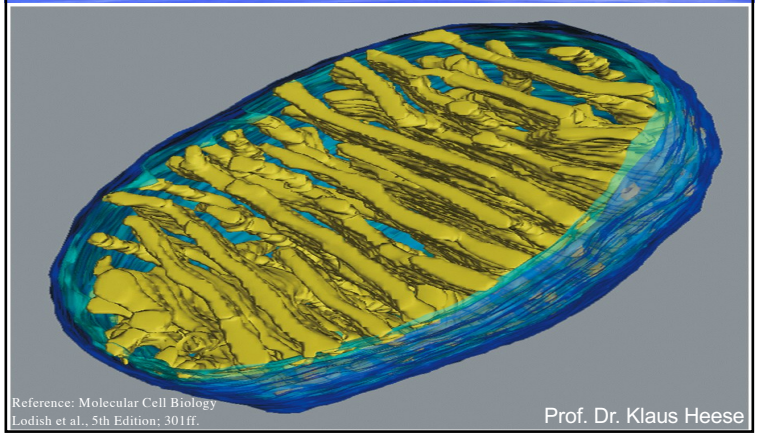
Molecular & Cell Biology

Bio-Energetics & ATP

Cellular Metabolism & Energetics

Prof. Dr. Klaus Heese

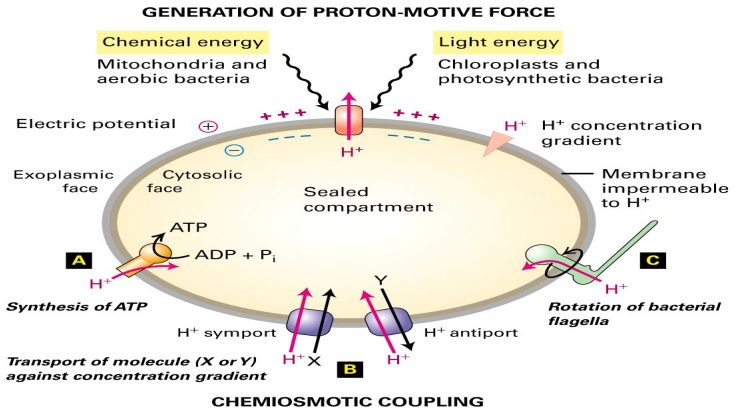
Biochemistry
Cellular Energetics



Reference: Molecular Cell Biology
Lodish et al., 5th Edition; 301ff.

Prof. Dr. Klaus Heese

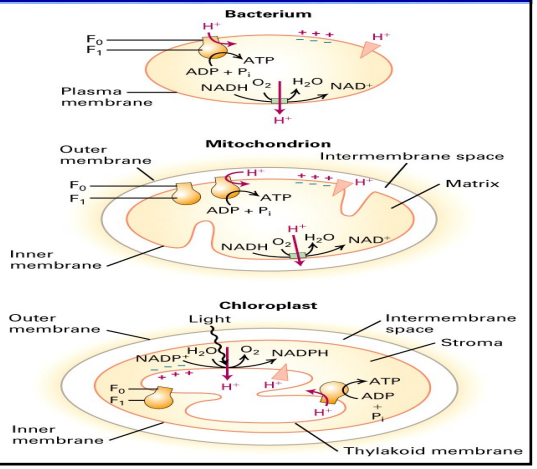
Overview of the generation and utilization of a proton-motive force



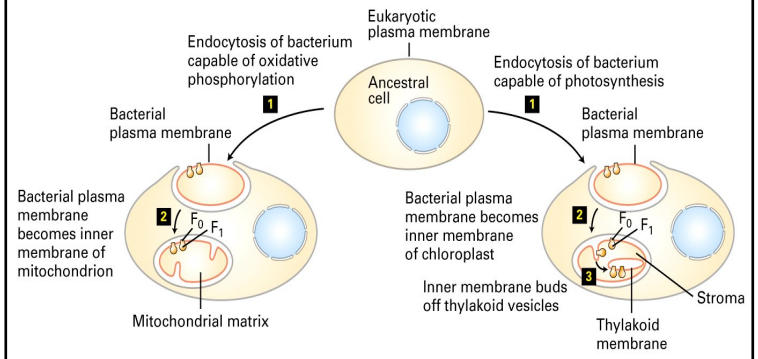
A transmembrane proton concentration gradient and a voltage gradient, collectively called the proton-motive force, are generated during photosynthesis and the aerobic oxidation of carbon compounds in mitochondria and aerobic bacteria. In chemiosmotic coupling a proton-motive force powers an energy-reqiring 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 thykoloid 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 synthesis (F0F1 complex), which protrudes from the cytosolic face in all cases.

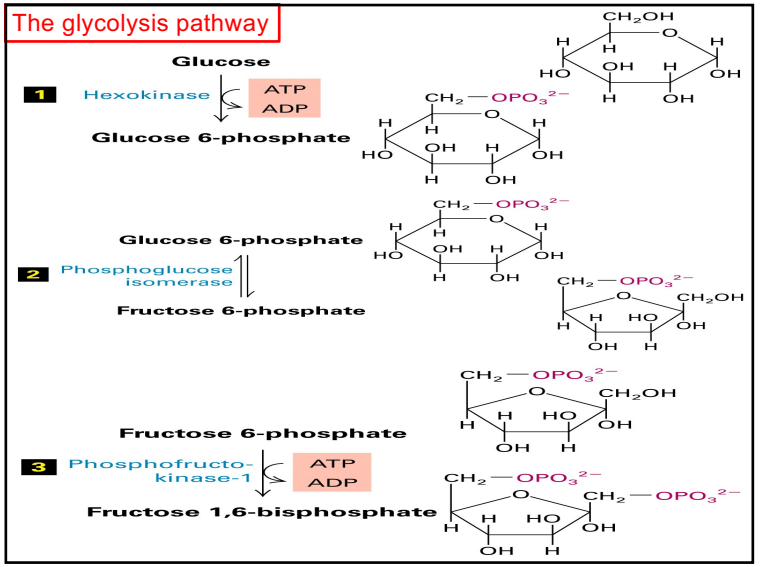


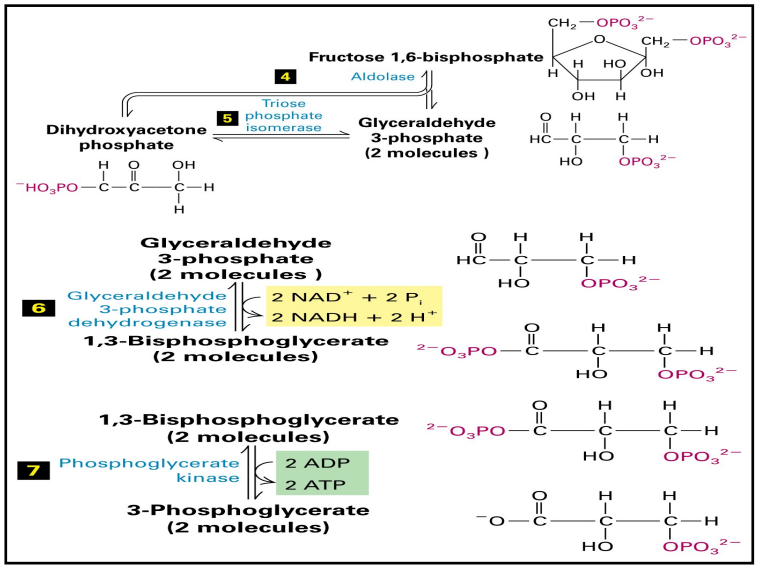
Evolutionary origin of mitochondria and chloroplasts according to endosymbiont hypothesis

