Cell Biology

Unit 3 · Cell Organelles and Cytoskeleton

A eukaryotic cell is divided into membrane-bound rooms, each with its own job, held in shape by a protein skeleton. After this unit you can draw and label every major organelle, trace a secreted protein from ribosome to cell surface, explain how mitochondria and chloroplasts make ATP, and describe how microtubules, actin and motor proteins move things inside the cell.

Taught at 7 of 10 | Usually Sem 3 | 9 chapters

About this unit

This unit walks through the parts of a eukaryotic cell one compartment at a time. It starts with the nucleus, where genes are kept and ribosome parts are made, then follows a new protein along the secretory road: endoplasmic reticulum, Golgi apparatus, and on to lysosomes and other destinations. After the ribosome itself, two energy organelles follow, mitochondria and chloroplasts. The last two chapters cover the cytoskeleton, the protein scaffold that gives the cell its shape, and the motors and centrioles that move things along it.

Chapters

1. Nucleus, nuclear envelope, nuclear pore and nucleolus
2. Endoplasmic reticulum
3. Golgi apparatus and protein sorting
4. Lysosomes, peroxisomes and vacuoles
5. Ribosomes
6. Mitochondria: structure and function
7. Chloroplasts: structure and function
8. Cytoskeleton: microtubules, actin and intermediate filaments
9. Molecular motors and centrioles

How to use this book

- Read the lead paragraph and the figure of each chapter first; together they carry the one idea of the chapter.
- Cover the solution of the worked example and do it yourself on paper, then compare step by step.

- Answer each "Check yourself" question in your head or in your notebook before you open it.

3.1 Nucleus, nuclear envelope, nuclear pore and nucleolus

The nucleus is the room where a eukaryotic cell keeps its DNA. A double membrane, the nuclear envelope, separates it from the cytoplasm, so that copying DNA and making RNA happen apart from making protein. Traffic between the two sides passes through nuclear pores, and inside the nucleus a dense patch called the nucleolus builds ribosome subunits. This separation is what lets eukaryotes process RNA (cut, cap and tail it) before any ribosome can read it.

You need: the prokaryote–eukaryote comparison from **Unit 1, Prokaryotic and eukaryotic cell organization**, and the lipid bilayer from **Unit 2, Plasma membrane structure**.

The nuclear envelope and lamina

The nuclear envelope is two lipid bilayers, an **outer nuclear membrane** and an **inner nuclear membrane**, with a thin gap between them called the **perinuclear space**. The outer membrane is continuous with the rough endoplasmic reticulum and carries ribosomes on its cytosolic face, so the perinuclear space is part of the ER lumen. The two membranes join only at the pores.

The inner membrane has its own proteins, which hold on to a mesh just beneath it: the **nuclear lamina**. The lamina is built from **lamins** (A, B and C), which are intermediate-filament proteins (see Chapter 3.8). It gives the nucleus its shape and strength and anchors chromatin. When a cell enters mitosis, kinases add phosphate groups to the lamins, the mesh comes apart and the envelope breaks into vesicles; it reassembles around each daughter set of chromosomes (cell division itself is in **Unit 4, Mitosis**). Mutations in the lamin A gene cause laminopathies, including the premature-ageing disease progeria.

The nuclear pore complex

A **nuclear pore complex** (NPC) is a large protein channel where the inner and outer membranes fuse. It is made of about 30 different proteins called **nucleoporins**, each present in many copies, arranged with **eightfold symmetry** around a central channel. Some nucleoporins carry long, disordered chains rich in phenylalanine–glycine repeats (**FG repeats**) that fill the channel like a gel. Ions, sugars, nucleotides and small proteins diffuse through freely. Molecules larger than roughly 40–60 kDa are held back by the FG mesh unless a transport receptor carries them.

1. **Import.** A protein meant for the nucleus carries a **nuclear localization signal** (NLS), a short stretch of basic amino acids (lysine, arginine). The classic example is PKKKRKV from the SV40 virus large T antigen. The adaptor **importin α** binds the NLS, and **importin β** binds importin α and slides through the FG mesh.
2. **Release.** Inside the nucleus, the small GTPase **Ran** in its GTP-bound form binds importin β, and the cargo is let go.
3. **Recycling.** The importin–Ran-GTP complex goes back out. In the cytosol a GTPase-activating protein (RanGAP) makes Ran hydrolyse its GTP, and importin is freed for another round.
4. **Export.** Proteins with a **nuclear export signal** (leucine-rich) bind an exportin together with Ran-GTP inside the nucleus and are released in the cytosol when GTP is hydrolysed. mRNA leaves bound to its own export proteins after processing.

The direction comes from the **Ran gradient**: the enzyme that loads Ran with GTP (RanGEF, also called RCC1) sits on chromatin inside the nucleus, while RanGAP sits in the cytosol. So Ran-GTP is high inside and low outside. Importins bind cargo where Ran-GTP is low; exportins bind cargo where it is high.

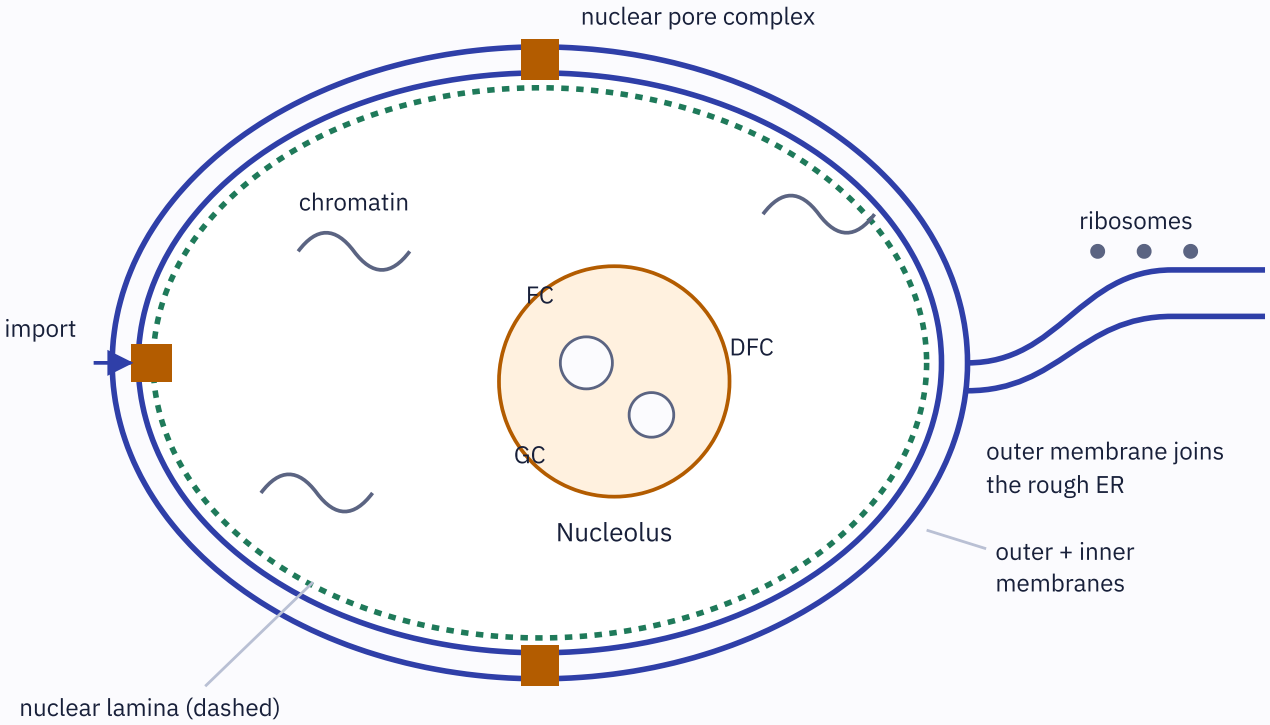


Figure 3.1.1 A nucleus in section. The envelope is two membranes; the outer one runs straight into the rough ER, so the space between them is ER lumen. Pores (orange) cross both membranes. Under the inner membrane lies the lamina. In the nucleolus, FC is the fibrillar centre (pale circles), DFC the dense fibrillar component around it, and GC the granular component outside.

The nucleolus: a ribosome factory

The **nucleolus** is not bounded by a membrane. It forms around the **nucleolar organizer regions** (NORs), the chromosome loops that carry many tandem copies of the ribosomal RNA (rRNA) genes. In humans the NORs sit on the short arms of the five acrocentric chromosomes, 13, 14, 15, 21 and 22. Under the electron microscope it shows three zones:

- **Fibrillar centre (FC):** holds the rRNA genes not being read, and RNA polymerase I waiting to start.
- **Dense fibrillar component (DFC):** where the rRNA genes are transcribed and the first processing begins.
- **Granular component (GC):** where rRNA folds and joins ribosomal proteins into maturing subunits.

RNA polymerase I makes one long precursor (45S in mammals). It is cut into the 18S, 5.8S and 28S rRNAs. The fourth rRNA, 5S, is made by RNA polymerase III from genes outside the nucleolus and brought in. Ribosomal proteins are made in the cytoplasm, imported through the pores, assembled with rRNA in the nucleolus, and the finished small and large subunits are exported separately. They join only in the cytoplasm, on an mRNA (see Chapter 3.5). A cell making a lot of protein, such as a plasma cell or a growing cancer cell, has a large and prominent nucleolus. The nucleolus disappears during mitosis and re-forms afterwards.

The rest of the nucleus holds **chromatin**, DNA wrapped on histones. Loosely packed, active chromatin is **euchromatin**; tightly packed, mostly silent chromatin is **heterochromatin**, often found against the lamina. That belongs to molecular biology and is not repeated here.

WORKED EXAMPLE

Question (8 marks). A researcher joins green fluorescent protein (GFP, about 27 kDa) to three different partners and looks at where the fusion sits in living cells. (a) GFP alone; (b) GFP joined to a 100 kDa cytosolic enzyme; (c) the same 100 kDa fusion with PKKKRKV added at one end. Predict where each fluoresces and explain. (d) What happens to (c) if the cells make a Ran mutant that cannot hydrolyse GTP?

Solution.

1. **(a)** 27 kDa is below the passive limit of about 40–60 kDa, so GFP diffuses both ways through the pores and is seen in both cytoplasm and nucleus.
2. **(b)** The fusion is about 127 kDa. It is too large to diffuse and has no NLS, so no importin carries it. It stays in the cytoplasm; the nucleus looks dark.
3. **(c)** PKKKRKV is the SV40 NLS. Importin α binds it, importin β carries the complex through the pore, Ran-GTP releases it inside. The nucleus glows and the cytoplasm is dim.
4. **(d)** Importin β stays locked to Ran-GTP after it returns to the cytosol, because GTP is never hydrolysed. Free importin runs out, import stops, and newly made fusion protein builds up in the cytoplasm.

Answer: (a) both compartments; (b) cytoplasm only; (c) nucleus; (d) import fails, protein stays cytoplasmic. The marks are for using size, the NLS and the Ran cycle, not just for the location.

EXAM TRAPS

- The nucleolus has no membrane. Students who draw a membrane around it lose the mark.
- 5S rRNA is not made in the nucleolus; it is made by RNA polymerase III elsewhere in the nucleus and brought in.
- The NLS is not cut off after import (unlike an ER signal sequence), so a protein can be imported again after every mitosis.
- Ran-GTP is high inside the nucleus, not outside. Reversing this gradient reverses every arrow in your answer.
- The perinuclear space is continuous with the ER lumen, not with the cytosol.

CHECK YOURSELF

– REMEMBER **Name the three zones of the nucleolus.** 2 marks

Fibrillar centre, dense fibrillar component and granular component.

– REMEMBER **What proteins make up the nuclear lamina?** 1 mark

Lamins (A, B and C), which are intermediate-filament proteins.

– UNDERSTAND **Why is the nuclear envelope described as part of the ER?** 2 marks

The outer nuclear membrane is continuous with the rough ER membrane and carries ribosomes, and the perinuclear space is continuous with the ER lumen.

– UNDERSTAND **How does the Ran gradient make nuclear transport one-way?** 4 marks

RanGEF on chromatin keeps Ran-GTP high in the nucleus; RanGAP in the cytosol keeps it low there. Importins release cargo when Ran-GTP binds them, which happens only inside; exportins bind cargo only together with Ran-GTP, inside, and release it when GTP is hydrolysed outside.

– APPLY **A 20 kDa protein with no NLS and a 90 kDa protein with an NLS are made in the cytosol. Where does each end up?** 3 marks

The 20 kDa protein diffuses through the pores and spreads over both compartments. The 90 kDa protein is too large to diffuse but is carried in by importins, so it collects in the nucleus.

– APPLY **Electron micrographs show a very large nucleolus in one cell type. What does that tell you about the cell?** 2 marks

It is making ribosomes fast, so it is making a lot of protein, as in a secretory cell, a plasma cell or a rapidly growing cell.

– ANALYZE **Explain how separating transcription from translation lets a eukaryote do things a bacterium cannot.** 4 marks

In bacteria ribosomes start reading an mRNA while it is still being made, so it cannot be edited first. Behind the envelope, a eukaryotic pre-mRNA can be capped, spliced and tailed, and only finished mRNA is exported. This allows alternative splicing and quality control, and the pore becomes another point where gene expression is controlled.

BEYOND THE SYLLABUS

Cryo-electron tomography and AlphaFold models have now given near-complete structures of the human nuclear pore, and the FG-repeat channel is studied as an example of liquid-like protein phases. The nucleolus is a classic example of a membraneless organelle formed by phase separation, a current research theme also linked to neurodegenerative disease. Nuclear import signals are used in gene therapy and CRISPR delivery to bring Cas9 into the nucleus. Search "nuclear pore complex cryo-ET" and "nucleolus phase separation" to read more. None of this is for the exam.

3.2 Endoplasmic reticulum

The endoplasmic reticulum (ER) is a network of membrane tubes and flat sacs that spreads from the nuclear envelope through the cytoplasm. Its inside, the **lumen**, is one connected space. The ER is the entry gate of the secretory pathway: every protein that will be secreted, or that will live in the plasma membrane, Golgi, lysosome or ER itself, enters here. The ER also makes most of the cell's membrane lipids, and in muscle it stores the calcium that triggers contraction.

You need: membrane lipids and proteins from **Unit 2, Membrane proteins and lipids**, and the nuclear envelope from Chapter 3.1.

Rough and smooth ER

Rough ER (RER) is studded with ribosomes on its cytosolic face and is usually built of flattened sacs (cisternae). It makes secretory and membrane proteins. Cells that secrete a lot of protein, such as pancreatic acinar cells (digestive enzymes) or plasma cells (antibodies), are packed with RER.

Smooth ER (SER) has no ribosomes and is usually tubular. Its jobs depend on the cell:

- **Lipid synthesis:** phospholipids and cholesterol in all cells; steroid hormones in the adrenal cortex, testis and ovary.
- **Detoxification:** in liver cells, cytochrome P450 enzymes add hydroxyl groups to drugs and toxins so they dissolve in water and leave in urine. Long use of drugs such as phenobarbital makes the liver SER expand.

