CC4: Cell Biology
Unit 3: Cytoplasmic organelles II
Mitochondrial Respiratory Chain

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Electron Transport System or Respiratory Chain:

The hydrogen and electron transport system comprise many hydrogen and electron acceptors (Fig. 17-6). In fact oxidation reduction reactions in a biological system involve hydrogen and electron acceptors. Both hydrogen and electrons are passed from one acceptor to another. Thus, there are intermediate and final hydrogen acceptors. The final hydrogen acceptor is molecular oxygen.

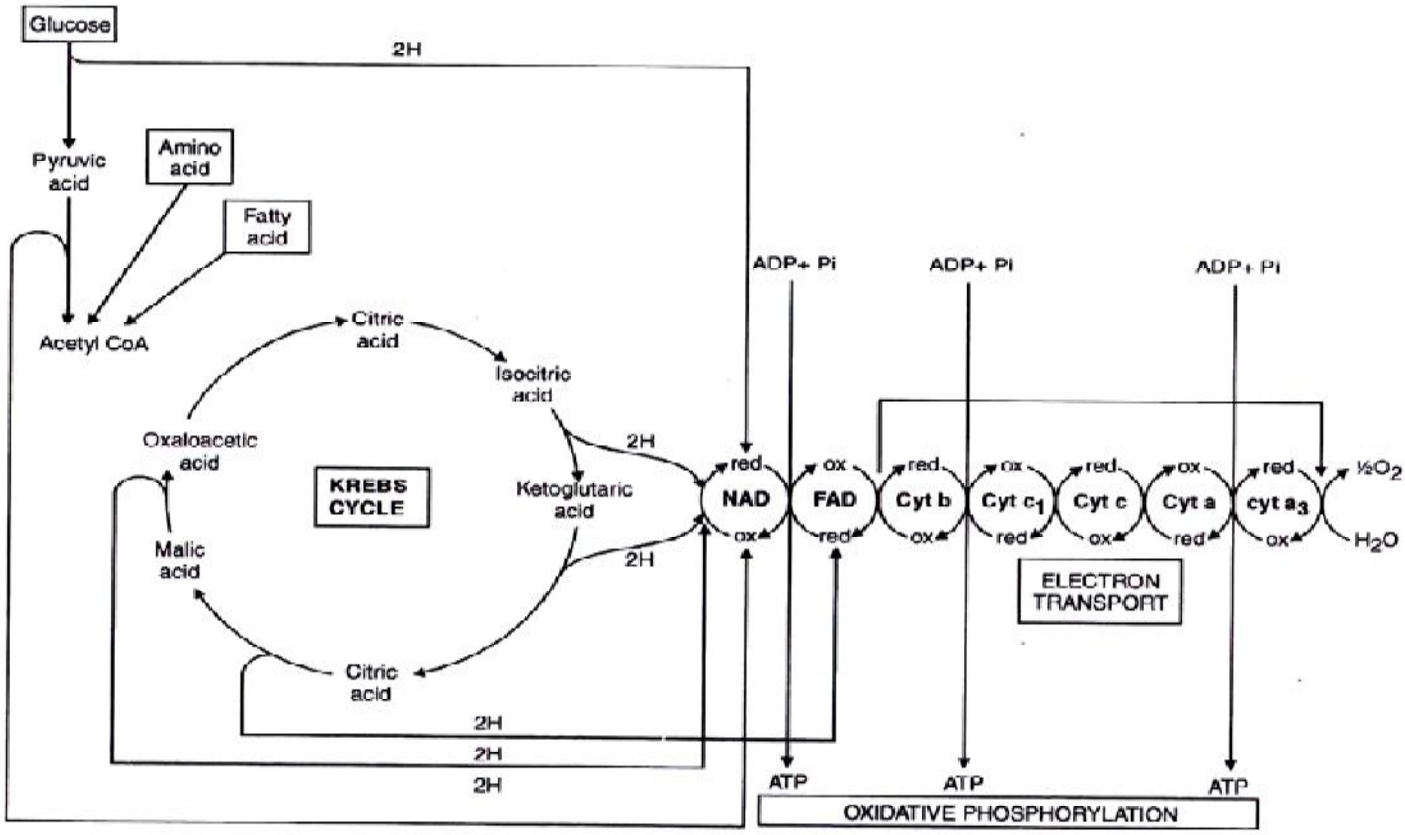


Fig. 17-6. Diagrammatic representation of steps involved in the oxidation of glucose to water and carbon dioxide. The energy thus released is conserved in the form of ATP mostly. ATP is formed during oxidative phosphorylation coupled to electron transport towards molecular oxygen.

