Oxidative Phosphorylation

1.1 Introduction and Overview

Oxidative phosphorylation (OXPHOS) represents the culmination of energy extraction from foodstuffs in aerobic organisms. It is the process wherein the reducing equivalents, NADH and FADH2, generated by the citric acid cycle, fatty acid β-oxidation, and glycolysis, are utilized to drive the synthesis of ATP from ADP and inorganic phosphate (Pi). This process is responsible for the vast majority of ATP production in most eukaryotic cells, dwarfing the yield from substrate-level phosphorylation. The elegance of OXPHOS lies in its coupling mechanism: the exergonic flow of electrons through a series of membrane-bound proteins is used to create an electrochemical gradient, the energy of which is then harnessed by a remarkable molecular machine to phosphorylate ADP. This chapter will dissect the components, mechanisms, and regulation of this vital process, with a strong emphasis on its clinical and pathological implications.

1.2 Mitochondrial Structure: Compartmentalization for Energy Transduction

The mitochondrion is not merely a bag of enzymes; its structure is exquisitely tailored for its role in OXPHOS. The organelle is bounded by two functionally distinct membranes. The **outer mitochondrial membrane** is permeable to small molecules and ions due to the presence of porin channels, making the intermembrane space biochemically similar to the cytosol for small solutes. The **inner mitochondrial membrane** is the site of oxidative phosphorylation. It is highly impermeable to most ions and small molecules, especially protons (H+). This impermeability is absolutely critical, as it allows for the establishment of an electrochemical gradient. The inner membrane is extensively folded into cristae, which dramatically increases its surface area, thereby maximizing the capacity for the electron transport chain (ETC) and ATP synthase complexes. The internal aqueous compartment, the **matrix**, contains the pyruvate dehydrogenase complex, the enzymes of the citric acid cycle, the fatty acid β-oxidation pathway, and the mitochondrial genome (mtDNA). It is here that NADH and FADH2 are primarily generated.

1.3 The Electron Transport Chain: A Thermodynamic Cascade

The electron transport chain (ETC) is a series of four protein complexes (I-IV) embedded in the inner mitochondrial membrane, along with two mobile electron carriers (ubiquinone and cytochrome c). The fundamental principle governing the ETC is thermodynamics: electrons are passed from donors with a more negative reduction potential (E'°) to acceptors with a more positive potential, releasing free energy at each step. The chain begins with the strong reductant NADH (E'° = -0.32 V) and terminates with the strong oxidant O2 (E'° = +0.82 V). This large, negative overall ΔG'° (-nFΔE'°) provides the driving force for the entire process. The released energy is not lost as heat but is conserved through the coupled pumping of protons across the inner membrane, from the matrix to the intermembrane space.

1.3.1 Complex I (NADH:Ubiquinone Oxidoreductase)

Complex I is a massive, L-shaped complex. The oxidation of one NADH molecule initiates the process. NADH binds and transfers two electrons as a hydride ion (H-) to the flavin mononucleotide (FMN) prosthetic group, reducing it to FMNH2. The electrons are then passed, one at a time, through a series of 8-9 iron-sulfur (Fe-S) clusters. These clusters act as electron conduits, exploiting the quantum mechanical phenomenon of electron tunneling for efficient transfer. The final Fe-S cluster reduces a molecule of **ubiquinone (Q)** to **ubiquinol (QH2)**. This reduction requires two electrons and is accompanied by the uptake of two protons from the matrix. The conformational changes associated with this electron transfer process drive the translocation of **four protons (H+)** from the matrix to the intermembrane space.

The reaction is: `NADH + H+ + Q + 4H+_matrix → NAD+ + QH2 + 4H+_IMS`.

1.3.2 Complex II (Succinate Dehydrogenase)

Complex II serves as a direct link between the citric acid cycle and the ETC. It catalyzes the oxidation of succinate to fumarate. This reaction reduces FAD, covalently bound to the enzyme, to FADH2. Electrons from FADH2 are then transferred via three Fe-S clusters to reduce ubiquinone to ubiquinol. Crucially, the free energy change for succinate oxidation (ΔG'° ≈ 0 kJ/mol) is insufficient to drive proton translocation.

Therefore, **Complex II does not pump protons**. This is the fundamental reason why FADH2, whose electrons enter the chain via Complex II, yields less ATP than NADH.