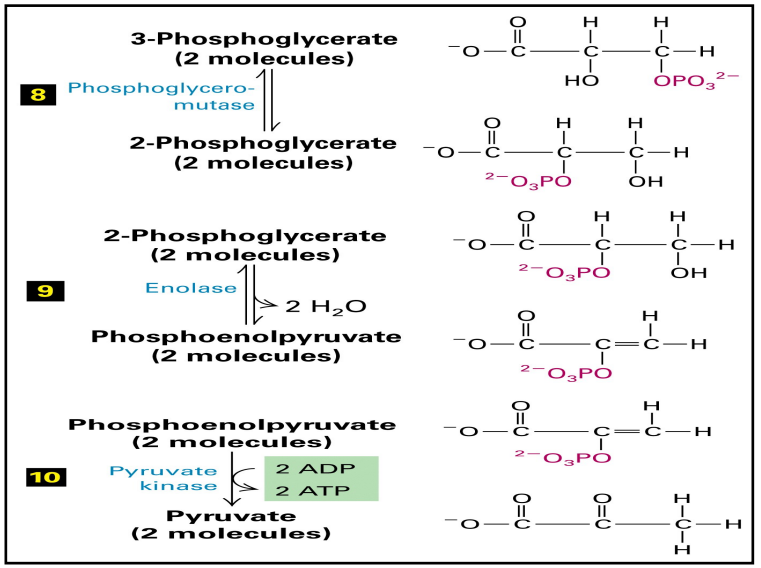


Membrane surfaces facing a shaded area are cytosolic faces; surfaces facing an unshaded area are exoplasmic faces. Endocytosis of a bacterium by an ancestral eukaryotic cell would generate an organelle with two membranes, the outer membrane derived from the eukaryotic plasma membrane and the inner one from the bacteiral 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 form 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







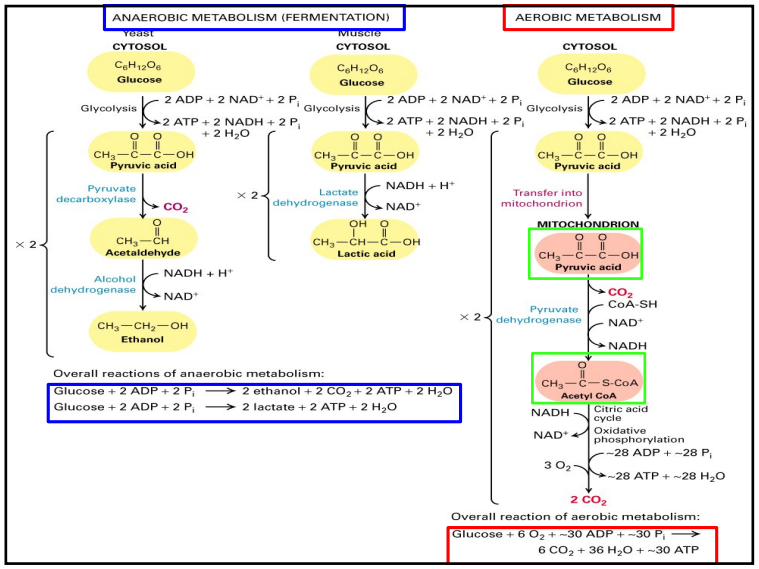
The glycolysis pathway by which glucose is degrade to pyruvic acid:

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).

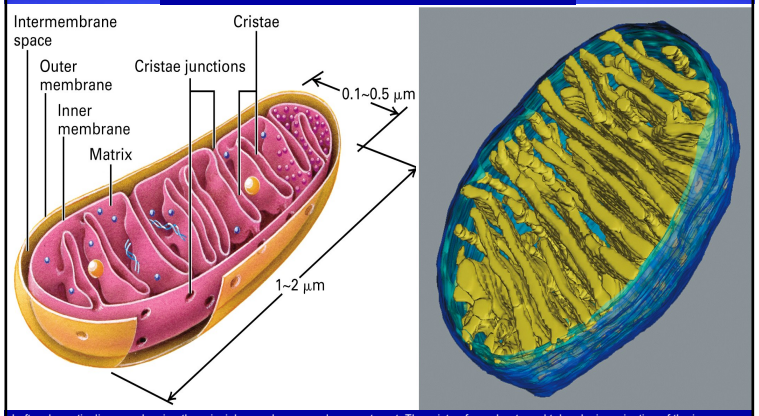
Note that all the intermediates between glucose and pyruvate are phosphorylated compounds. Reactions 1, 3, and 10, with single arrows, are essentially irreversible (large negative ΔG values) under conditions ordinarily obtaining in cells.



Anaerobic versus *Aerobic* Metabolism of Glucose

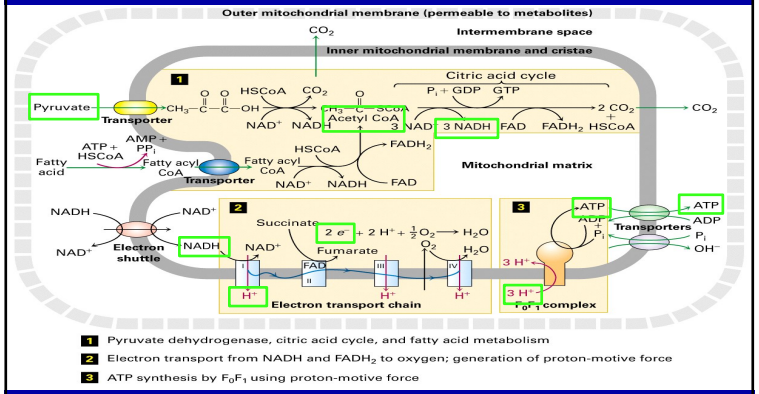
The ultimate fate of pyruvate formed during glycolysis depends on the presence or absence of oxygen. In the formation of pyruvate from glucose, one molecule of NAD^+ is reduced (by addition of two electrons) to NADH for each molecule pyruvate formed (see previous slides, reaction 6). (Left) In the absence 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 CO_2 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



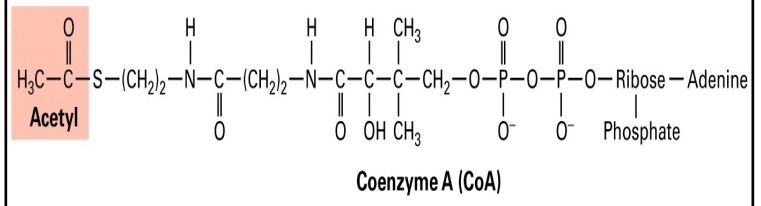
Left: schematic diagram showing the principle membranes and compartment. The cristae form sheets and tubes by invagination of the inner membrane and connect to the inner membrane through relatively small uniform tubular structures called crista junctions. The intermembrane space appears continous with the lumen of each cristae. The F_0F_1 complexes (small red spheres), which synthesize ATP, are intramembrane particles that protrude form the cristae and inner membrane into the matrix. The matrix contains the mitochondrial DNA (blue strand), ribosomes (small blue spheres), and granules (large yellow spheres). Right: computer generated model of a section of a mitochondrion from chicken brain. This model is based on a three-dimensional electron tomogram calculated from a series of two-dimensional electron micrographs recorded at regular angular intervals. This 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 P_i (purple) into the matrix and to export ATP (green). NADH generated in the cytosol is not transported directly to the matrix because the inner membrane is impermeable to NAD^+ and NADH; instead, a shuttle system (red) transports electrons from cytosolic NADH to NAD^+ in the matrix. O_2 diffuses into the matrix and CO_2 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 formation of NADH and $FADH_2$. Oxidation of acetyl CoA in the citric acid cycle generates NADH and $FADH_2$. Stage-2: electrons from these reduced coenzymes are transferred via electron transport complexes (blue boxes) to O_2 concomitant with transport of H^+ ions from the matrix to the intramembrane space, generating the proton-motive force. Electrons from NADH flow directly from complex I to complex III, bypassing complex II. Stage 3: ATP synthase, the F_0F_1 complex (ornage), harnesses the proton-motive force to synthesize ATP. Blue arrows indicate electron flow; red arrows transmembrane movement of protons; and green arrows indicate transport of metabolites.

