Organelles and compartmentalization

B2.2 · SL and HL

Why do a secretory cell and a muscle cell look different?

Cell contents support different demands

A pancreatic cell exports enzymes; a contracting muscle cell needs continuing ATP supply. Different internal arrangements support these different roles.

Common content: organelle classification, fractionation, nuclear separation, local reaction conditions and microscopy evidence.

AHL: mitochondrial/chloroplast adaptations, nuclear envelopes, ribosomes, rough ER, Golgi and vesicles.

Numerical datasets are invented teaching examples.

Use the course convention when classifying structures

Component	Classification and reason
Nucleus and vesicle	Organelles with enclosing membranes
Ribosome	Organelle without an enclosing membrane
Plasma membrane	Counts as an organelle in this course convention
Cell wall, cytoskeleton, cytoplasm	Do not count as organelles under this convention

Identify a structure before assigning activity to it

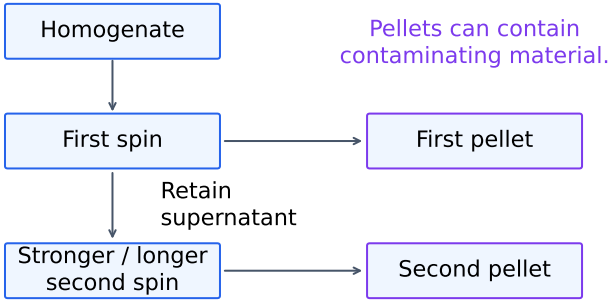
A micrograph supplies structural evidence

Look for membranes, folds, stacked sacs and location. A circular section may come from a tube; a schematic colour is not an identifying stain in every specimen.

Mature mammalian red blood cells lack nuclei and mitochondria. Secretory cells can have extensive rough ER and Golgi; active muscle cells can contain many mitochondria.

Organelle abundance supports a structure–function explanation but does not directly measure secretion rate or ATP production.

Follow the supernatant to the next spin



A pellet contains sedimented material; the next separation uses retained supernatant, and fractions can remain contaminated.

Preserve structures while separating them

Homogenize, centrifuge and test the resulting fractions

Break cell boundaries in a cold, suitable buffered/osmotic medium. Cooling slows unwanted reactions; the medium helps preserve pH and membrane-enclosed structures.

Collect an initial pellet and spin its supernatant under stronger or longer conditions. Sedimentation depends on density, shape, medium and time as well as size.

Use microscopy and marker assays to identify enrichment. High-speed centrifuges made functions of separated organelle fractions easier to investigate.

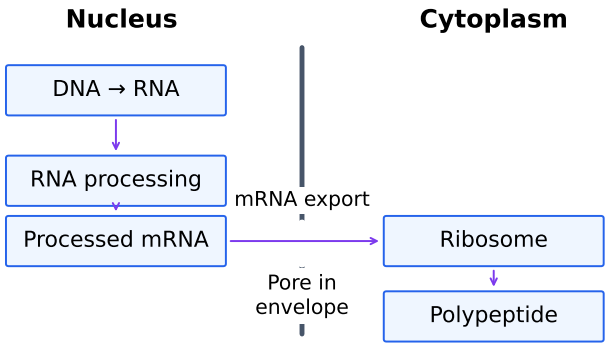
Read marker recoveries as an experimental distribution

Invented fraction	**Nuclear / mitochondrial / cytosolic marker recovery**
First pellet	80% / 2% / 5%
Second pellet	10% / 82% / 5%
Small-membrane pellet	5% / 10% / 10%
Final supernatant	5% / 6% / 80%

Separate enrichment from recovery

1. **Starting sample.** 30 enzyme units in 15 mg protein: specific activity $= 30/15 = 2$ units per mg.
2. **Collected fraction.** 20 units in 2 mg: $20/2 = 10$ units per mg. Enrichment $= 10/2 = 5$-fold.
3. **Recovery.** $20/30 \times 100 = 66.7\%$. Increased activity per protein mass does not mean all starting activity was recovered; compare the same assay conditions.

Export an RNA copy rather than the DNA gene



The nuclear envelope separates transcription and processing from cytoplasmic translation; processed mRNA crosses through pores.

A boundary allows processing before ribosomes meet mRNA

Compare eukaryotic separation with prokaryotic coupling

For a typical eukaryotic protein-coding transcript, splicing removes introns and joins retained exons. A cap and poly(A) tail are commonly added before export.

Ribosomes outside the nucleus translate processed mRNA. DNA remains in the nuclear compartment.

Prokaryotes lack this nuclear boundary, so translation may begin while transcription continues. They still have DNA and ribosomes; do not claim they lack all RNA processing.

