Overview of the generation and utilization of a proton-motive force

A transmembrane proton concentration gradient and a voltage gradient, collectively called the proton-motive force, are generated during photosynthesis and the aerobic oxidation of carbon compounds in mitochondria and aerobic bacteria. In chemiosmotic coupling a proton-motive force powers an energy-requiring process such as ATP synthesis (A), transport of metabolites across the membrane against their concentration gradient (B) or rotation of bacterial flagella (C).

Membrane orientation and the direction of proton movement during chemiosmotically coupled ATP synthesis in bacteria, mitochondria, and chloroplasts.

The membrane surface facing a shaded area is a cytosolic face, the surface facing an unshaded area is an exoplasmic face. Note that the cytosolic face of the bacterial plasma membrane, the matrix face of the inner mitochondrial membrane, and the stromal face of the thylakoid membrane are all equivalent. During electron transport, protons are always pumped from the cytosolic face to the exoplasmic face, creating a proton concentration gradient (exoplasmic face > cytosolic face) and an electric potential (negative cytosolic face and positive exoplasmic face) across the membrane. During the coupled synthesis of ATP, protons flow in the reverse directions (down their electrochemical gradient) through ATP synthase (F0F1 complex), which protrudes from the cytosolic face in all cases.
Evolutionary origin of mitochondria and chloroplasts according to endosymbiotic hypothesis

Membrane surfaces facing a shaded area are cytosolic faces; surfaces facing an unshaded area are exoplasmic faces. Endocytosis of a bacterium by an ancestral eukaryotic cell would generate an organelle with two membranes, the outer membrane derived from the eukaryotic plasma membrane and the inner one from the bacterial membrane. The F1 subunit of ATP synthase, localized to the cytosolic face of the bacterial membrane, would then face the matrix of the evolving mitochondrion (left) or chloroplast (right). Budding of vesicles from the inner chloroplast membrane, such as occurs during the development of chloroplasts in contemporary plants, would generate the thylakoid vesicles with the F1 subunit remaining on the cytosolic face, facing the chloroplast stroma.

The glycolysis pathway

1. Hexokinase ATP ADP
2. Hexokinase
3. Phosphofructokinase
4. Aldolase
5. Triose phosphate isomerase
6. Glyceraldehyde 3-phosphate dehydrogenase
7. Phosphoglycerate kinase
8. Phosphoglyceromutase
9. Enolase
10. Pyruvate kinase
The glycolysis pathway by which glucose is degrade to pyruvic acid:

2 reactions consume ATP, forming ADP and phosphorylated sugars (red);

2 reactions generate ATP from ADP by substrate-level phosphorylation (green);

1 reaction yields NADH by the reduction of NAD⁺ (yellow).

Note that all the intermediates between glucose and pyruvate are phosphorylated compounds. Reactions 1, 3, and 10, with single arrows, are essentially irreversible (large negative ΔG values) under conditions ordinarily obtaining in cells.

**Anaerobic versus Aerobic Metabolism of Glucose**

The ultimate fate of pyruvate formed during glycolysis depends on the presence or absence of oxygen. In the formation of pyruvate from glucose, one molecule of NAD⁺ is reduced (by addition of two electrons) to NADH for each molecule pyruvate formed (see previous slides, reaction 6). (Left) In the absence of oxygen (anaerobic metabolism) two electrons are transferred from each NADH molecule to an acceptor molecule to regenerate NAD⁺, which is required for continued glycolysis. In yeast, acetaldehyde is the acceptor and ethanol is the product. This process is called alcoholic fermentation. When oxygen is limiting in muscle cells, NADH reduces pyruvate to form lactic acid, regenerating NAD⁺. (Right) In the presence of oxygen, pyruvate is transported into mitochondria. First it is converted by pyruvate dehydrogenase into 1 molecule CO₂ and 1 of acetic acid, the latter linked to coenzyme-A (Co-A-SH) to form acetyl CoA, concomitant with the reduction of 1 molecule NAD⁺ to NADH. Further metabolism of acetyl CoA and NADH generated approximately an additional 28 molecules of ATP per glucose molecule oxidized.

Internal structure of a mitochondrion

(left) Schematic diagram showing the principle membranes and compartments. The cristae form sheets and tubes by invagination of the inner membrane and connect to the inner membrane through relatively small uniform tubular structures called cristae junctions. The intermembrane space separates the two, and is the location of each cristae. Mitochondria are sometimes referred to as the powerhouse of the cell, as it synthesizes ATP, the intramitochondria membrane contains theaturanadrenaline, which is synthesized from the inner membrane. The matrix contains the small mitochondria DNA (blue strand), ribosomes, and granules. The inner membrane contains the electron transport chain, which is responsible for the synthesis of ATP. The model is based on a three-dimensional electron tomogram calculated from a series of two-dimensional electron micrographs recorded at regular angular intervals. The technique is analogous to a three-dimensional X-ray tomograph (or CAT) scan. Note the tightly packed cristae (yellow-green), the inner membrane (light blue), and the outer membrane (dark blue).
Aerobic oxidation of pyruvate and fatty acids in mitochondria

The outer membrane is freely permeable to all metabolites, but specific transport proteins (colored ovals) in the inner membrane are required to import pyruvate (yellow), ADP (green), and Pi (purple) into the matrix. NADH generated in the cytosol is transported via a shuttle system (red) to the matrix. O2 diffuses into the matrix and CO2 diffuses out.

