

Mitochondria

Ultrastructure · Endosymbiotic Theory · Compartmentalized Metabolism · Electron Transport Chain · Chemiosmotic Theory · Functions

1. Ultrastructure of Mitochondria

Mitochondria are double-membrane-bound organelles (0.5–1 μm diameter, up to several μm long), often called the "powerhouse of the cell." The two membranes divide the organelle into four distinct functional compartments, each with its own enzyme content.

(a) Outer Mitochondrial Membrane (OMM)

- Smooth, $\sim 60\text{--}75\text{ \AA}$ thick; protein:lipid ratio $\approx 1:1$.
- Freely permeable to ions and small molecules ($<5\text{ kDa}$) due to **porins** (VDAC – Voltage-Dependent Anion Channels).
- Contains enzymes for fatty acid elongation, phospholipid synthesis, and monoamine oxidase.

(b) Intermembrane Space (IMS)

- Narrow space ($\sim 6\text{--}8\text{ nm}$) between outer and inner membranes.
- Similar ionic composition to cytosol (small solutes equilibrate through OMM porins).
- Houses proteins such as cytochrome c, adenylate kinase, and creatine kinase; release of cytochrome c into cytosol triggers apoptosis.

(c) Inner Mitochondrial Membrane (IMM)

- Highly impermeable (rich in cardiolipin); protein:lipid ratio $\approx 4:1$ – the most protein-rich membrane in the cell.
- Thrown into folds called **cristae**, which greatly increase surface area for oxidative phosphorylation.
- Embedded with the four ETC complexes, ATP synthase (F_1F_0 -ATPase/"lollipop" particles), and numerous transporters (ANT, phosphate carrier, etc.).
- Cristae junctions are stabilized by the **MICOS complex**; cristae shape/number changes with metabolic state (cristae remodeling).

(d) Matrix

- Gel-like interior enclosed by IMM; contains $\sim 50\%$ of total mitochondrial protein.
- Contains: TCA cycle enzymes, fatty acid β -oxidation enzymes, pyruvate dehydrogenase complex, mitochondrial ribosomes (55S/70S-type), circular mtDNA (multiple copies), tRNAs, and enzymes for mtDNA replication/transcription.
- mtDNA is circular, double-stranded, maternally inherited, and codes for 13 respiratory-chain proteins + rRNAs + tRNAs in humans (mitochondrial genetic code differs slightly from the universal code).

2. Endosymbiotic Theory

Proposed (and championed) by **Lynn Margulis (1967)**: mitochondria originated from a free-living α -proteobacterium (related to modern *Rickettsiales*) engulfed by an ancestral anaerobic/archaeal host cell roughly 1.5–2 billion years ago. The relationship became permanent and mutualistic — the bacterium supplied efficient aerobic ATP production, while the host provided protection and nutrients.

Key Supporting Evidence

- **Double membrane:** outer membrane derived from host phagocytic vesicle; inner membrane derived from the original bacterial plasma membrane.
- **Own genome:** circular, naked (histone-free) mtDNA resembling bacterial chromosomes, not linear nuclear chromatin.
- **Own protein-synthesis machinery:** 70S-type ribosomes (bacterial-like, sensitive to antibiotics such as chloramphenicol, unlike cytosolic 80S ribosomes).
- **Independent, binary-fission-like division,** uncoupled from the cell cycle, similar to bacterial fission.
- **Membrane biochemistry:** inner membrane contains cardiolipin, a phospholipid otherwise unique to bacterial plasma membranes.
- **Molecular phylogeny:** mitochondrial rRNA/protein sequences cluster with α -proteobacteria (e.g., *Rickettsia*), not with archaeal/eukaryotic nuclear genes.
- Over evolutionary time, most original bacterial genes were transferred to the host nucleus (endosymbiotic gene transfer), leaving only a minimal mitochondrial genome.