Use the stated event order rather than a universal delay

1. **Invented model.** Transcription ends at minute 3; processing ends at 5; export ends at 6.
2. **Prediction.** Cytoplasmic translation of this transcript cannot start at minute 2. In this model its processed mRNA arrives only at minute 6.
3. **Limit.** These numbers establish the order for one stated transcript. They do not give universal times for all genes or organisms.

Connect a boundary to the conditions it maintains

Concentration and separation offer different advantages

Keeping enzymes and substrates together can support encounters without increasing total amounts. Transport and enzyme properties still matter.

A lysosome supplies an acidic digestive environment while cytosol maintains different conditions. Its membrane helps contain digestive activity.

An engulfed particle remains within a phagocytic vacuole. Fusion with lysosomal compartments supports digestion; useful products can leave through suitable transport pathways.

Keep amount, volume and concentration separate

1. **Same amount.** Invented 12 pmol in 4 pL gives $12/4 = 3$ pmol per pL. In 12 pL it gives 1 pmol per pL.
2. **Compare.** The concentration is threefold greater in the smaller volume. Total amount remains 12 pmol in each case.
3. **Evaluate.** Compartments can separate incompatible conditions, but membranes, sorting and controlled transport require resources. This ratio model is not a measured concentration of a named metabolite.

Convert lengths before finding magnification

1. **Mitochondrion.** A 30 mm image represents 5.0 μm. $M = 30000/5.0 = 6000$: magnification $\times 6000$, without a unit.
2. **Vesicle.** A 4.0 mm image at the same scale gives actual diameter $4000/6000 = 0.667$ μm, about 0.67 μm.
3. **Image length.** A 2.0 μm structure at $\times 15000$ gives 30000 μm $=$ 30 mm. All stated examples are invented.

A scale bar preserves the ratio when an image is resized

1. **Original print.** A 2.0 μm bar measures 8.0 mm. A structure measures 18 mm: actual size $= (18/8)(2) = 4.5$ μm.
2. **Doubled print.** Bar 16 mm and structure 36 mm: $(36/16)(2) = 4.5$ μm. Resize the scale bar with its image.
3. **Evidence.** Locate at low power first; centre before increasing power and use fine focus. Draw observed outlines with straight labels and scale. Magnification does not by itself increase resolution or reveal movement.

Cristae provide a working membrane surface

Area, a restricted space and selective permeability work together

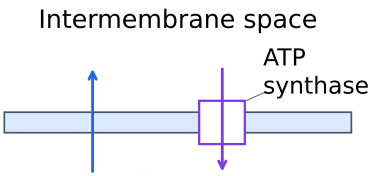
Cristae increase inner-membrane area for electron-transfer components and ATP synthase. Matrix enzymes and substrates support the Krebs cycle.

Energy from electron transfer supports hydrogen-ion transfer from matrix into the small intermembrane space. Restriction of uncontrolled ion return helps maintain an electrochemical gradient.

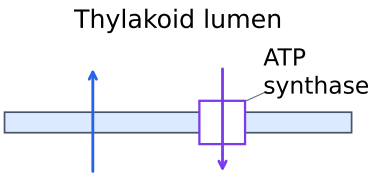
Hydrogen-ion return through ATP synthase toward matrix supports ATP production. More folds cannot replace substrates, oxygen in aerobic electron transfer or working proteins.

Name the compartment receiving hydrogen ions

Inner mitochondrial membrane



Chloroplast thylakoid membrane



Blue: energy-driven H^+ transfer.
Purple: H^+ return through ATP synthase.

Mitochondrial inner membrane and chloroplast thylakoid membrane separate different pairs of aqueous compartments.

Photosystems and carbon-fixing enzymes occupy different locations

Do not substitute the chloroplast envelope for thylakoids

Thylakoid membranes provide photosystem area. Light-dependent electron transfer supports hydrogen-ion accumulation in the small thylakoid lumen.

Return through ATP synthase toward stroma supports ATP production. Stroma enzymes and substrates support the Calvin cycle, incorporating carbon from carbon dioxide.

The envelope surrounds the chloroplast, while the thylakoid system supplies the internal membrane for light-dependent ATP synthesis. Membrane damage can dissipate a gradient.

Pores and envelope breakdown solve different access problems

Separation is compatible with regulated exchange

The nuclear envelope has two membranes; its outer membrane is continuous with ER. Pore complexes allow controlled RNA and protein traffic.

Processed mRNA can leave; proteins needed for nuclear functions can enter. Selectivity is more complex than excluding every large molecule.