Hydrogen (2H) is removed at various stages of glycolysis, oxidation of pyruvic acid and citric acid cycle. NAD is the initial compound for accepting hydrogen except in one instance, where the initial acceptor is FAD of flavoprotein.

The initial acceptors of hydrogen are coenzymes (NAD = nicotinamide adenine dinucleotide; FMN = flavin mononucleotide; CoQ = Co-enzyme Q; UQ = ubiquinone and cytochromes, b, Ci, c, and a, a_3. Some workers have postulated the occurrence of two flavoproteins between NAD and CoQ and these are designated as FPN_1 and FPN_2.

It may be noted that the former flavoprotein has the redox potential similar to that of NAD/NADH system while the latter has a much higher one. A reference may be made to the structure of the mitochondrion and it may be recalled that the respiratory chain is situated in the Inner membrane of the mitochondrion (Fig. 17-7A). When treated with chemicals, the chain breaks down into four complexes and also two mobile carriers.

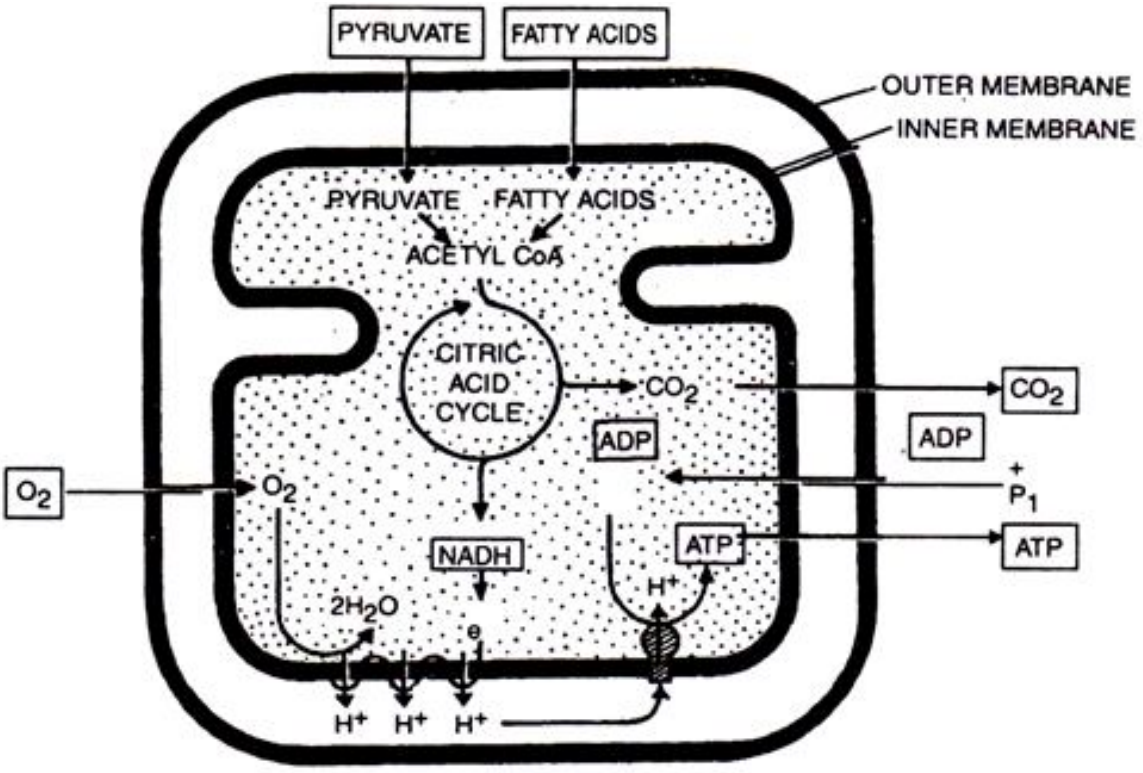


Fig. 17-7 A Summary of mitochondrial metabolism (redrawn from Alberts *et al.* Molecular Biology of the cell, 1994)