- **Calcium store:** in muscle the SER is called the **sarcoplasmic reticulum**; it pumps Ca^{2+} in and releases it on a nerve signal.
- **Glucose release:** in liver, glucose-6-phosphatase in the ER membrane frees glucose from glycogen breakdown.

The point where vesicles bud off towards the Golgi is a ribosome-free patch of RER called the **ER exit site** (transitional ER).

How a protein enters the ER: the signal hypothesis

All protein synthesis starts on free ribosomes in the cytosol. What sends a ribosome to the ER is the protein it is making. Günter Blobel's **signal hypothesis** (Nobel Prize 1999) explains how. A secretory protein begins with an **ER signal sequence**: about 15–30 amino acids at the N-terminus, with a core of 6–12 hydrophobic residues.

1. The signal sequence comes out of the ribosome's exit tunnel.
2. The **signal recognition particle** (SRP), a complex of six proteins and a 7S RNA, binds the signal sequence and the ribosome, and translation pauses.
3. SRP brings the ribosome to the **SRP receptor** on the ER membrane. Both bind GTP.
4. The ribosome is handed to the **translocon**, a channel called **Sec61**. GTP is hydrolysed, SRP and its receptor separate and SRP is recycled.
5. Translation restarts and the growing chain is threaded through Sec61 into the lumen. This is **cotranslational translocation**: the protein crosses while it is still being made.
6. **Signal peptidase** on the lumen side cuts off the signal sequence. The finished protein is released into the lumen when synthesis ends.

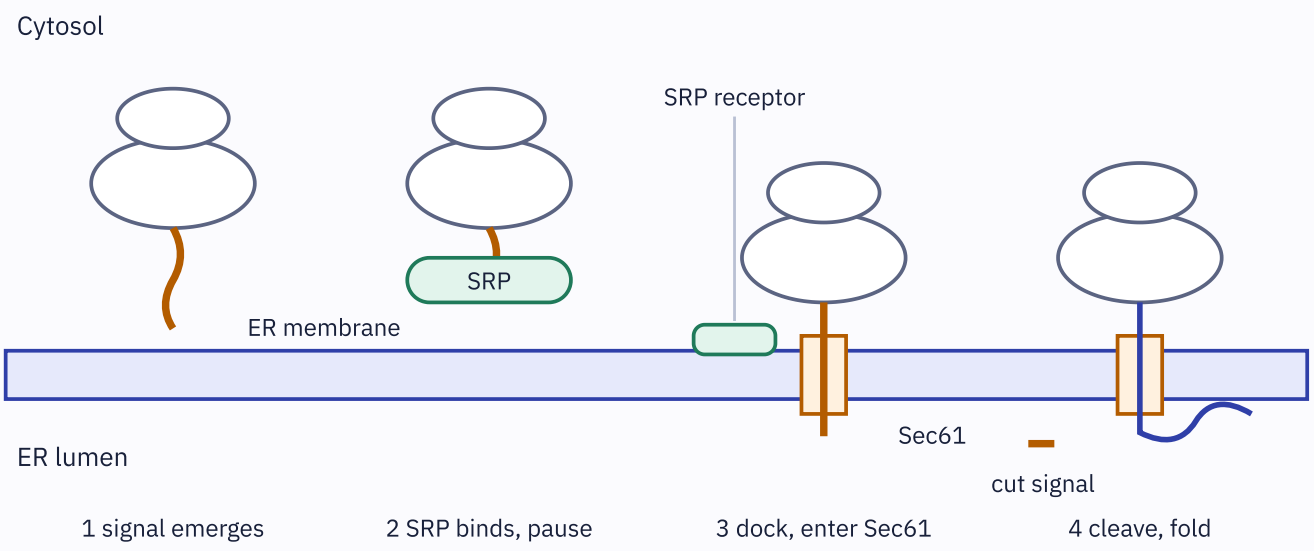


Figure 3.2.1 Cotranslational import into the ER. The signal sequence (orange) appears, SRP grabs it and pauses translation, the ribosome docks on the SRP receptor and Sec61 channel, and the chain enters the lumen; signal peptidase then cuts the signal off. Notice the ribosome stays on the cytosolic side the whole time.

Membrane proteins, folding and quality control

A protein that must stay in the membrane carries a second hydrophobic stretch, a **stop-transfer anchor sequence**. When it enters Sec61, the channel opens sideways and releases it into the bilayer as a membrane-spanning α-helix, while the rest of the chain finishes on the cytosolic side. An internal **signal-anchor sequence** (not cleaved) can start transfer in either orientation, and alternating start and stop sequences give proteins that cross the membrane many times.

Once in the lumen, proteins are folded and modified:

- **Chaperones** such as BiP hold unfolded chains so they do not clump.
- **Disulfide bonds** are made by protein disulfide isomerase (PDI); the lumen is oxidising, unlike the cytosol.
- **N-linked glycosylation:** the enzyme oligosaccharyltransferase moves a ready-made 14-sugar block ($Glc_3Man_9GlcNAc_2$, built on the lipid dolichol) on to asparagine in the sequence Asn–X–Ser/Thr, where X is any amino acid except proline.
- **Calnexin and calreticulin** check folding: a protein that is still misfolded gets a glucose added back and is held for another try.

Proteins that never fold are pulled back to the cytosol and destroyed by the proteasome; this is **ER-associated degradation** (ERAD). When misfolded proteins pile up, the ER sends the **unfolded protein response** (UPR) through three sensors, IRE1, PERK and ATF6: the cell makes more chaperones and slows translation, and if the stress lasts it triggers apoptosis (Unit 4).

Resident lumen proteins such as BiP carry the C-terminal signal **KDEL** (Lys-Asp-Glu-Leu); if they escape to the Golgi, a KDEL receptor returns them.

WORKED EXAMPLE

Question (6 marks). Predict the final location and orientation of each protein. (a) An N-terminal cleavable signal sequence, nothing else hydrophobic. (b) An N-terminal cleavable signal sequence and one stop-transfer sequence in the middle. (c) Same as (a) plus KDEL at the C-terminus. (d) No signal sequence at all.

Solution.

1. **(a)** SRP sends it to the ER, the whole chain is translocated and the signal is cut off. It is a soluble lumen protein; with no other signal it moves on to the Golgi and is secreted (the default route, see Chapter 3.3).
2. **(b)** Translocation starts at the N-terminus and stops at the stop-transfer sequence, which becomes the single membrane-spanning helix. The N-terminus is in the lumen and the C-terminus in the cytosol. This is a **single-pass type I** membrane protein. After the Golgi it reaches the plasma membrane with its N-terminus outside the cell, because the lumen side becomes the outside after vesicle fusion.
3. **(c)** It enters the lumen like (a), but the KDEL receptor keeps returning it from the Golgi. It is an ER-resident lumen protein.
4. **(d)** It is finished on a free ribosome and stays in the cytosol, unless it carries another signal (nuclear, mitochondrial or peroxisomal).

Answer: (a) secreted; (b) plasma membrane, type I, N-terminus outside; (c) ER lumen; (d) cytosol.

EXAM TRAPS

- Ribosomes are not permanently fixed to the RER. The same ribosome can be free or bound; the protein it makes decides.
- The ER signal sequence is cleaved; the nuclear localization signal is not. Do not mix them.
- N-linked sugars go on asparagine (Asn) in the ER. O-linked sugars go on serine or threonine, mostly in the Golgi.
- The lumen of the ER is topologically equivalent to the outside of the cell. This is why the lumen side of a membrane protein faces outward at the plasma membrane.
- Sarcoplasmic reticulum is smooth ER, not rough ER.

CHECK YOURSELF

- REMEMBER **Give two functions of the smooth ER.** 2 marks

  Any two of: lipid and steroid synthesis, detoxification of drugs by cytochrome P450, Ca^{2+} storage in muscle, glucose release in liver.

- REMEMBER **What is the consensus sequence for N-linked glycosylation?** 1 mark

  Asn–X–Ser/Thr, where X is any amino acid except proline.

- UNDERSTAND **Why does translation pause when SRP binds?** 2 marks

  So that the chain is not finished in the cytosol before the ribosome reaches the ER; the protein must cross the membrane while it is still unfolded.

- UNDERSTAND **What is the unfolded protein response and why does a cell need it?** 3 marks

  A signal sent by ER stress sensors (IRE1, PERK, ATF6) when misfolded proteins build up. It raises chaperone levels and slows new protein synthesis so the ER can catch up; if stress persists it triggers apoptosis to remove the damaged cell.

- APPLY **A liver cell is given phenobarbital for several days. Predict the change in its ER.** 2 marks

  The smooth ER expands and makes more cytochrome P450 enzymes to detoxify the drug.

- APPLY **A cell-free translation system has ribosomes and mRNA for a secreted protein but no ER membranes. What length of protein is made, and why?** 3 marks

  Without SRP, the full-length protein is made with its signal sequence still attached, because there is no signal peptidase. With SRP but no membranes, translation stalls. Adding microsomes (ER vesicles) gives the shorter, cleaved protein inside the vesicles. This was the original evidence for the signal hypothesis.

- ANALYZE **Compare how proteins enter the ER and the nucleus.** 4 marks

  ER: N-terminal hydrophobic signal, usually cotranslational, unfolded chain through Sec61, signal cleaved, GTP used by SRP and receptor. Nucleus: internal basic NLS, post-translational, folded protein through a large pore, signal kept, GTP used by Ran.

BEYOND THE SYLLABUS

Many therapeutic proteins, including insulin and monoclonal antibodies made in CHO cells, pass through the ER of the producing cell, and bioprocess engineers tune chaperone and UPR genes to raise yield. Signal-peptide choice is a standard design step when expressing a protein for secretion in yeast or mammalian cells. ER stress is studied in diabetes, where insulin-making β cells work near their ER limit. Search "signal peptide optimisation recombinant secretion" and "unfolded protein response diabetes". This is not for the exam.

3.3 Golgi apparatus and protein sorting

The Golgi apparatus is a stack of flat membrane sacs that receives proteins and lipids from the ER, finishes modifying them, and sorts them by destination, like a post office that stamps each parcel with an address. From the Golgi, cargo goes to the plasma membrane, out of the cell, or to lysosomes. Camillo Golgi saw it in nerve cells in 1898 with a silver stain. It is large in secretory cells such as goblet cells of the gut, which pour out mucus.

You need: the ER and signal sequences from Chapter 3.2, and vesicle fusion from **Unit 2, Endocytosis and exocytosis**.

Structure: a stack with two faces

A Golgi stack has 4–8 cisternae in most animal cells, and has a direction. The **cis face** faces the ER and receives cargo; the **trans face** faces the plasma membrane and ships it out. In order from entry to exit:

- **Cis Golgi network (CGN):** a network of tubes that takes in vesicles from the ER.
- **Cis, medial and trans cisternae:** each holds a different set of enzymes, so modifications happen in sequence.
- **Trans Golgi network (TGN):** the sorting station, where cargo is packed into different carriers for different destinations.

Animal cells usually have their Golgi stacks joined into one ribbon near the nucleus and the centrosome. Plant cells have hundreds of separate stacks (sometimes called dictyosomes) scattered in the cytoplasm, which also make the pectin and hemicellulose of the cell wall.

What the Golgi does to cargo

- **Trims and rebuilds N-linked sugars** added in the ER: mannose is removed, then N-acetylglucosamine, galactose and sialic acid are added, giving "complex" oligosaccharides. Some proteins keep "high-mannose" sugars.
- **O-linked glycosylation**: sugars added to serine or threonine, as in mucins and proteoglycans.
- **Sulfation** of tyrosines and of proteoglycan sugars, in the trans Golgi network.
- **Proteolytic processing** of some precursors, such as proinsulin to insulin, which starts in the TGN and goes on in secretory granules.
- **Lipid work**: sphingomyelin and glycolipids are made here.

Moving between compartments: coated vesicles

Cargo moves in small vesicles whose coat proteins both bend the membrane and pick the cargo. Three coats matter for the exam:

- **COPII**: ER → Golgi (forward, anterograde).
- **COPI**: Golgi → ER and between cisternae (backward, retrograde). This is how escaped KDEL proteins return to the ER.
- **Clathrin** (with adaptor proteins): TGN → endosomes, and plasma membrane → endosomes in endocytosis.

A vesicle finds the right target through **Rab** GTPases that mark each compartment, and fuses using matching **SNARE** proteins: a v-SNARE on the vesicle pairs with a t-SNARE on the target membrane. How cargo crosses the stack itself is debated. The **vesicular transport model** says the cisternae stay put and vesicles carry cargo forward. The **cisternal maturation model** says each cisterna moves forward and changes its identity as COPI vesicles carry enzymes backward. Evidence now favours maturation for large cargo such as procollagen, which is too big for a normal vesicle.

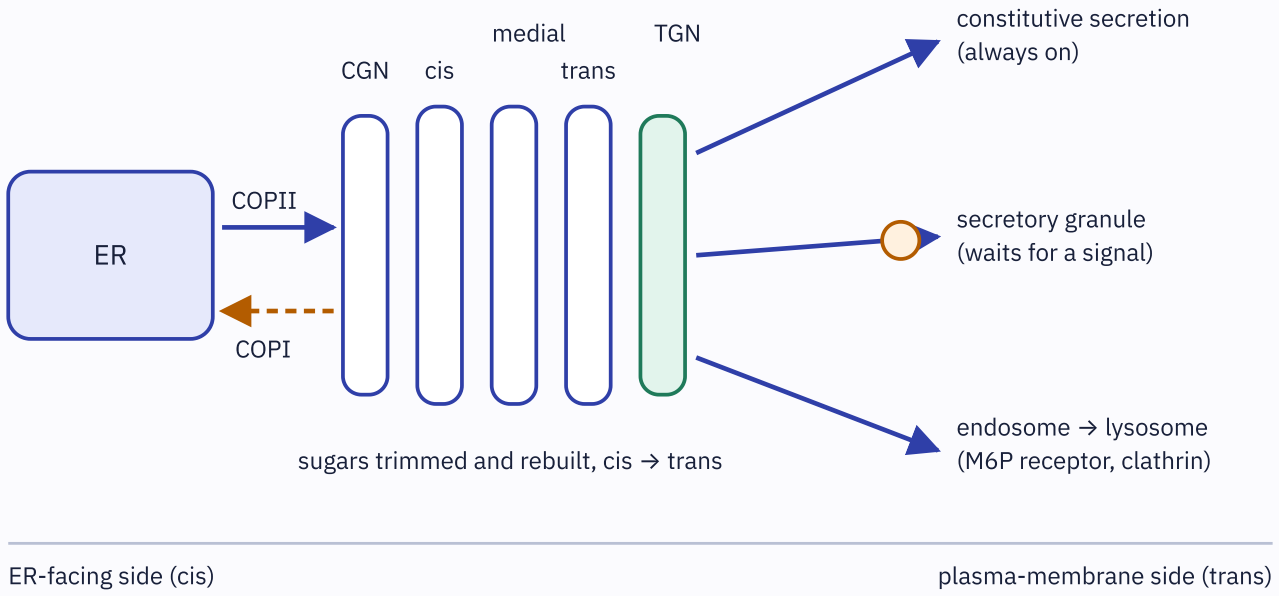


Figure 3.3.1 Cargo enters at the cis face in COPII vesicles, moves through the cisternae, and leaves the trans Golgi network by one of three routes. COPI (dashed) carries escaped ER proteins back. Notice that lysosomal enzymes are the only cargo here that needs a sugar tag to find its way.