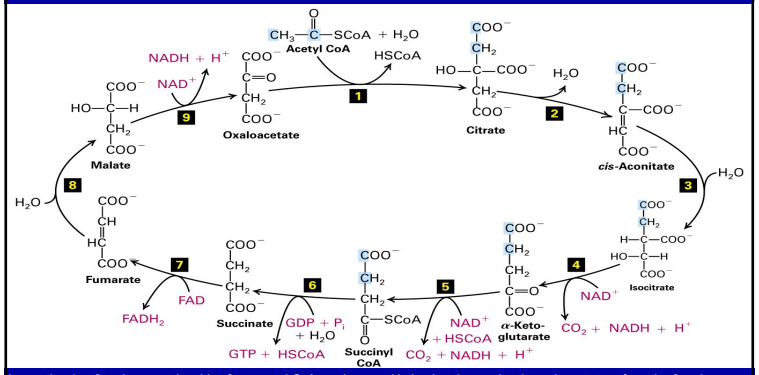
The structure of acetyl CoA



Coenzyme A (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 CO_2



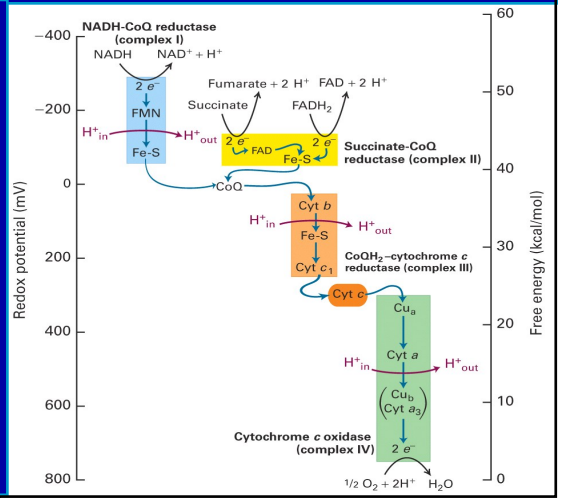
In reaction 1, a 2-carbon acetyl residue from acetyl CoA condenses with the 4-carbon molecule oxaloacetate to form the 6-carbon molecule citrate. In the remaining reactions (2-9) each molecule is eventually converted back to oxaloacetate, losing 2 CO_2 molecules in the process. In each turn of the cycle, 4 pairs of electrons are removed from carbon atoms, forming 3 molecules of NADH and 1 molecule of $FADH_2$. 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 CO_2 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

Reaction	CO_2 Molecules Produced	NAD^+ Molecules Reduced to NADH	FAD Molecules Reduced to $FADH_2$
1 glucose molecule to 2 pyruvate molecules	0	2	0
2 pyruvates to 2 acetyl CoA molecules	2	2	0
2 acetyl CoA to 4 CO_2 molecules	4	6	2
Total	6	10	2

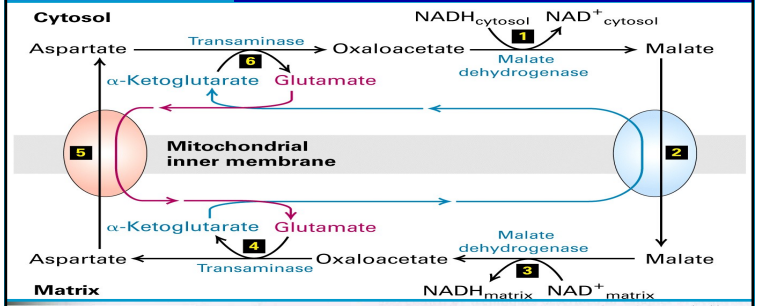
Changes in redox potential and free energy during stepwise flow of electrons through the respiratory chain

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 H^+ ions across the membrane, establishing the proton-motive force.



The Malate Shuttle

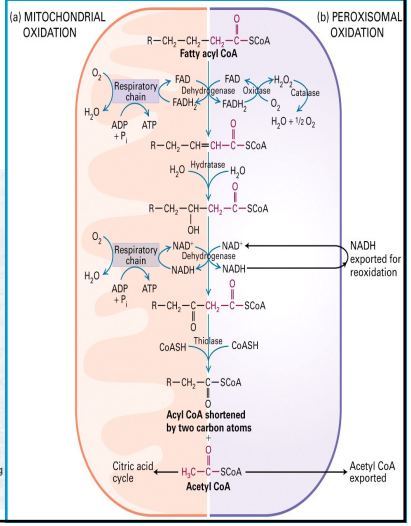
The net effect of the reactions constituting the malate aspartate shuttle is oxidation of cytosolic NADH to NAD+ and reduction of matrix NAD+ to NADH:
$NADH_{cytosol} + NAD^+_{matrix} \longrightarrow NAD^+_{cytosol} + NADH_{matrix}$



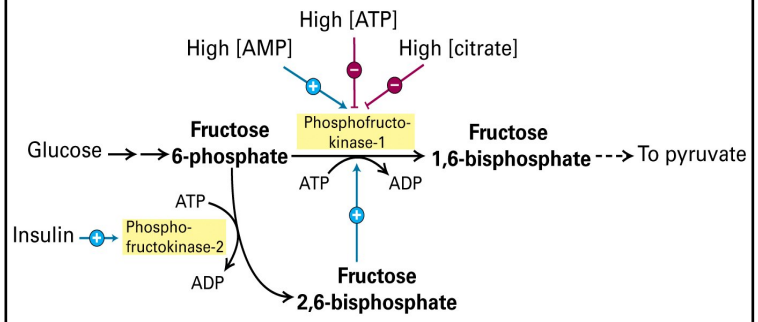
This cyclical series of reactions transfers electrons from NADH in the cytosol (intermembrane space) across the inner mitochondrial membrane, which is impermeable to NADH itself. Step 1: Cytosolic malate dehydrogenase transfers electrons from cytosolic NADH to oxaloacetate, forming malate. Step 2: An antiporter (blue oval) in the inner mitochondrial membrane transports malate into the matrix in exchange for α-ketoglutarate. Step 3: Mitochondrial malate dehydrogenase converts malate back to oxaloacetate, reducing NAD^+ in the matrix to NADH in the process. Step 4: Oxaloacetate, which cannot directly cross the inner membrane, is converted to aspartate by addition of an amino group from glutamate. In this transaminase-catalyzed reaction in the matrix, glutamate is converted to α-ketoglutarate. Step 5: A second antiporter (red oval) exports aspartate to the cytosol in exchange for glutamate. Step 6: A cytosolic transaminase converts aspartate to oxaloacetate, completing the cycle. The blue and red arrows reflect the movement of the α-ketoglutarate and glutamate, respectively. In step 4 glutamate is deaminated to α-ketoglutarate, which is transported to the cytosol by an antiporter (step 2); in step 6, the α-ketoglutarate is aminated, converting it back to glutamate, which is transported to the matrix by the antiporter in step 5.

Oxidation of fatty acids in mitochondria (coupled to ATP formation) and peroxisomes (generates no ATP)

Fatty acids are stored as triacylglycerols in adipose cells. ---> hydrolyzed to free fatty acids and glycerol and released into the blood ----> taken up and oxidized by other cells (e.g. heart muscle cells). In human 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 then the mitochondria. In both mitochondrial oxidation (a) and peroxisomal oxidation (b), four identical enzyme-catalyzed reactions (shown down the center of the figure) convert a fatty acyl CoA molecule to acetyl CoA and a fatty acyl CoA shortened by two carbon atoms. Concomitantly (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-bisphosphate, 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 is actively oxidizing glucose to CO_2. Phosphofructokinase-2 (PFK2) is a bifunctional enzyme: its kinase activity forms fructose 2,6-bisphosphate 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 glucolysis to fructose 2,6-bisphosphate. At low blood glucose, glucagon is released by the pancreas and promotes PFK2 phosphatase activity in the liver, indirectly modulating glycolysis.

---> Taking home message

Oxidation of Glucose and Fatty Acids to CO_2:

- 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 CO_2 (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 CO_2.
- Mitochondria have a permeable outer membrane and an inner membrane, which is the site of e^- 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 CO_2 and generate 3 NADH molecules, 1 $FADH_2$ molecule and 1 GTP.
- Although cytosolic NADH generated during glycolysis cannot enter mitochondria directly, the malate-aspartate shuttle indirectly transfers e^- from the cytosol to the mitochondrial matrix, thereby regenerating cytosolic NAD^+ for continued glycolysis.
- The flow of e^- from NADH and $FADH_2$ to O_2, via a series of e^- carriers in the inner mitochondrial membrane, is coupled to pumping the H^+ across the inner membrane. The resulting proton-motive force pmf 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 $FADH_2$. Subsequent oxidations of these metabolites is 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 glucogen) or fat when ATP is abundant.

