical segments with very little exposed to the aqueous surroundings, and the structure has become the model of globular membrane protein architecture.

The sodium–potassium pump is another integral protein whose hydrophobic core is built of α -helices, in this case spanning the bilayer ten times. By analogy with bacteriorhodopsin one would expect these segments to form a globular hydrophobic core anchoring the ATPase in the membrane, and the helices probably also account for the transport properties of the enzyme. Band 3, the anion exchanger of the erythrocyte, is a further multipass example, crossing the membrane at least a dozen times.

2.9.3 Porins — the β -barrel motif

The α -helix is not the only way to satisfy backbone hydrogen bonding in a hydrophobic environment. The **β -sheet** does it too, and the **porin** proteins of the outer membranes of Gram-negative bacteria such as *Escherichia coli* — and of the outer mitochondrial membrane of eukaryotic cells — span their membranes with large β -sheets rolled into barrels.

A good example is **malto porin**, also known as the LamB protein or lambda receptor, which participates in the entry of maltose and maltodextrins into *E. coli*. Malto porin is active as a trimer, and each 421-residue monomer is an 18-strand β -barrel. The strands are connected to their nearest neighbours either by long loops or by short β -turns: the long loops lie at the end of the barrel exposed to the cell exterior, the turns on the intracellular face, and three of the loops fold into the centre of the barrel.

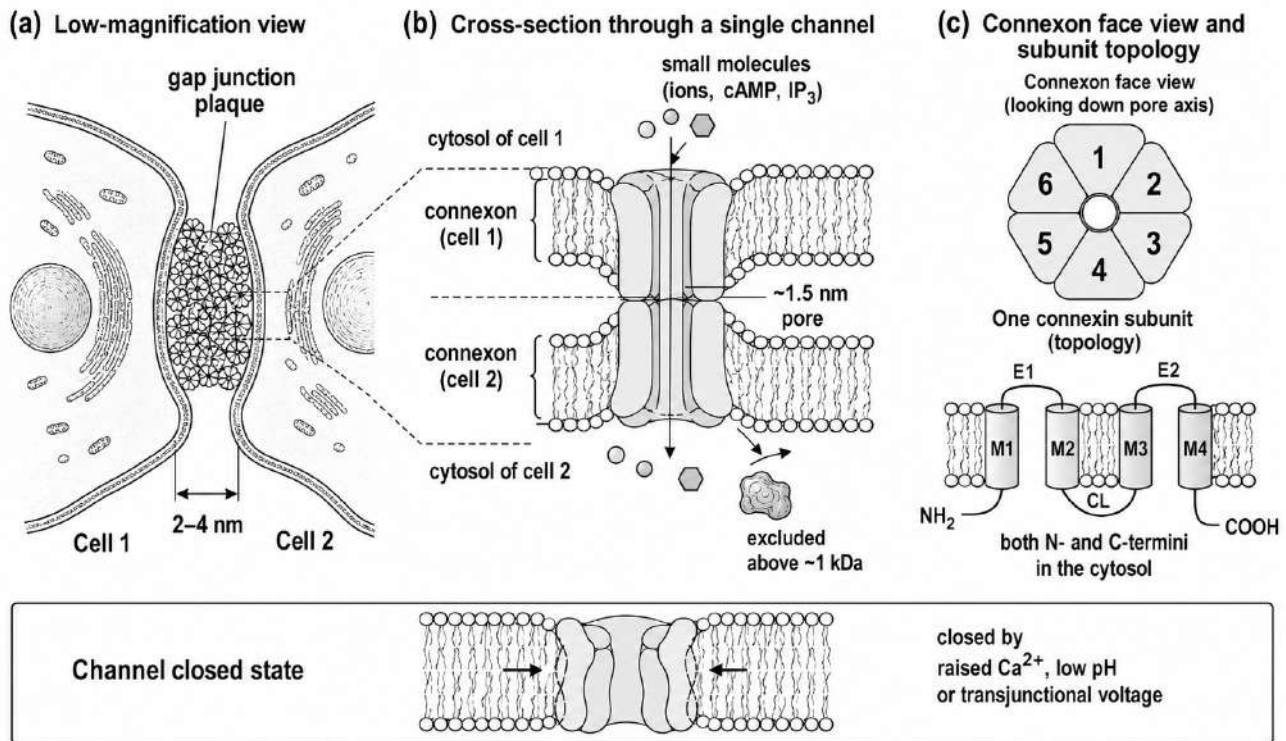


Figure 3.15 *The gap junction. Six connexin subunits assemble into a connexon, or hemichannel, which docks with a connexon in the adjacent cell to bridge the 2–4 nm intercellular gap. The resulting channel, about 1.5 nm wide, passes ions and molecules below about 1,000 daltons, and closes when cytosolic Ca^{2+} rises or pH falls.*

3.14 The Ionic Basis of the Membrane Potential

A **membrane potential** arises when the electrical charge on the two sides of a membrane differs — a slight excess of positive over negative ions on one side and a slight deficit on the other. Such charge differences can be produced in two ways: by active **electrogenic** pumping, and by passive ion **diffusion**.

