L2 Electron transport and oxidative phosphorylation

Key Notes

Overview

Electron transport and oxidative phosphorylation re-oxidize NADH and $FADH_2$ and trap the energy released as ATP. In eukaryotes, electron transport and oxidative phosphorylation occur in the inner membrane of mitochondria whereas in prokaryotes the process occurs in the plasma membrane.

Redox potential

The oxidation–reduction potential, E, (or redox potential) of a substance is a measure of its affinity for electrons. The standard redox potential (E_0') is measured under standard conditions, at pH 7, and is expressed in volts. The standard free energy change of a reaction at pH 7, $\Delta G^{0\prime}$, can be calculated from the change in redox potential $\Delta E_0'$ of the substrates and products. A reaction with a positive $\Delta E_0'$ has a negative $\Delta G^{0\prime}$ (i.e. is exergonic).

Electron transport from NADH

Electrons are transferred from NADH to oxygen along the electron transport chain (also called the respiratory chain). NADH passes electrons to NADH dehydrogenase, a large protein complex that contains FMN and two types of iron–sulfur (FeS) clusters in iron–sulfur proteins. The electrons are accepted by the FMN to produce $FMNH_2$ and then passed to the iron atoms of the FeS clusters which accept and donate electrons by alternating between Fe^{3+} and Fe^{2+} states. Electrons from NADH dehydrogenase are passed to ubiquinone (coenzyme Q, CoQ), converting it to ubiquinol (or $CoQH_2$), and then to the cytochrome bc_1 complex. This contains cytochrome b and cytochrome c_1, as well as an FeS protein. A cytochrome contains a heme group with a central iron atom which changes from the Fe^{3+} state to the Fe^{2+} state on accepting an electron. When the electron is donated to another component, the iron atom changes back to the Fe^{3+} state. The cytochrome bc_1 complex passes the electrons to cytochrome c which in turn passes them to cytochrome oxidase, a complex that contains two cytochromes (cytochrome a and a_3) paired with copper atoms (Cu_A and Cu_B , respectively). During electron transfer, the copper atoms cycle between the Cu^{2+} and Cu^{+} states. Finally, cytochrome oxidase passes four electrons to molecular oxygen to form two molecules of water.

Formation of an H^+ gradient

The change in redox potential along the chain is a measure of the free energy change at each step. At the steps involving NADH dehydrogenase, the cytochrome bc_1 complex and cytochrome oxidase, the free energy change is large enough to pump H^+ ions across the inner mitochondrial membrane, from the mitochondrial matrix into the intermembrane space, to create an H^+ gradient. Therefore, each of these complexes is an H^+ pump driven by electron transport.

Electron transport from $FADH_2$

$FADH_2$ is reoxidized to FAD by donating two electrons to succinate-CoQ reductase (complex II), a protein complex that contains FeS clusters. It passes the electrons on to ubiquinone in the main electron transport chain where their further transport leads to the formation of an H^+ gradient and ATP synthesis. However succinate-CoQ reductase does not itself pump H^+ ions.

Electron transport inhibitors

Rotenone and amytal inhibit electron transport at NADH dehydrogenase, antimycin A inhibits the cytochrome bc_1 complex, and cyanide (CN^-), azide (N_3^-) and carbon monoxide (CO) all inhibit cytochrome oxidase.

Oxidative phosphorylation

Oxidative phosphorylation is ATP synthesis linked to the oxidation of NADH and $FADH_2$ by electron transport through the respiratory chain. This occurs via a mechanism originally proposed as the chemiosmotic hypothesis. Energy liberated by electron transport is used to pump H^+ ions out of the mitochondrion to create an electrochemical proton (H^+) gradient. The protons flow back into the mitochondrion through the ATP synthase located in the inner mitochondrial membrane, and this drives ATP synthesis. Approximately three ATP molecules are synthesized per NADH oxidized and approximately two ATPs are synthesized per $FADH_2$ oxidized.

ATP synthase as a rotatory engine

ATP synthase is located in the inner mitochondrial membrane. It consists of two major components, F_1 ATPase [seen as spheres under the electron microscope and with a subunit structure of $(\alpha\beta)_3\gamma\delta\varepsilon$] attached to component F_0 (coupling factor 0) which is a proton channel spanning this membrane. Hence, ATP synthase is also known as F_0F_1 ATPase. In mitochondria, this complete complex uses the energy released by electron transport to drive ATP synthesis but, in isolation, F_1 ATPase hydrolyzes ATP. During ATP hydrolysis, and presumably also during ATP synthesis, subunit γ of F_1 ATPase rotates relative to $(\alpha\beta)_3$ and is the smallest rotatory engine known in nature.

Coupling and respiratory control

Electron transport is normally tightly coupled to ATP synthesis; electrons do not flow through the electron transport chain to oxygen unless ADP is simultaneously phosphorylated to ATP. If ADP is available, electron transport proceeds and ATP is made; as the ADP concentration falls, electron transport slows down. This process, called respiratory control, ensures that electron flow occurs only when ATP synthesis is required.