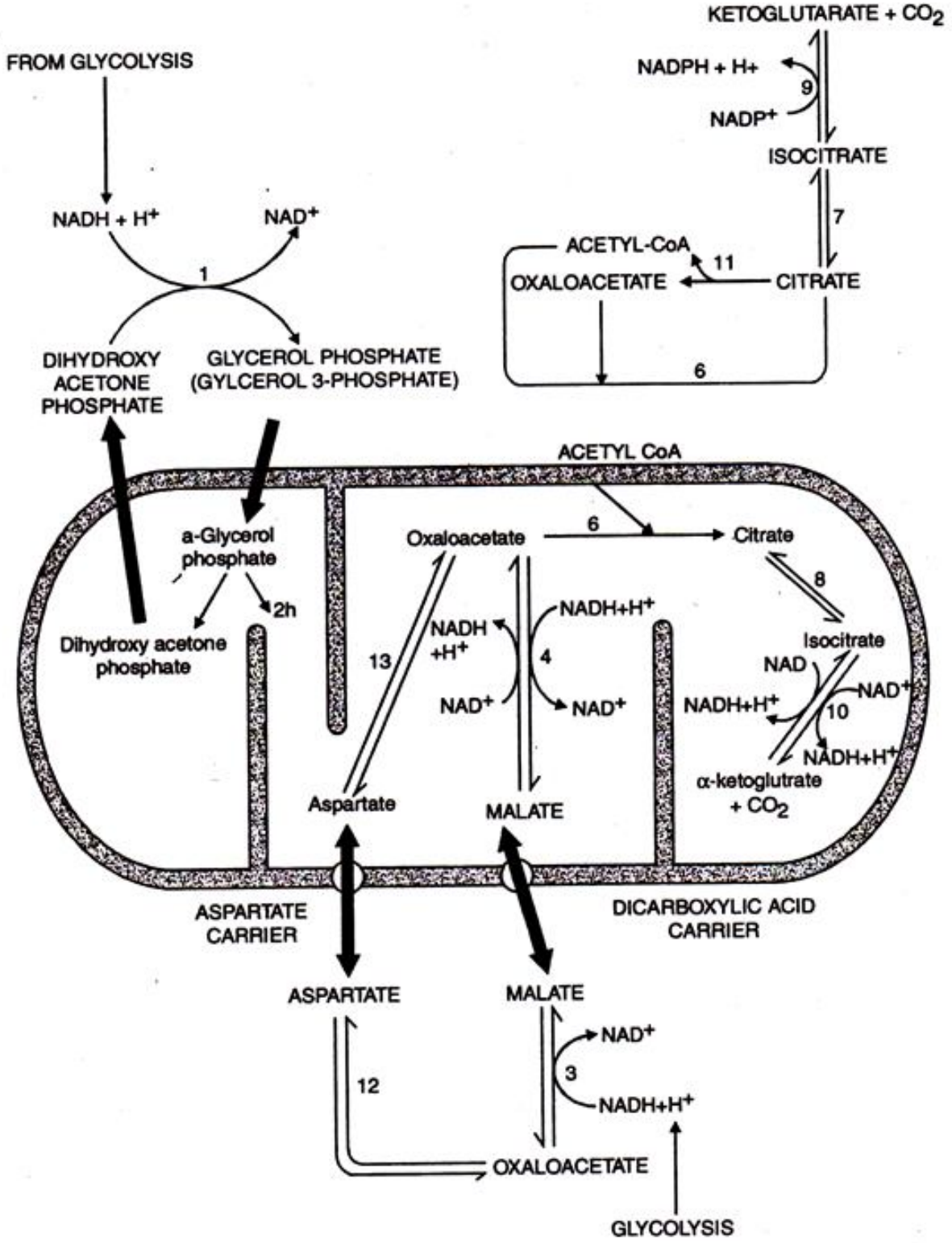


Fig. 17-7. Interchange of metabolites between the mitochondrion and cytoplasm. 1. α-glycerophosphate dehydrogenase 2. malate dehydrogenase (cytoplasmic) 3. citrate synthetase (mitochondrial), 4. aconitase (cytoplasmic) 4B, aconitase (mitochondria) 5. isocitrate dehydrogenase (cyto) 5a, isocitrate dehydrogenase (mit) 6. citrate lyase. 7a. transaminase (cytoplasmic) and 7b. transaminase (mitochondrial).

Complex I:

It consists of NADH dehydrogenase flavoprotein (FPN) with FMN as the prosthetic group. Flavoprotein is combined with non-heme iron of NADH dehydrogenase (Fe NHN).

Complex II:

This consists of succinate dehydrogenase, flavoprotein with FAD prosthetic group. The flavoprotein is combined with non-heme iron of succinate dehydrogenase (Fe NHS).

Mobile carriers exist between complexes I and III (CoQ) and II and III (UQ).