3. Metabolic Pathways in Different Mitochondrial Chambers

Because each compartment has a distinct protein/enzyme complement, metabolic pathways are physically segregated within the mitochondrion:

Compartment	Major Pathways / Processes
Outer membrane	Fatty acid activation (acyl-CoA synthetase) & elongation; phospholipid biosynthesis; conversion of biogenic amines (monoamine oxidase); tryptophan degradation (first step)
Intermembrane space	Oxidative phosphorylation-linked phosphotransfer: adenylate kinase ($2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$), creatine kinase (phosphocreatine shuttle); sulfhydryl oxidase (disulfide relay import pathway); site of cytochrome c prior to apoptotic release
Inner membrane	Electron Transport Chain (Complexes I-IV); ATP synthase (Complex V) – oxidative phosphorylation; substrate/ion transporters (ANT, phosphate carrier, carnitine-acylcarnitine translocase, Ca^{2+} uniporter)
Matrix	Pyruvate oxidative decarboxylation (PDH complex); Citric Acid (Krebs/TCA) cycle; β -oxidation of fatty acids; part of urea cycle (carbamoyl phosphate synthetase-I, ornithine transcarbamylase); mtDNA replication, transcription & translation; haem & some steroid precursor synthesis

This compartmentalization allows opposing/incompatible reactions (e.g., fatty acid synthesis in cytosol vs. β -oxidation in the matrix) to proceed without interference, and lets the cell couple substrate oxidation (matrix) tightly to the proton-motive force built across the inner membrane.

4. Electron Transport Chain (ETC)

Located on the inner mitochondrial membrane, the ETC is a series of four multi-subunit protein complexes plus two mobile carriers that pass electrons from reduced coenzymes (NADH, FADH₂) to molecular oxygen, releasing energy used to pump protons (H⁺) from the matrix into the intermembrane space.

Complex	Name	Electron Donor → Acceptor	H ⁺ pumped
I	NADH dehydrogenase (NADH-CoQ reductase)	NADH → Coenzyme Q (ubiquinone)	4 H ⁺
II	Succinate dehydrogenase (also TCA cycle enzyme)	FADH ₂ (succinate) → CoQ	0 (no pumping)
III	Cytochrome bc ₁ complex (CoQ-cytochrome c reductase)	CoQH ₂ → Cytochrome c (via Q-cycle)	4 H ⁺
IV	Cytochrome c oxidase	Cytochrome c → O ₂ (→ H ₂ O)	2 H ⁺
V	ATP synthase (F ₁ F ₀ -ATPase)	Not an electron carrier - uses H ⁺ flux to synthesize ATP	—

Key Points

- Overall reaction: $\text{NADH} + \frac{1}{2}\text{O}_2 + \text{H}^+ \rightarrow \text{NAD}^+ + \text{H}_2\text{O}$ (ΔG° strongly negative).
- Complex II does not pump protons - it feeds electrons from FADH₂ directly into CoQ, so FADH₂-linked substrates yield less ATP than NADH.
- Coenzyme Q (ubiquinone) and cytochrome c are mobile carriers that shuttle electrons between the fixed complexes.
- Net proton pumping (per NADH oxidized): Complex I (4H⁺) + Complex III (4H⁺) + Complex IV (2H⁺) ≈ 10 H⁺ per NADH; ~6 H⁺ per FADH₂.
- Inhibitors: rotenone (Complex I), malonate (competitive inhibitor of Complex II), antimycin A (Complex III), cyanide/azide/CO (Complex IV), oligomycin (ATP synthase).
- Uncouplers (e.g., 2,4-DNP, and physiologically, UCP1/thermogenin in brown fat) dissipate the H⁺ gradient as heat instead of ATP.

5. Chemiosmotic Theory

Proposed by **Peter Mitchell (1961, Nobel Prize 1978)**, this theory explains how the energy released during electron transport is coupled to ATP synthesis — not via a high-energy chemical intermediate (as earlier chemical-coupling hypotheses proposed), but via an electrochemical proton gradient.

Core Postulates

- As electrons flow through Complexes I, III and IV, H⁺ ions are actively pumped from the matrix into the intermembrane space.
- Because the inner membrane is impermeable to protons, this creates an electrochemical proton gradient (protonmotive force, PMF) - a combination of a pH gradient (ΔpH , matrix alkaline) and a membrane potential ($\Delta\psi$, matrix negative, ~-150 to -180 mV).
- The stored energy is the **proton-motive force**: $\Delta p = \Delta\psi - (2.3RT/F)\Delta\text{pH}$.
- Protons flow back into the matrix down their electrochemical gradient only through the F₀ channel of **ATP synthase**, and this flow drives rotation of the F₀ c-ring and the γ -subunit stalk.
- Rotation causes conformational changes in the three catalytic β subunits of F₁ (binding-change mechanism, Paul Boyer): Loose → Tight → Open, sequentially catalyzing ADP

+ Pi → ATP and releasing it.