Uncouplers

Some chemicals (e.g. 2,4-dinitrophenol; DNP) are uncoupling agents; they allow electron transport to proceed without ATP synthesis. They uncouple mitochondria by carrying H^+ ions across the inner mitochondrial membrane and hence dissipate the proton gradient. The energy derived from uncoupled electron transport is released as heat. Uncoupling also occurs naturally in some tissues (e.g. the mitochondria of brown adipose tissue are uncoupled by a protein called thermogenin). The resulting production of heat (nonshivering thermogenesis) by the adipose tissue serves to protect sensitive body tissues in newborn animals and to maintain body temperature during hibernation.

Reoxidation of cytosolic NADH

Cytosolic NADH cannot cross the inner mitochondrial membrane and enter mitochondria to be reoxidized. However, it can be reoxidized via the glycerol 3-phosphate shuttle. Cytosolic glycerol 3-phosphate dehydrogenase oxidizes the NADH and reduces dihydroxyacetone phosphate to glycerol 3-phosphate.

The glycerol 3-phosphate enters the mitochondrion and is converted back to dihydroxyacetone phosphate by mitochondrial glycerol 3-phosphate dehydrogenase (an FAD-linked enzyme). The dihydroxyacetone phosphate diffuses back to the cytosol. The enzyme-linked $FADH_2$ is reoxidized by transferring its electrons to ubiquinone in the electron transport chain. Since the electrons enter the electron transport chain from $FADH_2$, only about two ATPs are synthesized per molecule of cytosolic NADH. In heart and liver, cytosolic NADH can be reoxidized via the malate–aspartate shuttle. Oxaloacetate in the cytosol is reduced to malate by NADH and enters the mitochondrion via a malate–α-ketoglutarate carrier. In the matrix, the malate is reoxidized to oxaloacetate by NAD^+ which is converted to NADH, resulting in a net transfer of electrons from cytosolic NADH to matrix NADH. The oxaloacetate is converted to aspartate by transamination, leaves the mitochondrion and is reconverted to oxaloacetate in the cytosol, again by transamination.

Related topics

Glycolysis (J3)
Citric acid cycle (L1)
Photosynthesis (L3)

Overview

In eukaryotes, electron transport and oxidative phosphorylation occur in the inner membrane of mitochondria. These processes re-oxidize the NADH and $FADH_2$ that arise from the citric acid cycle (located in the mitochondrial matrix; Topic L2), glycolysis (located in the cytoplasm; Topic J3) and fatty acid oxidation (located in the mitochondrial matrix; Topic K2) and trap the energy released as ATP. Oxidative phosphorylation is by far the major source of ATP in the cell. In prokaryotes, the components of electron transport and oxidative phosphorylation are located in the plasma membrane (see Topic A1).

Redox potential

The oxidation of a molecule involves the loss of electrons. The reduction of a molecule involves the gain of electrons. Since electrons are not created or destroyed in a chemical reaction, if one molecule is oxidized, another must be reduced (i.e. it is an **oxidation–reduction reaction**). Thus, by definition, oxidation–reduction reactions involve the transfer of electrons. In the oxidation–reduction reaction:

$$NADH + H^+ + {}^1/_2\,O_2 \rightleftharpoons NAD^+ + H_2O$$

when the NADH is oxidized to NAD^+, it loses electrons. When the molecular oxygen is reduced to water, it gains electrons.

The **oxidation–reduction potential**, E, (or **redox potential**) is a measure of the affinity of a substance for electrons and is measured relative to hydrogen. A positive redox potential means that the substance has a higher affinity for electrons than does hydrogen and so would accept electrons from hydrogen. A substance with a negative redox potential has a lower affinity for electrons than does hydrogen and would donate electrons to H^+, forming hydrogen. In the example above, NADH is a strong reducing agent with a negative redox potential and has a tendency to donate electrons. Oxygen is a strong oxidizing agent with a positive redox potential and has a tendency to accept electrons.

For biological systems, the **standard redox potential** for a substance (E_0') is measured under standard conditions, at pH 7, and is expressed in volts. In an oxidation–reduction reaction, where electron transfer is occurring, the total

voltage change of the reaction (change in electric potential, ΔE) is the sum of the voltage changes of the individual oxidation–reduction steps. The standard free energy change of a reaction at pH 7, $\Delta G^{0\prime}$, can be readily calculated from the change in redox potential $\Delta E_0'$ of the substrates and products:

$$\Delta G^{0\prime} = -nF\,\Delta E_0'$$

where n is the number of electrons transferred, $\Delta E_0'$ is in volts (V), $\Delta G^{0\prime}$ is in kilocalories per mole (kcal mol^{-1}) and F is a constant called the Faraday (23.06 kcal V^{-1} mol^{-1}). Note that a reaction with a **positive** $\Delta E_0'$ has a **negative** $\Delta G^{0\prime}$ (i.e. is exergonic).