Stage 1: Fatty acyl groups are transferred from fatty acyl CoA and transported across the inner membrane via a special carrier (blue oval) and then reattached to CoA on the matrix side. Pyruvate is converted to acetyl CoA with the formation of NADH, and fatty acids attached to CoA are also converted to acetyl CoA with the formation of NADH and FADH2. Stage 2: electrons from these reduced coenzymes are transferred via electron transport complexes (blue boxes) to O2, and protons are transported from the matrix to the intermembrane space, generating the proton-motive force. Electrons from NADH flow directly from complex I to complex III, bypassing complex II. Stage 3: ATP synthase (orange) harnesses the proton-motive force to synthesize ATP. Blue arrows indicate electron flow, red arrows indicate transmembrane movement of protons, and green arrows indicate transport of metabolites.

The structure of acetyl CoA

This compound is an important intermediate in the aerobic oxidation of pyruvate, fatty acids, and many amino acids. It also contributes acetyl groups in many biosynthetic pathways.

The citric acid cycle (also known as the tricarboxylic acid cycle (TCA cycle), the Krebs cycle, or the Szent-Györgyi–Krebs cycle), in which acetyl groups transferred from CoA are oxidized to CO2

In reaction 1, a 2-carbon acetyl residue from acetyl CoA condenses with the 4-carbon molecule oxaloacetate to form the 6-carbon molecule citrate. In the remaining reactions (2-9), each molecule is eventually converted back to oxaloacetate, losing 2 CO2 molecules in the process. In each turn of the cycle, 4 pairs of electrons are removed from carbon atoms, forming 3 molecules of NADH and 1 molecule of FADH2. The 2 carbon atoms that enter the cycle with acetyl CoA are highlighted in blue through succinyl CoA. In succinate and fumarate, which are symmetric molecules, they can no longer be specifically denoted. Isotope labeling studies have demonstrated that these carbon atoms are not lost in the turn of the cycle in which they enter; on average, 1 will be lost as CO2 during the next turn of the cycle and the other in the subsequent turns.

Net Result of the Glycolytic Pathway and the Citric Acid Cycle

<table>
<thead>
<tr>
<th>Reaction</th>
<th>CO2 Molecules Produced</th>
<th>NAD+ Molecules Reduced to NADH</th>
<th>FAD Molecules Reduced to FADH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 glucose molecule to 2 pyruvate molecules</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2 pyruvates to 2 acetyl CoA molecules</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2 acetyl CoA to 4 CO2 molecules</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>
Changes in redox potential and free energy during stepwise flow of electrons through the respiratory chain

Blue arrows indicate electron flow; red arrows, translocation of protons across the inner mitochondrial membrane. Four large multiprotein complexes located in the inner membrane contain several electron-carrying prosthetic groups. Coenzyme Q (CoQ) and cytochrome c transport electrons between the complexes. Electrons pass through the multiprotein complexes from those at lower reduction potential to those with higher (more positive) potential (left scale), with a corresponding reduction in free energy (right scale). The energy released as electrons flow through three of the complexes is sufficient to power the pumping of $\text{H}^+$ ions across the membrane, establishing the proton-motive force.

**The Malate Shuttle**

The net effect of the reactions constituting the malate-aspartate shuttle is oxidation of cytosolic NADH to NAD$^+$ and reduction of matrix NAD$^+$ to NADH:

$$\text{NADH}_{\text{cytosol}} + \text{NAD}^+_{\text{matrix}} \rightarrow \text{NAD}^+_{\text{cytosol}} + \text{NADH}_{\text{matrix}}$$

Fatty acids are stored as triacylglycerols in adipose cells. They are metabolized to free fatty acids and glycerol and released into the blood, then taken up and oxidized by other cells (e.g., heart muscle cells). Inhuman, the oxidation of fats is quantitatively more important than oxidation of glucose as a source of ATP. Fat may generate 6 times more ATP per gram fat compared to hydrated glycogen (stored in muscle and liver). In cytosol, fatty acids are converted to fatty acyl CoA under consumption of ATP. Fatty Acyl CoA enters the mitochondria. In both mitochondrial oxidative and peroxisomal oxidation (ii), fatty acids are converted to acetyl CoA by the following reactions (shown down the center of the figure) via a fatty acyl CoA molecule to acetyl CoA and a fatty acyl CoA shortened by two carbon atoms. Concomitantly (i.e., in reactions moving to the left of center for mitochondria and to the right of center for peroxisomes), one FAD molecule is reduced to FADH$_2$, and one NAD$^+$ molecule is reduced to NADH. The cycle is repeated on the shortened acyl CoA until fatty acids with an even number of carbon atoms are completely converted to acetyl CoA. In mitochondria, electrons from FADH$_2$ and NADH enter the respiratory chain and ultimately are used to generate ATP; the acetyl CoA generated is oxidized in the citric acid cycle, resulting in synthesis of additional ATP. Because peroxisomes lack the electron-transport complexes composing the respiratory chain and the enzymes of the citric acid cycle, oxidation of fatty acids in these organelles yields no ATP.