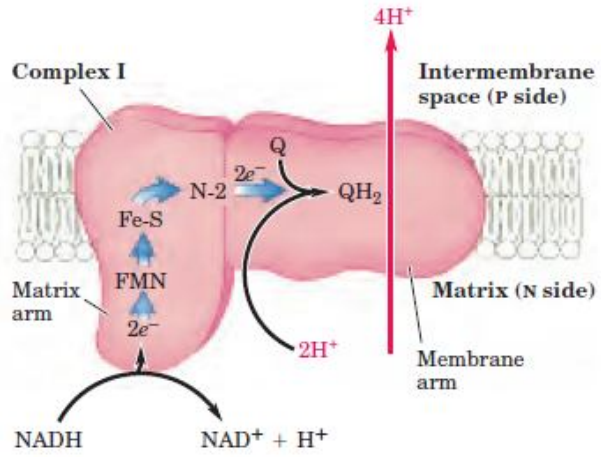


FIGURE 19–9 NADH:ubiquinone oxidoreductase (Complex I). Complex I catalyzes the transfer of a hydride ion from NADH to FMN, from which two electrons pass through a series of Fe-S centers to the iron-sulfur protein N-2 in the matrix arm of the complex. Electron transfer from N-2 to ubiquinone on the membrane arm forms QH_2, which diffuses into the lipid bilayer. This electron transfer also drives the expulsion from the matrix of four protons per pair of electrons. The detailed mechanism that couples electron and proton transfer in Complex I is not yet known, but probably involves a Q cycle similar to that in Complex III in which QH_2 participates twice per electron pair (see Fig. 19–12). Proton flux produces an electrochemical potential across the inner mitochondrial membrane (N side negative, P side positive), which conserves some of the energy released by the electron-transfer reactions. This electrochemical potential drives ATP synthesis.

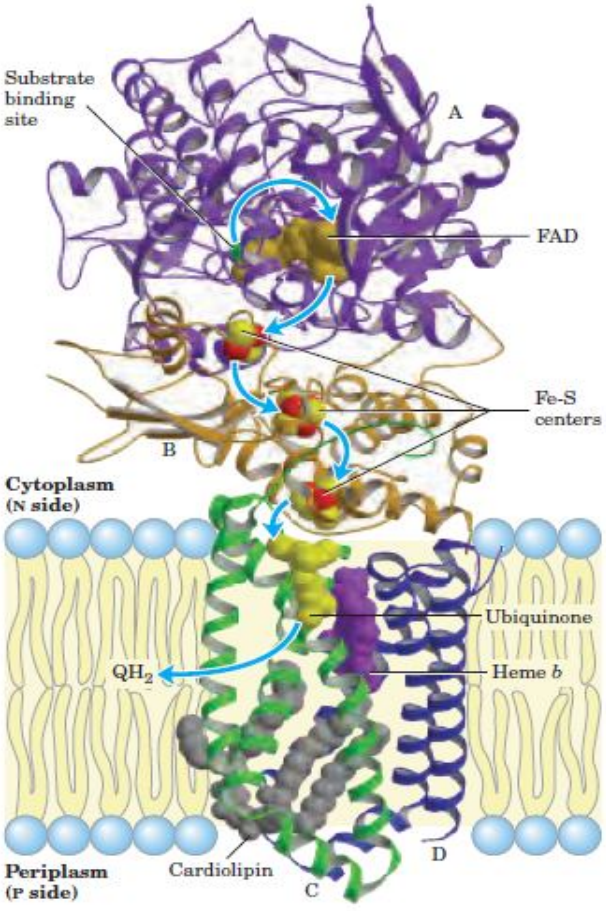


FIGURE 19–10 Structure of Complex II (succinate dehydrogenase) of *E. coli* (PDB ID 1NEK). The enzyme has two transmembrane subunits, C (green) and D (blue); the cytoplasmic extensions contain subunits B (orange) and A (purple). Just behind the FAD in subunit A (gold) is the binding site for succinate (occupied in this crystal structure by the inhibitor oxaloacetate, green). Subunit B has three sets of Fe-S centers (yellow and red); ubiquinone (yellow) is bound to subunit C; and heme *b* (purple) is sandwiched between subunits C and D. A cardiolipin molecule is so tightly bound to subunit C that it shows up in the crystal structure (gray spacefilling). Electrons move (blue arrows) from succinate to FAD, then through the three Fe-S centers to ubiquinone. The heme *b* is not on the main path of electron transfer but protects against the formation of reactive oxygen species (ROS) by electrons that go astray.

Complex III:

It comprises cytochrome b and cytochrome CI. Non-heme iron of complex III (FeNHR) is associated with cytochrome b.