Which mechanism dominates depends on the membrane. Most of the potential across the inner mitochondrial membrane is generated by electrogenic H^+ pumps, and electrogenic pumps also generate most of the potential across the plasma membrane of plants and fungi. In typical **animal** cells, however, it is passive ion movement that makes the largest contribution.

The argument is worth following slowly, because it is the foundation of the rest of the chapter. The Na^+/K^+ pump keeps the intracellular Na^+ concentration low, which helps maintain osmotic balance. But a low intracellular Na^+ cannot balance the charge carried by the cell's **fixed anions** — the negatively charged organic molecules trapped inside. That balancing is done largely by K^+ , which the pump drives in and which can also move freely out through **K^+ leak channels**.

Now suppose that at the start there is no voltage across the membrane, but K^+ is high inside and low outside. K^+ leaves through the leak channels, driven by its concentration gradient. As it goes it leaves behind unbalanced negative charge, creating an electrical field — a membrane potential — that opposes further efflux. Net efflux stops when the potential reaches the value at which the electrical force on K^+ exactly balances the concentration gradient, that is, when the **electrochemical** gradient for K^+ is zero. Chloride equilibrates too, but the membrane potential keeps most Cl^- out of the cell because its charge is negative.

CHAPTER

5

The Endomembrane System

A bacterium is a single room. A eukaryotic cell is a house — and the reason it can be a thousand times larger without descending into chaos is that its cytoplasm is divided into rooms, each holding a different set of enzymes at a different pH, and each connected to the others by a traffic system of membrane vesicles. This chapter follows a protein from the moment its first few amino acids emerge from a ribosome, through the endoplasmic reticulum where it is folded and sugared, through the Golgi stack where the sugars are rebuilt and the address is written, to the lysosome, the vacuole, the cell surface or the outside world. It then turns the traffic around and follows the cell's own worn-out parts into the lysosome to be broken up for scrap. The peroxisome, which stands slightly apart because it imports its proteins directly rather than by vesicle, closes the chapter.

LEARNING OBJECTIVES

- Define the endomembrane system and state which organelles belong to it and why.
- Describe the structure of rough and smooth ER and distinguish their functions.
- Explain co-translational translocation, the signal hypothesis, SRP and the Sec61 translocon.
- Describe protein folding, quality control and the unfolded protein response
- Account for lipid and steroid synthesis and calcium storage in the smooth ER.
- Describe the organization and polarity of the Golgi apparatus.
- Compare the vesicular transport and cisternal maturation models of Golgi traffic.
- Describe N-linked and O-linked glycosylation and where each step occurs.
- Explain sorting and export from the trans-Golgi network
- Describe the lysosome, its biogenesis and the mannose-6-phosphate pathway.
- Account for the major lysosomal storage disorders, including I-cell disease.
- Describe the plant vacuole and the tonoplast.
- Distinguish macroautophagy, microautophagy and chaperone-mediated autophagy.
- Describe peroxisomes, their biogenesis by PTS1 and PTS2, and the peroxisomal disorders.

5.1 The Endomembrane System — An Overview

In addition to a nucleus, eukaryotic cells are distinguished from prokaryotic cells by **membrane-enclosed organelles** in the cytoplasm. These organelles provide discrete compartments in which specific cellular activities take place, and the resulting subdivision of the cytoplasm allows eukaryotic cells to function efficiently in spite of their large size — about a thousand times the volume of a bacterium.

A subset of these organelles is functionally continuous. The **endoplasmic reticulum**, the Golgi **apparatus**, the **endosomes**, the **lysosome** (or the **vacuole** in plants and fungi), the **secretory vesicles** and the **plasma membrane** itself are distinguished from other cytoplasmic organelles by their common involvement in protein processing and by their connection through **vesicular transport**. Together they are called the **endomembrane system**. Membrane and cargo move between them by budding and fusion, so that a patch of membrane made in the ER can end up in the plasma membrane without ever crossing the cytosol.

Because of this complex internal organization, sorting and targeting proteins to their correct destinations is a considerable task, and the cell solves it with three mechanistically distinct kinds of transport, set out in Table 5.1. Knowing which is which resolves a great many questions at a stroke.

Table 5.1 The three modes of protein transport into organelles

Mode	How the protein crosses	Destinations
Gated transport	Through an aqueous pore that stays open to both sides; the protein is fully folded and may even be an assembled complex	Nucleus, through the nuclear pore complex
Transmembrane transport	Through a protein translocator in the membrane; the protein is usually unfolded as it crosses	ER, mitochondrion, chloroplast — and, exceptionally, the peroxisome, which takes folded protein
Vesicular transport	The protein never crosses a membrane at all; it is enclosed in a vesicle that buds from one compartment and fuses with the next	ER → Golgi → endosome → lysosome → plasma membrane, and the reverse routes

Two consequences follow that are worth fixing now. First, the **mitochondrion**, the **chloroplast**, the **peroxisome** and the **nucleus** do **not** receive their proteins by vesicular transport; they are outside the endomembrane system even though they sit inside the same cytoplasm. Second, transport from the cytoplasm into the ER lumen is transmembrane transport, whereas transport from the ER to the Golgi is vesicular — the same protein experiences both on one journey.