Electron Transport and generation of the Proton-Motive Force PMF

During respiration: (oxidative phosphorylation)

$NADH + H^+ + 1/2\ O_2$ ---> $NAD^+ + H_2O$ $\quad \Delta G^{o\prime}$ -52.6 kcal/mol

$FADH_2 + 1/2\ O_2$ ---> $FAD + H_2O$ $\quad \Delta G^{o\prime}$ -43.4 kcal/mol

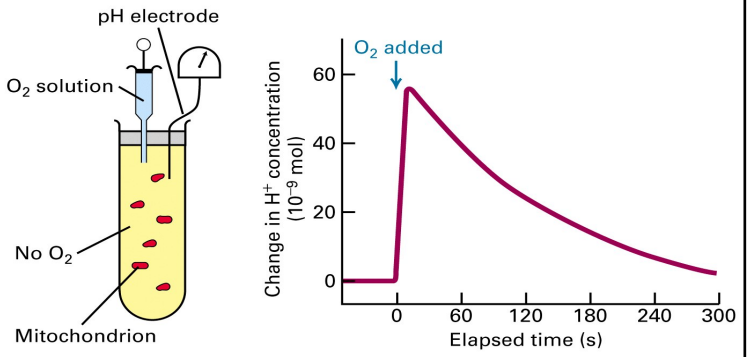
Both reactions are strongly exergonic. Conversion of 1 glucose molecule to CO_2 via glycolytic pathway and citric acid cycle yields 10 NADH and 2 $FADH_2$. Oxidation of these reduced coenzymes has thus a total $\Delta G^{o\prime}$ of -613 kcal/mol. Thus, of the potential free energy present in the chemical 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 $FADH_2$ molecule to O_2 is sufficient to drive the synthesis of several molecules of ATP from ADP and P_i, a reaction with a $\Delta G^{o\prime}$ of + 7.3 kcal/mol.

Electron Transport and generation of the Proton-Motive Force PMF

During respiration:

At several sites during electron transport from NADH to O_2, protons from the mitochondrial matrix are pumped across the inner mitochondrial membrane; this "uphill" transport generates a proton concentration gradient across the inner membrane (previous slide). Because the outer membrane is freely permeable to protons, whereas the inner membrane is not, this pumping causes the pH of the mitochondrial matrix to become higher (= $[H^+]$ is lower) than that of the cytosol and intermembrane space. An electric potential across the inner membrane also results from the uphill pumping of H^+ outward from the matrix, which becomes negative with respect to the intermembrane space. Thus, 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_i by the ATP synthase.

Electron transfer from NADH or $FADH_2$ to O_2 is coupled to proton transport across the mitochondrial membrane



If NADH is added to a suspension of mitochondria depleted of O_2, no NADH is oxidized. When a small amount of O_2 is added to the system (arrow), the pH of the surrounding medium drops sharply – a change that corresponds to an increase in protons outside the mitochondria. (the presence of a large amount of valinomycin and K^+ in the reaction dissipates the voltage gradient generated by the H^+ translocation, so that all pupped H^+ ions contribute to the pH change). Thus the oxidation of NADH by O_2 is coupled to the movement of protons out of the matrix. Once the O_2 is depleted, the excess protons slowly move back into mitochondria (powering the synthesis of ATP) and the pH of the extracellular medium returns to its initial value.

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_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^+.

pmf = Ψ - (RT/F x ΔpH) = Ψ -59 ΔpH

R = gas constant of 1.987 cal/(degree mol); T degree in Kelvin, F = Faraday constant of 23,062 cal/(V mol); and Ψ is the transmembrane electric potential, pmf and Ψ are measured in millivolt. In respiring mitochondria Ψ across the inner membrane is about -160 mV, ΔpH 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

$E = -59 \log [K^+_{in}]/[K^+_{out}] = -59 \log 500 = -160$ mV

TABLE 8-2 Electron-Carrying Prosthetic Groups in the Respiratory Chain

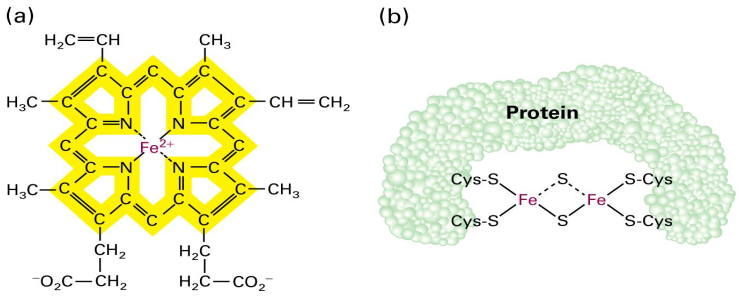
Protein Component	Prosthetic Groups*
NADH-CoQ reductase (complex I)	FMN Fe-S
Succinate-CoQ reductase (complex II)	FAD Fe-S
$CoQH_2$–cytochrome *c* reductase (complex III)	Heme b_L Heme b_H Fe-S Heme c_1
Cytochrome *c*	Heme *c*
Cytochrome *c* oxidase (complex IV)	Cu_a^{2+} Heme *a* Cu_b^{2+} Heme a_3

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

SOURCE: J. W. De Pierre and L. Ernster, 1977, *Ann. Rev. Biochem.* 46:201.

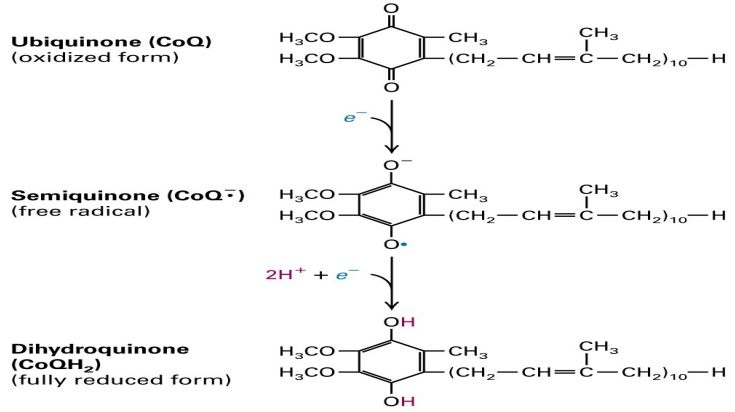
Heme and iron-sulfur prosthetic groups in the respiratory (electron-transport) chain

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



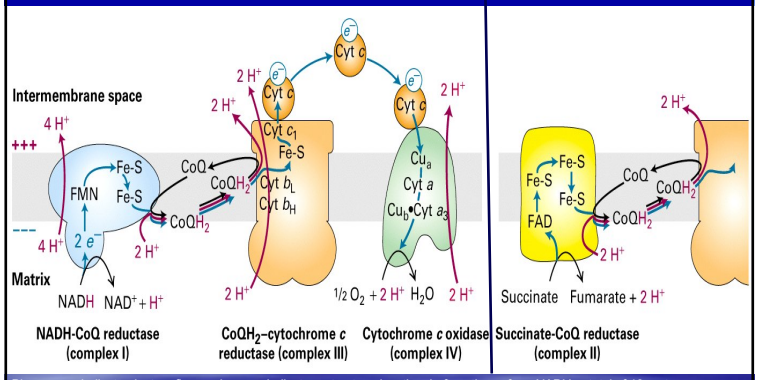
(a) Heme portion of cytochromes b_L and b_H, which are components of the $CoQH_2$-cytochrome reductase complex. The same porphyrin ring (yellow) is present in all hemes. The chemical substituents attached to the porphyrin ring differ in the other cytochromes in the respiratory chain. All hemes accept and release one electron at a time. (b) Dimeric iron-sulphur 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 at time.

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



Because of its long hydrocarbon 'tail' of isoprene unites, CoQ is soluble in the hydrophobic core of phospholipid bilayers and is very mobile. Reduction of CoQ to the fully reduced form, QH_2, occurs in two steps with a half-reduced free-radical intermediate, called semichinone.