Thus for the reaction:

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons NAD^+ + H_2O$$

$$\Delta E_0' = +1.14\ V$$

$$\Delta G^{0\prime} = -52.6\ kcal\ mol^{-1}.$$

Electron transport from NADH

Comparing the energetics of the oxidation of NADH:

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons NAD^+ + H_2O \qquad \Delta G^{o\prime} = -52.6\ kcal\ mol^{-1}$$

and the synthesis of ATP:

$$ADP + P_i + H^+ \rightleftharpoons ATP + H_2O \qquad \Delta G^{0\prime} = +7.3\ kcal\ mol^{-1}$$

it is clear that the oxidation of NADH releases sufficient energy to drive the synthesis of several molecules of ATP. However, NADH oxidation and ATP synthesis do not occur in a single step. Electrons are not transferred from NADH to oxygen directly. Rather the electrons are transferred from NADH to oxygen along a chain of electron carriers collectively called the **electron transport chain** (also called the **respiratory chain**).

The main part of the electron transport chain consists of three large protein complexes embedded in the inner mitochondrial membrane, called **NADH dehydrogenase, the cytochrome bc_1 complex** and **cytochrome oxidase.** Electrons flow from NADH to oxygen through these three complexes as shown in *Fig. 1*. Each complex contains several electron carriers (see below) that work sequentially to carry electrons down the chain. Two small electron carriers are also needed to link these large complexes; **ubiquinone**, which is also called **coenzyme Q** (abbreviated here as **CoQ**), and **cytochrome c** (*Fig. 1*).

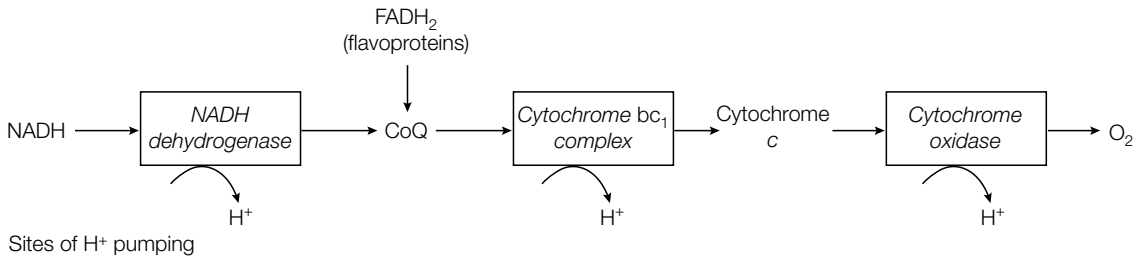

Fig. 1. Overview of the electron transport chain (respiratory chain).

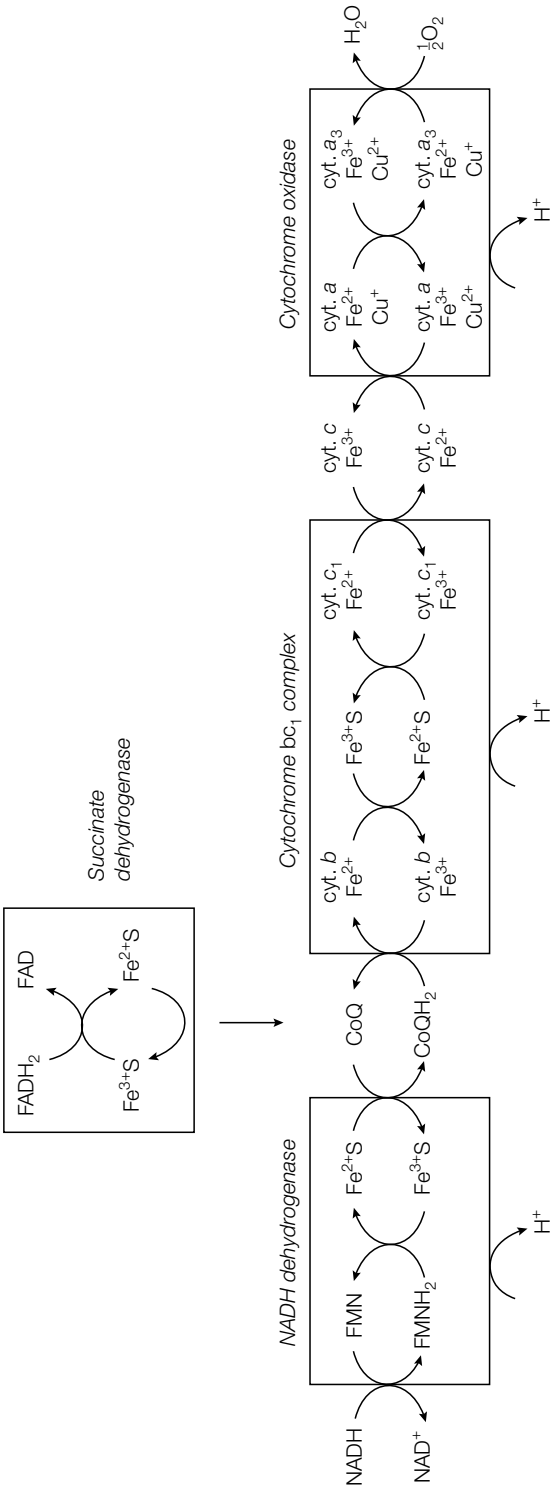

Fig. 2. Details of electron transport.