**Allosteric control of glucose metabolism in the cytosol at the level of fructose 6-phosphate**

The key regulatory enzyme in glycolysis, phosphofructokinase-1, is allosterically activated by AMP and fructose 2,6-biphosphate, which are elevated when the cell’s energy stores are low. The enzyme is inhibited by ATP and citrate, which are elevated when the cells are actively oxidizing glucose to CO$_2$. Phosphofructokinase-2 (PFK2) is a bifunctional enzyme: its kinase activity forms fructose 2,6-biphosphate from fructose 6-phosphate, and its phosphatase activity catalyzes the reverse reaction. Insulin, which is released by the pancreas when blood glucose levels are high, promotes PFK2 kinase activity and thus stimulates glycolysis to fructose 2,6-biphosphate. At low blood glucose, glucose-6-phosphate is released by the pancreas and promotes PFK2 phosphatase activity in the liver, indirectly modulating glycolysis.
Oxidation of Glucose and Fatty Acids to CO₂

- In the cytosol of eukaryotic cells, glucose is converted to pyruvate via the glycolytic pathway, with the net formation of 2 ATPs and the net reduction of 2 NAD⁺ molecules to NADH. ATP is formed by 2 substrate-level phosphorylation reactions in the conversion of glyceraldehyde-3-phosphate to pyruvate.
- In anaerobic conditions, cells can metabolize pyruvate to lactate or to ethanol plus CO₂ (in the case of yeast), with the reoxidation of NADH. In aerobic conditions, pyruvate is transported into the mitochondrion, where pyruvate dehydrogenase converts it into acetyl CoA and CO₂.
- Mitochondria have a permeable outer membrane and an inner membrane, which is the site of electron transport and ATP synthesis.
- In each turn of the citric acid cycle, acetyl CoA condenses with the 4-carbon molecule oxaloacetate to form the 6-carbon citrate, which is converted back to oxaloacetate by a series of reactions that release 2 molecules of CO₂ and generate 3 NADH molecules, 1 FADH₂ molecule and 1 GTP.
- Although cytosolic NAD⁺ generated during glycolysis cannot enter mitochondria directly, the malate-aspartate shuttle indirectly transfers electrons from the cytosol to the mitochondrial matrix, thereby regenerating cytosolic NAD⁺ for continued glycolysis.
- The flow of electrons from NADH and FADH₂ to O₂, via a series of electron carriers in the inner mitochondrial membrane, is coupled to pumping the H⁺ across the inner membrane. The resulting proton-motive force powers ATP synthesis and generates most of the ATP resulting from aerobic oxidation of glucose.
- Oxidation of fatty acids in mitochondria yields acetyl CoA, which enters the citric acid cycle, and the reduced coenzymes NADH and FADH₂. Subsequent oxidations of these metabolites are coupled to formation of ATP.
- In most eukaryotic cells, oxidation of fatty acids, especially very long chain fatty acids, occurs primarily in peroxisomes and is not linked to ATP production; the related energy is converted to heat.
- The rate of glucose oxidation via glycolysis and the citric acid cycle is controlled by the inhibition of stimulation of several enzymes, depending on the cell's need for ATP. This complex regulation coordinates the activities of the glycolytic pathway and the citric acid cycle and results in the storage of glucose (as glycogen) or fat when ATP is abundant.

--- Taking home message

**Oxidation of Glucose and Fatty Acids to CO₂**

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**Electron Transport and generation of the Proton-Motive Force PMF**

**During respiration: (oxidative phosphorylation)**

\[
\text{NADH} + \text{H}^+ + 1/2 \text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O} \quad \Delta G^{\circ} = -52.6 \text{ kcal/mol}
\]

\[
\text{FADH}_2 + 1/2 \text{O}_2 \rightarrow \text{FAD} + \text{H}_2\text{O} \quad \Delta G^{\circ} = -43.4 \text{ kcal/mol}
\]

Both reactions are strongly exergonic. Conversion of 1 glucose molecule to CO₂ via glycolytic pathway and citric acid cycle yields 10 NADH and 2 FADH₂. Oxidation of these reduced coenzymes has thus a total \(\Delta G^{\circ}\) of -613 kcal/mol. Thus, of the potential free energy present in the chemical bonds of glucose (-680 kcal/mol), about 90% is conserved in the reduced coenzymes.

The free energy released during the oxidation of a single NADH or FADH₂ molecule to O₂ is sufficient to drive the synthesis of several molecules of ATP from ADP and Pᵢ, a reaction with a \(\Delta G^{\circ} = +7.3\) kcal/mol.
Electron Transport and generation of the Proton-Motive Force PMF

During respiration:
Free energy released during the oxidation of NADH or FADH$_2$ is stored both as an electric potential and a proton concentration gradient - collectively, the proton-motive force - across the inner membrane; driven by this force, is coupled to the synthesis of ATP from ADP and P$\text{i}$, by the ATP synthase.
Relative contribution of the 2 components to the total PMF depends on the permeability of the membrane to other ions than H$^+$. $\text{pmf} = \Psi - (RT/F x \Delta pH) = \Psi -59 \Delta pH$

R = gas constant of 1.987 cal/(degree mol); T degree in Kelvin, F = Faraday constant of 23,062 cal/(V mol); and $\Psi$ is the transmembrane electric potential, pmf and $\Psi$ are measured in millivolt. In respiring mitochondria $\Psi$ across the inner membrane is about -160 mV, $\Delta pH$ about 1.0 (~ 60 mV) --> pmf = -220 mV with the transmembrane electric potential responsible for about 73%.