Sorting at the TGN: three exits

1. **Constitutive secretion** is the default. A soluble protein with no sorting signal is carried in vesicles that fuse with the plasma membrane continuously. Collagen made by fibroblasts and serum albumin made by liver cells leave this way.
2. **Regulated secretion**: in specialised cells, some proteins clump together in the acidic TGN and are packed into dense **secretory granules** that wait near the plasma membrane. They fuse only on a signal, usually a rise in cytosolic Ca^{2+}. Insulin from pancreatic β cells, digestive zymogens and neurotransmitters are released this way.
3. **To lysosomes** via the **mannose-6-phosphate (M6P)** tag. In the cis Golgi, the enzyme GlcNAc-phosphotransferase recognises a 3-D **signal patch** on each lysosomal enzyme and adds GlcNAc-phosphate to its mannose. A second enzyme removes the GlcNAc, exposing M6P. In the TGN, **M6P receptors** bind the tagged enzymes and are gathered into clathrin-coated vesicles that go to endosomes. The endosome is acidic, so the enzyme falls off, its phosphate is removed, and the receptor returns to the Golgi for reuse.

Membrane proteins are sorted by short signals in their cytosolic tails, and in epithelial cells the TGN also separates proteins for the apical and the basolateral surfaces. The same material answers that heading.

WORKED EXAMPLE

Question (8 marks). In I-cell disease (mucolipidosis II), a child's cells show large inclusions full of undigested material, and lysosomal enzyme levels in the blood are many times normal. Explain both findings from the molecular defect. Why does adding the missing enzymes to the culture medium of the child's fibroblasts, taken from a healthy person's cells, correct the inclusions only if the enzymes carry M6P?

Solution.

1. **Defect:** the child lacks working GlcNAc-phosphotransferase, so no lysosomal enzyme gets the M6P tag in the cis Golgi.
2. **Sorting fails:** without M6P, the receptors in the TGN cannot capture the enzymes. The enzymes follow the default constitutive route and are secreted.
3. **Blood finding:** secreted enzymes reach the blood, so their blood levels are high.
4. **Inclusions:** lysosomes lack most of their hydrolases, so material taken in by endocytosis and autophagy is not broken down and piles up as inclusions (hence "inclusion cell").
5. **Rescue:** some M6P receptors also sit on the plasma membrane. Enzymes carrying M6P bind them, are taken in by clathrin-mediated endocytosis and are delivered to lysosomes, so the inclusions clear. Enzymes without M6P cannot be picked up this way.

Answer: missing M6P tag → enzymes secreted instead of sorted → high blood enzymes and empty lysosomes; rescue works through surface M6P receptors. This uptake route is also the basis of enzyme replacement therapy for some lysosomal storage diseases.

EXAM TRAPS

- COPII goes forward (ER → Golgi); COPI goes backward. A memory aid: "two goes to" the Golgi.
- The M6P tag is added in the cis Golgi but read in the trans Golgi network. Examiners ask "where" for each step.
- Constitutive secretion needs no signal; it is the default. Regulated secretion and lysosomal sorting need signals.
- Lysosomal enzymes are recognised by a 3-D signal patch, not a linear sequence like KDEL.

CHECK YOURSELF

– REMEMBER **Name the faces of the Golgi and say which one receives cargo.** 2 marks

Cis face (receives cargo from the ER) and trans face (ships cargo out through the TGN).

– REMEMBER **Which coat protein forms vesicles at the TGN bound for endosomes?** 1 mark

Clathrin, with adaptor proteins.

– UNDERSTAND **Distinguish constitutive and regulated secretion with one example each.** 4 marks

Constitutive: continuous, no signal needed, in all cells; for example albumin from liver cells. Regulated: cargo stored in granules and released only on a signal such as a Ca^{2+} rise, in specialised cells; for example insulin from β cells.

– UNDERSTAND **State the two models of transport through the Golgi stack.** 3 marks

Vesicular transport: cisternae are fixed and vesicles carry cargo forward. Cisternal maturation: cisternae move forward and mature from cis to trans while COPI vesicles return enzymes backward.

– APPLY **A drug blocks Ca^{2+} entry into pancreatic β cells. What happens to insulin secretion and to albumin secretion by the same patient's liver?** 2 marks

Insulin release falls, because regulated secretion needs the Ca^{2+} signal. Albumin secretion carries on, because it is constitutive.

– APPLY **A lysosomal enzyme is engineered to carry KDEL at its C-terminus. Where will most of it end up?** 2 marks

In the ER: the KDEL receptor returns it from the cis Golgi in COPI vesicles before it can reach the TGN.

– ANALYZE **Why does it make sense for the M6P receptor to release its cargo in the endosome rather than in the Golgi?** 3 marks

Binding is pH-dependent: the receptor holds the enzyme at the near-neutral pH of the TGN and lets go at the lower pH of the endosome. That delivers the enzyme to the right place and frees the receptor to return and carry more, so few receptors serve many enzymes.

BEYOND THE SYLLABUS

Golgi glycosylation decides how long a therapeutic antibody lasts in the blood and how strongly it activates immune cells, so glycoengineering of CHO cell lines (for example removing core fucose) is an industrial specialty. Enzyme replacement therapies for Gaucher and Pompe disease rely on mannose or M6P receptors for uptake. Live-cell imaging in yeast is used to watch cisternal maturation directly. Search "antibody glycoengineering afucosylation" and "enzyme replacement therapy M6P". Not for the exam.

3.4 Lysosomes, peroxisomes and vacuoles

These three organelles are the cell's digestion, oxidation and storage rooms. **Lysosomes** are acidic sacs of digestive enzymes that break down worn-out parts and material brought in from outside. **Peroxisomes** carry out oxidation reactions that make hydrogen peroxide, and destroy it safely in the same room. **Vacuoles**, large in plant and fungal cells, store water, ions and wastes and keep the plant cell firm. All three are single-membrane compartments, and lysosomes and vacuoles are close relatives: the plant vacuole does the lysosome's job too.

You need: M6P sorting from Chapter 3.3, and endocytosis from **Unit 2, Endocytosis and exocytosis**.

Lysosomes

Christian de Duve found lysosomes in the 1950s while fractionating rat liver, and later found peroxisomes too; he shared the 1974 Nobel Prize. A lysosome holds several dozen kinds of **acid hydrolases**: proteases, nucleases, lipases, glycosidases, phosphatases and sulfatases. Together they can break down every major class of large molecule.

- **Acidic inside:** a **V-type H^+-ATPase** in the membrane uses ATP to pump protons in, keeping the lumen near pH 4.5–5. The cytosol is about pH 7.2.
- **Self-protection:** the membrane proteins (such as LAMPs) are heavily glycosylated on the inside, so the enzymes cannot digest the membrane.
- **Safety catch:** the hydrolases work poorly at neutral pH. If a lysosome leaks, the enzymes do little harm in the cytosol.

Material reaches lysosomes by three routes:

1. **Endocytosis:** outside material in endocytic vesicles → early endosome → late endosome → lysosome.
2. **Phagocytosis:** large particles such as bacteria are engulfed by macrophages and neutrophils into a phagosome, which fuses with lysosomes.
3. **Autophagy:** worn-out organelles or cytosol are wrapped in a double membrane (the autophagosome), which fuses with a lysosome. Starved cells use it to recycle their own parts. Yoshinori Ohsumi won the 2016 Nobel Prize for the autophagy genes.

Older textbooks call the newly made organelle the **primary lysosome** and the one that has fused with its cargo the **secondary lysosome**; undigested remains form **residual bodies**, such as the lipofuscin pigment of ageing heart and nerve cells.

Lysosomal storage diseases are inherited loss of one hydrolase, so its substrate builds up. Three examples: **Tay–Sachs disease** (hexosaminidase A missing, GM2 ganglioside builds up in neurons), **Gaucher disease** (glucocerebrosidase), and **Pompe disease** (acid α-glucosidase, glycogen builds up in muscle). I-cell disease, where all enzymes are missorted, is in Chapter 3.3.

Peroxisomes and other microbodies

Peroxisomes are small (about 0.2–1 µm) single-membrane organelles with no DNA or ribosomes. Their key pair of enzymes:

```
oxidases:  RH₂ + O₂  →  R + H₂O₂
catalase:  2 H₂O₂    →  2 H₂O + O₂
           or  H₂O₂ + R′H₂ → R′ + 2 H₂O   (peroxidative use: detoxifies phenols, etha
```

Here RH_2 is an organic substrate that gives up two hydrogens. Catalase is often so abundant that it forms a crystal core visible in electron micrographs. Functions:

- **β-oxidation of very-long-chain fatty acids** (more than about 22 carbons), which mitochondria cannot handle first. The shortened chains go to mitochondria to finish.
- **Plasmalogen synthesis**, a lipid abundant in myelin.
- **Detoxification** in liver and kidney; a good share of the ethanol people drink is oxidised to acetaldehyde here.
- **Glyoxysomes** in germinating oily seeds (castor, groundnut) run the **glyoxylate cycle**, which turns stored fat into sugar. Animals lack this cycle.
- In leaves, peroxisomes take part in **photorespiration** with chloroplasts and mitochondria (see Chapter 3.7).

Peroxisomal proteins are made on free ribosomes and imported **fully folded**. Most carry **PTS1**, the C-terminal tripeptide Ser–Lys–Leu (SKL). The import machine is made of proteins called

peroxins (PEX genes). Peroxisomes multiply by growing and dividing; new membrane can also come from the ER. When import fails, cells have empty peroxisome "ghosts": this is **Zellweger syndrome**, a fatal disorder of infancy.

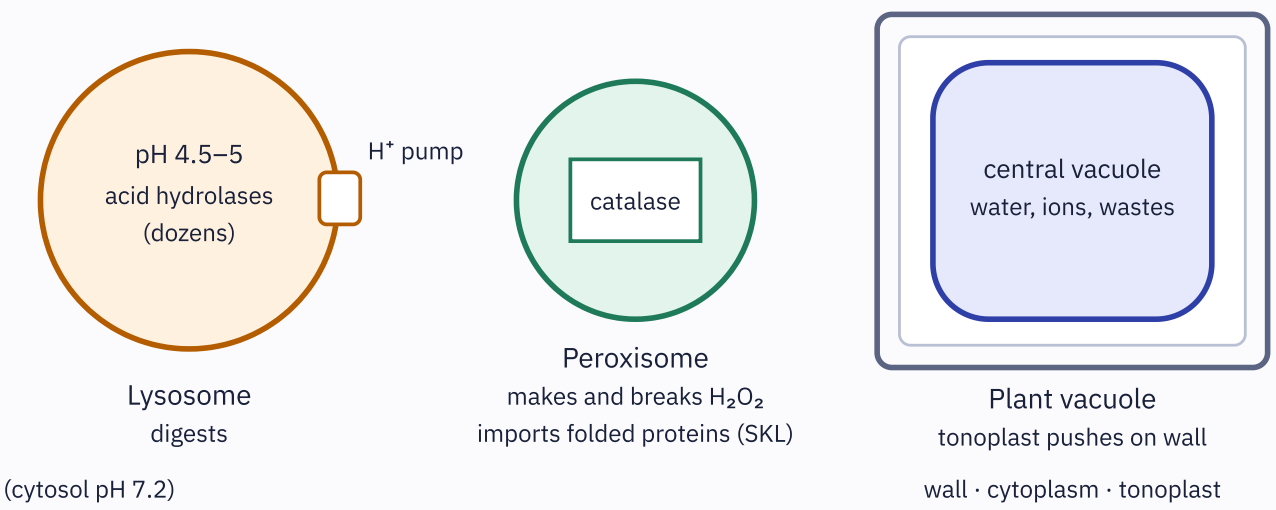


Figure 3.4.1 The three single-membrane organelles of this chapter, not to scale. The lysosome keeps its pH low with a proton pump; the peroxisome keeps catalase next to the oxidases that make H_2O_2; the plant vacuole, bounded by the tonoplast, fills most of the cell and presses the thin cytoplasm against the wall.

Vacuoles

A mature plant cell usually has one large **central vacuole** that can fill most of its volume, often up to about 90%. Its membrane is the **tonoplast**, which has its own proton pumps and transporters. The vacuole:

- takes in water by osmosis and pushes the cytoplasm against the wall; this **turgor pressure** keeps leaves and young stems firm, and its loss is why a plant wilts;
- lets the cell grow large cheaply, by adding water rather than cytoplasm;
- stores sugars, ions, pigments (anthocyanins give red and purple colours), and defensive or waste compounds such as alkaloids;
- contains acid hydrolases and acts as the plant cell's lysosome.

Freshwater protists such as *Paramecium* have **contractile vacuoles** that collect excess water and pump it out, so the cell does not burst. **Food vacuoles** in protists are phagosomes that fuse with lysosomes.

WORKED EXAMPLE

Question (6 marks). (a) A lysosome is at pH 4.7 and the cytosol at pH 7.2. How many times higher is the H^+ concentration inside? (b) Peroxisomes in a liver sample make 0.50 µmol of H_2O_2 per minute through oxidases. If catalase removes all of it by the catalatic reaction, how much O_2 does catalase release per minute, and what is the net O_2 use of the whole oxidase–catalase pair?

Solution.

1. pH = $-\log_{10}[H^+]$, so the ratio of concentrations is 10^(pH difference). Difference = 7.2 − 4.7 = 2.5.
2. 10^2.5 = 10^2 × 10^0.5 = 100 × 3.16 ≈ 316. The lumen has about **316 times** more H^+ than the cytosol.
3. Catalase: 2 H_2O_2 → 2 H_2O + O_2. So 0.50 µmol H_2O_2 gives 0.50 ÷ 2 = **0.25 µmol O_2 per minute**.
4. The oxidases used 1 O_2 per H_2O_2 made, so 0.50 µmol O_2 per minute. Net use = 0.50 − 0.25 = **0.25 µmol O_2 per minute**.

Answer: (a) about 316-fold; (b) 0.25 µmol O_2 released, net 0.25 µmol O_2 consumed per minute. The proton gradient is held against a 316-fold difference, which is why the V-ATPase needs ATP.

EXAM TRAPS

- Lysosomal enzymes are made in the rough ER and sorted by the Golgi; peroxisomal enzymes are made on free ribosomes and imported directly. Many students say peroxisomes "bud from the Golgi"; they do not.
- Peroxisomes have no DNA, unlike mitochondria and chloroplasts.
- Do not call the vacuole membrane the plasma membrane; it is the tonoplast.
- Glyoxysomes are found in plant seeds, not in animal cells.
- "Suicide bags" is an old nickname for lysosomes; their enzymes are mostly inactive at cytosolic pH, so a single leak does not kill the cell.