NADH to NADH dehydrogenase

NADH dehydrogenase (also called **NADH-Q reductase** or **complex I**) consists of at least 30 polypeptides. It binds the NADH and re-oxidizes it to NAD^+, passing the two electrons from NADH to a prosthetic group called **FMN** (**flavin mononucleotide**) (*Fig. 2*) to produce **$FMNH_2$** (see Topic C1 for structure of FMN). Each electron is accepted together with a hydrogen ion, H^+, such that two electrons and two H^+ are accepted in total. The electrons are then transferred, within the NADH dehydrogenase complex, to **iron–sulfur clusters** (FeS) in **iron–sulfur proteins** (also called **nonheme iron proteins**). Several types of FeS clusters exist but in each case the iron atoms are coordinated to inorganic sulfur atoms and the sulfur of cysteine side chains in the protein. Within an FeS cluster, an electron is carried by the iron atom which, on accepting the electron, changes from the Fe^{3+} (ferric) state to the Fe^{2+} (ferrous) state (*Fig. 2*). As the electron is passed to another electron carrier, the iron atom of the FeS cluster changes back again to the Fe^{3+} state.

NADH dehydrogenase to ubiquinone (CoQ)

Electrons from the FeS clusters of NADH dehydrogenase are passed on to ubiquinone (CoQ), a small lipid-soluble molecule in the inner mitochondrial membrane. This molecule can act as an electron carrier by accepting up to two electrons and two H^+ ions. In so doing, ubiquinone (CoQ) is converted to ubiquinol ($CoQH_2$).

Ubiquinol to cytochrome **bc_1** *complex*

When ubiquinol ($CoQH_2$) donates its two electrons to the next carrier in the chain, the **cytochrome bc_1 complex** (also called **cytochrome reductase** or **complex III**), the H^+ ions are released once more. The cytochrome bc_1 complex contains two types of cytochromes, **cytochrome *b*** and **cytochrome c_1**, as well as an FeS protein (*Fig. 2*). A cytochrome is a protein with a bound **heme group** that contains an iron atom (see Topic M4, *Fig. 1*). Different cytochromes have different heme groups, but all cytochromes have the ability to act as electron carriers. As the electron is accepted, the iron atom of the heme group changes from the Fe^{3+} (ferric) state to the Fe^{2+} (ferrous) state. *Figure 2* shows the electrons passing from ubiquinol (QH_2) through the cytochrome *b*, FeS and cytochrome c_1 components of the cytochrome bc_1 complex to the next electron carrier, cytochrome *c*. Since ubiquinol is a two-electron carrier whereas cytochromes are one-electron carriers, the pathway of electron transfer within the cytochrome bc_1 complex is complicated and involves ubiquinol ($CoQH_2$) releasing first one electron and an H^+ ion to become ubisemiquinone ($CoQH^{\bullet}$) and then the second electron and H^+ ion to become ubiquinone (CoQ).

Cytochrome **bc_1** *complex to cytochrome* **c** *to cytochrome oxidase*

Cytochrome *c* is a peripheral membrane protein that is loosely bound to the outer surface of the inner mitochondrial membrane. It binds to the cytochrome bc_1 complex and accepts an electron via an Fe^{3+} to Fe^{2+} transition. Then it binds to cytochrome oxidase and donates the electron, with the iron atom of the heme of cytochrome *c* then reverting to the Fe^{3+} state (*Fig. 2*).

Cytochrome oxidase to oxygen

Cytochrome oxidase (also called **complex IV**) contains two cytochromes (cytochrome *a* and a_3). Cytochrome *a* is paired with a copper atom, Cu_A, and cytochrome a_3 is paired with a different copper atom, Cu_B. During electron

transfer, the iron atoms of the cytochromes cycle between the Fe^{3+} and Fe^{2+} states whilst the copper atoms cycle between Cu^{2+} and Cu^{+}. The cytochrome oxidase reaction is complex; it transfers four electrons from four cytochrome *c* molecules and four H^+ ions to molecular oxygen to form two molecules of water:

$$4 \text{ cyt. } c\ (Fe^{2+}) + 4\ H^+ + O_2 \xrightarrow{\textit{Cytochrome oxidase}} 4 \text{ cyt. } c\ (Fe^{3+}) + 2\ H_2O$$

Formation of an H^+ gradient

All of the electron carriers in the electron transport chain interact according to their redox potentials. Every time that an electron transfer occurs, the accepting carrier has a higher affinity for electrons than the donating carrier. Thus there is a net flow of electrons from NADH (most negative redox potential, least affinity for electrons) to oxygen (most positive redox potential, highest affinity for electrons). This ensures a unidirectional flow of electrons. However, note that each cytochrome, each FeS center and each copper atom can carry only one electron but each NADH donates two electrons. Furthermore, each molecule of oxygen (O_2) needs to accept four electrons to be reduced to a molecule of water, H_2O. The various components are arranged in such a manner as to allow their different electron-handling properties to work in harmony.