Using $K^+$, the electric potential $E$ across the inner membrane of respiring mitochondria can be determined to
$E = -59 \log [K^+\text{in}] / [K^+\text{out}] = -59 \log 500 = -160$ mV

Heme and iron-sulfur prosthetic groups in the respiratory (electron-transport) chain

Fe$^{3+} + e^- \leftrightarrow Fe^{2+}$

(a) Heme portion of cytochromes b$_1$ and b$_{551}$, which are components of the CoQH$_2$-cytochrome reductase complex. The same porphyrin ring (yellow) is present in all hemes. The chemical substituents attached to the porphyrin ring differ in the other cytochromes in the respiratory chain. All hemes accept and release one electron at a time. (b) Dimeric iron-sulfur cluster (2Fe-2S).
Each Fe atom is bonded to four S atoms: two are inorganic sulfur and two are in cysteine side chains of the associated protein. (note that only the two inorganic S atoms are counted in the chemical formula). All Fe-S clusters accept and release one electron at time.

Oxidized and reduced forms of coenzyme Q (CoQ; also called ubiquinone), which carries two protons and two electrons

*Not included is coenzyme Q, an electron carrier that is not permanently bound to a protein complex.

Overview of multiprotein complexes, bound prosthetic groups, and associated mobile carriers in the respiratory chain

**NADH-CoQ Reductase (Complex I)**

In the NADH-CoQ reductase complex, electrons first flow from NADH to FMN (flavin mononucleotide), a cofactor related to FAD, then to an iron-sulfur cluster, and finally to CoQ. FMN, like FAD, can accept 2 electrons, but does so one electron at a time.

The overall reaction catalyzed by this complex is:

\[
\text{NADH}^{\text{reduced}} + \text{CoQ}^{\text{oxidized}} + 2 \text{H}^+ \rightarrow \text{NAD}^{\text{oxidized}} + \text{H}_2\text{O} + \text{CoQH}_2^{\text{reduced}}
\]

Each transported electron undergoes a drop in potential of \(-360\) mV, equivalent to a \(\Delta G^\circ\) of \(-16.6\) kcal/mol for the two electrons transported. Much of this released energy is used to transport four protons across the inner membrane per molecule of NADH oxidized by the NADH-CoQ reductase complex.

**CoQH$_2$-Cytochrome c Reductase (Complex III)**

A CoQH$_2$ generated either by complex I or –II donates two electrons to the CoQH$_2$-cytochrome c reductase complex, regenerating oxidized CoQ. Concomitantly it releases two protons picked up on the cytosolic face into the intermembrane space, generating part of the pmf. Within complex III, the released electrons first are transferred to an iron-sulfur cluster within complex III and then to two b-type cytochromes (b$_L$ and b$_H$) or cytochrome c$_1$. Finally, the two electrons are transferred to two molecules of the oxidized form of cytochrome c, a water soluble peripheral protein that diffuses in the intermembrane space. For each pair of electrons transferred, the overall reaction catalyzed by this CoQH$_2$-cytochrome c reductase complex is:

\[
\text{CoQH}_2^{\text{reduced}} + 2 \text{Cyt c}^{3+} \rightarrow \text{CoQ}^{\text{oxidized}} + 2 \text{H}^+ + 2 \text{Cyt c}^{2+}\text{oxidized}
\]

The \(\Delta G^\circ\) for this reaction is sufficiently negative, that two additional protons are translocated from the mitochondrial matrix across the inner membrane for each pair of electrons transferred; this involves the proton-motive Q cycle.

**Succinate-CoQ Reductase (Complex II)**

Succinate dehydrogenase, the enzyme that oxidize a molecule of succinate to fumarate in the citric acid cycle, is an integral component of the succinate-CoQ reductase complex. The two electrons released in conversion of succinate to fumarate are transferred first to FAD, then to an iron-sulfur cluster, and finally to CoQ.

The overall reaction catalyzed by this complex is:

\[
\text{Succinate}^{\text{reduced}} + \text{CoQ}^{\text{oxidized}} \rightarrow \text{fumarate}^{\text{oxidized}} + \text{CoQH}_2^{\text{reduced}}
\]

Although the \(\Delta G^\circ\) for this reaction is negative, the released energy is insufficient for proton pumping. Thus, no protons are translocated across the membrane by succinate-CoQ reductase complex, and no pmf is generated in this part of the respiratory chain.
Cytochrome c Oxidase (Complex IV)

Cytochrome c, after being reduced by the CoQH$_2$-cytochrome c reductase complex, transports electrons, one at a time, to the cytochrome c oxidase complex. Within this complex, electrons are transferred, again one at a time, first to a pair of copper ions called Cu$_{a}^{2+}$, then to cytochrome $a$, next to a complex of another copper ion (Cu$_{b}^{2+}$) and cytochrome $a_3$, and finally to O$_2$, the ultimate electron acceptor, yielding H$_2$O.

For each pair of electrons transferred, the overall reaction catalyzed by this cytochrome c oxidase complex is:

$$2 \text{Cyt c}^{2+}_{(\text{reduced})} + 2 \text{H}^+ + 1/2 \text{O}_2 \rightarrow 2 \text{Cyt c}^{3+}_{(\text{oxidized})} + \text{H}_2\text{O}$$

During transport of each pair of electrons through the cytochrome c oxidase complex, two protons are translocated across the membrane.

Molecular structure of the core of the cytochrome c oxidase complex in the inner mitochondrial membrane

Mitochondrial cytochrome c oxidases contain 13 different subunits, but the catalytic core of the enzyme consists of only three subunits (I green, II blue, and III yellow). The function of the remaining subunits (white) is rather unknown. Bacterial cytochrome c oxidase contain only the three catalytic subunits. Heme $a$ and $a_3$ are shown as purple and orange space-filling models, respectively; the three copper atoms are dark blue spheres.