CHECK YOURSELF

– REMEMBER **What is the pH inside a lysosome, and what keeps it there?** 2 marks

About 4.5–5, kept by a V-type H^+-ATPase pumping protons in.

– REMEMBER **What is the peroxisomal targeting signal PTS1?** 1 mark

The C-terminal tripeptide Ser–Lys–Leu (SKL).

– UNDERSTAND **Why are catalase and the oxidases kept in the same organelle?** 2 marks

The oxidases make H_2O_2, which is toxic; catalase next to them breaks it down at once before it can damage the rest of the cell.

– UNDERSTAND **Explain how a plant wilts in terms of the vacuole.** 3 marks

When water is short, water leaves the vacuole by osmosis, the vacuole shrinks, turgor pressure on the wall falls and the cells go limp, so leaves and stems droop.

– APPLY **A drug raises lysosomal pH from 4.8 to 6.3. By what factor does $[H^+]$ fall, and what happens to digestion?** 3 marks

10^1.5 ≈ 32-fold fall. Acid hydrolases lose most of their activity, so digestion of endocytosed and autophagic material slows. Chloroquine acts partly in this way.

– APPLY **A patient's cells cannot break down very-long-chain fatty acids, and peroxisomes look like empty membranes. Name the likely disorder and the defect.** 2 marks

Zellweger syndrome: peroxisomal protein import fails because of a PEX gene defect.

– ANALYZE **Compare lysosomes and peroxisomes on origin, enzymes and one disease each.** 4 marks

Lysosomes: from the ER–Golgi–endosome route, acid hydrolases sorted by M6P; disease, Tay–Sachs. Peroxisomes: proteins made on free ribosomes and imported folded via PTS1, organelle grows and divides; oxidases and catalase; disease, Zellweger syndrome. Both have one membrane and no DNA.

BEYOND THE SYLLABUS

Autophagy is a major drug target: boosting it may clear toxic protein clumps in Parkinson's and Alzheimer's disease, while blocking it may starve some tumours. Enzyme replacement and gene therapies are approved or in trials for several lysosomal storage diseases. Engineered yeast peroxisomes are being used as compartments for metabolic engineering, keeping toxic pathway intermediates away from the cytosol. Search "autophagy neurodegeneration" and "peroxisome compartmentalization metabolic engineering". Not for the exam.

3.5 Ribosomes

A ribosome is the machine that reads an mRNA and joins amino acids into a protein. It is made of two subunits of ribosomal RNA (rRNA) and protein, has no membrane, and is found in every living cell, which is why it is often counted as an organelle even in bacteria. The key fact for modern biology is that the ribosome is a **ribozyme**: the bond between amino acids is made by its RNA, not by its proteins. Because bacterial and human ribosomes differ, many antibiotics can block the first without harming the second.

You need: the nucleolus from Chapter 3.1. The genetic code and the steps of translation in full belong to molecular biology; this chapter covers the ribosome as a structure.

Size, subunits and the Svedberg unit

Ribosomes are named by how fast they sink in an ultracentrifuge. The **sedimentation coefficient** s is the sinking speed divided by the centrifugal acceleration, and is measured in **Svedberg units** (S), where 1 S = 10^{-13} s. A larger s means faster sinking. It depends on mass *and* shape, so values do not add up: a 50S and a 30S subunit together make a 70S ribosome, not 80S, because the joined particle is more compact than two separate ones.

FEATURE	PROKARYOTIC (70S)	EUKARYOTIC CYTOSOLIC (80S)
Large subunit	50S: 23S + 5S rRNA, about 33 proteins	60S: 28S + 5.8S + 5S rRNA, about 47 proteins
Small subunit	30S: 16S rRNA, 21 proteins	40S: 18S rRNA, about 33 proteins
Where made	in the cytoplasm	rRNA and assembly in the nucleolus
Also found in	mitochondria and chloroplasts have bacteria-like ribosomes	free in cytosol or bound to rough ER

Protein counts vary by a few between species and sources, so learn them as "about". In both kinds, rRNA forms most of the mass and the core of the active sites; the proteins sit mostly on the surface. The 16S rRNA of the small subunit pairs with the Shine–Dalgarno sequence of a bacterial mRNA to place the start codon, and its sequence is the standard tool for identifying bacteria.

Ribosomes need magnesium ions to hold the two subunits together. If Mg^{2+} is removed in the lab, the ribosome falls apart into its subunits.

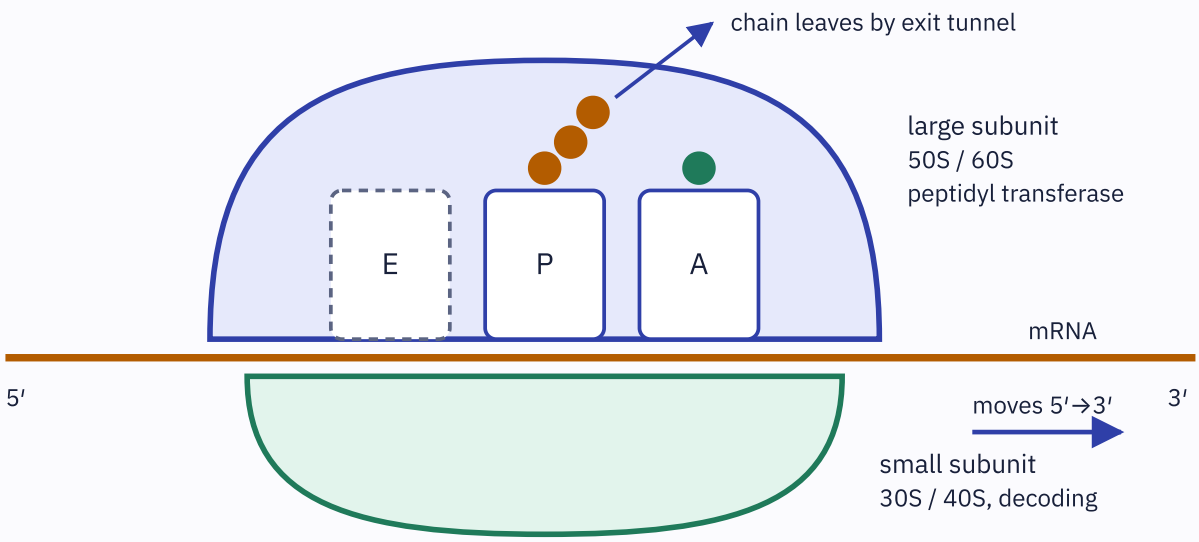
The working parts: three tRNA sites and a tunnel

The two subunits clamp the mRNA between them. The **small subunit** holds the mRNA and checks that each codon pairs correctly with the anticodon of its tRNA (decoding). The **large subunit** holds the **peptidyl transferase centre**, which makes the peptide bond. Three tRNA sites lie across both subunits:

- **A site** (aminoacyl): the incoming tRNA carrying the next amino acid binds here.
- **P site** (peptidyl): the tRNA holding the growing chain sits here.
- **E site** (exit): the empty tRNA leaves from here.

1. An aminoacyl-tRNA, brought by an elongation factor with GTP, enters the A site if its anticodon matches the codon.
2. The peptidyl transferase centre moves the whole growing chain from the P-site tRNA on to the amino acid of the A-site tRNA, making a new peptide bond.
3. Another elongation factor, using GTP, moves the ribosome three nucleotides along the mRNA (translocation): the A-site tRNA moves to P, the P-site tRNA to E.
4. The empty tRNA leaves from E and the A site is free for the next codon. A stop codon brings a release factor instead of a tRNA, and the chain is freed.

The new chain leaves through an **exit tunnel** in the large subunit. This is where SRP meets the signal sequence for ER targeting (Chapter 3.2). Several ribosomes can read one mRNA at the same time, spaced along it like beads; this string is a **polysome** (polyribosome).



Boxes are tRNA sites; orange = growing chain, green = next amino acid.

Figure 3.5.1 A ribosome during elongation. The mRNA runs between the two subunits; the E, P and A sites span both. The new peptide bond forms in the large subunit, and the chain threads out through the exit tunnel. The ribosome steps toward the 3′ end of the mRNA, one codon at a time.

Antibiotics and the endosymbiont link

Because 70S and 80S ribosomes differ, many antibiotics hit only the bacterial kind:

- **Streptomycin** binds the 30S subunit and causes misreading of codons.
- **Tetracycline** binds the 30S subunit and blocks aminoacyl-tRNA from entering the A site.
- **Chloramphenicol** binds the 50S subunit and blocks peptidyl transferase.
- **Erythromycin** (a macrolide) binds the 50S subunit and blocks the exit tunnel.
- **Cycloheximide** blocks eukaryotic 80S ribosomes and is used in the lab, not as a drug.

Mitochondria and chloroplasts carry their own ribosomes, which resemble bacterial ones and are sensitive to some of these antibiotics. This is one piece of evidence for their bacterial origin (Chapter 3.6), and it explains some side effects of chloramphenicol.

WORKED EXAMPLE

Question (6 marks). The coding region of an mRNA is 1,203 nucleotides long, including the start codon AUG and one stop codon. (a) How many amino acids are in the protein? (b) Assume a eukaryotic ribosome adds 6 amino acids per second. How long does one ribosome take to make the protein? (c) If ribosomes start on this mRNA every 4 seconds and it stays in use for 10 minutes, about how many protein copies does it give?

Solution.

1. Codons = 1,203 ÷ 3 = 401. The stop codon codes for no amino acid, so the protein has **400 amino acids** (the first is methionine, from AUG).
2. Time = 400 amino acids ÷ 6 per second ≈ **67 s**, a little over one minute.
3. Ten minutes = 600 s. A new ribosome starts every 4 s, so about 600 ÷ 4 = 150 ribosomes start. Allowing the last ones about 67 s to finish, about **150 copies** are made from the single mRNA, and at any moment about 67 ÷ 4 ≈ 17 ribosomes sit on it as a polysome.

Answer: 400 amino acids; about 67 s; about 150 copies. The polysome is how one mRNA becomes many proteins quickly.

EXAM TRAPS

- 50S + 30S = 70S, and 60S + 40S = 80S. Svedberg values are not additive; say why (shape and mass, not mass alone).
- Mitochondrial ribosomes are not 80S. They are bacteria-like (in mammals about 55S).
- The peptide bond is made by rRNA (23S or 28S), not by a ribosomal protein. This is the most asked "ribozyme" question.
- The 5.8S rRNA is found only in eukaryotes, in the large subunit, paired with the 28S rRNA.
- Free and ER-bound ribosomes are the same; only the protein being made decides where they sit.

CHECK YOURSELF

– REMEMBER **List the rRNAs of the eukaryotic large subunit.** 2 marks

28S, 5.8S and 5S rRNA.

– REMEMBER **What do the A, P and E sites hold?** 3 marks

A: the incoming aminoacyl-tRNA. P: the tRNA carrying the growing peptide. E: the empty tRNA about to leave.

– UNDERSTAND **Why is the ribosome called a ribozyme?** 2 marks

Its catalytic step, forming the peptide bond, is carried out by rRNA in the large subunit. Atomic structures show that the active site is built of rRNA.

– UNDERSTAND **Explain why a 70S ribosome dissociates into 50S and 30S rather than into two 35S halves.** 2 marks

The subunits are different particles of different mass and composition; the S value of each reflects its own mass and shape, and the joined particle's S is not the sum.

– APPLY **A protein has 250 amino acids. What is the minimum length of its coding region including the stop codon?** 2 marks

(250 + 1) × 3 = 753 nucleotides.

– APPLY **Why can tetracycline treat a bacterial infection without stopping protein synthesis in the patient's cytosol?** 2 marks

It binds the 30S subunit of the bacterial 70S ribosome; the human cytosolic 80S ribosome has a different 40S small subunit that it does not block effectively.

– ANALYZE **What would you expect if a drug blocked the exit tunnel in a pancreatic acinar cell? Reason about the ER.** 4 marks

Chains could not leave the tunnel, so no signal sequence would appear for SRP to bind. Ribosomes would not be targeted to the ER, digestive enzymes would not enter the secretory pathway, and secretion would stop. Synthesis of all other proteins would also stall.

BEYOND THE SYLLABUS

The 2009 Nobel Prize in Chemistry went to Ramakrishnan, Steitz and Yonath for atomic structures of the ribosome, which are now used to design antibiotics that avoid resistance. 16S rRNA sequencing is the standard method in microbiome studies, from gut bacteria to soil. Ribosome profiling (Ribo-seq) maps which mRNAs are being translated in a cell at a given moment. Search "ribosome profiling" and "16S rRNA amplicon sequencing". Not for the exam.

3.6 Mitochondria: structure and function

Mitochondria are the organelles that make most of a eukaryotic cell's ATP by using oxygen to burn food molecules. They have two membranes, their own small genome and their own ribosomes, because they descend from bacteria that lived inside an ancient host cell. Their central trick, the **chemiosmotic** mechanism, is to pump protons across the inner membrane and let them flow back through ATP synthase, much like water stored behind a dam turning a turbine. Beyond ATP, mitochondria also control apoptosis, buffer calcium and make heme.

You need: active transport and ATP pumps from **Unit 2, Active transport**. The detailed reactions of glycolysis and the citric acid cycle belong to your biochemistry course.

Structure: four compartments

A mitochondrion is often drawn as a bean about 0.5–1 µm wide and a few µm long, but in living cells many mitochondria join into a branching network that keeps splitting (**fission**) and joining (**fusion**). The number per cell follows energy demand: heart muscle and liver cells hold hundreds to thousands. Mature red blood cells have none.

- **Outer membrane:** smooth and leaky. Channel proteins called **porins** (VDAC) let molecules up to about 5 kDa pass, so ions and small metabolites cross freely.
- **Intermembrane space:** the narrow gap between the membranes; it holds cytochrome c and becomes rich in protons when the electron transport chain runs.
- **Inner membrane:** folded into **cristae**, which raise its area. It is about three-quarters protein, holds the electron transport chain and ATP synthase, and is rich in the lipid **cardiolipin**. It is impermeable to most ions, including H^+; every metabolite crosses on a specific carrier (for example the ADP/ATP translocase).
- **Matrix:** the inner space, a dense gel holding the enzymes of pyruvate oxidation, the **citric acid cycle** and fatty-acid **β-oxidation**, plus mitochondrial DNA, ribosomes and tRNAs.