Protein sorting begins while translation is still in progress. Proteins destined for the ER, the Golgi, the lysosome, the plasma membrane or the outside of the cell are synthesised on ribosomes bound to the **rough** ER; the growing polypeptide is threaded into the ER for folding and processing, vesicles bud from the ER carrying the cargo to the Golgi, and further sorting at the Golgi despatches it to its final address.

Everything else — cytosolic proteins, and proteins for the nucleus, mitochondrion, chloroplast and peroxisome — is made on **free** ribosomes in the cytosol and imported after translation is complete. Membrane-bound and free ribosomes are structurally identical; at any given moment they differ only in the **nascent protein** they happen to be making.

KEY POINT

Endomembrane system = ER + Golgi + endosomes + lysosome/vacuole + secretory vesicles + plasma membrane, connected by **vesicular** transport. Mitochondria, chloroplasts, peroxisomes and the nucleus are **not** part of it and receive proteins by transmembrane or gated transport instead. Free and membrane-bound ribosomes are identical; the destination is decided by the signal sequence on the protein being made.

5.2 Rough Endoplasmic Reticulum and Protein Secretion

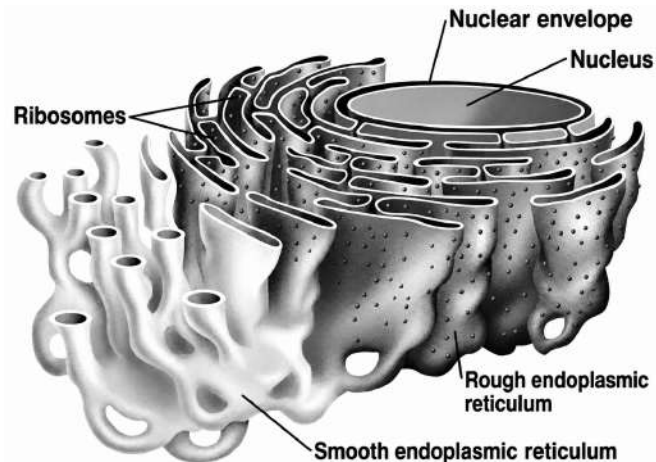
The **endoplasmic reticulum** is a network of membrane-enclosed tubules and flattened sacs, or **cisternae**, that extends from the nuclear envelope throughout the cytoplasm. The entire ER is enclosed by one continuous membrane and is the largest organelle of most eukaryotic cells: its membrane may account for about **half** of all cell membrane, and the space it encloses — the lumen, or cisternal space — for about 10 per cent of total cell volume. The outer nuclear membrane is continuous with the ER membrane, so the perinuclear space and the ER lumen are one compartment.

Two structurally and functionally distinct regions are recognised, and Table 5.2 sets them against one another.

Table 5.2 Rough and smooth endoplasmic reticulum compared

Feature	Rough (granular) ER	Smooth (agranular) ER
Ribosomes	Studded with ribosomes on the cytosolic face	None
Shape	Flattened, stacked cisternae	Branching tubules
Principal role	Synthesis, translocation, folding and glycosylation of proteins	Lipid and steroid synthesis, detoxification, carbohydrate metabolism, calcium storage
Abundant in	Pancreatic acinar cells, plasma cells, goblet cells	Hepatocytes, adrenal cortex, Leydig cells, muscle (as sarcoplasmic reticulum)
Marker enzyme	Ribophorins; ribosome-studded microsomes on fractionation	Glucose-6-phosphatase; smooth microsomes

Figure 5.1 *Rough and smooth endoplasmic reticulum. The rough ER is continuous with the outer nuclear membrane and is studded with ribosomes on its cytosolic surface; the smooth ER is a tubular network without ribosomes. The two are continuous with one another, so the lumen is a single compartment.*



5.2.1 Targeting proteins to the ER

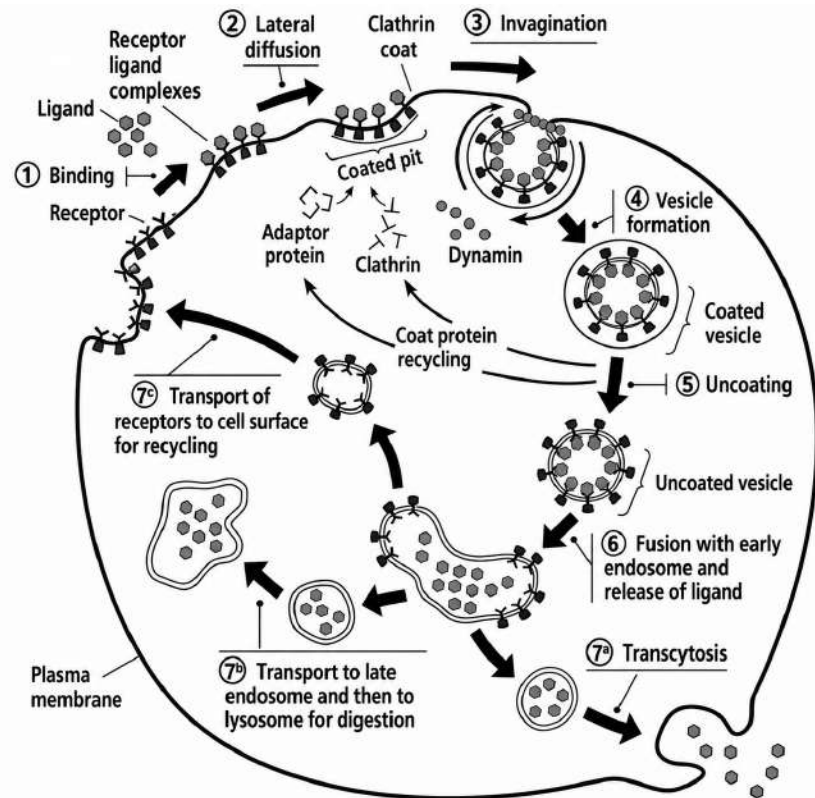
Proteins can be translocated into the ER either **during** their synthesis on membrane-bound ribosomes — **CO-translational** translocation — or **after** translation has been completed on free cytosolic ribosomes — **Post-translational** translocation. In mammalian cells most proteins enter the ER co-translationally; yeast uses both routes freely.