CoQ and Cytochrome c as Mobile Electron Shuttles

The four electron-transport complexes described in the previous slides are laterally mobile in the inner mitochondrial membrane; moreover, they are present in unequal amounts and do not form stable contacts with one another. These properties preclude the direct transfer of electrons from one complex to the next. Instead, electrons are transported from one complex to another by diffusion of CoQ in the membrane and by cytochrome c in the intermembrane space.

Reduction Potentials of Electron Carriers Favor Electron Flow from NADH to O$_2$

The reduction potential $E$ for a partial reduction reaction:

$$\text{oxidized molecule} + e^{-} \leftarrow \rightarrow \text{reduced molecule}$$

is a measure of the equilibrium constant of that partial reaction. With the exception of the $b$ cytochromes in the CoQH$_2$-cytochrome c reductase complex, the standard reduction potential $E^{\circ}$ of the carriers in the mitochondrial respiratory chain increases steadily from NADH to O$_2$. For instance, for the partial reaction

$$\text{NAD}^{+} + \text{H}^+ + 2 \text{e}^{-} \leftarrow \rightarrow \text{NADH}$$

The value of the standard reduction potential is -320 mV, which is equivalent to a $\Delta G^{\circ}$ of +14.8 kcal/mol for transfer of two electrons. Thus this partial reaction tends to proceed toward the left, that is, toward the oxidation of NADH to NAD$^+$. 
Reduction Potentials of Electron Carriers Favor Electron Flow from NADH to O$_2$

By contrast, the standard reduction potential for the partial reaction:

\[
\text{cytochrome } c_{\text{ox}}(\text{Fe}^{3+}) + e^- \rightarrow \text{cytochrome } c_{\text{red}}(\text{Fe}^{2+})
\]

is +220 mV, which is equivalent to a $\Delta G^\circ'$ of $-5.1$ kcal/mol for transfer of one electron. Thus this partial reaction tends to proceed toward the right, that is, toward the reduction of cytochrome $c_{\text{ox}}(\text{Fe}^{3+})$ to cytochrome $c_{\text{red}}(\text{Fe}^{2+})$.

Electron transfer from reduced cytochrome c ($\text{Cyt } c^{2+}$) to O$_2$ via the cytochrome c oxidase complex is coupled to proton transport

Monitoring of the medium pH reveals a sharp drop in pH following addition of O$_2$. As the reduced cytochrome c becomes fully oxidized, H$^+$ leak back into the vesicles, and the pH of the medium returns to its initial value. Measurements show that 2 H$^+$ are transported per O atom, but cytochrome c transfers only 1 e$^-$; thus two molecules of Cyt $c^{2+}$ are oxidized for each O reduced.
CoQ and three Electron-Transfer Complexes Pump Protons out of the Mitochondrial Matrix

Studies as shown in the previous slides show that the NADH-CoQ reductase complex translocates four H⁺ per pair of e⁻ transported, whereas the cytochrome c oxidase complex translocates 2 H⁺ per e⁻ pair transported (or, equivalently, for every molecules of cytochrome c oxidized). Current evidence suggests that a total of 10 H⁺ are transported from the matrix space across the inner mitochondrial membrane for every e⁻ pair that is transferred from NADH to O₂. Since the succinate-CoQ reductase complex does not transport H⁺, only 6 H⁺ are transported across the membrane for every e⁻ pair that is transferred from succinate (or FADH₂) to O₂. Relatively little is known about the coupling of e⁻ flow and H⁺ translocation by the NADH-CoQ reductase complex. More is known about the operation of the cytochrome c oxidase complex (which we discuss here). The coupled e⁻ and H⁺ movements mediated by the CoQ₇₂-cytochrome c reductase complex, which involves a unique mechanism, are described separately.

CoQ and three Electron-Transfer Complexes Pump Protons out of the Mitochondrial Matrix

After cytochrome c is reduced by the QH₂-cytochrome c reductase complex, it is reoxidized by the cytochrome c oxidase complex, which transfers e⁻ to oxygen. Cytochrome c oxidase contains three copper ions and two heme groups. The flow of e⁻ through these carriers is depicted in the next slide. 4 molecules of reduced cytochrome c, first to Cu₅⁺₂ bound to subunit II, then to the heme a bound to subunit I, and finally to Cu₅⁺₂ and heme a₃ that make up the oxygen reduction center. The cyclic oxidation and reduction of the iron and copper in the oxygen reduction center of cytochrome c oxidase, together with the uptake of 4 H⁺ from the matrix space, are coupled to the transfer of the 4 e⁻ to oxygen and the formation of water. Proposed intermediates in oxygen reduction include the peroxide anion O₂⁻ and probably the hydroxyl radical OH⁻ as well as unusual complexes of iron and oxygen atoms. These intermediates would be harmful to the cell if they escaped from the reaction center, but they do so only rarely.

Schematic depiction of the cytochrome c oxidase complex showing the pathway of e⁻ flow from reduced cytochrome c to O₂.

Heme groups are denoted by red diamonds. Blue arrows indicate e⁻ flow. 4 e⁻, sequentially released from 4 molecules of reduced cytochrome c together with 4 H⁺ from the matrix, combine with one O₂ molecule to form 2 H₂O molecules. Additionally, for each e⁻ transferred from cytochrome c to oxygen, one H⁺ is transported from the matrix to the intermembrane space, or a total of 4 for each O₂ molecule reduced to 2 H₂O molecules.