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



Blue arrows indicate electron flow; red arrows indicate proton translocation. Left: pathway from NADH: a total of 10 protons are translocated per pair of electrons that flow from NADH to O_2. The protons released into the matrix space during oxidation of NADH by NADH-CoQ reductase are consumed in the formation of water from O_2 by cytochrome c oxidase, resulting in no net proton translocation from these reactions. Right: pathway from succinate. During oxidation of succinate to fumarate and reduction of CoQ by succinate-CoQ reductase complex, no protons are translocated across the membrane. The remainder of electron transport from CoQH proceeds by the same pathway as in the left diagram. Thus for every pair of electrons transported from succinate to O_2, six protons are translocated from the matrix into the intermembrane space. Coenzyme Q and cytochrome c function as mobile carriers in electron transport from both NADH and succinate.

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:

$$NADH_{(reduced)} + CoQ_{(oxidized)} + 2\ H^+ \text{ ---> } NAD^+_{(oxidized)} + H^+ + CoQH_{2(reduced)}$$

Each transported electron undergoes a drop in potential of ~360 mV, equivalent to a $\Delta G^{o\prime}$ 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.

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:

$$Succinate_{(reduced)} + CoQ_{(oxidized)} \text{ ---> } fumarate_{(oxidized)} + CoQH_{2(reduced)}$$

Although the $\Delta G^{o\prime}$ 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.

$CoQH_2$-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:

$$CoQH_{2(reduced)} + 2\ Cyt\ c^{3+}_{(oxidized)} \text{ ---> } CoQ_{(oxidized)} + 2\ H^+ + 2\ Cyt\ c^{2+}_{(reduced)}$$

The $\Delta G^{o\prime}$ 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.

Cytochrome *c* Oxidase (Complex IV)

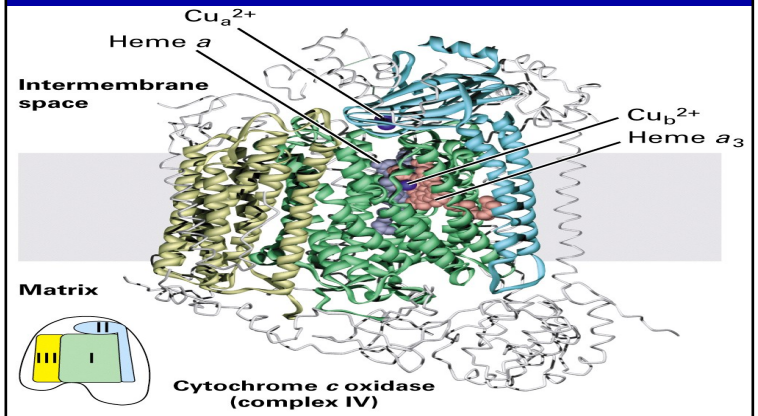
Cytochrome *c*, after being reduced by the $CoQH_2$-cytochrome *c* reductase complex, transports electrons, one at a time, to the cytochrome *c* oxidase complex. Within this complex, electrons are transferred , again one ata time, first to a pair of copper ions called Cu_a^{2+}, then to cytochrome *a*, 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_2O.

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

$$2\ \text{Cyt}\ c^{2+}_{(\text{reduced})} + 2\ H^+ + 1/2\ O_2 \longrightarrow 2\ \text{Cyt}\ c^{3+}_{(\text{oxidized})} + H_2O$$

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:

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

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 $E^{o\prime}$ of the carriers in the mitochondrial respiratory chain increases steadily from NADH to O_2. For instance, for the partial reaction

$$NAD^+ + H^+ + 2\ e^- \longleftrightarrow NADH$$

The value of the standard reduction potential is -320 mV, which is equivalent to a $\Delta G^{o\prime}$ 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:

cytochrome c_{ox} (Fe^{3+}) + e^- <----> cytochrome c_{red} (Fe^{2+})

Is +220 mV, which is equivalent to a $\Delta G^{o\prime}$ 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_{ox} (Fe^{3+}) to cytochrome c_{red} (Fe^{2+}).

Reduction Potentials of Electron Carriers Favor Electron Flow from NADH to O_2

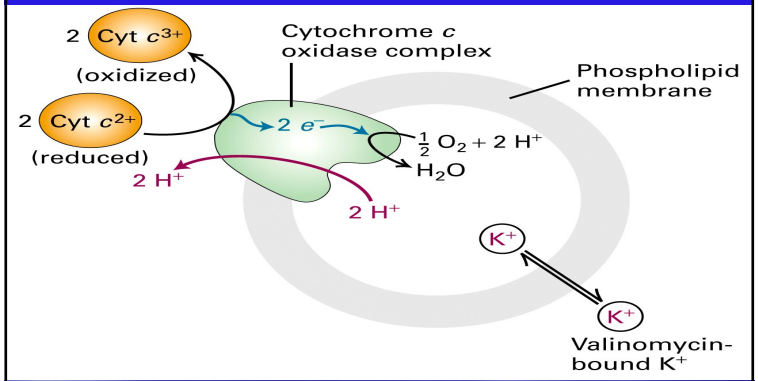
The final reaction in the respiratory chain, the reduction of O_2 to H_2O:

$2\ H^+ + \frac{1}{2}\ O_2 + 2\ e^-$ ----> H_2O

has a standard reduction potential of +816 mV, which is equivalent to a $\Delta G^{o\prime}$ of – 37.8 kcal/mol for transfer of one electron, the most positive in the whole series; thus, this reaction tends to proceed toward the right.

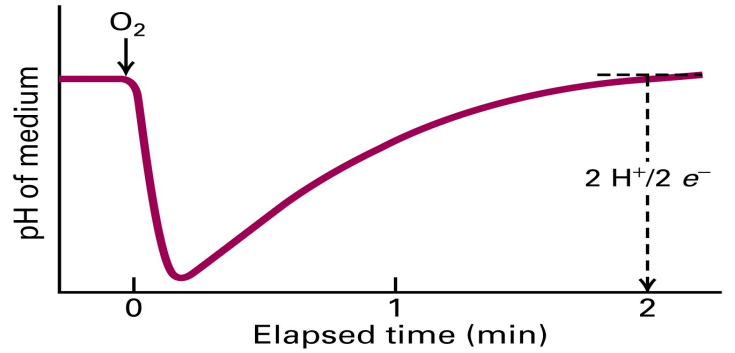
The steady increase in a $E^{o\prime}$ values and the corresponding decrease in $\Delta G^{o\prime}$ values of the carriers in the respiratory chain favors the flow of electrons from NADH and succinate to oxygen.

Electron transfer from reduced cytochrome c (Cyt c^{2+}) to O_2 via the cytochrome *c* oxidase complex is coupled to proton transport



The oxidase complex is incorporated into liposomes with the binding site for cytochrome *c* positioned on the outer surface. When O_2 and reduced cytochrome *c* are added, electrons are transferred to O_2 to form H_2O and H^+ are transported from the inside to the outside of the vesicles. Valinomycin and K^+ are added to the medium to dissipate the voltage gradient generated by the translocation of H^+, which would otherwise reduce the number of H^+ moved across the membrane.

Electron transfer from reduced cytochrome c (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

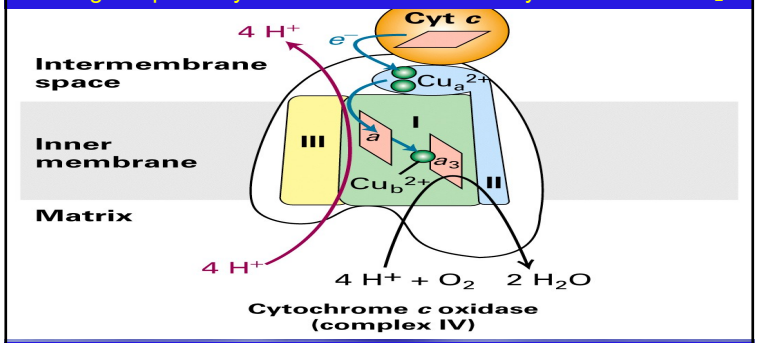
Studies as shown in the previous slides show that the NADH-CoQ reductase complex translocates four H^+ per pair of e^- transported, whereas the cytochomre *c* oxidase complex translocates 2 H^+ per e^- pair transported (or, equivalently, for every molecules of cytochrome *c* oxidized). Current evidence 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 O_2. 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 $FADH_2$) to O_2. 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 $CoQH_2$-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 Cu_a^{2+} bound to subunit II, then to the heme *a* bound to subunit I, and finally to Cu_b^{2+} and heme a_3 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 O_2^{2-} 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.