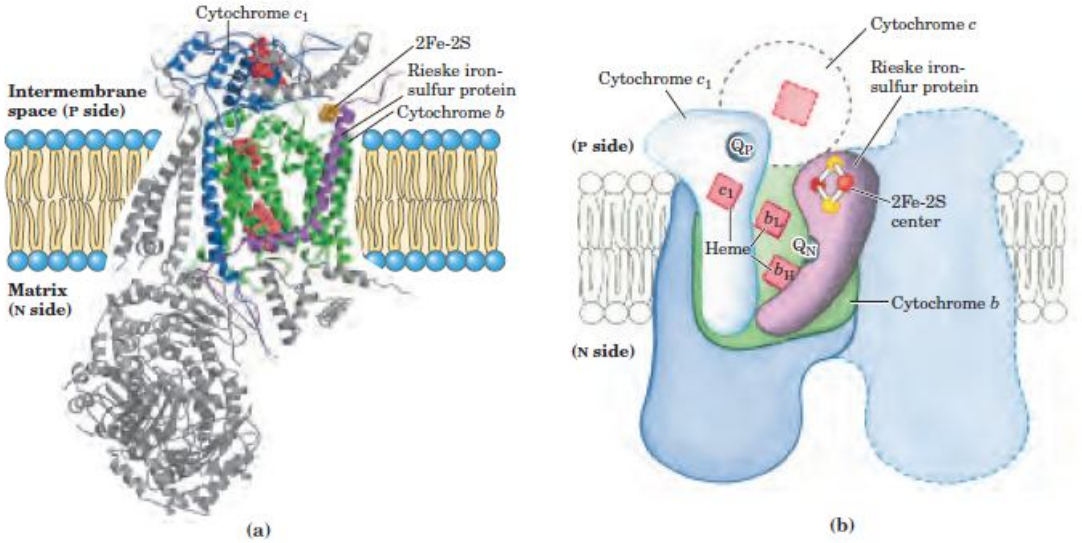


FIGURE 19–11 Cytochrome bc_1 complex (Complex III). The complex is a dimer of identical monomers, each with 11 different subunits. **(a)** Structure of a monomer. The functional core is three subunits: cytochrome b (green) with its two hemes (b_H and b_L, light red); the Rieske iron-sulfur protein (purple) with its 2Fe-2S centers (yellow); and cytochrome c_1 (blue) with its heme (red) (PDB ID 1BGY). **(b)** The dimeric functional unit. Cytochrome c_1 and the Rieske iron-sulfur protein project from the P surface and can interact with cytochrome c (not part of the functional complex) in the intermembrane space. The complex has two distinct binding sites for ubiquinone, Q_N and Q_P, which correspond to the sites of inhibition by two drugs that block oxidative phosphorylation. Antimycin A, which blocks electron flow from heme b_H to Q, binds at Q_N, close to heme b_H on the N (matrix) side of the membrane. Myxothiazol, which prevents electron flow from QH_2 to the Rieske iron-sulfur protein, binds at Q_P, near the 2Fe-2S center and heme b_L on the P side. The dimeric structure is essential to the function of Complex III. The interface between monomers forms two pockets, each containing a Q_P site from one monomer and a Q_N site from the other. The ubiquinone intermediates move within these sheltered pockets.

Complex III crystallizes in two distinct conformations (not shown). In one, the Rieske Fe-S center is close to its electron acceptor, the heme of cytochrome c_1, but relatively distant from cytochrome b and the QH_2-binding site at which the Rieske Fe-S center receives electrons. In the other, the Fe-S center has moved away from cytochrome c_1 and toward cytochrome b. The Rieske protein is thought to oscillate between these two conformations as it is reduced, then oxidized.

Complex IV:

Cytochrome a and a3 and bound copper constitute this complex.

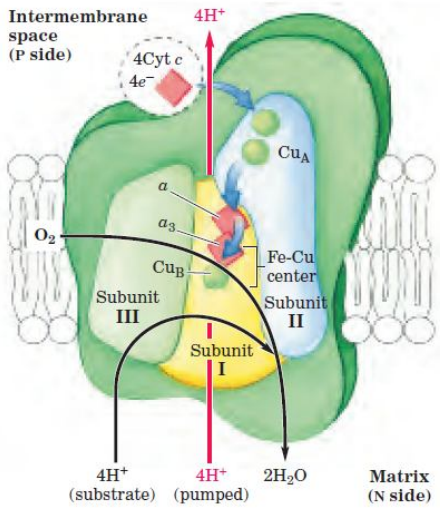


FIGURE 19–14 Path of electrons through Complex IV. The three proteins critical to electron flow are subunits I, II, and III. The larger green structure includes the other ten proteins in the complex. Electron transfer through Complex IV begins when two molecules of reduced cytochrome c (top) each donate an electron to the binuclear center Cu_A. From here electrons pass through heme a to the Fe-Cu center (cytochrome a_3 and Cu_B). Oxygen now binds to heme a_3 and is reduced to its peroxy derivative (O_2^{2-}) by two electrons from the Fe-Cu center. Delivery of two more electrons from cytochrome c (making four electrons in all) converts the O_2^{2-} to two molecules of water, with consumption of four "substrate" protons from the matrix. At the same time, four more protons are pumped from the matrix by an as yet unknown mechanism.

It is pertinent to mention that electrons follow either the pathway of complexes I, III and IV or II or III and IV.