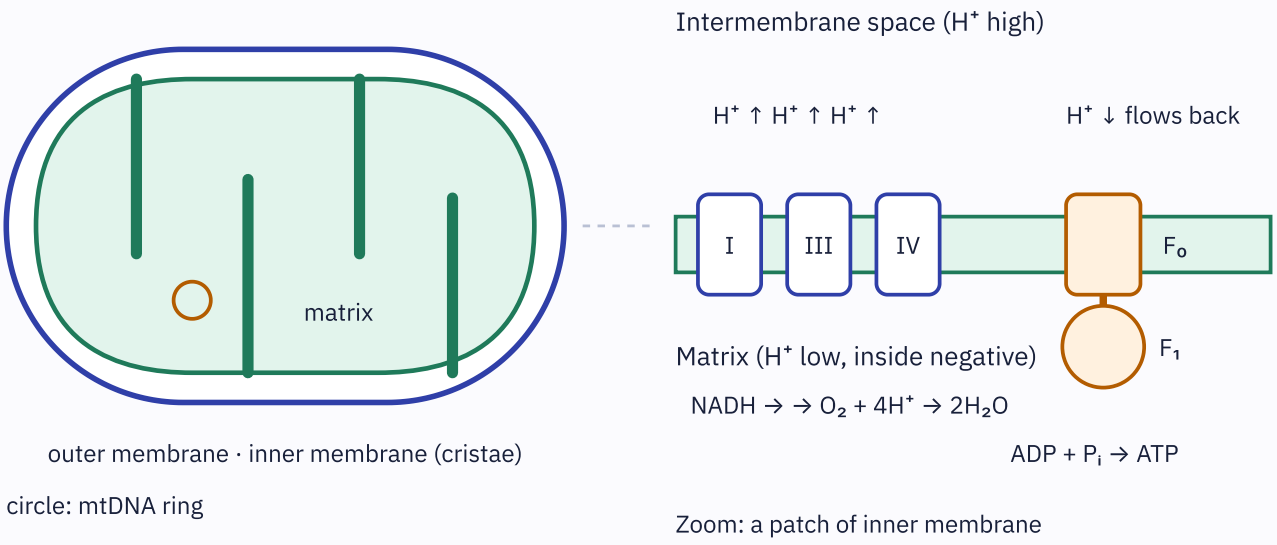


Figure 3.6.1 Left, a mitochondrion in section. Right, a patch of inner membrane: complexes I, III and IV pump protons into the intermembrane space as electrons pass from NADH to oxygen; protons return through the F_o part of ATP synthase, and its F_1 head in the matrix makes ATP. Complex II, which feeds electrons from $FADH_2$, pumps none and is left out.

How mitochondria make ATP

1. **Fuel in.** Pyruvate from glycolysis and fatty acids enter the matrix and are oxidised to acetyl-CoA, which the citric acid cycle burns to CO_2. The energy is captured as the reduced carriers NADH and $FADH_2$.
2. **Electron transport.** NADH gives electrons to **complex I**; $FADH_2$ (from succinate) gives them to **complex II**. Ubiquinone carries them to **complex III**, cytochrome c carries them to **complex IV**, which passes them to O_2, forming water. Oxygen is the final electron acceptor.
3. **Proton pumping.** Complexes I, III and IV use the energy released to move protons from the matrix to the intermembrane space: about 10 H^+ for each NADH and 6 for each $FADH_2$.
4. **Proton-motive force.** The result is a pH difference (matrix more alkaline) and a voltage (matrix negative). Together they form the **proton-motive force**, Δp.
5. **ATP synthesis.** Protons flow back through **ATP synthase**. Its membrane part F_o includes a ring of c subunits that rotates as protons pass; the rotation turns a central stalk inside the F_1 head (three α and three β subunits), and each β subunit in turn binds ADP and P_i, makes ATP and releases it. This is Paul Boyer's **binding-change mechanism**.

Peter Mitchell proposed the chemiosmotic hypothesis in 1961 and received the 1978 Nobel Prize for it. In modern accounting, one NADH gives about 2.5 ATP and one $FADH_2$ about 1.5 ATP (the P/O ratios), and complete oxidation of one glucose gives about 30–32 ATP.

$$\Delta p = \Delta\psi + (2.303\ RT / F) \cdot \Delta pH$$

Here $\Delta\psi$ is the membrane potential (size of the voltage, matrix negative), ΔpH = pH(matrix) − pH(intermembrane space), R = 8.314 J mol^{-1} K^{-1}, T is the temperature in kelvin, and F = 96,485 C mol^{-1} (Faraday constant). At 37 °C, 2.303RT/F ≈ 61.5 mV per pH unit. The energy released when one mole of H^+ flows back is $\Delta G = -F \cdot \Delta p$.

Blocking the chain. Rotenone blocks complex I, antimycin A complex III, and cyanide, azide and carbon monoxide complex IV; oligomycin blocks ATP synthase. **Uncouplers** such as 2,4-dinitrophenol carry protons across the inner membrane, so oxygen is used but no ATP is made and the energy is lost as heat. Brown fat does this on purpose with **UCP1** (thermogenin) to keep newborns warm.

Genome, origin and other roles

Human mitochondrial DNA (mtDNA) is a circle of 16,569 base pairs with 37 genes: 13 for proteins of the respiratory chain and ATP synthase, 22 for tRNAs and 2 for rRNAs. All other mitochondrial proteins, more than a thousand, are coded in the nucleus, made in the cytosol and imported through the **TOM** (outer) and **TIM** (inner) complexes, guided by an N-terminal presequence. mtDNA is inherited from the mother, and mutations in it cause disorders such as Leber hereditary optic neuropathy.

The **endosymbiotic theory**, argued strongly by Lynn Margulis from 1967, says mitochondria came from α-proteobacteria taken in by an ancestral host cell. Evidence: two membranes, circular DNA without histones, 70S-type ribosomes sensitive to some antibiotics, and division by fission. Mitochondria also release **cytochrome c** to start apoptosis (Unit 4), take up Ca^{2+}, and make heme and iron–sulfur clusters.

WORKED EXAMPLE

Question (8 marks). In a liver mitochondrion at 37 °C, the membrane potential is 160 mV (matrix negative) and the matrix pH is 0.75 units higher than the intermembrane space. (a) Find the proton-motive force. (b) Find the free energy released per mole of H^+ flowing into the matrix. (c) Making one mole of ATP inside the cell needs about 50 kJ. What is the minimum number of protons per ATP?

Solution.

1. Chemical part: 61.5 mV × 0.75 = 46.1 mV.
2. Δp = 160 + 46.1 = **206 mV** = 0.206 V.
3. $\Delta G = -F \cdot \Delta p = -96{,}485\ \text{C mol}^{-1} \times 0.206\ \text{V} = -19{,}876\ \text{J mol}^{-1} \approx$ **−19.9 kJ per mol H^+** (C × V = J).
4. Minimum protons = 50 ÷ 19.9 ≈ 2.5, so at least **3 protons** must flow per ATP (a whole number above 2.5).

Answer: Δp ≈ 206 mV; about 19.9 kJ per mole of H^+; at least 3 H^+ per ATP. The real figure is a little higher, because protons are also spent bringing ADP and phosphate into the matrix; this is why NADH gives 2.5 ATP and not more. Note that the voltage contributes more than the pH gradient in mitochondria.

EXAM TRAPS

- Protons are pumped into the intermembrane space, not into the matrix. The matrix is the alkaline, negative side.
- Glycolysis happens in the cytosol, not in the mitochondrion. The citric acid cycle is in the matrix; the electron transport chain is in the inner membrane.
- Complex II pumps no protons, which is why $FADH_2$ gives less ATP than NADH.
- The outer membrane is permeable to small molecules; the inner membrane is the barrier. Students often swap them.
- Most mitochondrial proteins are coded by the nucleus, not by mtDNA.

CHECK YOURSELF

– REMEMBER **Name the four compartments of a mitochondrion.** 2 marks

Outer membrane, intermembrane space, inner membrane (with cristae) and matrix.

– REMEMBER **What does human mtDNA code for?** 2 marks

37 genes: 13 proteins of oxidative phosphorylation, 22 tRNAs and 2 rRNAs.

– UNDERSTAND **Why are cristae important?** 2 marks

They fold the inner membrane and raise its area, so more electron transport chains and ATP synthases fit in one mitochondrion; very active cells have densely packed cristae.

– UNDERSTAND **Explain how an uncoupler causes fever-like heat.** 3 marks

It lets protons leak back across the inner membrane without passing through ATP synthase. The chain keeps running and using oxygen to rebuild the gradient, but the energy is released as heat instead of being stored in ATP.

– APPLY **At 37 °C, $\Delta\psi$ = 150 mV and ΔpH = 0.5. Find Δp.** 2 marks

61.5 × 0.5 = 30.8 mV; Δp = 150 + 30.8 ≈ 181 mV.

– APPLY **A cell is poisoned with cyanide. What happens to oxygen use and ATP synthesis, and why?** 3 marks

Both stop: cyanide blocks complex IV, so electrons cannot reach O_2, the chain backs up, no protons are pumped and the gradient that drives ATP synthase collapses.

– ANALYZE **Evaluate the evidence that mitochondria were once bacteria.** 5 marks

Supporting: double membrane, circular DNA without histones, 70S-type ribosomes sensitive to chloramphenicol, division by fission, and gene sequences closest to α-proteobacteria. Complication: most of their genes have moved to the nucleus, so they cannot live alone; this is explained by gene transfer after the endosymbiosis.

BEYOND THE SYLLABUS

Mitochondrial replacement therapy ("three-parent IVF") lets a mother with an mtDNA disease have a child with healthy mitochondria from a donor egg; the UK made it legal in 2015. Cryo-EM structures of the full respiratory supercomplexes and the rotating ATP synthase are a fast-moving research area. In bioprocessing, the mitochondrial activity of CHO and yeast cells limits how much protein they make. Search "mitochondrial replacement therapy" and "respirasome cryo-EM". Not for the exam.

3.7 Chloroplasts: structure and function

Chloroplasts are the organelles of plants and algae that capture light energy and use it to turn carbon dioxide and water into sugar, releasing oxygen. Like mitochondria they have two envelope membranes, their own DNA and bacteria-like ribosomes, and they also make ATP with a proton gradient and an ATP synthase. The difference is the energy source (light instead of food) and a third membrane system inside, the **thylakoids**, where the light is caught. Almost all the food and oxygen on Earth come from this one organelle.

> You need: the proton-motive force and ATP synthase from Chapter 3.6.

Structure

A typical leaf chloroplast is a lens-shaped disc about 5–10 μm long, and a leaf mesophyll cell holds dozens of them. From outside in:

- **Envelope:** an outer membrane, permeable to small molecules, and an inner membrane with specific transporters (for example the triose-phosphate/phosphate translocator that exports sugar). The inner envelope has **no** electron transport chain, unlike the inner mitochondrial membrane.
- **Stroma:** the fluid inside the envelope. It holds the enzymes of the **Calvin cycle**, including RuBisCO, plus chloroplast DNA, 70S ribosomes and starch grains.
- **Thylakoids:** flattened membrane sacs. Stacks of them are **grana** (one stack is a granum); single thylakoids joining the stacks are **stroma lamellae** (intergranal thylakoids). The space inside every thylakoid is one connected compartment, the **thylakoid lumen**.

The thylakoid membrane carries the pigments: **chlorophyll a** (the main pigment), **chlorophyll b** and **carotenoids**, held by proteins in light-harvesting complexes around two **photosystems**. The membrane is rich in galactolipids rather than phospholipids.

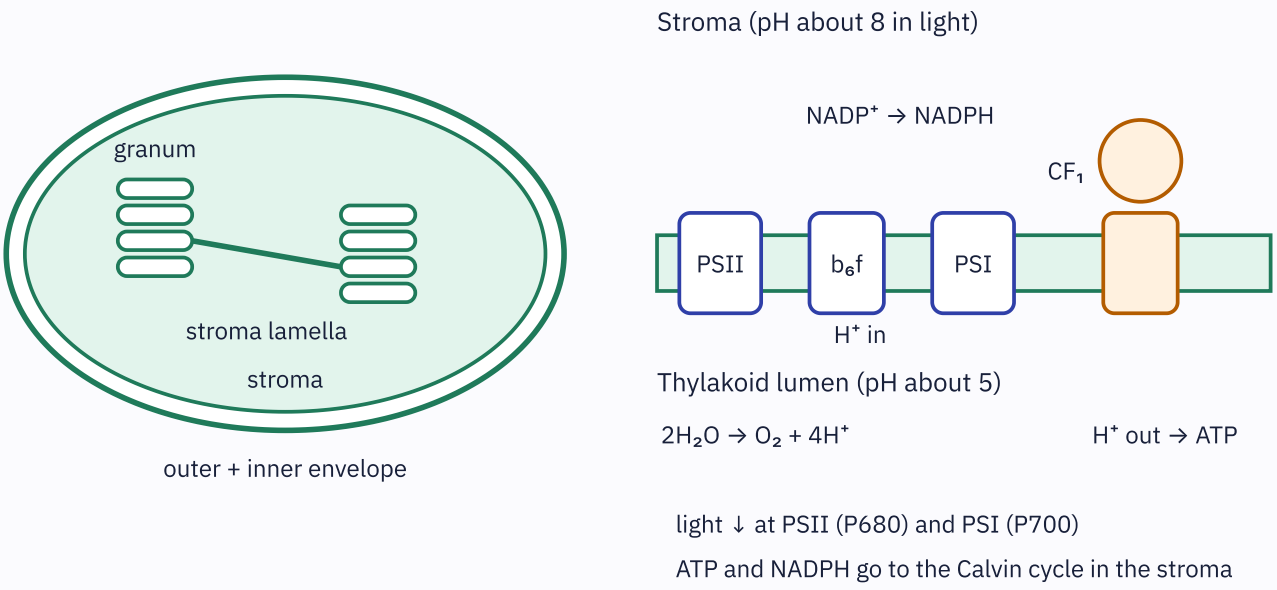


Figure 3.7.1 Left, a chloroplast with grana joined by stroma lamellae. Right, a patch of thylakoid membrane: water is split on the lumen side, protons collect in the lumen, NADPH is made on the stroma side, and protons flow out through ATP synthase whose CF_1 head faces the stroma. Compare with Figure 3.6.1: the head points into the matrix there and into the stroma here.

The light reactions in the thylakoid

1. **Light capture.** Pigments in the antenna absorb photons and pass the energy to a special chlorophyll pair at a reaction centre: **P680** in photosystem II (PSII) and **P700** in photosystem I (PSI), named for the wavelength in nm they absorb best.
2. **Water splitting.** Excited P680 gives up an electron. It takes a replacement from water through the manganese-containing oxygen-evolving complex: $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$. The O_2 we breathe comes from water, and the protons stay in the lumen.
3. **Electron flow.** Electrons pass through plastoquinone, the **cytochrome b_6f complex** (which pumps more protons into the lumen) and plastocyanin to PSI. Light excites P700, and its electrons go via ferredoxin to the enzyme ferredoxin–$NADP^+$ reductase, which makes **NADPH** in the stroma. Drawn on an energy scale this path is the **Z-scheme**.
4. **ATP synthesis.** Protons flow from the lumen back to the stroma through **CF_oCF_1 ATP synthase**, making ATP in the stroma. This is **photophosphorylation**. When only PSI runs and electrons cycle back to b_6f (cyclic flow), the cell makes extra ATP without NADPH or O_2.