For every four e⁻ transferred from reduced cytochrome c through cytochrome c oxidase (i.e., for every molecule of O₂ reduced to 2 H₂O molecules), 4 H⁺ are translocated from the matrix space to the intermembrane space (2 H⁺ per e⁻ pair). However, the mechanism by which these H⁺ are translocated is not known.
The Q cycle

CoQH₂ binds to the Qₛ site on the intermembrane space (outer) side of CoQ-cytochrome c reductase complex and CoQ binds to the Qi site on the matrix (inner) side. One e⁻ from the CoQH₂ binds to Qₛ, travels directly to cytochrome c via an Fe-S cluster and cytochrome c₁. The other e⁻ moves through the b cytochromes to CoQ at the Qₛ site, forming the partially reduced semiquinone (Q⁻). Simultaneously, CoQH₂ releases its 2 H⁺ into the intermembrane space. The CoQ now at the Qₛ site dissociates and a second CoQH₂ binds there. As before, 1 e⁻ moves directly to cytochrome c₁ and the other to the Qᵢ at the Qi site, forming, together with 2 H⁺ picked up from the matrix space, CoQH₂, which then dissociates. The net result is that 4 H⁺ are translocated from the matrix to the intermembrane space for each pair of e⁻ transported through the CoQH₂-cytochrome c reductase complex.

Alternative 3-D conformations of the Fe-S subunit of the CoQ-cytochrome c reductase complex

In the dimeric complex, cytochrome b₃ and b₁, are associated with one subunit and the 2Fe·2S cluster is shown in its two alternative conformational states, which differ primarily in the position of the protein toward the intermembrane space. In one conformation (yellow), the 2Fe·2S cluster (green) is positioned near the Qₛ site on the intermembrane side of the protein, able to pick up an e⁻ from CoQH₂. In the alternative (blue), the 2Fe·2S cluster is located adjacent to the c₁ heme on the cytochrome c and able to transfer an e⁻ to it.

Electron Transport and Generation of the Proton-Motive Force PMF:
- The pmf is a combination of a [H⁺] (pH)-gradient (exoplasmic face > cytosolic face) and an electric potential (negative cytosolic face) across the membrane.
- In the mitochondrion, the pmf generated by coupling the e⁻ flow from NADH and FADH₂ to O₂ to the uphill transport of H⁺ from the matrix across the inner membrane to the intermembrane space.
- The major components of the mitochondrial respiratory chain are four inner membrane multiprotein complexes: NADH-CoQ reductase (I), succinate-CoQ reductase (II), CoQH₂-cytochrome c reductase (III), and cytochrome c oxidase (IV).
- The last complex transfers the e⁻ to O₂ to form H₂O.
- Each complex contains one or more e⁻-carrying prosthetic groups: iron-sulfur clusters, flavins, heme groups, and copper ions. Cytochrome c, which contain heme, and coenzyme Q (CoQ) are mobile e⁻ carriers.
- Each e⁻ carrier accepts an e⁻ or e⁻ pair from a carrier with a less positive reduction potential and transfers the e⁻ to a carrier with a more positive reduction potential. Thus the reduction potentials of e⁻ carriers favor unidirectional e⁻ flow from NADH and FADH₂ to O₂.
- A total of 10 H⁺ ions are translocated from the matrix across the inner membrane per e⁻ pair flowing from NADH to O₂.
- The Q cycle allows four H⁺ (rather than two) to be translocated per pair of e⁻ moving through the CoQH₂-cytochrome c reductase complex.
Synthesis of ATP by $F_0F_1$ depends on a pH gradient across the membrane.

Isolated chloroplast thylakoid vesicles containing $F_0F_1$ particles were equilibrated in the dark with a buffered solution at pH 4.0. When the pH in the thylakoid lumen became 4.0, the vesicles were readily mixed with a solution at pH 8.0 containing ADP and $P_i$. A burst of ATP synthesis accompanied the transmembrane movement of $H^+$ driven by the 10,000-fold [$H^+$] gradient ($10^{-4}$ versus $10^{-8}$ M). In similar experiments using “inside-out” preparations of submitochondrial vesicle, an artificial generated membrane electric potential also resulted in ATP synthesis.

Chemiosmosis: The Energy-Coupling Mechanism

- ATP synthase – is the enzyme that actually makes ATP

A rotor (c) within the intermembrane space spins clockwise when $H^+$ flows past it down the $H^+$ gradient.

A stator anchored in the mitochondrial inner membrane holds the rotor stationary.

A rod (for "stalk") extending from the stator across the intermembrane space to the inner membrane.

Three catalytic sites in the rotary head join intergenic phosphorylation to make ATP.
Rotation of the $F_1 \gamma$ subunit, driven by the $H^+$ movement through $F_0$, powers ATP synthesis

The $F_0$ portion is built of three integral membrane proteins: one copy $a$, two copies of $b$, and on average 10 copies of $c$ arranged in a ring in the plane of the membrane. Two $H^+$ half-channels lie at the interface between the $a$ subunit and the $c$ ring. Half-channel I allows $H^+$ to move one at a time from the exoplasmic medium and bind to $F_0$-61 in the center of a $c$ subunit near the middle of the membrane. Half-channel II (after rotation of the $c$ ring) permits $H^+$ to dissociate from the aspartate and move into the cytosolic medium. The $F_1$ portion contains three copies each of subunits $\alpha$ and $\beta$ that form a hexamer resting atop the single rod-shaped $\gamma$ subunit, which is inserted into the $c$ ring of $F_0$.