The change in redox potential along the chain is a measure of the free energy change occurring (see above). The potential falls (i.e. becomes more positive) throughout the chain but mainly in three large steps that correspond to the three main protein complexes: the NADH dehydrogenase complex, the cytochrome bc_1 complex and the cytochrome oxidase complex. The large free energy change at each of these three steps, and only these three steps, is large enough to pump H^+ ions from the mitochondrial matrix across the inner mitochondrial membrane and into the intermembrane space. Thus, each of these three complexes is an **H^+ pump** driven by electron transport (*Figs 1* and *2*). Overall, therefore, electron transport along the chain from NADH releases energy that is used to create an **H^+ gradient**.

Electron transport from $FADH_2$

Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate in the citric acid cycle (Topic L1). The succinate dehydrogenase contains bound FAD that is reduced to $FADH_2$ in the reaction. The re-oxidation of the $FADH_2$ occurs via **succinate–coenzyme Q reductase** (also called **complex II**), an integral protein of the inner mitochondrial membrane. Succinate dehydrogenase is part of this complex but it also contains FeS clusters. During re-oxidation of $FADH_2$, the two electrons pass from the $FADH_2$ to the FeS clusters and are then passed on to ubiquinone (CoQ; see *Fig. 2*). They then enter the main electron transport chain and cause H^+ ions to be pumped out of the mitochondrion as for the oxidation of NADH. However, succinate–CoQ reductase itself is *not* an H^+ pump because the free energy change of the overall reaction is too small. The $FADH_2$ of other **flavoproteins,** such as mitochondrial glycerol 3-phosphate dehydrogenase in the glycerol 3-phosphate shuttle (see below) and fatty acyl CoA dehydrogenase in fatty acid oxidation (Topic K2), also feed their electrons into the electron transport chain at ubiquinone.

Electron transport inhibitors

Several inhibitors of specific electron carriers are known and were used in the original studies to determine the order of the components in the respiratory chain. For example:

- **rotenone** and **amytal** inhibit electron transport at NADH dehydrogenase and

so prevent NADH oxidation but the oxidation of $FADH_2$ can still occur since this feeds electrons into the chain at CoQ (see *Fig. 1*) (i.e. past the point of inhibition);

- **antimycin A** inhibits electron transport at the cytochrome bc_1 complex;
- **cyanide** (CN^-), **azide** (N_3^-) and **carbon monoxide** (CO) all inhibit cytochrome oxidase.

Oxidative phosphorylation

Oxidative phosphorylation is the name given to the synthesis of ATP (*phosphorylation*) that occurs when NADH and $FADH_2$ are oxidized (hence *oxidative*) by electron transport through the respiratory chain. Unlike substrate level phosphorylation (see Topics J3 and L1), it does not involve phosphorylated chemical intermediates. Rather, a very different mechanism was proposed by Peter Mitchell in 1961, the **chemiosmotic hypothesis**. This proposes that energy liberated by electron transport is used to create a proton gradient across the mitochondrial inner membrane and that it is this that is used to drive ATP synthesis. Thus the proton gradient couples electron transport and ATP synthesis, not a chemical intermediate. The evidence is overwhelming that this is indeed the way that oxidative phosphorylation works. The actual synthesis of ATP is carried out by an enzyme called **ATP synthase** located in the inner mitochondrial membrane (*Fig. 3*).

In summary, the process is as follows. Electron transport down the respiratory chain from NADH oxidation causes H^+ ions to be pumped out of the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space by the three H^+ pumps; NADH dehydrogenase, the cytochrome bc_1 complex and cytochrome oxidase (see above). [Because $FADH_2$ is reoxidized via ubiquinone (see *Figs 1* and *2*), its oxidation causes H^+ ions to be pumped out only by the cytochrome bc_1 complex and cytochrome oxidase and so the amount of ATP made from $FADH_2$ is less than from NADH.] The free energy change in transporting an electrically charged ion across a