The Calvin cycle in the stroma

The ATP and NADPH power carbon fixation. **RuBisCO** (ribulose-1,5-bisphosphate carboxylase/oxygenase) joins CO_2 to ribulose-1,5-bisphosphate (RuBP), giving two molecules of

3-phosphoglycerate. These are reduced to glyceraldehyde-3-phosphate (G3P), and most G3P is used to rebuild RuBP. To make one G3P (3 carbons) the cycle fixes 3 CO_2 and uses **9 ATP and 6 NADPH**. RuBisCO also reacts with O_2, starting **photorespiration**, which wastes carbon and involves chloroplasts, peroxisomes and mitochondria together (Chapter 3.4). RuBisCO is thought to be the most abundant protein on Earth.

Genome, origin and other plastids

Chloroplast DNA is a circle of roughly 120–160 kb in most land plants, coding for rRNAs, tRNAs and around a hundred proteins, including the large subunit of RuBisCO; the small subunit is coded in the nucleus and imported. Chloroplasts came from a **cyanobacterium** taken in by an early eukaryote, the same endosymbiotic story as mitochondria. All plastids grow from **proplastids**: **chloroplasts** (green, photosynthetic), **chromoplasts** (carotenoid colours of tomato and chilli fruit), **amyloplasts** (starch stores, as in potato), and other **leucoplasts**. Seedlings grown in the dark have **etioplasts**, which turn into chloroplasts in light.

FEATURE	MITOCHONDRION	CHLOROPLAST
Energy source	oxidation of food	light
Where the chain sits	inner membrane	thylakoid membrane
Protons pumped into	intermembrane space	thylakoid lumen
ATP made in	matrix	stroma
Main part of Δp	voltage (Δψ)	pH difference
Ancestor	α-proteobacterium	cyanobacterium

WORKED EXAMPLE

Question (6 marks). (a) Find the energy of one photon of 680 nm light and of one mole of such photons. (b) In bright light the thylakoid lumen is 3.0 pH units more acidic than the stroma, with almost no voltage across the membrane. Find the free energy released per mole of H^+ flowing out at 25 °C. Take h = 6.626×10^{-34} J s, c = 3.00×10^{8} m s^{-1}, N_a = 6.022×10^{23} mol^{-1}, R = 8.314 J mol^{-1} K^{-1}.

Solution.

1. E = hc/λ, where λ is the wavelength in metres: λ = 680×10^{-9} m.
2. E = $(6.626 \times 10^{-34} \times 3.00 \times 10^{8}) \div (6.80 \times 10^{-7}) = 1.988 \times 10^{-25} \div 6.80 \times 10^{-7}$ = **2.92×10^{-19} J per photon**.
3. Per mole: $2.92 \times 10^{-19} \times 6.022 \times 10^{23} = 1.76 \times 10^{5}$ J = **176 kJ per mole of photons**.
4. For a pure pH gradient, ΔG = −2.303 RT × ΔpH. T = 298 K, so 2.303 × 8.314 × 298 = 5,706 J ≈ 5.71 kJ per pH unit.
5. ΔG = −5.71 × 3.0 = **−17.1 kJ per mole of H^+**.

Answer: 2.92×10^{-19} J per photon, 176 kJ mol^{-1}; about 17 kJ per mole of H^+. One mole of red photons carries roughly ten times the energy of one mole of protons crossing the membrane, which is why each photon can drive several steps.

EXAM TRAPS

- Protons collect in the thylakoid lumen, not in the stroma, and not between the two envelope membranes.
- The light reactions happen on the thylakoid membrane; the Calvin cycle happens in the stroma. "Dark reactions" do not need darkness; several of their enzymes are activated by light.
- The O_2 released comes from water, not from CO_2.
- PSII acts before PSI in the electron path; the numbering follows the order of discovery.
- Chloroplasts have bacteria-like 70S ribosomes, not the 80S ribosomes of the cytosol.

CHECK YOURSELF

– REMEMBER **Name the three membrane systems of a chloroplast.** 2 marks

Outer envelope membrane, inner envelope membrane and thylakoid membrane.

– REMEMBER **What are the reaction-centre chlorophylls of PSII and PSI?** 1 mark

P680 (PSII) and P700 (PSI).

– UNDERSTAND **Why is ATP made in the stroma and not in the lumen?** 2 marks

The CF_1 head of ATP synthase faces the stroma; protons flow from the lumen out to the stroma through it, and the Calvin cycle that uses ATP is also in the stroma.

– UNDERSTAND **What is the advantage of cyclic electron flow?** 2 marks

It pumps protons and makes ATP without making NADPH, adjusting the ATP:NADPH ratio to the 9:6 the Calvin cycle needs.

– APPLY **How much ATP and NADPH are needed to make one glucose (6 carbons) from CO_2?** 2 marks

Two G3P are needed, so 2 × 9 = 18 ATP and 2 × 6 = 12 NADPH, fixing 6 CO_2.

– APPLY **Find the energy per mole of photons at 700 nm.** 2 marks

$E = 1.988 \times 10^{-25} \div 7.00 \times 10^{-7} = 2.84 \times 10^{-19}$ J; × 6.022×10^{23} = about 171 kJ mol^{-1}.

– ANALYZE **Compare how mitochondria and chloroplasts build and use the proton gradient.** 5 marks

Both pump protons across an inner membrane with an electron transport chain and use an F-type ATP synthase. Mitochondria pump from matrix to intermembrane space using food oxidation and make ATP in the matrix; Δp is mostly voltage. Chloroplasts pump into the thylakoid lumen using light and make ATP in the stroma; Δp is mostly pH, because ions such as Cl^- and Mg^{2+} move to cancel the voltage.

BEYOND THE SYLLABUS

Engineering RuBisCO, or adding bypasses for photorespiration, is a major goal in crop science; field trials in tobacco reported higher biomass. Projects to move C4 photosynthesis into rice aim at higher yields for Asian farmers. Chloroplast transformation is used to make vaccines and enzymes in plants because the plastid genome is not spread by pollen in most crops. Search "photorespiration bypass" and "C4 rice project". Not for the exam.

3.8 Cytoskeleton: microtubules, actin and intermediate filaments

The cytoskeleton is a network of protein filaments that runs through the cytoplasm of every eukaryotic cell. It gives the cell its shape, resists stretching, places the organelles, lays tracks for transport, pulls chromosomes apart in mitosis and makes the cell crawl. There are three kinds of filament, each built from many copies of one small protein subunit: **microtubules** (tubulin), **actin filaments** or microfilaments (actin), and **intermediate filaments** (a family of fibrous proteins). Unlike a building's skeleton, two of the three are rebuilt all the time, in seconds to minutes.

You need: protein structure (α-helix, subunits) from your biochemistry course. Motor proteins that move along these filaments are in Chapter 3.9.

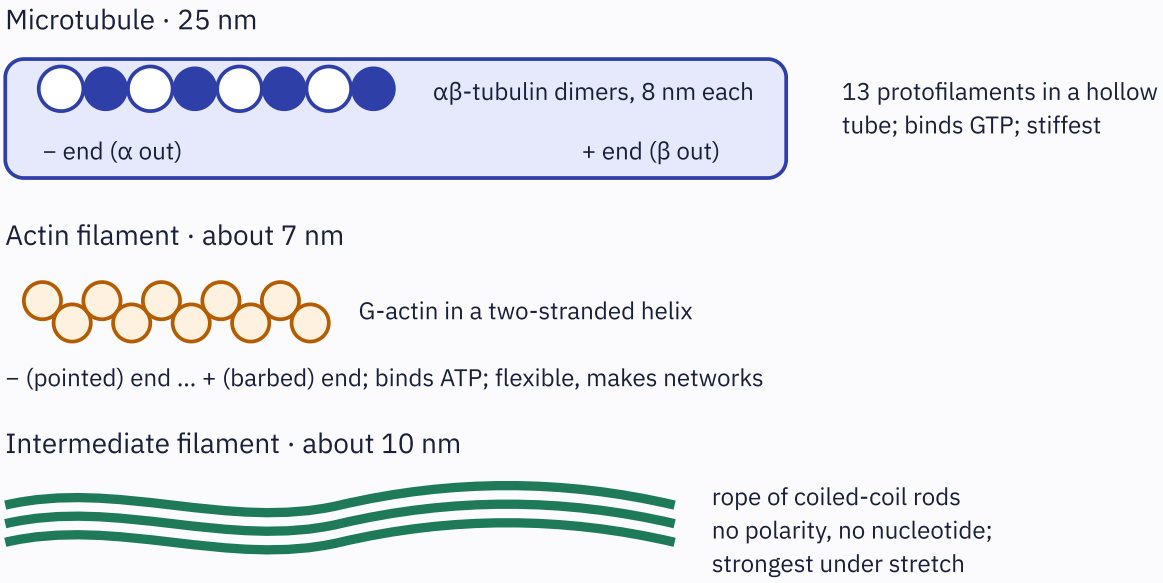


Figure 3.8.1 The three filaments drawn side by side (not to scale along their length). Microtubules and actin filaments have a plus and a minus end because their subunits all point the same way; intermediate filaments do not. Notice that only the first two bind a nucleotide, which is what lets them grow and shrink quickly.

Microtubules

A microtubule is a hollow tube about 25 nm across, made of **αβ-tubulin dimers** lined up head-to-tail into **protofilaments**; usually 13 protofilaments form the wall. Since every dimer points the same way, the tube has a **plus end** (β-tubulin exposed, grows fast) and a **minus end** (α-tubulin

exposed). Each tubulin binds GTP. The GTP on β-tubulin is hydrolysed to GDP soon after the dimer joins the tube.

In animal cells most microtubules grow out from the **centrosome**, the main **microtubule-organising centre** (MTOC), where **γ-tubulin ring complexes** start new tubes and hold the minus ends. Plus ends point out towards the cell edge. Microtubules show **dynamic instability** (Mitchison and Kirschner, 1984):

1. A growing plus end keeps a **GTP cap** of newly added dimers, which bind each other tightly.
2. If hydrolysis catches up with addition and the cap is lost, the GDP-tubulin protofilaments curl outward and the tube shrinks rapidly. This switch is a **catastrophe**.
3. If GTP-tubulin adds again before the tube is gone, it starts growing once more: a **rescue**.

This lets microtubules search the cell and be captured where they are needed, for example by chromosomes in mitosis. **Microtubule-associated proteins** (MAPs) tune this: tau and MAP2 stabilise microtubules in neurons, while proteins such as stathmin and kinesin-13 take them apart. Microtubules form the mitotic spindle (Unit 4), tracks for organelle transport, and the core of cilia and flagella (Chapter 3.9). **Drugs:** colchicine and nocodazole stop assembly; vinblastine and vincristine also block assembly and are used in cancer treatment; **paclitaxel** (taxol) does the opposite, freezing microtubules so they cannot shrink, which also stops mitosis.

Actin filaments

Actin is one of the most abundant proteins in many animal cells. A globular monomer (**G-actin**) binds ATP; monomers join into a two-stranded helical filament (**F-actin**) about 7 nm thick. Its fast-growing plus end is called the **barbed end** and its minus end the **pointed end**, after the arrowhead pattern myosin heads make when they bind it.

Assembly starts slowly because a nucleus of three monomers is unstable (the **lag phase**), then grows until free monomer falls to the **critical concentration** C_c: the free-monomer concentration at which adding and losing subunits balance. Above C_c an end grows; below it, the end shrinks. With ATP-actin, C_c is lower at the plus end than at the minus end. At monomer levels between the two, the filament adds at the plus end and loses at the minus end at the same rate, so its length stays the same while subunits flow through it: **treadmilling**.

Cells control actin with helper proteins:

- **Nucleators:** the **Arp2/3 complex** starts branches on existing filaments, building the meshwork of the **lamellipodium**; **formins** make long unbranched filaments for **filopodia**, stress fibres and the contractile ring of cell division.

- **Monomer binders:** profilin helps monomers add at plus ends; thymosin β4 holds a reserve of monomers.
- **Cappers and severers:** capping protein stops plus-end growth; cofilin cuts old ADP-actin filaments.

Actin forms the **cell cortex** under the plasma membrane, microvilli in the gut lining, and with myosin it drives muscle contraction and cytokinesis. A crawling cell such as a neutrophil or a fibroblast moves in three steps: it **protrudes** its front by actin polymerisation, **attaches** it to the surface through integrins at focal adhesions, and **contracts** its rear with myosin II. **Drugs:** cytochalasins cap barbed ends, latrunculin binds monomers, and phalloidin (a mushroom toxin) stabilises filaments; fluorescent phalloidin is the standard stain for F-actin.

Intermediate filaments

Intermediate filaments are about 10 nm thick, between actin and microtubules, hence the name. Each subunit has a long central **α-helical rod**. Two rods wind into a **coiled-coil dimer**; two dimers pair side by side in opposite directions (**antiparallel tetramer**); about eight tetramers pack into a **unit-length filament**, which joins end to end. Because the tetramer is antiparallel, the filament has **no polarity**, binds no nucleotide and carries no motors. It bends easily but is very hard to break, so it protects cells from mechanical stress.

CLASS	PROTEIN	WHERE
I and II	acidic and basic keratins	epithelial cells, hair, nails
III	vimentin, desmin, GFAP	fibroblasts; muscle; astrocytes
IV	neurofilament proteins	nerve axons
V	lamins	nuclear lamina (Chapter 3.1)

Cells regulate them mainly by phosphorylation, as with lamins at mitosis. Mutant keratins 5 or 14 cause **epidermolysis bullosa simplex**, in which skin blisters from slight rubbing. Pathologists use the intermediate filament type to tell which tissue a tumour came from.

WORKED EXAMPLE

Question (8 marks). (a) For ATP-actin, C_c = 0.12 μM at the plus end and 0.60 μM at the minus end. Predict what each end does when the free G-actin concentration is held at 0.05 μM, 0.30 μM and 1.0 μM. (b) A microtubule is 10 μm long and has 13 protofilaments. Each αβ-dimer is 8 nm long. How many dimers does it contain?

Solution.

1. **0.05 μM:** below both C_c values, so both ends lose subunits and the filament shrinks.
2. **0.30 μM:** above 0.12 (plus end grows) but below 0.60 (minus end shrinks). The filament treadmills.
3. **1.0 μM:** above both, so both ends grow, the plus end faster.
4. Dimers per protofilament = 10 μm ÷ 8 nm = 10,000 nm ÷ 8 nm = 1,250.
5. Total = 1,250 × 13 = **16,250 dimers** (32,500 tubulin monomers).

Answer: (a) shrinks; treadmills; grows at both ends. (b) 16,250 αβ-dimers. In a steady state the free monomer settles between the two C_c values, which is why treadmilling is seen in cells.