- ~3-4 H⁺ pass through ATP synthase per ATP synthesized; the same gradient also drives secondary active transport (e.g., ADP/ATP antiport, phosphate symport, Ca²⁺ uniport).

Evidence: ATP synthesis requires an intact, sealed inner membrane; artificially imposing a pH/voltage gradient across a membrane (even without electron transport) can drive ATP synthesis; uncouplers that make the membrane leaky to H⁺ abolish ATP synthesis without stopping electron flow.

6. Marker / Representative Enzymes by Chamber

Location	Representative Enzymes (Markers)
Outer membrane	Monoamine oxidase (classic marker enzyme); NADH-cytochrome b5 reductase (rotenone-insensitive); fatty acyl-CoA synthetase; phospholipase A ₂ ; kynurenine hydroxylase
Intermembrane space	Adenylate kinase (marker enzyme); creatine kinase; nucleoside diphosphokinase; sulfhydryl oxidase (Mia40/Erv1 import relay)
Inner membrane	Succinate dehydrogenase (marker enzyme - only TCA enzyme bound to IMM); NADH dehydrogenase, cytochrome c oxidase, ATP synthase; carnitine acyltransferase I/II
Matrix	Malate dehydrogenase, citrate synthase (marker enzyme), aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, fumarase; pyruvate dehydrogenase complex; enzymes of β-oxidation (acyl-CoA dehydrogenase, etc.); carbamoyl phosphate synthetase-I

Exam tip: Succinate dehydrogenase is the only TCA-cycle enzyme embedded in the inner membrane (all others are matrix-soluble) – a favourite distinguishing fact in competitive exams.

7. Functions of Mitochondria

(a) Bioenergetics

- Principal site of aerobic ATP production via the TCA cycle, ETC and oxidative phosphorylation (~30–32 ATP per glucose molecule, vs. 2 ATP from glycolysis alone).
- β -oxidation of fatty acids and amino acid catabolism also feed reducing equivalents into the ETC.

(b) Calcium Homeostasis & Signalling

- Mitochondrial Ca^{2+} uniporter (MCU) sequesters cytosolic Ca^{2+} , shaping the amplitude/duration of Ca^{2+} signals and regulating Ca^{2+} -dependent matrix dehydrogenases (PDH, isocitrate DH, α -KG DH) to match ATP supply with demand.

(c) Apoptosis (Programmed Cell Death)

- Intrinsic (mitochondrial) apoptotic pathway: permeabilization of the outer membrane (via Bax/Bak) releases cytochrome c into the cytosol, which nucleates the apoptosome (with Apaf-1, procaspase-9), activating the caspase cascade.

(d) Thermogenesis (Heat Production)

- In brown adipose tissue, uncoupling protein-1 (UCP1/thermogenin) dissipates the proton gradient as heat instead of ATP (non-shivering thermogenesis).

(e) Biosynthetic Roles

- Steroidogenesis: first, rate-limiting step of steroid hormone synthesis (cholesterol $\rightarrow$ pregnenolone) occurs in the mitochondrial matrix (via CYP11A1).
- Haem synthesis: initial and final steps occur in mitochondria (ALA synthase; ferrochelatase).
- Urea cycle: first two steps (carbamoyl phosphate synthetase-I, ornithine transcarbamylase) occur in the matrix.
- Contributes intermediates for gluconeogenesis and amino acid biosynthesis (via TCA cycle anaplerosis/cataplerosis).

(f) Reactive Oxygen Species (ROS) Generation & Redox Signalling

- Complexes I and III leak electrons to O_2 , generating superoxide (O_2^- , superoxide radical anion), managed by mitochondrial SOD2/MnSOD; excess ROS causes oxidative damage and is implicated in ageing and disease, but controlled ROS also acts as a signalling molecule.

(g) Other Roles

- Iron-sulfur cluster biogenesis (essential for numerous cellular enzymes).
- Autonomous replication and semi-independent genetic system, allowing rapid adjustment of mitochondrial mass/number (biogenesis) to cellular energy demand (regulated via PGC-1 α).
- Maternal inheritance of mtDNA is exploited in phylogenetics and forensic/lineage studies.