The co-translational route is the one to know in detail. Its logic was worked out by Günter Blobel and David Sabatini as the **signal hypothesis**, and it runs as follows. The N-terminus of a secretory protein carries a **signal peptide** — about 16 to 30 residues with a positively charged N-terminal region, a central core of six to twelve **hydrophobic** residues, and a cleavage site. As soon as this signal emerges from the ribosome it is bound by the **signal recognition particle**.

SRP is a rod-shaped **ribonucleoprotein**. In mammalian cells it consists of **six** protein subunits assembled on a single 7S RNA of about 300 nucleotides. Two of its ends do different jobs: one domain binds the signal peptide and also contacts the ribosome so as to **arrest** or slow further elongation, and the other engages the SRP **receptor** (docking protein) in the ER membrane. Both SRP and its receptor are GTPases, and the reciprocal GTP hydrolysis that follows docking releases the signal peptide from SRP and hands the ribosome to the translocation channel.

That channel is the **translocon**, built around the SEC61 complex — Sec61 $\alpha\beta\gamma$ in mammals, SecYEG in bacteria. It is an aqueous, gated pore. The signal peptide inserts as a loop, translation resumes, and the growing chain is threaded directly into the lumen. A luminal enzyme, **signal peptidase**, then cleaves the signal peptide from most soluble secretory proteins while translocation is still under way; the mature protein never exists in the cytosol at all.

Figure 7.35 *Receptor-mediated endocytosis. Receptor–ligand complexes gather in a clathrin-coated pit by lateral diffusion; adaptors, clathrin and dynamin drive invagination and scission; the vesicle uncoats and fuses with an early endosome, from which cargo is recycled, sent on to the late endosome and lysosome, or carried across the cell.*



7.17 The LDL Receptor and Familial Hypercholesterolaemia

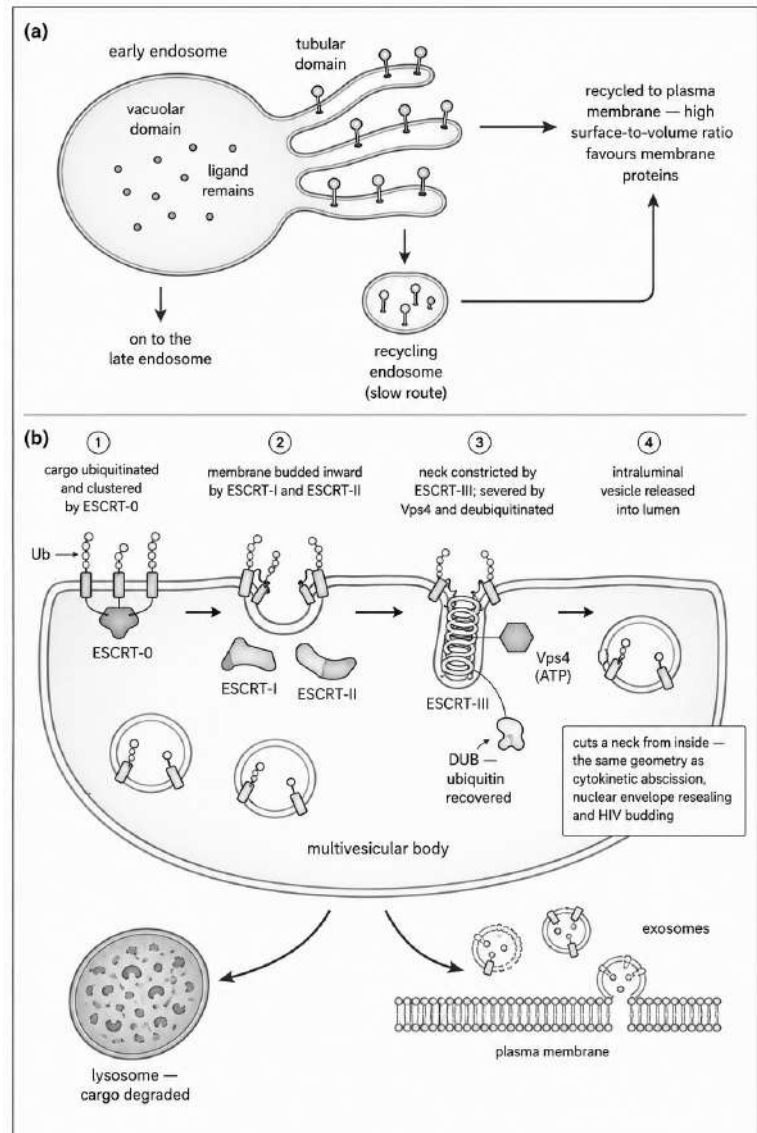
The uptake of cholesterol by mammalian cells provided the model on which the whole understanding of receptor-mediated endocytosis was built. Cholesterol travels in the bloodstream as **low-density lipoprotein**. Each LDL particle is a sphere 20 to 25 nm across with a core of cholesteryl esters, surrounded by a shell of cholesterol and phospholipid and a **single** molecule of the apolipoprotein APOB-100. Most mammalian cells make cell-surface receptors that bind apoB-100 specifically and internalise the particle. The receptor is **recycled** to the plasma membrane while the LDL is carried to the lysosome, where cholesterol is released for the cell to use. LDL cholesterol is called 'bad cholesterol' because it can clog arteries and lead to heart attacks, whereas HDL is 'good cholesterol' because it removes cholesterol from the arterial wall.