EXAM TRAPS

- Actin binds ATP; tubulin binds GTP. Intermediate filaments bind neither.
- Plus end does not mean "the end that only grows". It is the faster end; both ends can grow or shrink depending on monomer level.
- Colchicine and taxol both stop mitosis, but in opposite ways: one prevents assembly, the other prevents disassembly.
- Lamins are intermediate filaments, even though they sit in the nucleus.
- Cytochalasin acts on actin, colchicine on microtubules. Examiners like to swap them.

CHECK YOURSELF

– REMEMBER **Give the diameter and subunit of each cytoskeletal filament.** 3 marks

Microtubule about 25 nm, αβ-tubulin; actin filament about 7 nm, G-actin; intermediate filament about 10 nm, proteins such as keratin, vimentin or lamin.

– REMEMBER **What is the main MTOC of an animal cell?** 1 mark

The centrosome.

– UNDERSTAND **Explain dynamic instability.** 4 marks

Growing microtubules carry a GTP cap at the plus end. If GTP hydrolysis catches up and the cap is lost, GDP protofilaments peel apart and the tube shrinks quickly (catastrophe). Regaining GTP-tubulin restarts growth (rescue). Individual microtubules therefore switch between growth and shrinkage.

– UNDERSTAND **Why do intermediate filaments have no polarity?** 2 marks

The two coiled-coil dimers in each tetramer point in opposite directions, so both ends of the tetramer, and of the filament, are the same.

– APPLY **A cell is treated with cytochalasin D. Which of these stop: mitosis, crawling, cytokinesis?** 3 marks

Crawling and cytokinesis stop because both need actin. The spindle (microtubules) still forms, so chromosomes separate, but the cell cannot divide and may end with two nuclei.

– APPLY **How many tubulin dimers are in a 4 µm microtubule with 13 protofilaments?** 2 marks

4,000 ÷ 8 = 500 per protofilament; × 13 = 6,500 dimers.

– ANALYZE **Explain why paclitaxel is useful against cancer but causes nerve damage as a side effect.** 4 marks

It stabilises microtubules so the spindle cannot work and dividing cancer cells arrest in mitosis. Neurons do not divide, but they depend on microtubules for long-distance transport in axons; freezing them disturbs transport, which gives peripheral neuropathy.

BEYOND THE SYLLABUS

Super-resolution microscopy (STORM, PALM, expansion microscopy) now shows single cytoskeletal filaments inside cells, and cryo-electron tomography shows their arrangement in 3-D. Tau that falls off microtubules forms the tangles of Alzheimer's disease. Microtubule drugs such as eribulin and antibody-drug conjugates carrying tubulin poisons are current cancer treatments. Search "expansion microscopy cytoskeleton" and "antibody-drug conjugate tubulin". Not for the exam.

3.9 Molecular motors and centrioles

Molecular motors are proteins that turn the chemical energy of ATP into movement along a cytoskeletal track. Three families do almost all the work: **kinesins** and **dyneins** walk on microtubules, and **myosins** walk on actin. They carry vesicles and organelles, pull chromosomes apart, beat cilia and contract muscle. **Centrioles** are small barrels of microtubules that organise the centrosome and form the base of every cilium, so they decide where many of the motors' tracks begin.

You need: microtubule and actin polarity from Chapter 3.8.

How a motor works

Every motor has a **head** (motor domain) that binds both the track and ATP, and a **tail** that binds the cargo. One ATP cycle drives one step:

1. The head binds ATP (or, for myosin, ADP + P_i after hydrolysis) and its grip on the track changes.
2. Hydrolysis and release of phosphate cause a shape change in the head, the **power stroke**, which swings a lever or linker.
3. The head lets go of ADP and the cycle resets.

Because the track is polar, each motor family moves in a fixed direction. A motor is **processive** if it takes many steps without falling off; this needs two heads working in turn, so that one is always attached.

The three families

Kinesins mostly move towards the **plus end** of microtubules, from the cell centre outward. Conventional **kinesin-1** has two heads that walk **hand over hand**, each step 8 nm (the length of one tubulin dimer) and using one ATP. It carries vesicles, mitochondria and mRNA particles down nerve axons (**anterograde transport**). Other kinesins work in mitosis: kinesin-5 pushes spindle poles apart, and kinesin-13 does not walk at all but takes microtubule ends apart. A few kinesins (kinesin-14) move towards the minus end.

Dyneins move towards the **minus end**. They are very large; the motor is a ring of six ATPase domains with a long stalk that touches the microtubule. **Cytoplasmic dynein**, working with the adaptor complex **dynactin**, carries cargo back to the cell centre (**retrograde transport** in axons),

keeps the Golgi near the centrosome and helps position the spindle. **Axonemal dynein** forms the arms on the microtubules of cilia and flagella and makes them beat.

Myosins move along actin, almost all towards the **plus (barbed) end**. **Myosin II** has two heads and long tails that bundle into thick filaments; in muscle it pulls actin thin filaments to shorten the sarcomere, and in dividing cells it closes the contractile ring. In its **cross-bridge cycle**, ATP binding releases the head from actin, hydrolysis cocks it, the head rebinds and releasing P_i triggers the power stroke. Muscle structure is in **Unit 6, Muscle cells**. **Myosin V** is a processive cargo carrier with large steps of about 36 nm; **myosin VI** is unusual in walking towards the minus end.

MOTOR	TRACK	DIRECTION	TYPICAL JOB
Kinesin-1	microtubule	plus end (outward)	anterograde axonal transport
Cytoplasmic dynein	microtubule	minus end (inward)	retrograde transport, Golgi position
Axonemal dynein	microtubule doublets	minus end	beating of cilia and flagella
Myosin II	actin	plus end	muscle contraction, cytokinesis
Myosin V	actin	plus end	vesicle and pigment transport

Centrioles, centrosomes, basal bodies and cilia

A **centriole** is a short cylinder about 0.2 µm across and roughly twice as long, whose wall is made of **nine triplets** of microtubules (called A, B and C, with only A a complete tube). A cartwheel-like structure at one end sets the ninefold pattern. Two centrioles lying at right angles, surrounded by a cloud of **pericentriolar material** rich in γ-tubulin ring complexes, form the **centrosome**, the main MTOC of animal cells (Chapter 3.8). The centrioles organise the material; the material starts the microtubules.

Centrioles **duplicate once per cell cycle**, starting around the G1-to-S transition under control of the kinase PLK4. Each old centriole grows one new daughter at right angles, so each of the two centrosomes that separate in mitosis has one old and one new centriole (Unit 4). Higher plants and most fungi have no centrioles and still build spindles, which shows that centrioles are not needed for the spindle itself.

When a centriole moves to the plasma membrane and anchors a cilium, it is called a **basal body**. The cilium's core, the **axoneme**, grows from it: its triplets continue as **nine doublets**. A **motile** cilium or flagellum has **9 + 2**: nine outer doublets around a central pair of single microtubules. Axonemal dynein arms on each doublet walk on the next doublet, and **nexin links** stop the doublets sliding past each other, so the sliding turns into bending. A **primary cilium** has **9 + 0**

(no central pair) and does not beat; it acts as a sensor and an antenna for signals, and most human cells carry one.

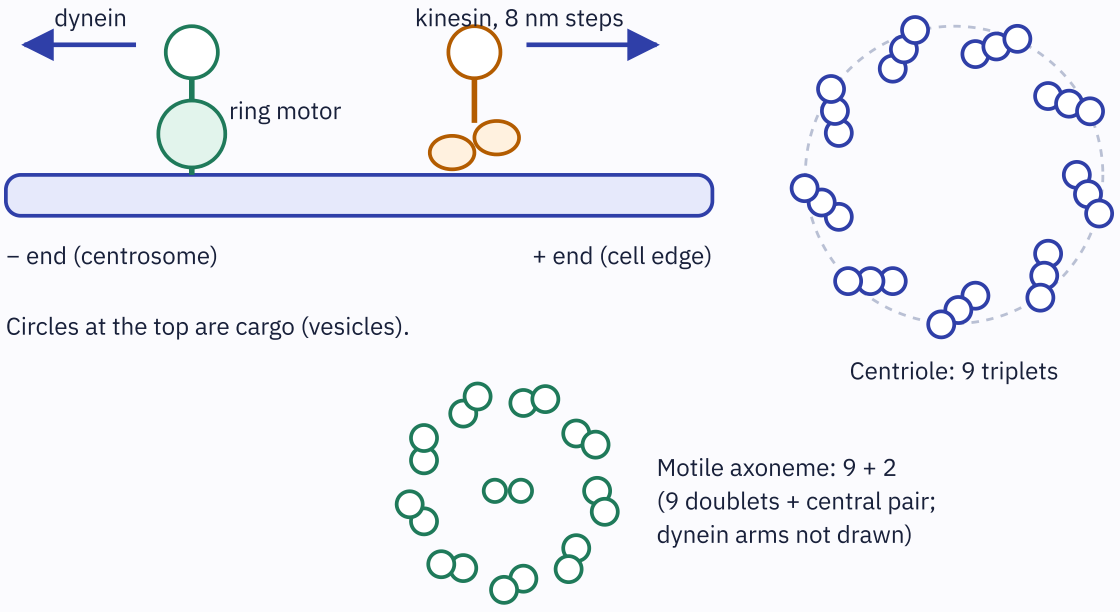


Figure 3.9.1 Top left, two motors on one microtubule carry cargo in opposite directions: kinesin towards the plus end, dynein towards the minus end. Right, a centriole in cross-section with its nine triplets. Bottom, a motile axoneme with nine doublets and a central pair; the triplets of the basal body continue as these doublets.

WORKED EXAMPLE

Question (6 marks). A motor neuron runs from the spinal cord to a toe, an axon about 1.0 m long. A kinesin-1 motor takes 8 nm steps, uses one ATP per step, and takes 100 steps per second. (a) What is its speed? (b) How long does it take to carry a vesicle the full length? (c) How many ATP molecules does one motor use for the trip? (d) Which motor brings worn-out material back, and in which direction relative to microtubule polarity?

Solution.

1. Speed = 8 nm × 100 s^{-1} = 800 nm s^{-1} = **0.8 µm s^{-1}**.
2. 1.0 m = 1.0 × 10^6 µm. Time = 1.0 × 10^6 ÷ 0.8 = 1.25 × 10^6 s. One day is 86,400 s, so the time is about **14.5 days**.
3. Steps = 1.0 m ÷ 8 × 10^{-9} m = 1.25 × 10^8, so **1.25 × 10^8 ATP**.
4. In axons, microtubule plus ends point away from the cell body. Kinesin goes out towards the plus ends; **cytoplasmic dynein** brings material back towards the minus ends in the cell body.

Answer: 0.8 µm s^{-1}; about 14.5 days; 1.25 × 10^8 ATP; dynein, minus-end directed. Diffusion of a vesicle over a metre would take far longer than a lifetime, which is why long cells depend on motors.

EXAM TRAPS

- Kinesin to plus, dynein to minus, myosin on actin. A memory aid: "dynein drives down to the centre".
- A cilium's axoneme is 9 + 2 doublets; a centriole or basal body is 9 + 0 triplets. Do not draw triplets in a cilium.
- Centrioles are not needed to make a spindle: plant cells have none.
- Myosin binds actin, not microtubules; the only common minus-end myosin is myosin VI.
- Kartagener syndrome (primary ciliary dyskinesia) comes from defective axonemal dynein: chronic chest infections, male infertility, and in about half of patients organs on the wrong side.

CHECK YOURSELF

– REMEMBER **Name the three motor families and their tracks.** 3 marks

Kinesins and dyneins on microtubules; myosins on actin filaments.

– REMEMBER **What is the microtubule pattern of a motile cilium and of a centriole?** 2 marks

Motile cilium: 9 + 2 (nine doublets, central pair). Centriole: nine triplets, no central pair.

– UNDERSTAND **Why does a processive motor need two heads?** 2 marks

One head must stay bound to the track while the other steps forward; with one head the motor would fall off after each step.

– UNDERSTAND **How does dynein sliding turn into bending of a cilium?** 3 marks

Dynein arms on one doublet walk on the next, trying to slide it. Nexin links and the basal body hold the doublets together, so they cannot slide freely and the axoneme bends instead.

– APPLY **A myosin V takes 36 nm steps at 10 steps per second. How far does it move in a minute?** 2 marks

36 nm × 10 × 60 = 21,600 nm ≈ 21.6 µm.

– APPLY **A drug stops cytoplasmic dynein. Predict the effect on the Golgi and on retrograde axonal transport.** 3 marks

The Golgi, normally held near the centrosome by dynein, scatters through the cytoplasm. Retrograde transport stops, so material from the axon tip, including signals for the cell body, is not returned.

– ANALYZE **A patient has chronic sinus and lung infections and immotile sperm. Link the symptoms to one cellular defect.** 4 marks

Motile cilia of the airway sweep mucus out, and the sperm tail is a flagellum; both use the 9 + 2 axoneme with dynein arms. A dynein-arm defect (primary ciliary dyskinesia) stops both, so mucus and bacteria stay in the airways and sperm cannot swim.

BEYOND THE SYLLABUS

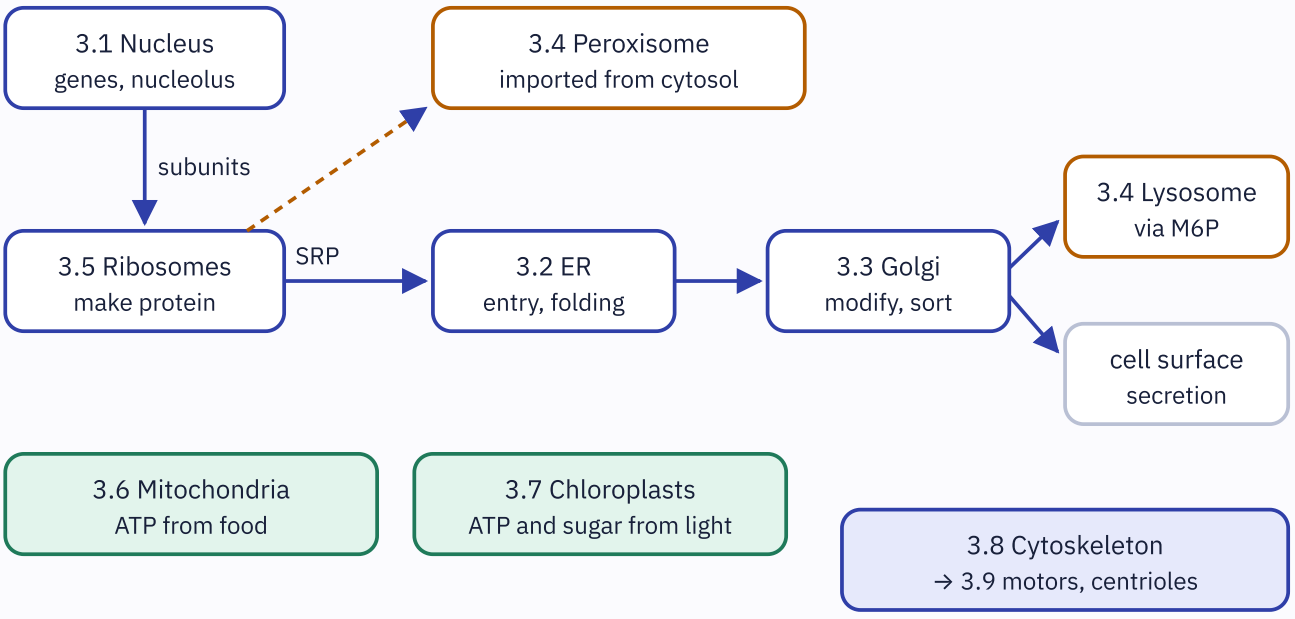
Single-molecule methods such as optical tweezers measured the 8 nm kinesin step directly, and they are now used to test new motor drugs. Diseases of primary cilia, called ciliopathies, affect the kidney, eye and brain, and are an active field. Synthetic biologists are building artificial motors from DNA and engineering kinesins for nanoscale transport on chips. Search "optical tweezers kinesin" and "ciliopathies". Not for the exam.