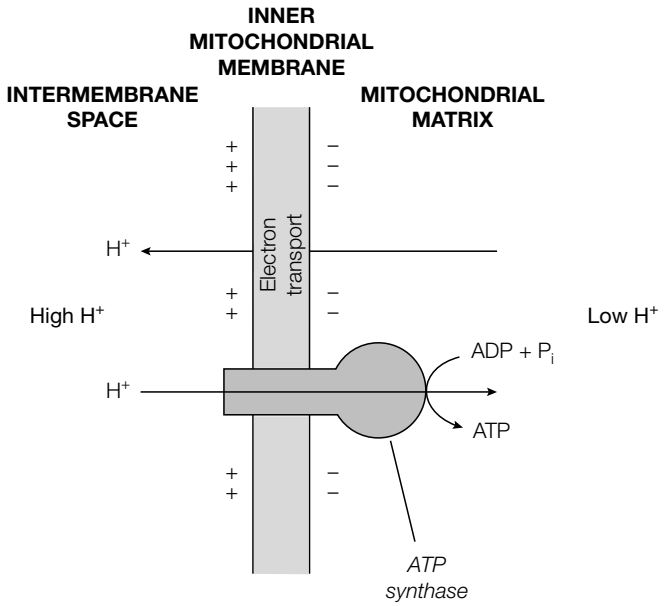

Fig. 3. The mechanism of oxidative phosphorylation.

membrane is related both to its electrical charge and the concentration of the species. The pumping out of the H^+ ions generates a higher concentration of H^+ ions in the intermembrane space and an electrical potential, with the side of the inner mitochondrial membrane facing the intermembrane space being positive (*Fig. 3*). Thus, overall, an **electrochemical proton gradient** is formed. The protons flow back into the mitochondrial matrix through the ATP synthase and this drives ATP synthesis. The ATP synthase is driven by **proton-motive force**, which is the sum of the pH gradient (i.e. the chemical gradient of H^+ ions) and the membrane potential (i.e. the electrical charge potential across the inner mitochondrial membrane). There is some debate over the exact stoichiometry of ATP production; in past years it was believed that 3 ATP were generated per NADH and 2 ATP per $FADH_2$ but some recent measurements have indicated that the numbers of ATP molecules generated may be 2.5 and 1.5, respectively.

ATP synthase as a rotatory engine

The ATP synthase can be seen as spherical projections from the inner membrane (*Fig. 4a*). If mitochondria are subjected to sonic disruption,

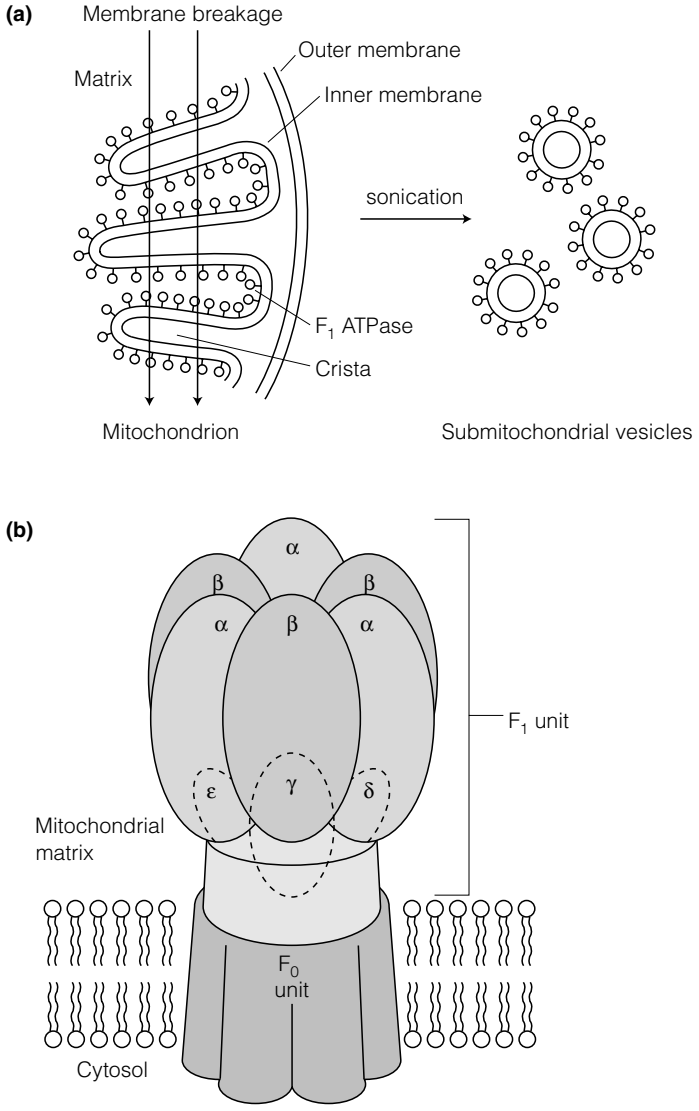

Fig. 4. (a) Sonic disruption (sonication) of mitochondria produces submitochondrial vesicles, (b) schematic representation of the ATP synthase complex.

submitochondrial vesicles are formed in which the spheres of the ATP synthase point outward (*Fig. 4a*). In 1960, Racker showed that the spheres can be removed and that the isolated spheres *hydrolyze* ATP, that is, the spheres have ATPase activity (called **F_1 ATPase**; *Fig. 4b*). F_1 ATPase contains five types of polypeptide in the ratio $(\alpha\beta)_3\gamma\delta\varepsilon$. The stripped submitochondrial vesicles, devoid of the F_1 ATPase, can still transport electrons along the electron transport chain but cannot synthesize ATP. These stripped submitochondrial vesicles contain the other major part of the ATP synthase, called **F_0** (coupling factor 0) which is a proton channel that spans the inner mitochondrial membrane (*Fig. 4b*). Since it is composed of these two major component parts, ATP synthase is also known as **F_0F_1 ATPase**. The stalk between F_0 and F_1 (*Fig. 4b*) contains several additional polypeptides. The complete complex harnesses the energy released by electron transport to drive ATP synthesis whereas alone, without coupling to electron transport, the F_1 component hydrolyzes ATP.