1.3.3 Ubiquinone (Coenzyme Q)

Ubiquinone is a small, hydrophobic benzoquinone with a long isoprenoid tail that allows it to diffuse freely within the lipid bilayer of the inner membrane. It is a mobile electron carrier that can accept one electron to form the semiquinone radical (Q•-) or two electrons to form the fully reduced ubiquinol (QH2). Its primary role is to collect electrons from Complex I and Complex II and deliver them to Complex III.

1.3.4 Complex III (Ubiquinol:Cytochrome c Oxidoreductase) and the Q Cycle

Complex III faces a mechanistic challenge: it must transfer two electrons from ubiquinol (a two-electron carrier) to two molecules of cytochrome c (a one-electron carrier). This is elegantly solved by the **Q Cycle**, a complex but efficient mechanism. The cycle involves two distinct ubiquinone-binding sites (Qo and Qi). In essence, for every two QH2 molecules oxidized at the Qo site, one Q molecule is reduced at the Qi site.

The net result is: `QH2 + 2 Cyt c_(ox) + 2H+_matrix → Q + 2 Cyt c_(red) + 4H+_IMS`.

This process results in the net translocation of **4 H+** to the IMS per two electrons transferred. The Q cycle thus amplifies proton pumping and is a key site for regulation and inhibition (e.g., by Antimycin A).

1.3.5 Cytochrome c

Cytochrome c is a small, water-soluble hemoprotein located in the intermembrane space, loosely associated with the outer surface of the inner membrane. It is a mobile carrier that shuttles electrons, one at a time, from Complex III to Complex IV. Its release from mitochondria into the cytosol is a critical step in the initiation of apoptosis.

1.3.6 Complex IV (Cytochrome c Oxidase)

Complex IV catalyzes the final and most thermodynamically favorable step: the four-electron reduction of molecular oxygen to water. Four molecules of reduced cytochrome c sequentially donate one electron each to the enzyme. The electrons pass through a

CuA center, then a heme a, and finally to a binuclear center composed of heme a3 and a copper ion (CuB). Dioxygen (O2) binds tightly to this binuclear center. In a meticulously controlled reaction, the O2 molecule is split, and the oxygen atoms are reduced to water without the release of potentially dangerous partially reduced oxygen intermediates like superoxide or peroxide. This four-electron reduction consumes four "chemical" or "substrate" protons from the matrix to form water. The energy released by this reaction drives the translocation of **2 protons** from the matrix to the IMS. The overall reaction is:

`4 Cyt c_(red) + 8H+_matrix + O2 → 4 Cyt c_(ox) + 2 H2O + 4H+_IMS`.

This complex is a major site of toxic inhibition; both **cyanide (CN-)** and **carbon monoxide (CO)** bind irreversibly to the ferric iron of heme a3, blocking cellular respiration.

1.4 The Chemiosmotic Theory: The Coupling Link

Proposed by Peter Mitchell, the chemiosmotic theory was a revolutionary concept that unified the processes of electron transport and ATP synthesis. It posits that the free energy released by electron transport is stored as an **electrochemical proton gradient**, or **proton motive force (PMF)**, across the inner mitochondrial membrane. The PMF has two components: 1) A **chemical gradient (ΔpH)** due to a difference in proton concentration ([H+] is higher in the IMS). 2) An **electrical gradient (Δψ)** due to a separation of charge (the IMS side is positive relative to the matrix). The total PMF (Δp) is expressed by the equation: $\Delta p = \Delta\psi - (2.3RT/F)\Delta pH$. In most cells, Δψ is the dominant component (~150-180 mV), while ΔpH contributes a smaller amount (~0.5-1 pH unit). The energy stored in the PMF is the immediate driving force for ATP synthesis.

1.5 ATP Synthase: The Molecular Turbine

ATP synthase (also called Complex V or F0F1 ATPase) is the enzyme that converts the energy of the PMF into the chemical energy of ATP. It is a molecular motor with two main components:

- **F0 Unit:** An integral membrane complex that forms a transmembrane proton channel. It consists of a c-ring (composed of 8-15 c-subunits, depending on the species) and a stationary a-subunit. The a-subunit contains two half-channels that do not connect, forcing protons to bind to and rotate the c-ring to traverse the membrane.
- **F1 Unit:** A peripheral membrane complex that protrudes into the matrix and contains the catalytic sites for ATP synthesis/hydrolysis. It is composed of an α3β3 hexamer, with the three β-subunits each containing a catalytic site. A central γ-subunit extends from the c-ring up into the center of the α3β3 hexamer.