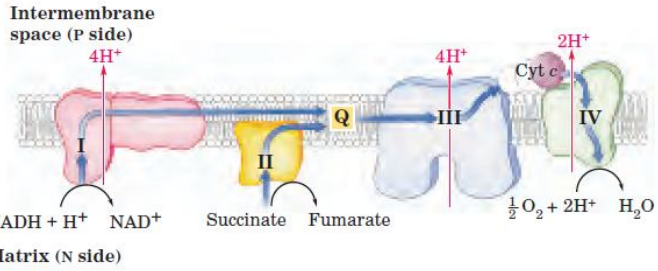
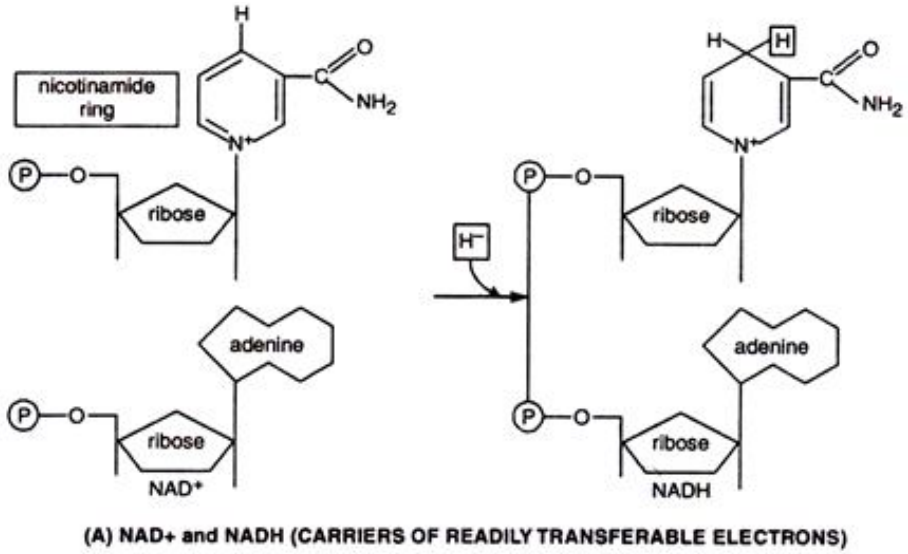


FIGURE 19–15 Summary of the flow of electrons and protons through the four complexes of the respiratory chain. Electrons reach Q through Complexes I and II. QH_2 serves as a mobile carrier of electrons and protons. It passes electrons to Complex III, which passes them to another mobile connecting link, cytochrome *c*. Complex IV then transfers electrons from reduced cytochrome *c* to O_2. Electron flow through Complexes I, III, and IV is accompanied by proton flow from the matrix to the intermembrane space. Recall that electrons from β oxidation of fatty acids can also enter the respiratory chain through Q (see Fig. 19–8).

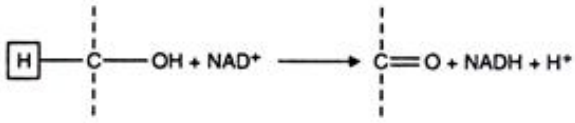
In the following a brief account of the various initial hydrogen acceptors is given:

Nicotinamide Adenine Dinucleotide (NAD):

Hydrogen released from substrates other than succinate is accepted by NAD^+ or NADP. The latter is phosphorylated form of NAD. The former was once referred to as Coenzyme I and the latter as Coenzyme II. Two nucleotides are there in NAD: nicotinamide ribose phosphate and adenine ribose phosphate (Fig. 17-7B).



(A) NAD+ and NADH (CARRIERS OF READILY TRANSFERABLE ELECTRONS)



(B) AN EXAMPLE OF A REACTION INVOLVING NAD+ AND NADH

Fig. 17-7 B Structures and function of NAD^+ (nicotinamide adenine dinucleotide) and NADH (reduced form of NAD^+)

It is worth remembering that N of nicotinamide is positively charged. Thus, in many instances NAD is also written as NAD^+. Hydrogen atoms from the substrate NAD^+ or $NADP^+$ are reduced to $NADH^+ + H^+$. NAD is also referred to as acceptor molecule.

Further NADH is referred to as a reduced form of NAD. Similarly NADP is reduced to $NADPH_2$. The FAD of flavoprotein also accepts hydrogen atoms from succinic acid and is converted to $FADH_2$.

Flavoproteins:

Flavoproteins are present in the complexes I and II. The flavoprotein of complex I is referred to as NADH dehydrogenase while the flavoprotein of complex II is called succinate dehydrogenase. The two complexes contain prosthetic groups FMN and FAD, respectively. FAD accepts 2H from succinate and is thus reduced to $FADH_2$. Likewise FMN also accepts 2H from $NADH_2$ and is reduced to $FMNH_2$.