The receptor itself has about 700 extracellular amino acids, a single transmembrane helix of 22 residues, and a cytoplasmic tail of 50 residues. The N-terminal 292 residues carry the **LDL-binding domain**. Six residues within the cytoplasmic tail define the **internalisation signal**, first recognised through a familial hypercholesterolaemia patient in whom a **tyrosine** had mutated to **cysteine** and the receptor was no longer concentrated in coated pits.

7.17.1 The Brown and Goldstein experiment

Studies of the inherited disorder **familial hypercholesterolaemia** led to the discovery of the LDL receptor and to the first understanding of the endocytic pathway. Patients have elevated serum cholesterol and suffer heart attacks early in life; homozygotes may have myocardial infarction in childhood. Michael Brown and Joseph Goldstein found that the cells of these patients **failed** to internalise LDL from the extracellular fluid, which is why the serum cholesterol rises.

Figure 7.39 *The early endosome as a sorting station, and the ESCRT pathway. Receptors are recycled from the tubular domain while released ligands remain in the vacuolar domain. Ubiquitinated membrane proteins are clustered by ESCRT-0, budded inward by ESCRT-I and ESCRT-II and severed by ESCRT-III and Vps4, forming intraluminal vesicles and converting the endosome into a multivesicular body destined for the lysosome — or, if it fuses with the plasma membrane, releasing its contents as exosomes.*



7.20 Transcytosis, Polarized Sorting and Exocytosis

7.20.1 Polarized sorting and transcytosis

An epithelial cell has two faces — an **apical** surface facing the lumen and a **basolateral** surface facing the blood — separated by **tight junctions** that prevent membrane proteins from diffusing from one domain to the other. Different proteins must therefore be delivered to each. Two strategies are used. In **direct** sorting, vesicles leaving the trans-Golgi network are addressed to one face or the other: GPI **anchors** and certain N-glycans act as apical signals, while cytoplasmic tail motifs, often tyrosine- or dileucine-based, act as basolateral signals read by the epithelium-specific adaptor AP-1B. In **indirect** sorting, everything goes first to the basolateral surface and is then retrieved and carried across (Figure 7.40).