The $c$ subunit is rigidly attached to the $\gamma$ subunit and also to several of the $c$ subunits. The $\delta$ subunit permanently links one of the $\alpha$ subunits in the $F_1$ complex to the $b$ subunit of $F_0$. Thus the $F_0$ $a$ and $b$ subunits and the $F_1 \delta$ subunit and the $(\alpha\beta)_3$ hexamer form a rigid structure anchored in the membrane (orange). During $H^+$ flow, the $c$ ring and the attached $F_1 \gamma$ and $\gamma$ subunits rotate as a unit (green), causing conformational changes in the $F_1 \beta$ subunits leading to ATP synthesis.

The binding-change mechanism of ATP synthesis from ADP and P_i by the $F_0F_1$ complex

Mitochondrial $F_1$ particles are required for ATP synthesis, but not for electron transport

*inside-out* membrane vesicles that lack $F_1$ and retain the $e^+$ transport complexes are prepared as indicated. Although these can transfer $e^+$ from NADH to $O_2$, they cannot synthesize ATP. The subsequent addition of $F_1$ particles reconstitutes the native membrane structure, restoring the capacity for ATP synthesis. When detached from the membrane, $F_1$ particles exhibit ATPase activity.

Rotation of the $\gamma$ subunit of the $F_1$ complex relative to the $(\alpha\beta)_3$ hexamer can be observed microscopically.

$F_1$ complexes were engineered that contained $\beta$ subunits with an additional His6 sequence, which causes them to adhere to a glass plate coated with a metal reagent that binds histidine. The $\gamma$ subunit in the engineered $F_1$ complexes was linked covalently to a fluorescently labeled actin filament. When viewed in a fluorescence microscope, the actin filaments were seen to rotate counterclockwise in discrete 120° steps in the presence of ATP, powered by ATP hydrolysis by the $\beta$ subunits.
The phosphate and ATP/ADP transport system in the inner mitochondrial membrane

ATP is utilized to power many energy-requiring reactions. The coordinated action of two antiporters (purple and green) results in the uptake of one ADP\(^3\) and one HPO\(_4^{2-}\) in exchange for one H\(^+\) during e\(-\) transport. The outer membrane is not shown here because it is permeable to molecules smaller than 5kDa.

ATP-ADP Exchange Across the Inner Mitochondrial Membrane Is Powered by the Proton-Motive Force PMF

In addition to powering ATP synthesis, the pmf across the inner mitochondrial membrane also powers the exchange of ATP formed by oxidative phosphorylation inside the mitochondrion for ADP and P\(_i\) in the cytosol. This exchange, which is required for oxidative phosphorylation to continue, is mediated by two proteins in the inner membrane: a phosphate transporter (HPO\(_4^{2-}\)/OH\(^-\) antiporter) and an ATP/ADP antiporter (shown in previous figure-slide). Each OH\(^-\) transported outward combines with H\(^+\) to form H\(_2\)O. This drives the overall reaction in the direction of ATP export and ADP and P\(_i\) import. Because some of the H\(^+\) translocated out of the mitochondrion during e\(-\) transport provide the power (by combining with the exported OH\(^-\)) for the ATP-ADP exchange, fewer H\(^+\) are available for the ATP synthesis. It is estimated that for every four H\(^+\) translocated out, three are used to synthesize one ATP molecule and one is used to power the export of ATP from the [H\(^+\)] gradient mitochondrion in exchange for ADP and P\(_i\). This expenditure of energy from the mitochondrial in exchange of ADP and P\(_i\) ensures a high ratio of ATP to ADP in the cytosol, where hydrolysis of the high-energy phosphoanhydride bond of ATP is utilized to power many energy-requiring reactions.

Number of Translocated H\(^+\) required for ATP Synthesis

A simple calculation indicates that the passage of more than 1 H\(^+\) is required to synthesize 1 molecule of ATP from ADP and P\(_i\). Although the \(\Delta G\) for this reaction under standard condition is +7.3 kcal/mol, at the concentrations of reactants in the mitochondrion, \(\Delta G\) is probably higher (+10 to + 12 kcal/mol). We can calculate the amount of free energy released by the passage of 1 mole of H\(^+\) down an electrochemical gradient of 220 mV (0.22 V) from the Nernst equation, setting \(n = 1\) and \(\Delta E = 0.22\) V in volts:

\[
\Delta G \text{ (cal/mol)} = -nF\Delta E = -(23,062 \text{ cal V}^{-1} \text{ mol}^{-1}) \times 0.22 \\
= 5074 \text{ cal/mol} = 5.1 \text{ kcal/mol}
\]

Since the downhill movement of 1 mol of H\(^+\) releases just over 5 kcal of free energy, the passage of at least 2 H\(^+\) is required for the synthesis of each molecule of ATP from ADP and P\(_i\).