Coenzyme Q or Ubiquinone:

This is the carrier between flavoproteins and cytochrome. It is found in the mitochondria in the form of oxidized quinone under aerobic conditions. However, under anaerobic conditions it is found as reduced quinone. The reduced form is also called hydroquinone. Coenzyme Q has a polyisoprenoid side chain and the number of isoprenoid units vary from 6-10 in coenzyme Q.

Cytochromes:

As many as 30 cytochromes have been identified and they are named after their resemblance to the original three types e.g., a_t, a_3, c_1, c_2, c_3, etc. It is generally viewed that cytochromes a and a_3 may be separate pigments or may be a single protein with two prosthetic groups.

Cytochrome oxidase represents a_3. The cytochromes are large complex molecules of porphyrins containing heme, a tetrapyrrole compound with Fe. Ionic iron exists as ferrous (Fe^{++}) or ferric (Fe^{+++}) and these are interconvertible by a loss or gain of an electron. Fe of cytochrome oxidase acts as an electron donor or acceptor and thus the atom is reduced or oxidized.

Redox Potential:

Redox potential is the tendency for the release or acceptance of electrons. The world is derived from reduction-oxidation and represents the ratio of the reduced form to the oxidized form. It is expressed as volts.

Different compounds of the hydrogen transport system possess different redox potentials. As a general rule electrons flow from the high electronegative components to the high electropositive components. Thus, a compound which is a reducing agent in one reaction becomes an oxidizing agent in another.

Redox System of the Respiratory Chain:

Based on the redox potential of coenzymes and prosthetic groups, the enzymes of the respiratory chain may be arranged. This sequence (Fig. 17-8) is well documented in animals while in plants it still remains to be confirmed.

As will be made out the first redox system is $NADH^+ H^+ /NAD^+$ which has a strong negative potential (—0.32 volt), shows the highest electron pressure and thus the largest reduction potential. Hydrogen is therefore, easily passed on to flavoproteins which occupy lower position on the redox scale and they accept hydrogen (E_0 = 0.00 volt). This decrease of the electron pressure equals a change in the free energy of ΔG^0 = 12000 cal/mole.

By following the energy gradient hydrogen has moved to lower or more positive carrier. It is believed that $NADH^+H^+$ emanating from mitochondria can donate its hydrogen directly to the respiratory chain.

The hydrogen of $NADH+H^+$ obtained during glycolysis in the cytoplasm in all probability enters the mitochondria having been attached to some metabolites which deliver it to the respiratory chain. NAD system seems to be present to a relatively large extent in mitochondria. The proportion of NAD to mitochondria is 40: 1 in plant mitochondria.

NADP system appears to be missing. The flavoprotein contains FMN or FAD. They enable the enzymes to transfer hydrogen. Hydrogen may be removed directly from the substrate or accepted from $NADH+H^+$. There is also NADH-cytochrome C reductase which is a complex enzyme system.

It is firmly attached to mitochondria membranes and is difficult to isolate and characterize. Possibly there are several NADH reductases which differ in their prosthetic groups, in the number of iron atoms, active SH group, etc. The reduced flavoproteins are oxidized by a quinone and converted to hydroquinone. A coenzyme Q seems also to be active in the respiratory chain.

Cytochromes of several types cause oxidation of reduced quinones or reduced flavoproteins. They are c, b, a and are apparently groups of closely related components. As will be seen from Fig. 17-9 the transfer of hydrogen atoms changes to pure electron transport because hydrogen is ionized.

In general two cytochrome equivalents are needed for one transfer step. No specific function is ascribed to cytochrome b and it is believed to have specific role in plants. Cytochrome a finally catalyzes the transfer of electrons to oxygen. This enzyme can directly react with oxygen and