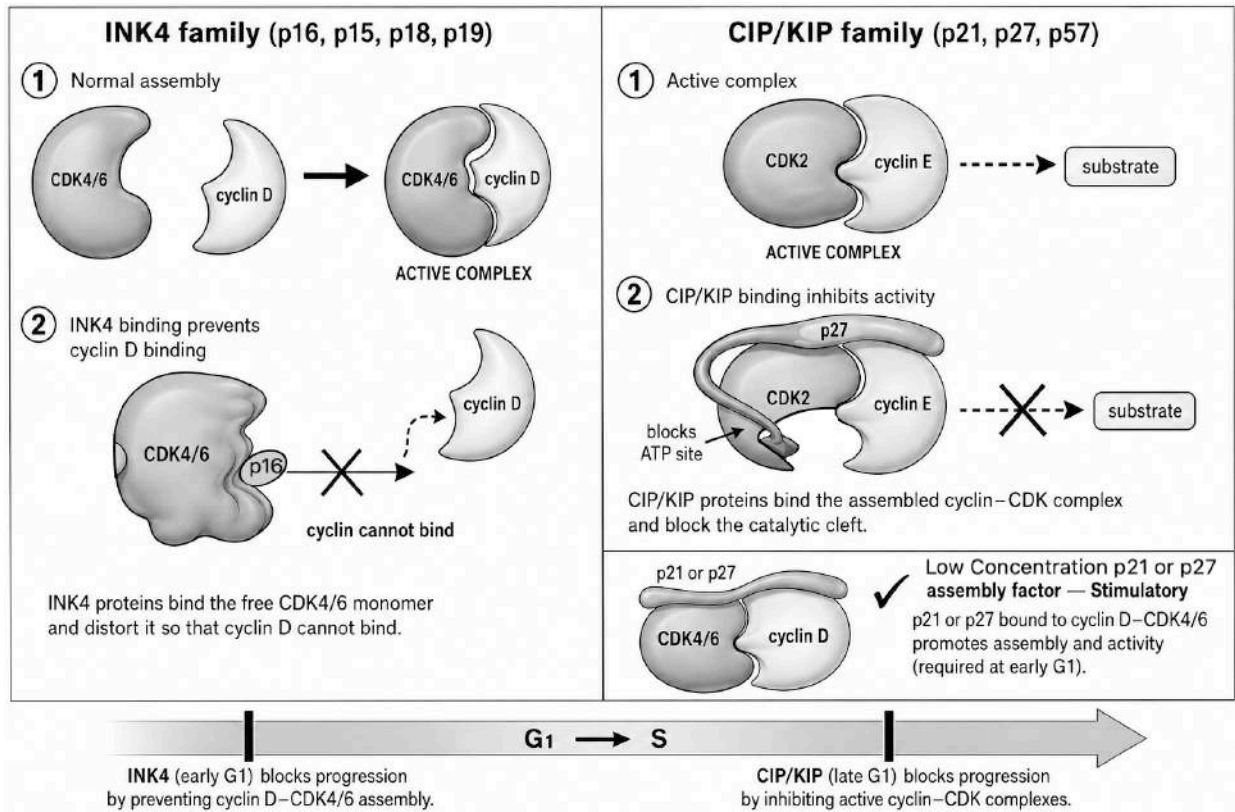


Figure 12.13 The two families of CDK inhibitor. INK4 proteins bind the free CDK4 or CDK6 monomer and distort it so that cyclin D cannot bind; CIP/KIP proteins bind the assembled cyclin-CDK complex and block the catalytic cleft.

The CIP/KIP family — CDK-interacting protein and kinase inhibitory protein — comprises p21 (also called CIP1 or WAF1), p27 (KIP1) and p57 (KIP2). These have a much broader range: they bind the **assembled cyclin-CDK complex**, contacting **both subunits**, and inhibit CDK1 and CDK2 complexes with cyclins A, B and E. A CIP/KIP protein lies along the complex like a strap, one end docking on the cyclin and the other inserting into the catalytic cleft of the kinase to displace ATP.

Table 12.5 The CDK inhibitory proteins and their targets

Inhibitor	Family	Interacts with	Notes
p16 (INK4a)	INK4	CDK4, CDK6	Encoded at the <i>CDKN2A</i> locus; frequently deleted in cancer; accumulates in senescence
p15 (INK4b)	INK4	CDK4	Induced by transforming growth factor β
p18 (INK4c)	INK4	CDK4, CDK6	Chiefly developmental
p19 (INK4d)	INK4	CDK4, CDK6	Chiefly developmental
p21 (CIP1 / WAF1)	CIP/KIP	Cyclin E-CDK2 and other complexes	Transcriptional target of p53; the effector of the G1 DNA-damage arrest
p27 (KIP1)	CIP/KIP	Cyclin D3-CDK4; cyclin E1-CDK2	High in G0; degraded via SCF-Skp2 after phosphorylation on Thr187
p57 (KIP2)	CIP/KIP	Cyclin E1-CDK2	Imprinted gene; mutated in Beckwith-Wiedemann syndrome

There is a wrinkle here that is genuinely important and frequently examined. CIP/KIP proteins are **not purely inhibitory**. At low concentrations p21 and p27 act as **assembly factors** for cyclin D with CDK4 and CDK6, promoting the formation of the complex and its import into the nucleus, and they do not inhibit that complex appreciably.

Cell Death

A multicellular organism is built as much by deletion as by addition. About sixty thousand million cells die in an adult human every day, and are replaced; the fingers of a human embryo are carved out of a solid paddle by the deliberate killing of the cells between them; roughly half of the neurons ever generated are destroyed during development because they failed to reach a target; and every self-reactive lymphocyte that would otherwise attack its owner is eliminated before it leaves the thymus. None of this is damage. It is a genetically encoded suicide programme, present in every cell, held in check by inhibitors and executed by a set of proteases that the cell has been carrying all along. The programme was worked out in a worm with exactly 131 doomed cells, and the genes found there turned out to have human counterparts that are mutated in lymphoma. Understanding cell death is therefore not a specialised corner of the subject: it is where development, immunity, cancer and neurodegeneration all meet.

LEARNING OBJECTIVES

- Define programmed cell death and describe its physiological roles.
- Describe the *Caenorhabditis elegans* death pathway and its mammalian counterparts.
- Distinguish apoptosis from necrosis morphologically and biochemically.
- Describe caspase structure and the difference between initiator and executioner caspases.
- Explain the intrinsic pathway — the Bcl-2 family, MOMP and the apoptosome.
- Explain the extrinsic pathway and the difference between type I and type II cells.
- Describe the IAPs and their neutralisation by Smac/DIABLO.
- Explain phosphatidylserine exposure and the clearance of apoptotic cells.
- Describe the methods used to detect apoptosis and what each of them measures.
- Describe necroptosis, pyroptosis and ferroptosis and the molecules that execute each.
- Compare apoptosis, autophagy and necrosis.
- Relate defects in cell death to cancer, autoimmunity and neurodegeneration

14.1 Programmed Cell Death — Definition and Physiological Roles

Programmed cell death is the death of a cell by an **orderly, genetically encoded process** carried out by machinery that the cell itself possesses. It is not the same thing as damage. A cell that is crushed, frozen or poisoned dies passively; a cell undergoing programmed death is spending energy to dismantle itself in a controlled sequence, and can in principle be stopped by mutating a single gene.