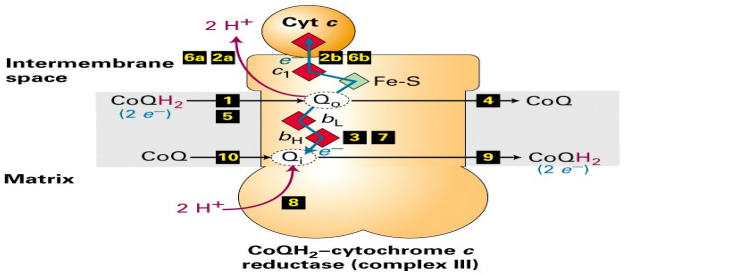
Schematic depiction of the cytochrome *c* oxidase complex showing the pathway of e^- flow from reduced cytochrome *c* to O_2.



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 O_2 molecule to form 2 H_2O molecules. Additionally, for each e^- transferred from cytochrome *c* to oxygen, one H^+ is transported from the matrix to the intermembrae space, or a total of 4 for each O_2 molecule reduced to 2 H_2O molecules.

For every four e^- transferred from reduced cytochrome *c* through cytochrome *c* oxidase (i.e., for every molecule of O_2 reduced to 2 H_2O 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



$CoQH_2$–cytochrome *c* reductase (complex III)

At Q_o site: $2\ CoQH_2 + 2\ Cyt\ c^{3+} \longrightarrow 2\ CoQ + 2\ Cyt\ c^{2+} + 2\ e^- + 4\ H^+_{(outside)}$
$(4\ H^+, 4\ e^-)$ $(2\ e^-)$

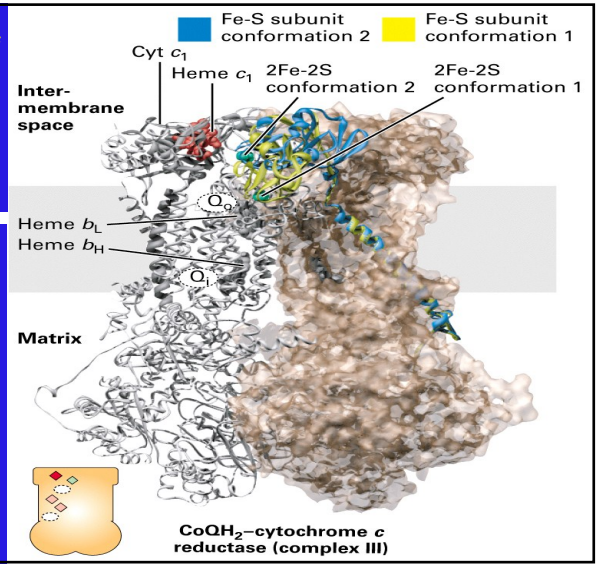
At Q_i site: $CoQ + 2\ e^- + 2\ H^+_{(matrix\ side)} \longrightarrow CoQH_2$
$(2\ H^+, 2\ e^-)$

Net Q cycle (sum of reactions at Q_o and Q_i):
$CoQH_2 + 2\ Cyt\ c^{3+} + 2\ H^+_{(matrix\ side)} \longrightarrow CoQ + 2\ Cyt\ c^{2+} + 4\ H^+_{(outside)}$
$(2\ H^+, 2\ e^-)$ $(2\ e^-)$

Per $2\ e^-$ transferred through complex III to cytochrome *c*, $4\ H^+$ released to the intermembrane space

$CoQH_2$ binds to the Q_o 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 $CoQH_2$ bound to Q_o travels directly to cytochrome *c* via an Fe-S cluster and cytochrome c_1. The other e^- moves through the *b* cytochromes to CoQ at the Q_i site, forming the partially reduced semiquinone ($Q^{-\cdot}$). Simultaneously, CoQH2 releases its 2 H^+ into the intermembrane space. The CoQ now at the Q_o site dissociates and a second $CoQH_2$ binds there. As before, 1 e^- moves directly to cytochrome c_1 and the other to the $Q^{-\cdot}$ at the Qi site, forming, together with 2 H^+ picked up from the matrix space, $CoQH_2$, 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 $CoQH_2$-cytochrome *c* reductase complex.

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



$CoQH_2$–cytochrome *c* reductase (complex III)

In the dimeric complex, cytochrome b_L and b_H,are associated with one subunit and the 2Fe-2S cluster is shown in its two alternative conformational states, which differ primarily in the portion of the protein toward the intermembranse space. In one conformation (yellow), the 2Fe-2S cluster (green) is positioned near the Q_o site on the intermembrane side of the protein, able to pick up an e^- from $CoQH_2$. In the alternative conformation (blue), the 2Fe-2S cluster is located adjacent to the c_1 heme on the cytochrome c_1 and able to transfer an e^- to it.

---> Taking home message

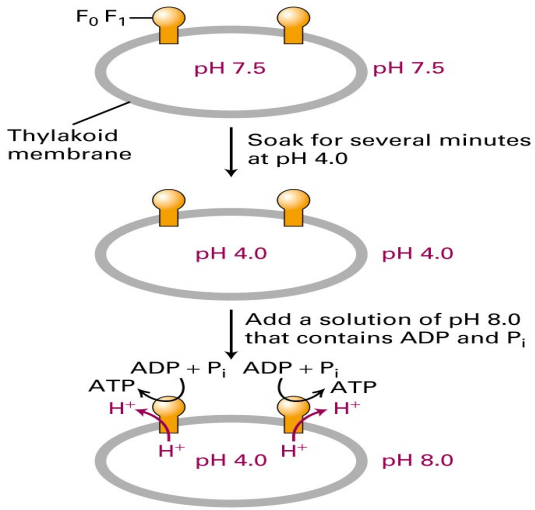
Electron Transport and Generation of the Proton-Motive Force PMF:

- 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 $FADH_2$ to O_2 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 multiprotien 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 O_2 to form H_2O.
- 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 form NADH and $FADH_2$ to O_2.
- A total of 10 H+ ions are translocated from the matrix across the inner membrane per e^- pair flowing from NADH to O_2.
- The Q cycle allows four H^+ (rather than two) to be translocated per pair of e^- moving through the CoQH-cytochrome *c* reductase complex.

Harnessing the Proton-Motive Force (PMF) for Energy-Requiring Processes

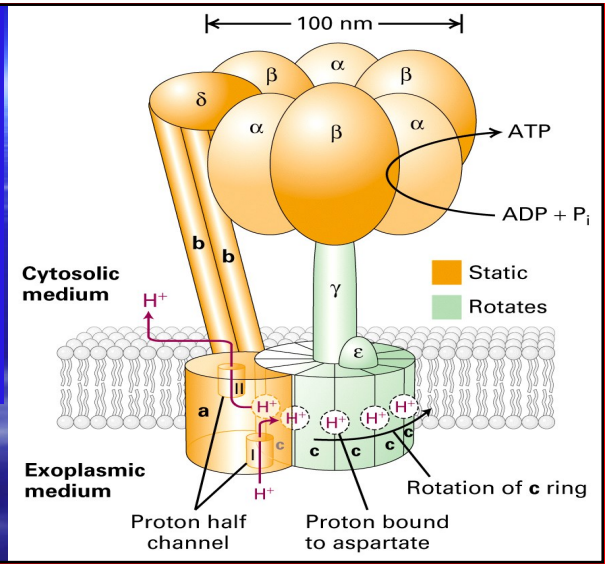
Synthesis of ATP by F_0F_1 depends on a pH gradient across the membrane.

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.