Amazingly it has recently been shown that the F_1 portion of ATP synthase behaves as a rotatory engine; during ATP hydrolysis (and presumably also during ATP synthesis) subunit γ of the F_1 ATPase rotates relative to $(\alpha\beta)_3$. In fact, this is the smallest rotatory engine so far discovered in nature!

Coupling and respiratory control

Electron transport is normally tightly **coupled** to ATP synthesis (i.e. electrons do not flow through the electron transport chain to oxygen unless ADP is simultaneously phosphorylated to ATP). Clearly, it also follows that ATP is not synthesized unless electron transport is occurring to provide the proton gradient. Thus oxidative phosphorylation needs NADH or $FADH_2$, oxygen, ADP and inorganic phosphate. The actual rate of oxidative phosphorylation is set by the availability of ADP. If ADP is added to mitochondria, the rate of oxygen consumption rises as electrons flow down the chain and then the rate of oxygen utilization falls when all the ADP has been phosphorylated to ATP; a process called **respiratory control**. This mechanism ensures that electrons flow down the chain only when ATP synthesis is needed. If the level of ATP is high and the ADP level is low, no electron transport occurs, NADH and $FADH_2$ build up, as does excess citrate, and the citric acid cycle (Topic L1) and glycolysis (Topic J3) are inhibited.

Uncouplers

Some chemicals, such as 2,4-dinitrophenol (DNP), act as **uncoupling agents**, that is, when added to cells, they stop ATP synthesis but electron transport still continues and so oxygen is still consumed. The reason is that DNP and other uncoupling agents are lipid-soluble small molecules that can bind H^+ ions and transport them across membranes (i.e. they are **H^+ ionophores**). Electron transport occurs and pumps out H^+ ions across the inner mitochondrial membrane but DNP in the same membrane carries the H^+ ions back into the mitochondrion, preventing formation of a proton gradient. Since no proton gradient forms, no ATP can be made by oxidative phosphorylation. Rather the energy derived from electron transport is released as heat.

The production of heat by uncoupling is called nonshivering **thermogenesis.** It is important in certain biological situations. For example, uncoupling occurs naturally in brown adipose tissue. This tissue is rich in mitochondria, the inner mitochondrial membranes of which contain a protein called **thermogenin** (or **uncoupling protein**). Thermogenin allows H^+ ions to flow back into mitochondria without having to enter via the ATP synthase and so uncouples electron transport and oxidative phosphorylation, generating heat. The importance of this natural phenomenon is that brown adipose tissue is found in

sensitive body areas of some newborn animals (including humans) where the heat production provides protection from cold conditions. In addition, thermogenesis by brown adipose tissue plays a role in maintaining body temperature in hibernating animals.

Reoxidation of cytosolic NADH

The inner mitochondrial membrane is impermeable to NADH. Therefore NADH produced in the cytoplasm during glycolysis must be reoxidized via a **membrane shuttle**, a combination of enzyme reactions that bypass this impermeability barrier. *Figure 5* shows the **glycerol 3-phosphate shuttle**. Dihydroxyacetone phosphate in the cytosol is reduced to glycerol 3-phosphate, and NADH reoxidized to NAD^+, by cytosolic glycerol 3-phosphate dehydrogenase. The glycerol 3-phosphate diffuses across the inner mitochondrial membrane where it is converted back to dihydroxyacetone phosphate by mitochondrial glycerol 3-phosphate dehydrogenase, a transmembrane protein of the inner mitochondrial membrane. The dihydroxyacetone phosphate then diffuses back to the cytosol. The mitochondrial glycerol 3-phosphate dehydrogenase does not use NAD^+ but instead uses FAD. The enzyme-linked $FADH_2$ ($E.FADH_2$) is then reoxidized by transferring its electrons to ubiquinone in the same inner mitochondrial membrane (see above). Note that the shuttle does not allow cytoplasmic NADH to enter the mitochondrion but its operation effectively transports the two electrons from the NADH into the mitochondrion and feeds them into the electron transport chain. Since the electrons from cytoplasmic NADH actually enter the electron transport chain from $FADH_2$, only about two ATPs are synthesized instead of approximately three ATPs from each NADH that arises inside the mitochondrion from the citric acid cycle (Topic L1) and fatty acid oxidation (Topic K2).