The commonest form of programmed cell death is **apoptosis**. The name was coined in 1972 by Kerr, Wyllie and Currie from the Greek for the falling of petals from a flower or of leaves from a tree, and it was chosen deliberately to convey that the process is natural, orderly and seasonal rather than catastrophic. What made their paper important was the observation that cells dying in this way in completely different tissues all looked the **same**, which implied a common underlying mechanism.

CED-9 is the **inhibitor** that binds CED-4 and holds it inactive, and it is the counterpart of BCL-2 — indeed the human *BCL2* gene, when introduced into the worm, will substitute for *ced-9*. EGL-1 is a BH3-**only** protein that binds CED-9 and releases CED-4. The logic is a **double negative**: an inhibitor of an inhibitor. A cell dies when EGL-1 appears; it lives when CED-9 is free.

Two further groups of genes act after death. The **engulfment** genes — *ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7*, *ced-10* and *ced-12* — direct neighbouring cells to swallow the corpse, and *nuc-1* encodes the nuclease that degrades its DNA inside the engulfing cell.

DO YOU KNOW?

The mammalian pathway contains one step the worm's does not: the release of **cytochrome C** from the mitochondrion. A protein whose day job is to carry electrons between complexes III and IV of the respiratory chain has been recruited as the trigger for apoptosis, and the same molecule therefore both keeps the cell alive and, when it escapes into the cytosol, orders its destruction.

14.2 The Morphology of Apoptosis and Necrosis

The two classical forms of cell death were distinguished long before either mechanism was understood, purely by how the dying cells looked, and the morphological description remains the quickest way to tell them apart (Figure 14.2).

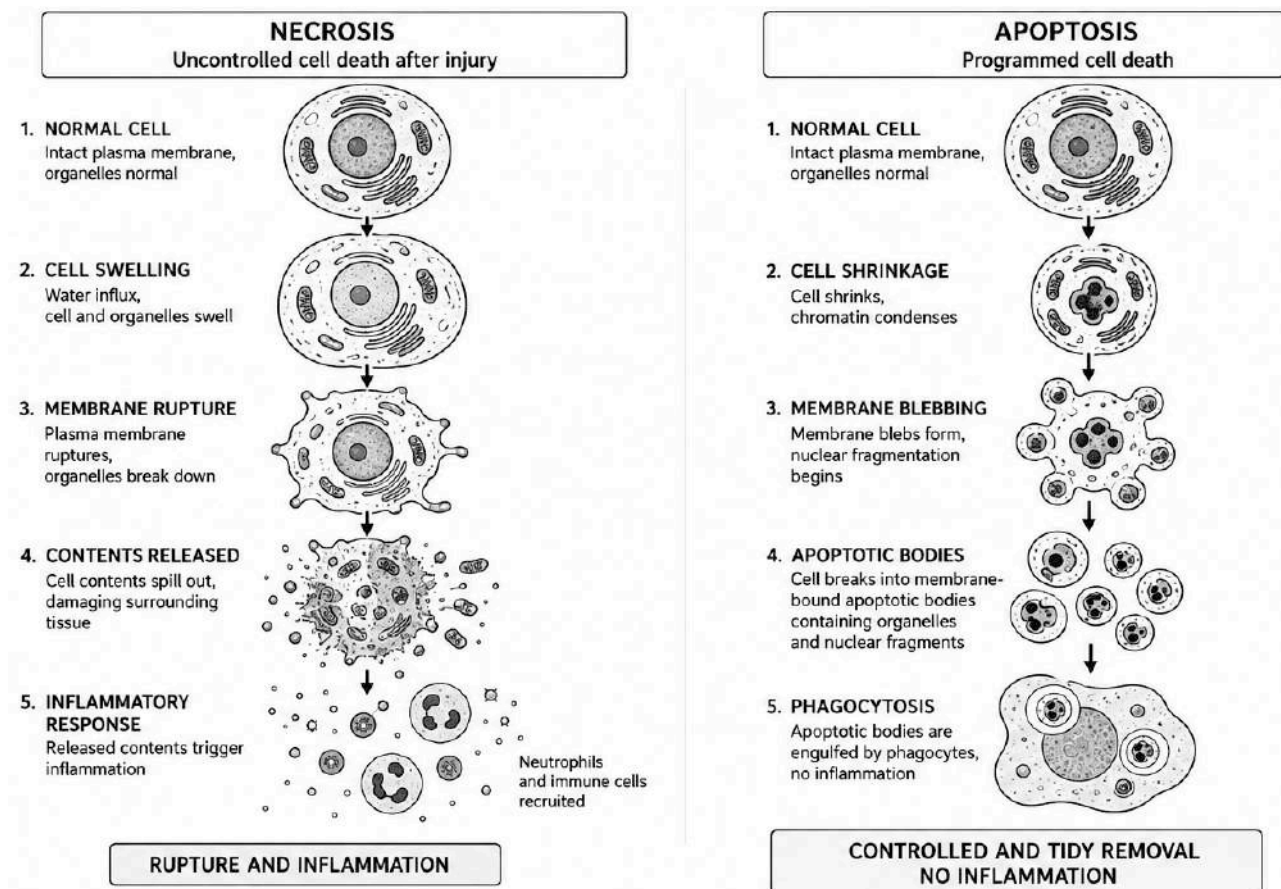


Figure 14.2 The morphology of apoptosis compared with necrosis. Apoptosis is a controlled, tidy demolition that provokes no inflammation; necrosis is a rupture that spills the cell contents and inflames the tissue.