Unit summary

KEY TERMS AND FORMULAS

TERM	MEANING OR FORMULA	CHAPTER
Nuclear localization signal	short basic sequence (e.g. PKKKRKV) read by importin α; not cleaved	3.1
Ran gradient	Ran-GTP high in nucleus (RanGEF on chromatin), low in cytosol (RanGAP)	3.1
Nucleolus	membraneless site of rRNA synthesis and subunit assembly: FC, DFC, GC	3.1
Signal sequence	N-terminal hydrophobic stretch read by SRP; cut by signal peptidase	3.2
N-linked glycosylation	14-sugar block added to Asn in Asn–X–Ser/Thr (X ≠ Pro), in the ER	3.2
KDEL	C-terminal retention signal returning ER lumen proteins from the Golgi	3.2
COPII / COPI / clathrin	ER→Golgi / Golgi→ER / TGN and plasma membrane→endosomes	3.3
Mannose-6-phosphate	tag added in cis Golgi, read by M6P receptor in TGN; sends enzymes to lysosomes	3.3
Lysosome pH	4.5–5, kept by V-type H^+-ATPase; $[H^+]$ ratio = 10^(ΔpH)	3.4
Catalase	$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$; PTS1 = C-terminal SKL	3.4
Ribosomes	70S = 50S + 30S; 80S = 60S + 40S; S values not additive	3.5
Proton-motive force	$\Delta p = \Delta\psi + (2.303RT/F)\cdot\Delta pH$; 61.5 mV per pH unit at 37 °C; $\Delta G = -F\Delta p$	3.6
Photon energy	$E = hc/\lambda$; 680 nm → 176 kJ mol^{-1}	3.7
Calvin cycle cost	$3\ CO_2$ → 1 G3P uses 9 ATP + 6 NADPH	3.7
Critical concentration	free monomer level where an end neither grows nor shrinks; treadmilling between Cc(+) and Cc(–)	3.8
Motor directions	kinesin → plus end; dynein → minus end; myosin on actin	3.9

TERM	MEANING OR FORMULA	CHAPTER
Centriole and axoneme	centriole 9 triplets; motile cilium 9 + 2; primary cilium 9 + 0	3.9



Green: proton gradient and ATP synthase. Cytoskeleton carries and places all of them.

Figure 3.S.1 How the chapters connect. The nucleus supplies ribosome subunits; ribosomes making secretory proteins are sent by SRP to the ER, then to the Golgi, which sorts cargo to lysosomes or the cell surface. Peroxisomal proteins (dashed) are imported straight from the cytosol. Mitochondria and chloroplasts share one ATP-making mechanism. The cytoskeleton and its motors move and position everything.

Takeaways

1. The nuclear envelope is two membranes continuous with the ER; nuclear pores pass small molecules freely and large ones only with an NLS or NES, driven by the Ran-GTP gradient.
2. The nucleolus has no membrane; it makes 18S, 5.8S and 28S rRNA and assembles ribosome subunits.
3. Secretory and membrane proteins enter the ER while being made, guided by a signal sequence, SRP and Sec61; the ER folds them, adds N-linked sugars and destroys failures.
4. The Golgi modifies cargo from cis to trans and sorts it at the TGN: default secretion, regulated granules, or lysosomes through the M6P tag.
5. Lysosomes digest at pH 4.5–5; peroxisomes make and destroy H_2O_2 and import folded proteins with SKL; plant vacuoles give turgor and store.

6. Ribosomes are ribozymes; 70S and 80S differences make antibiotics selective, and organelle ribosomes are bacteria-like.
7. Mitochondria and chloroplasts both pump protons and use ATP synthase; $\Delta p = \Delta\psi + 61.5\ mV \times \Delta pH$ at 37 °C, and both organelles began as bacteria.
8. Microtubules (GTP, 25 nm) and actin (ATP, 7 nm) are polar and dynamic; intermediate filaments (10 nm) are non-polar, stable and strong.
9. Kinesin walks to plus ends, dynein to minus ends, myosin along actin; centrioles (9 triplets) build centrosomes and basal bodies, and motile cilia are 9 + 2.

Exam question bank

Remember and understand

- **Q1. With a labelled diagram, describe the structure of the nucleus and the functions of the nuclear envelope, nuclear pore complex and nucleolus.** `12 marks`

  Diagram: double envelope continuous with RER, perinuclear space, pores, lamina, chromatin (eu- and heterochromatin), nucleolus with FC, DFC, GC. Envelope separates transcription from translation. NPC: about 30 nucleoporins, eightfold symmetry, FG repeats, passive limit about 40–60 kDa, NLS/importin/Ran import and NES/exportin export. Nucleolus: NORs, Pol I makes 45S precursor → 18S, 5.8S, 28S; 5S imported; subunit assembly and export.

- **Q2. Explain the signal hypothesis of protein targeting to the ER.** `10 marks`

  Signal sequence features; SRP binding and translational pause; SRP receptor; GTP use; Sec61 translocon; cotranslational transfer; signal peptidase; the cell-free experiment with and without microsomes; stop-transfer sequences for membrane proteins.

- **Q3. Describe the structure of the mitochondrion and relate each part to its function.** `10 marks`

  Outer membrane with porins; intermembrane space with cytochrome c; inner membrane with cristae, cardiolipin, ETC and ATP synthase, impermeable to ions; matrix with TCA cycle, β-oxidation, mtDNA (16,569 bp, 37 genes) and ribosomes. Labelled diagram; fission and fusion; other roles (apoptosis, Ca^{2+}, heme).

- **Q4. Write short notes on (a) lysosomes, (b) peroxisomes (microbodies), (c) vacuoles.** 12 marks

  (a) acid hydrolases, pH 4.5–5, V-ATPase, glycosylated membrane, three routes in, storage diseases. (b) oxidases and catalase reactions, VLCFA β-oxidation, plasmalogens, glyoxysomes, PTS1 import, Zellweger syndrome. (c) central vacuole, tonoplast, turgor, storage, lytic role, contractile vacuoles.

Apply

- **Q5. Trace the journey of a lysosomal enzyme from its gene to the lysosome, naming every compartment, signal and receptor. Explain what goes wrong in I-cell disease.** 14 marks

  Nucleus (transcription, mRNA export through pore) → free ribosome → SRP → RER (signal cleaved, N-glycosylation, folding) → COPII → cis Golgi (GlcNAc-phosphotransferase reads signal patch, M6P exposed) → TGN (M6P receptor, clathrin vesicle) → endosome (low pH releases enzyme, receptor recycles) → lysosome. I-cell: no phosphotransferase → enzymes secreted → inclusions and high blood enzyme levels. Spans Chapters 3.1, 3.2, 3.3, 3.4 and 3.5.

- **Q6. At 37 °C a mitochondrion has $\Delta\psi = 170$ mV and $\Delta pH = 0.6$. Calculate Δp and the energy per mole of H^+. If ATP synthesis needs 52 kJ mol^{-1}, how many protons are needed at minimum?** 8 marks

  $61.5 \times 0.6 = 36.9$ mV; $\Delta p = 206.9$ mV ≈ 207 mV. $\Delta G = 96{,}485 \times 0.207 \approx 20.0$ kJ per mol H^+. $52 \div 20.0 = 2.6$, so at least 3 H^+ per ATP. State the sign convention and the role of the ADP/P_i transport cost.

- **Q7. Predict the effect of each drug on a dividing animal cell and explain: colchicine, paclitaxel, cytochalasin D, phalloidin.** 8 marks

  Colchicine: no spindle, mitotic arrest. Paclitaxel: microtubules frozen, spindle cannot work, arrest. Cytochalasin D: spindle forms but no contractile ring, binucleate cell. Phalloidin: actin cannot depolymerise; cytokinesis and crawling impaired (it does not enter living cells easily, so mostly used on fixed cells).

- **Q8. A 1.2 m axon carries vesicles by kinesin at 1.0 μm s^{-1}. How long is the journey in days? Which motor returns material, and what would happen to the neuron if it failed?** 8 marks

  1.2×10^6 μm ÷ 1.0 = 1.2×10^6 s ≈ 13.9 days. Cytoplasmic dynein with dynactin, minus-end directed, returns material to the cell body. Failure: debris and signals pile up at the tip, the cell body misses survival signals, and the axon degenerates (seen in some motor neuron diseases).

Analyze and evaluate

- **Q9. Compare the structure and energy conversion of mitochondria and chloroplasts, and evaluate the evidence that both arose by endosymbiosis.** 16 marks

  Table: membranes, where the chain sits, where protons go, where ATP is made, main part of Δp, products. Shared features: F-type ATP synthase, chemiosmosis. Evidence: double membranes, circular genomes, 70S ribosomes, antibiotic sensitivity, fission, gene sequences close to α-proteobacteria and cyanobacteria. Limits: most genes moved to the nucleus. Spans Chapters 3.5, 3.6 and 3.7.

- **Q10. Compare how proteins are imported into the nucleus, the ER, mitochondria and peroxisomes.** 12 marks

  For each: signal (NLS; N-terminal hydrophobic; N-terminal amphipathic presequence; C-terminal SKL), timing (post- or cotranslational), folded or unfolded, machinery (importins and Ran; SRP and Sec61; TOM/TIM; peroxins), signal cleaved or not, energy source. Spans Chapters 3.1, 3.2, 3.4 and 3.6.

- **Q11. Compare microtubules, actin filaments and intermediate filaments, and explain why only two of them show rapid turnover.** 12 marks

  Diameter, subunit, structure, polarity, nucleotide, motors, drugs, functions. Turnover: nucleotide hydrolysis after assembly weakens the polymer (GTP cap, ATP-actin vs ADP-actin), giving dynamic instability and treadmilling; IFs bind no nucleotide and are regulated by phosphorylation instead.

- **Q12. "Centrioles are essential for cell division." Evaluate this statement.** 8 marks

  For: centrioles organise the centrosome, the main MTOC, and duplicate once per cycle to give two spindle poles in animal cells. Against: higher plants and most fungi have no centrioles and still form spindles; animal cells with centrioles removed can still divide, though less reliably. Conclusion: important for accuracy and essential for cilia, not strictly for the spindle.

Glossary

Acid hydrolase	Digestive enzyme of the lysosome that works best at low pH.
Axoneme	The microtubule core of a cilium or flagellum; 9 + 2 in motile cilia.
Basal body	A centriole at the base of a cilium, from which the axoneme grows.
Catalase	Peroxisomal enzyme that breaks H_2O_2 into water and oxygen.

Centrosome	Two centrioles with pericentriolar material; the main microtubule-organising centre of animal cells.
Cisterna	A flattened membrane sac of the ER or Golgi.
Cristae	Folds of the inner mitochondrial membrane.
Critical concentration	Free-subunit concentration at which a filament end neither grows nor shrinks.
Dynamic instability	Switching of microtubule ends between growth and rapid shrinkage.
Endosymbiotic theory	The idea that mitochondria and chloroplasts descend from bacteria living inside an ancestral cell.
Granum	A stack of thylakoids in a chloroplast.
Importin	Transport receptor that carries NLS proteins into the nucleus.
Lamina	Mesh of lamin filaments under the inner nuclear membrane.
Mannose-6-phosphate	Sugar tag that targets enzymes from the Golgi to lysosomes.
Microbody	Older name for peroxisomes and glyoxysomes.
Nucleolus	Membraneless nuclear region where rRNA is made and ribosome subunits assembled.
Nucleoporin	One of the proteins that make up the nuclear pore complex.
Polysome	Several ribosomes translating the same mRNA at once.
Processive motor	A motor that takes many steps without letting go of its track.
Proton-motive force	Energy stored in a proton gradient across a membrane, from voltage and pH difference.
Sec61	The channel (translocon) through which proteins cross the ER membrane.
Signal recognition particle	RNA–protein complex that brings ribosomes making signal-sequence proteins to the ER.
Stroma	Fluid inside the chloroplast envelope, site of the Calvin cycle.
Svedberg unit (S)	Unit of sedimentation coefficient, 10^{-13} s.
Thylakoid	Membrane sac inside a chloroplast carrying pigments, photosystems and ATP synthase.
Tonoplast	Membrane around the plant vacuole.
Trans Golgi network	Exit face of the Golgi where cargo is sorted to its destinations.
Treadmilling	Steady addition at the plus end and loss at the minus end, keeping filament length constant.
Unfolded protein response	ER stress signal that raises chaperones and slows translation.

Sources and further reading

Standard textbooks

- Bruce Alberts and others, *Molecular Biology of the Cell* (W. W. Norton / Garland Science): chapters on intracellular compartments and protein sorting, intracellular membrane traffic, energy conversion in mitochondria and chloroplasts, and the cytoskeleton. (from memory; check)
- Harvey Lodish and others, *Molecular Cell Biology* (W. H. Freeman / Macmillan). (from memory; check)
- Geoffrey M. Cooper, *The Cell: A Molecular Approach* (Sinauer / Oxford University Press). (from memory; check)
- Gerald Karp, *Cell and Molecular Biology: Concepts and Experiments* (Wiley). (from memory; check)
- P. S. Verma and V. K. Agarwal, *Cell Biology, Genetics, Molecular Biology, Evolution and Ecology* (S. Chand), widely prescribed in Indian universities. (from memory; check)

Open resources

- OpenStax, *Biology 2e*, Chapter 4 "Cell Structure", section 4.3 "Eukaryotic Cells" and 4.4 "The Endomembrane System and Proteins" (CC BY 4.0): an open textbook covering the organelles of this unit at an introductory level. Reached through the course pages below; section titles from memory; check.
- Penn State DuBois, Biology 110 course pages built on OpenStax *Biology 2e*: psu.pb.unizin.org/biol110/part/cell-structure/
- City Tech OpenLab, Biology 1101 Course Hub, Lecture 11 "The Eukaryotic Cell", with readings and slides: openlab.citytech.cuny.edu/bio1101coursehub/?p=3597

Numbers in this book (pore size limit, ribosome protein counts, mtDNA size, filament diameters, motor step sizes) are standard textbook values written from memory; check them against your prescribed book. Worked-example inputs such as step rates and translation speeds are given in the question and are for practice.

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