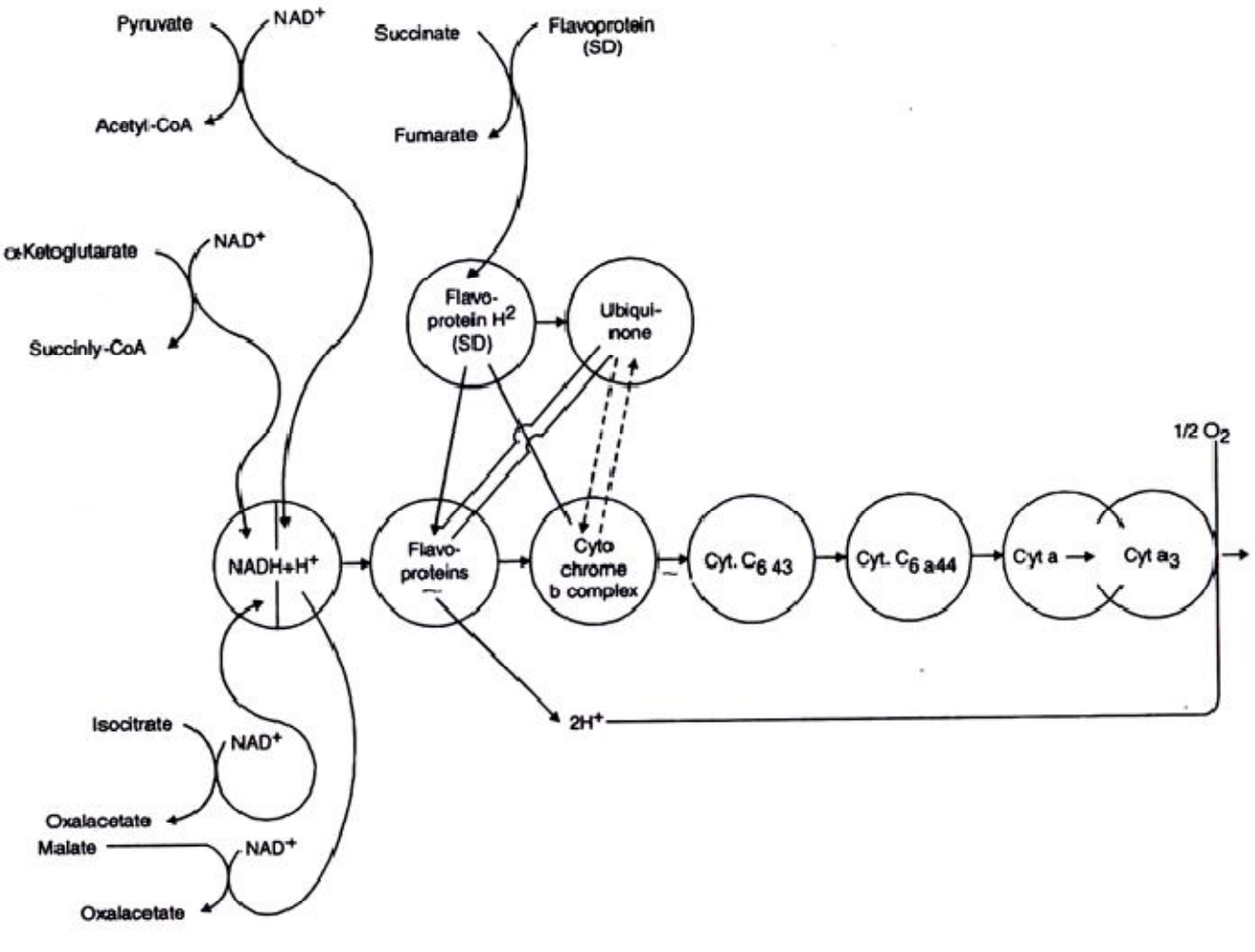


Fig. 17-9. The details of the respiratory chain of higher plants. Ubiquinone appears to serve as electron reservoir.

acts like anendoxidase. The oxygen ion (Cr) reacts with two hydrogen ions (H^+) and water is formed.

In plants the knowledge of the plant mitochondria respiratory chain is limited. The possible mechanism is shown in Fig. 17-9. There are, however, differences with regard to redox enzymes involved especially of the cytochrome components.

Hydrogen and Electrons Transfer:

From the substrate the hydrogen atoms are transferred to NAD and from the latter they are transferred to FMN of flavoprotein 1. Then the hydrogen atom undergoes ionization and thus it is spilt into electron and proton.

In further stages only electrons are transferred to coenzyme Q. From coenzymes Q it goes to cytochromes b, c_1, c, a, and a_3. In this process the proton is released free. Following is the sequence of compounds in the hydrogen transport system:

NAD → FMN → CoQ → Cyt.b → Cyt. c1 → Cyt.c → Cyt. a → Cyt. a_3

As the hydrogen atom or electron passes down the chain, at each step, there is simultaneous oxidation of one coenzyme and reduction of another. The compounds of the hydrogen transfer system act as oxidizing and then reducing agents. Thus, substance in the respiratory chain is alternately oxidized and reduced. $NADH_2$ and $FMNH_2$ lose hydrogen.

But coenzyme Q and different cytochromes lose electrons to become oxidized. Two electrons are released at one time. The different cytochromes accept one electron at one time. Consequently two molecules each of the enzymes and the cytochrome system accept the two molecules.

After transfer from the cytochrome a_3, both an electron and a proton are combined to produce hydrogen. This hydrogen is ultimately accepted by oxygen molecule and thus water is formed. The need and the essentiality of oxygen for respiration would be clear soon after.