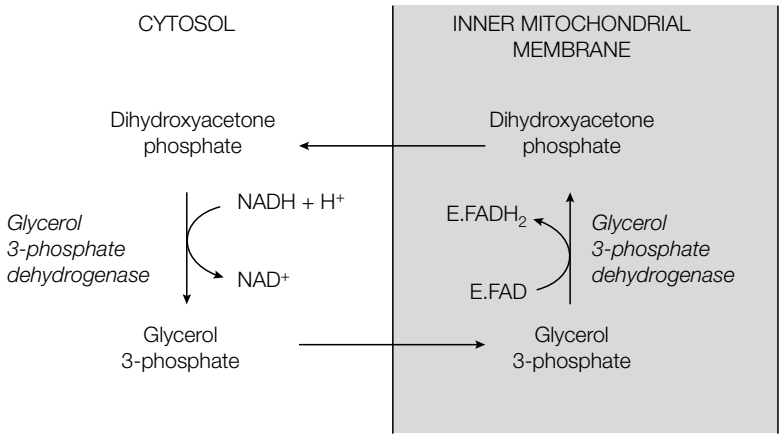

Fig. 5. The glycerol 3-phosphate shuttle.

A similar shuttle, the **malate–aspartate shuttle**, operates in heart and liver (*Fig. 6*). Oxaloacetate in the cytosol is converted to malate by cytoplasmic malate dehydrogenase, reoxidizing NADH to NAD^+ in the process. The malate enters the mitochondrion via a **malate–α-ketoglutarate carrier** in the inner mitochondrial membrane. In the matrix the malate is reoxidized to oxaloacetate by NAD^+ to form NADH. Oxaloacetate does not easily cross the inner mitochondrial membrane and so is transaminated to form aspartate which then exits from the mitochondrion

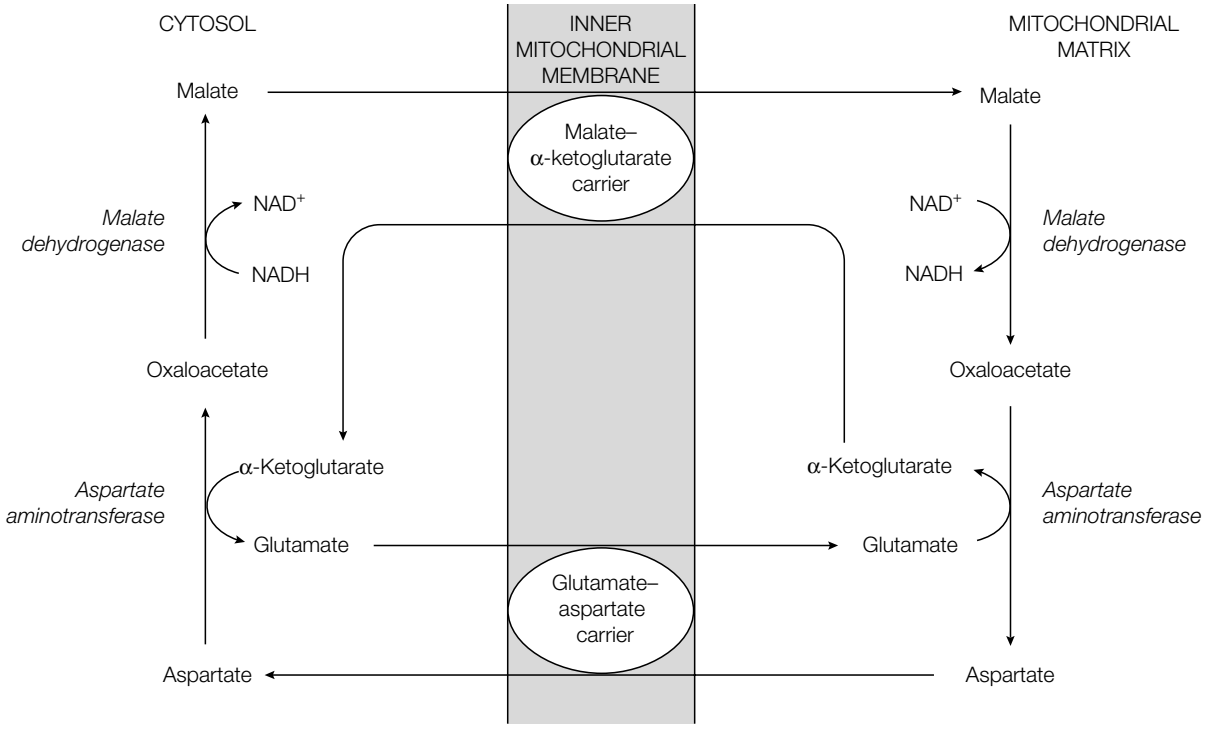

Fig. 6. The malate–aspartate shuttle.

and is reconverted to oxaloacetate in the cytosol, again by transamination. The net result of this cycle of reactions is to transfer the electrons from NADH in the cytosol to NADH in the mitochondrial matrix which is then reoxidized by the electron transport chain.