Model of the structure and function of ATP synthase (the F_0F_1 complex) in the bacterial plasma membrane

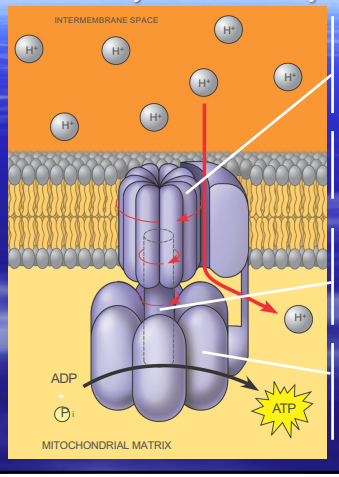
F_0 = a, b, c
F_1 = α, β, ε, γ δ



Chemiosmosis: The Energy-Coupling Mechanism

- ATP synthase – is the enzyme that actually makes ATP

The proton motive force (pmf)



A **rotor** within the membrane spins clockwise when H^+ flows past it down the H^+ gradient.

A **stator** anchored in the membrane holds the knob stationary.

A **rod** (for "stalk") extending into the knob also spins, activating catalytic sites in the knob.

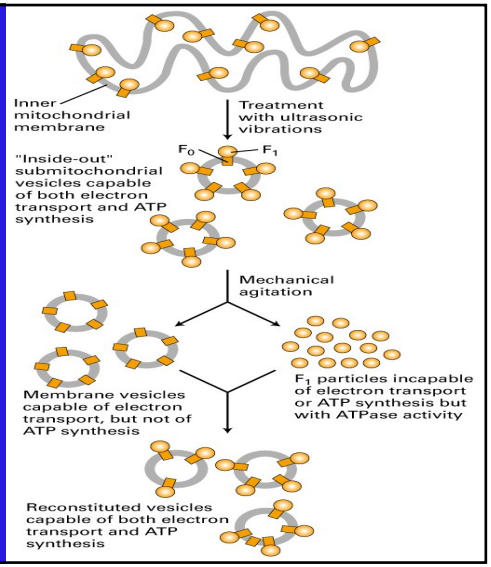
Three catalytic sites in the stationary **knob** join inorganic Phosphate to ADP to make ATP.

Rotation of the F_1 γ subunit, driven by the H^+ movement through F_0, powers ATP synthesis

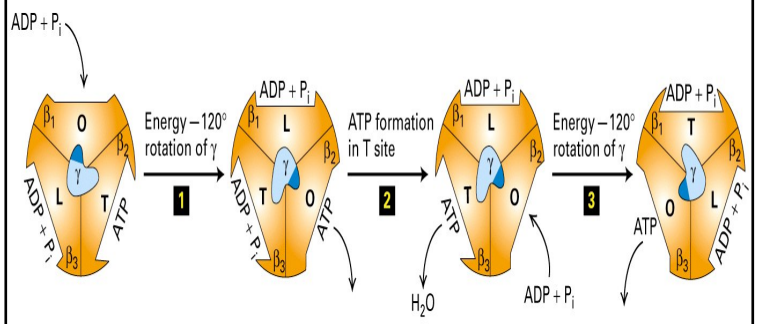
The F_0 portion is built of three integral membrane proteins: one copy **a**, two copies of **b**, and on average 10 copies of **c** arranged in a ring in the plane of the membrane. Two H^+ half-channels lie at the interface between the **a** subunit and the **c** ring. Half-channel I allows H^+ to move one at a time from the exoplasmic medium and bind to aspartate-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 α and β that form a hexamer resting atop the single rod-shaped γ subunit, which is inserted into the **c** ring of F_0. The ε subunit is rigidly attached to the γ subunit and also to several of the **c** subunits. The δ subunit permanently links one of the α 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 δ 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 ε and γ subunits rotate as a unit (green), causing conformation changes in the F_1 β subunits leading to ATP synthesis.

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 reconstitute the native membrane structure, restoring the capacity for ATP synthesis. When detached from the membrane, F_1 particles exhibit ATPase activity.

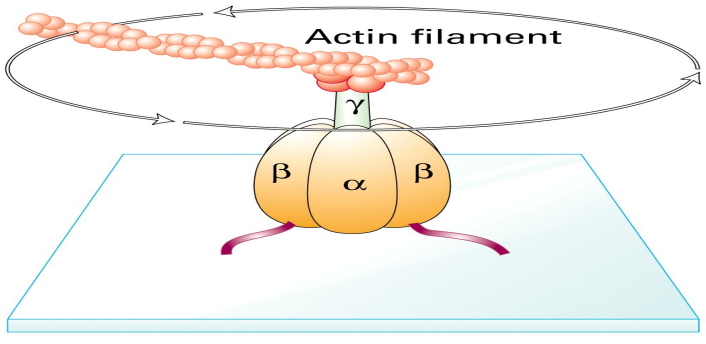


The binding-change mechanism of ATP synthesis from ADP and P_i by the F_0F_1 complex



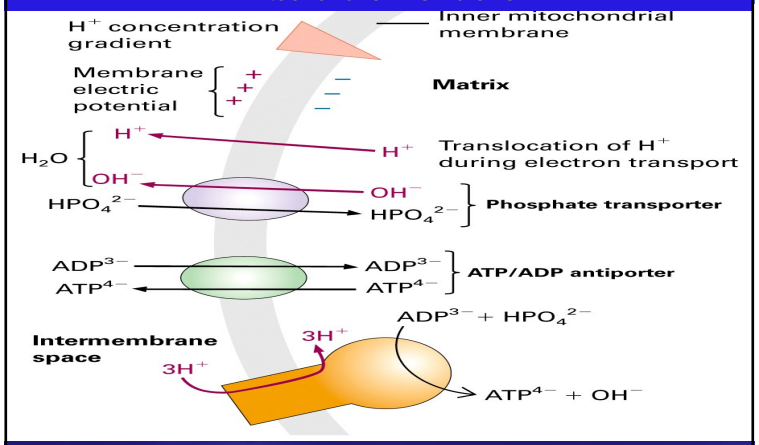
This view is looking up at F_1 from the membrane surface. Each of the F_1 β subunits alternate between three conformational states that differ in their binding affinity to ATP, ADP and P_i. Step-1: After ADP and P_i bind to one of the three β subunits (here, arbitrarily designated as β_1) whose nucleotide-binding site is in the O (open) conformation, H^+ powers a 120° rotation of the γ subunit (relative to the fixed β subunits). This causes an increase in the binding affinity of the β_1 subunit for ADP and P_i to L (low), an increase in the binding affinity of the β_3 subunit for ADP and Pi from L to T (tight), and a decrease in the binding affinity of the β_2 subunit for ATP from T to O, causing release of the bound ATP. Step-2: The ADP and P_i in the T site (here in the β_3 subunit) form ATP, a reaction that does not require an input of energy and ADP and P_i bind to the β_2 subunit, which is in the O state. This generates an F_1 complex identical with that which started the process (left) except that it is rotated 120°. Step-3: Another 120° rotation of γ again causes the O-->L-->T-->O conformational changes in the β subunits described above. Repetition of steps-1 and -2 leads to formation of three ATP molecules for every 360° rotation of γ.

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



F_1 complexes were engineered that contained β subunits with an additional His6 sequence, which causes them to adhere to a glass plate coated with a metal reagent that binds histidine. The γ 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 β subunits.

The phosphate and ATP/ADP transport system in the inner mitochondrial membrane



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.

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 ΔG for this reaction under standard condition is +7.3 kcal/mol, at the concentrations of reactants in the mitochondrion, ΔG 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 ΔE (= 0.22 V) in volts:

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

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.

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_2O. 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 mitochondrion 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.

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 Pi (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.