Rate of Mitochondrial Oxidation Normally Depends on ADP Levels

If intact isolated mitochondria are provided with NADH (or FADH\(_2\)), O\(_2\), and P\(_i\), but not with ADP, the oxidation of NADH and the reduction to O\(_2\) rapidly cease, as the amount of endogenous ADP is depleted by ATP formation. If ADP is then added, the oxidation of NADH is rapidly restored. Thus mitochondria can oxidize FADH\(_2\) and NADH only as long as there is a source of ADP and P\(_i\) to generate ATP. This phenomenon, termed respiratory control, occurs because oxidation of NADH, succinate, or FADH\(_2\) is obligatorily coupled to proton transport across the inner mitochondrial membrane. If the resulting proton-motive force pmf is not dissipated in the synthesis of ATP from ADP and P\(_i\) (or for some other purpose), both the transmembrane [H\(^+\)] gradient and the membrane electric potential will increase to very high levels. At this point, pumping of additional H\(^+\) across the inner membrane requires so much energy that it eventually ceases, thus blocking the coupled oxidation of NADH and other substrates.

Certain poisons, called uncouplers, render the inner mitochondrial membrane permeable to H\(^+\). One example is the lipid-soluble chemical 2,4-dinitrophenol (DNP), which can reversibly bind and release H\(^+\) and shuttle H\(^+\) across the inner membrane from the intermembrane space into the matrix. As a result, DNP dissipates the pmf by short-circuiting both the transmembrane [H\(^+\)] gradient and the membrane electric potential. Uncouplers such as DNP abolish ATP synthesis and overcome respiratory control, allowing NADH oxidation to occur regardless of the ADP level. The energy released by the oxidation of NADH in the presence of DNP is converted to heat.
Harnessing the PMF for Energy-Required Processes:
- The multiprotein F$_0$F$_1$ complex catalyzes ATP synthesis as H$^+$ flow back through the inner mitochondrial membrane (plasma membrane in bacteria) down their electrochemical proton gradient.
- F$_0$ contains a ring of 10-14 c subunits that is rigidly linked to the rod-shaped γ subunit and the ε subunit of F$_1$. Resting atop the γ subunit is the hexameric knob of F$_1$ (εβ)$_6$, which protrudes into the mitochondrial matrix (cytosol in bacteria). The three β subunits are the sites of ATP synthesis.
- Movement of H$^+$ across the membrane via two half-channels at the interface of the F$_0$ a subunit and the c ring powers rotation of the ε ring with its attached F$_1$ c and γ subunits.
- Rotation of the F$_1$ γ subunit leads to changes in the conformation of the nucleotide-binding sites in the F$_1$ β subunits. By means of this binding-change mechanism, the β subunits bind ADP and P$_i$, condense them to form ATP, and then release ATP.
- The PMF also powers the uptake of P$_i$ and ADP from the cytosol in exchange for mitochondrial ATP and OH$^-$, thus reducing some of the energy available for ATP synthesis.
- Continued mitochondrial oxidation of NADH and the reduction of O$_2$ are dependent on sufficient ADP being present. This phenomenon, termed respiratory control, is an important mechanism for coordinating oxidation and ATP synthesis in mitochondria.
- In brown fat, the inner mitochondrial membrane contains thermogenin, a H$^+$ transporter that converts the pmf into heat. DNP has the same effect, uncoupling the oxidative phosphorylation from ATP synthesis.

--- Taking home message

At certain steps along the electron transport chain
- Electron transfer causes protein complexes to pump H$^+$ from the mitochondrial matrix to the intermembrane space.

The resulting H$^+$ gradient
- stores energy
- drives chemiosmosis in ATP synthase
- is referred to as a proton-motive force (pmf)

Chemiosmosis
- Is an energy-coupling mechanism that uses energy in the form of a H$^+$ gradient across a membrane to drive cellular work.

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Chemiosmosis and the electron transport chain

Aerobic oxidation of pyruvate and fatty acids in mitochondria

An Accounting of ATP Production by Cellular Respiration

During respiration, most energy flows in this sequence glucose to NADH to electron transport chain to proton-motive force to ATP.
The phosphate and ATP/ADP transport system in the inner mitochondrial membrane

There are three main processes in this metabolic enterprise

- About 40% of the energy in a glucose molecule is transferred to ATP during cellular respiration, making approximately 38 ATP.

Types of Fermentation

- Fermentation consists of glycolysis plus reactions that regenerate NAD+, which can be reused by glycolysis.

  In alcohol fermentation:
  - pyruvate is converted to ethanol in two steps, one of which releases CO₂.

  During lactic acid fermentation:
  - pyruvate is reduced directly to NADH to form lactate as a waste product.

Fermentation enables some cells to produce ATP without the use of oxygen.

Cellular respiration:
- relies on oxygen to produce ATP.
- In the absence of oxygen, cells can still produce ATP through fermentation.

Glycolysis:
- can produce ATP with or without oxygen, in aerobic or anaerobic conditions.
- couples with fermentation to produce ATP.
Fermentation and Cellular Respiration Compared

- Both fermentation and cellular respiration use glycolysis to oxidize glucose and other organic fuels to pyruvate.
- Fermentation and cellular respiration differ in their final electron acceptor.
- Cellular respiration produces more ATP.
- Pyruvate is a key juncture in catabolism.

Glycolysis and the citric acid cycle connect to many other metabolic pathways

The Versatility of Catabolism

- Catabolic pathways
  - Funnel electrons from many kinds of organic molecules into cellular respiration.
  - The catabolism of various molecules from food.

Biosynthesis (Anabolic Pathways)

- The body uses small molecules to build other substances.
- These small molecules may come directly from food or through glycolysis or the citric acid cycle.

Regulation of Cellular Respiration via Feedback Mechanisms

- Cellular respiration is controlled by allosteric enzymes at key points in glycolysis and the citric acid cycle.