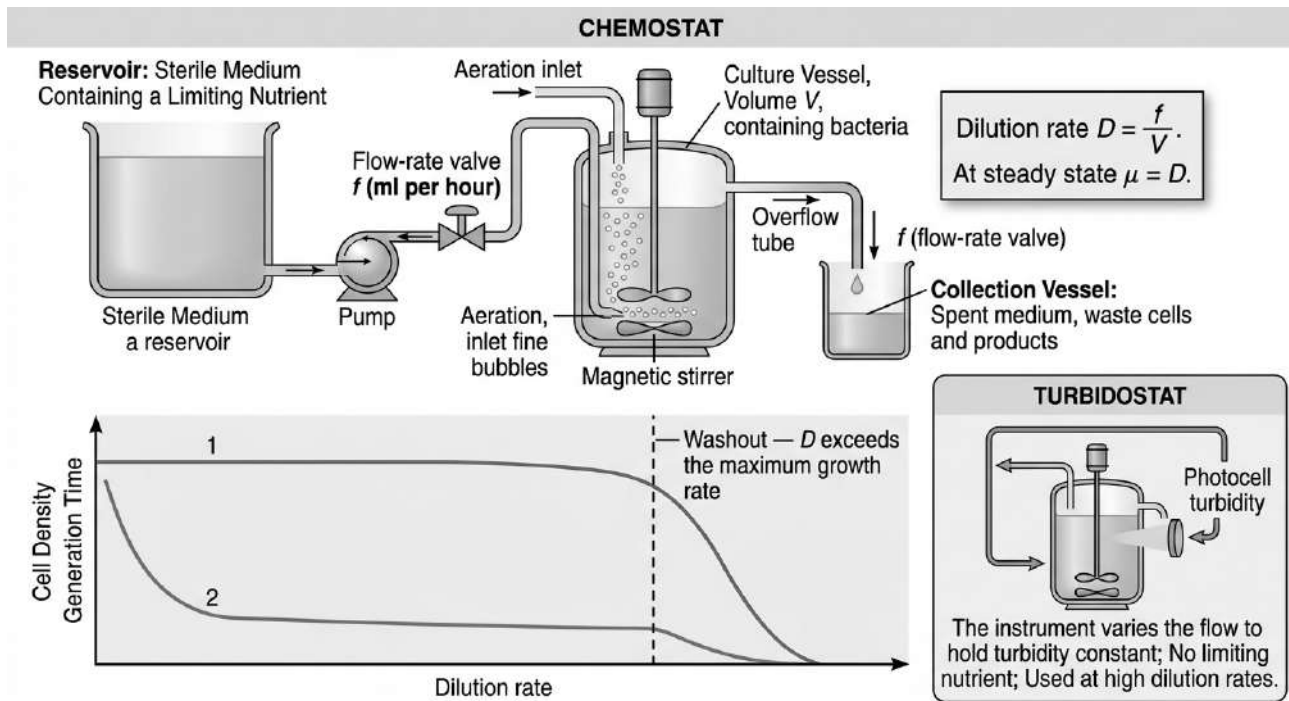


Figure 15.6 The chemostat. The dilution rate set by the operator determines the growth rate, and the concentration of the limiting nutrient in the reservoir determines the cell density.

The **turbidostat** works the other way round. There is **no limiting nutrient** at all; instead a photocell continuously monitors the turbidity of the culture and adjusts the flow rate to hold the cell density constant. It therefore operates best at **high** dilution rates, where the chemostat is unstable, and the organism grows at close to its maximum rate.

Continuous culture is used to study growth at low nutrient concentrations, which is the condition in most natural environments; to obtain a large uniform population for physiological or genetic study; and industrially, in the manufacture of vitamins, amino acids, enzymes and single-cell protein, and in the aerobic stage of sewage treatment.

DO YOU KNOW?

The chemostat has an elegant unintended use: it is a machine for running evolution. Because it can be kept going for thousands of generations under a single, precisely defined selective pressure, mutants that use the limiting nutrient more efficiently take over the culture and can be recovered and sequenced. The longest-running such experiment has followed *E. coli* for more than 75,000 generations.

15.5 The Influence of Environmental Factors on Growth

Microorganisms must respond to variations in nutrient levels, and their growth is also greatly affected by the chemical and physical nature of their surroundings. The ability of some of them to adapt to extreme and inhospitable environments is remarkable: prokaryotes are present anywhere life can exist, and many of their habitats would kill any other organism. *Bacillus infernus* appears to live more than 1.5 miles below the Earth's surface, without oxygen and at temperatures above 60 °C. Organisms growing in such conditions are called **extremophiles**. Table 15.4 summarises the classes.