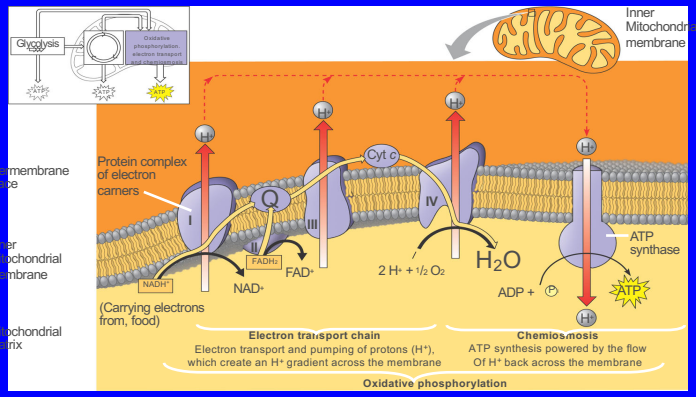
---> Taking home message

Harnessing the PMF for Energy-Requiring Processes:

- The multiprotein F_0F_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 $[(\alpha\beta)_3]$, 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 **c** ring with its attached F_1 ε 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 e^- transport.

- 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

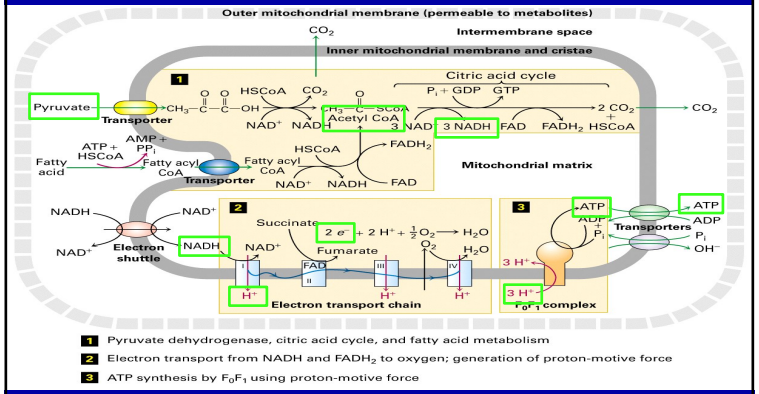
Chemiosmosis and the electron transport chain



An Accounting of ATP Production by Cellular Respiration

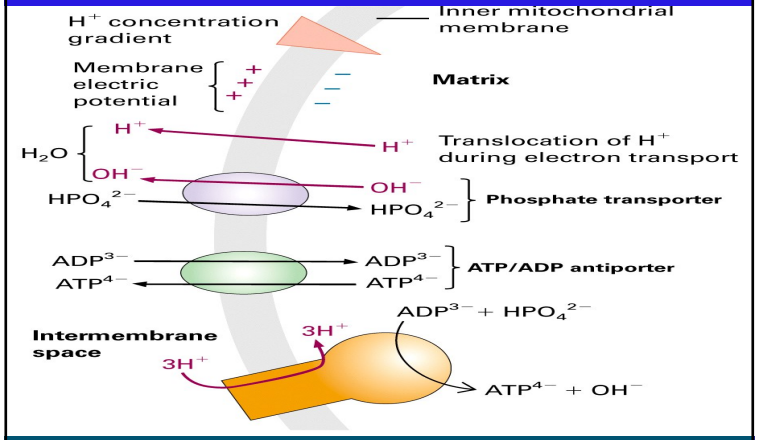
During respiration, most energy flows in this sequence glucose to NADH to electron transport chain to proton-motive force to ATP

Aerobic oxidation of pyruvate and fatty acids in mitochondria



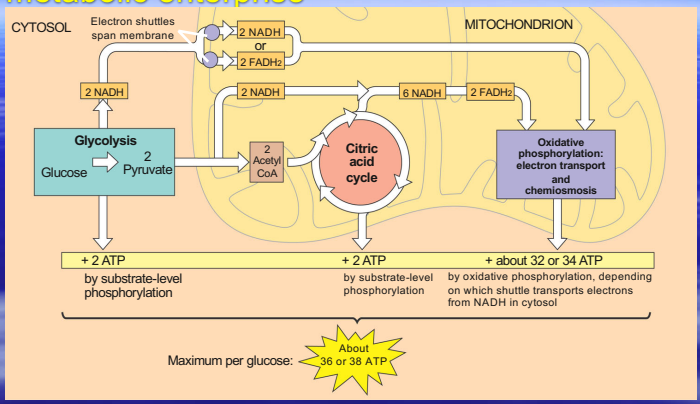
The outer membrane is freely permeable to all metabolites, but specific transport proteins (colored ovals) in the inner membrane are required to import pyruvate (yellow), ADP (green), and P_i (purple) into the matrix and to export ATP (green). NADH generated in the cytosol is not transported directly to the matrix because the inner membrane is impermeable to NAD^+ and NADH; instead, a shuttle system (red) transports electrons from cytosolic NADH to NAD^+ in the matrix. O_2 diffuses into the matrix and CO_2 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 formation of NADH and $FADH_2$. Oxidation of acetyl CoA in the citric acid cycle generates NADH and $FADH_2$. Stage-2: electrons from these reduced coenzymes are transferred via electron transport complexes (blue boxes) to O_2 concomitant with transport of H^+ ions from the matrix to the intramembrane space, generating the proton-motive force. Electrons from NADH flow directly from complex I to complex III, bypassing complex II. Stage 3: ATP synthase, the F_0F_1 complex (ornage), harnesses the proton-motive force to synthesize ATP. Blue arrows indicate electron flow; red arrows transmembrane movement of protons; and green arrows indicate transport of metabolites.

The phosphate and ATP/ADP transport system in the inner mitochondrial membrane



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.

- 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

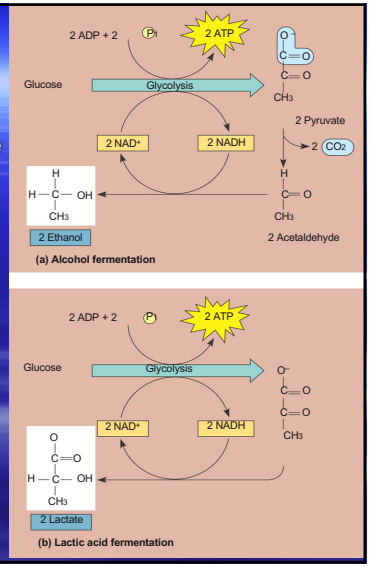
- **Fermentation** enables some cells to produce **ATP without the use of oxygen**
- **Cellular respiration**
  - relies on oxygen to produce ATP
- In the absence of oxygen
  - cells can still produce ATP through fermentation

- **Glycolysis**
  - can produce ATP with or without oxygen, in aerobic or anaerobic conditions
  - couples with fermentation to produce ATP

Types of Fermentation

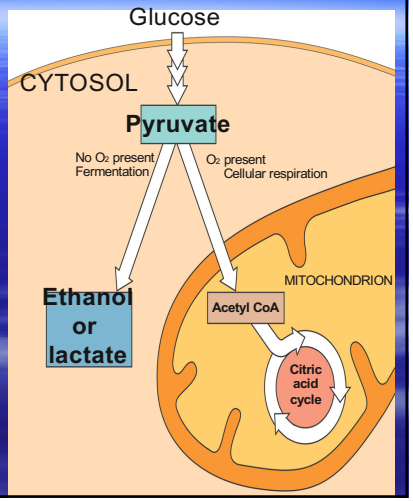
- Fermentation consists of
- - glycolysis plus reactions that regenerate NAD^+, which can be reused by glycolysis

- In alcohol fermentation
  - pyruvate is converted to ethanol in two steps, one of which releases CO_2
- During lactic acid fermentation
  - pyruvate is reduced directly to NADH to form lactate as a waste product



Fermentation and Cellular Respiration Compared

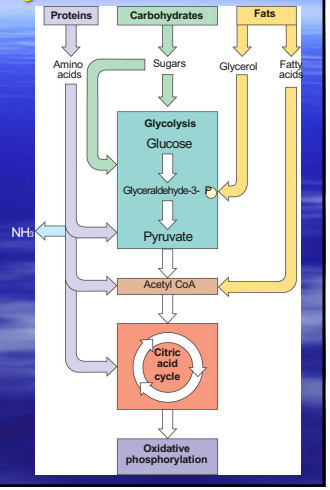
- Both fermentation and cellular respiration
  - use glycolysis to oxidize glucose and other organic fuels to pyruvate
- Fermentation and cellular respiration
  - differ in their final electron acceptor
- Cellular respiration produces more ATP
- pyruvate is a key juncture in catabolism



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