The mechanism of ATP synthesis, known as the **binding change mechanism** (elucidated by Paul Boyer), involves rotational catalysis. The flow of protons through the F0 unit causes the c-ring and the attached γ-subunit to rotate. This rotation drives sequential conformational changes in the three β-subunits. Each subunit cycles through three distinct states:

- **Open (O) state:** Very low affinity for nucleotides, releases ATP.
- **Loose (L) state:** Binds ADP and Pi.
- **Tight (T) state:** Catalyzes the formation of ATP from the bound ADP and Pi. The energy input is used primarily to release the tightly bound ATP, not to form the phosphoanhydride bond per se.
  With each 120° rotation of the γ-subunit, one ATP is synthesized and released. Approximately 4 protons are required for a full 360° rotation, which produces 3 ATP molecules.

1.6 Stoichiometry, Shuttles, and Regulation

1.6.1 Stoichiometry (P/O Ratios)

The theoretical maximum yield of ATP can be calculated from the proton tally. For NADH, 10 H+ are pumped (4 from CI, 4 from CIII, 2 from CIV). For FADH2, 6 H+ are pumped (0 from CII, 4 from CIII, 2 from CIV). If ~4 H+ are required to synthesize one ATP, the P/O ratios are ~2.5 for NADH and ~1.5 for FADH2. These values supersede the older textbook values of 3 and 2, which did not account for the proton cost of transporting metabolites (e.g., the ATP-ADP translocase uses the PMF).

1.6.2 Shuttles for Cytosolic NADH

The inner membrane is impermeable to NADH, so reducing equivalents from cytosolic glycolysis must be shuttled in. Two primary shuttles exist:

- **Glycerol-3-Phosphate Shuttle:** Predominant in muscle and brain. Cytosolic NADH reduces dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate (G3P). G3P is re-oxidized by a mitochondrial membrane-bound dehydrogenase, which passes electrons to ubiquinone via FAD. **Net result: Cytosolic NADH -> mitochondrial FADH2 (Yield: ~1.5 ATP).**
- **Malate-Aspartate Shuttle:** Predominant in heart and liver. This is a more energy-efficient shuttle that uses specific metabolite transporters (antiporters). **Net result: Cytosolic NADH -> mitochondrial NADH (Yield: ~2.5 ATP).**

1.6.3 Regulation: Acceptor Control

The rate of OXPHOS is tightly regulated by cellular energy demand, a phenomenon known as **acceptor or respiratory control**. The key regulator is the level of ADP. In a resting cell (State 4 respiration), ADP levels are low. ATP synthase is idle, the proton gradient is high, and this "back-pressure" inhibits further proton pumping by the ETC, slowing electron flow and O2 consumption. When ATP is hydrolyzed (State 3 respiration), ADP levels rise. ATP synthase is activated, dissipating the proton gradient. The loss of back-pressure allows the ETC to operate at maximum capacity. Thus, the system is perfectly responsive; **electron transport is coupled to and controlled by ATP utilization.**

1.7 Clinical and Pharmacological Correlations

1.7.1 Uncouplers

Uncouplers dissociate electron transport from ATP synthesis by dissipating the proton gradient. They are weak acids that can carry protons across the inner membrane. This causes the ETC to run freely, consuming O2 and burning fuels, but all energy is released as heat.

- **Physiological Uncoupling:** Brown adipose tissue expresses **UCP1 (thermogenin)**, which creates a regulated proton leak to generate heat in newborns and hibernating mammals.
- **Toxicological Uncoupling: 2,4-Dinitrophenol (DNP)** was once used as a weight-loss drug. It is extremely dangerous, causing hyperthermia that can be fatal.

1.7.2 Inhibitors

Specific inhibitors block distinct steps in OXPHOS (see Slide 32 table). Understanding their effects is diagnostically and toxicologically critical.

1.7.3 Reactive Oxygen Species (ROS)

The ETC is a major intracellular source of ROS, primarily at Complex I and III, where single electrons can leak and reduce O2 to superoxide (O2•-). ROS cause oxidative damage and are implicated in aging, neurodegeneration, and many other diseases.

1.7.4 Mitochondrial Diseases

Mutations in either nuclear or mitochondrial genes encoding ETC/OXPHOS components lead to a heterogeneous group of disorders. Due to the high energy demand of nerve and muscle cells, these tissues are most commonly affected. Examples include **Leber's Hereditary Optic Neuropathy (LHON)** and **MELAS syndrome**. Diagnosis often involves muscle biopsy, measurement of blood lactate, and genetic testing.