During open division the envelope disassembles into rearranging membrane fragments/vesicles and later reforms around daughter chromosome sets. DNA instructions are retained.

Some organisms retain a nuclear boundary during division. Envelope breakdown is not universal.

Compare ribosome locations without inventing permanent types

Location	**Protein destinations and mechanism**
Free in cytosol	Many cytosolic proteins, plus proteins later imported into nucleus, mitochondria or chloroplasts
Attached to rough ER	Appropriate growing chains enter ER lumen or membrane; destinations include secretion and the endomembrane system
Shared feature	Two subunits contain RNA and proteins; targeting information can lead a translating ribosome to ER

Ribosomes attach at the cytosolic face of rough ER

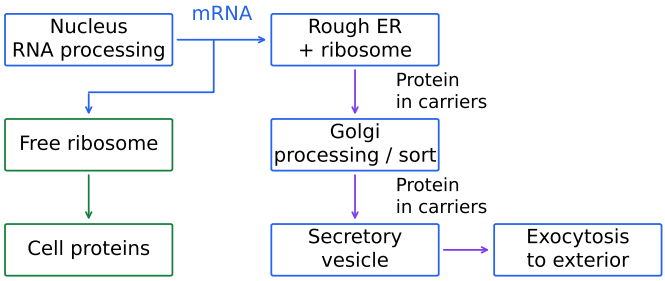
A rough-ER product can remain within the cell

The ER is a membrane system, not a pile of ribosomes. The growing chain can enter its lumen or insert into its membrane.

Folding and early processing occur in the ER route. A lysosomal enzyme is an intracellular destination, so rough ER does not mean export from the cell in every case.

Free-ribosome products are not restricted to staying forever in cytosol; suitable proteins can be imported into other compartments.

Keep the mRNA route separate from the secretory protein route



Blue: RNA route. Purple: secretory protein route. Green: other protein products.

The nucleus supplies an RNA copy; rough ER, Golgi and vesicles handle the appropriate protein pathway.

Vesicles preserve compartments while delivering selected cargo

Sorting and membrane fusion maintain distinct destinations

Golgi sacs receive appropriate cargo, modify and sort proteins, then package them for destinations. Golgi does not translate mRNA.

Vesicle budding, movement, recognition and fusion deliver selected cargo. Exocytosis joins vesicle membrane to plasma membrane and releases soluble contents.

Clathrin helps form some buds and organize cargo through associated proteins on the cytosolic side. The coat is removed before later fusion steps.

Clathrin is not a lipid membrane or a permanent coat on every vesicle. Other transport carriers use other coat systems.

Common practice: fractions, nuclear separation and containment

1. **Questions 1–5.** Use the course organelle convention; explain specialization and fractionation. A mitochondrial marker is 82% in the second pellet, leaving 18% elsewhere.
2. **Enzyme calculations.** Specific activities 2 and 10 units per mg; fivefold enrichment; 66.7% recovery. A marker-enriched fraction is not automatically pure.
3. **Questions 6–9.** Export processed mRNA, not DNA. Apply the stated timing model and distinguish concentration from amount. Lysosomes and phagocytic vacuoles combine containment with controlled exchange.

Common practice: microscopy arithmetic and limitations

1. **Question 10.** Magnification $\times 6000$; vesicle about 0.67 μm; image of a 2.0 μm structure at $\times 15000$ is 30 mm.
2. **Question 11.** Scale-bar calculation gives 4.5 μm before and after proportional resizing.
3. **Method.** Begin at low power, use fine focus at high power, and draw observed structures with labels and scale. Section orientation, preparation and lack of time information limit conclusions.

AHL practice: boundaries and protein destinations

1. **Question 12.** Contrast intermembrane space $\rightarrow$ matrix with thylakoid lumen $\rightarrow$ stroma for hydrogen-ion return through ATP synthase. Link folds to proteins, not energy creation.
2. **Question 13.** Explain pores, open-division envelope dynamics and both ribosome locations. Give an intracellular rough-ER destination and an imported free-ribosome product.
3. **Question 14.** Trace mRNA export, translation, ER entry, Golgi processing, vesicle delivery and exocytosis. Many vesicles can also indicate blocked transport; measure flux to distinguish explanations.

Check yourself

Name the structure, mechanism, evidence and limitation

Distinguish recovery from enrichment and concentration from total amount. Use the same assay conditions and units.

A scale bar must belong to the same image and resize with it. A static micrograph does not directly measure a process rate.

AHL: keep nuclear RNA export separate from secretory protein delivery, and name both aqueous sides of an ATP-producing membrane.