5

Mitochondria are the power station of the cell

In the process of biological oxidation, substrates such as carbohydrates are oxidized to form water and CO_2. Biological oxidation can be seen as a reversal of the photosynthesis process. It evolved only after oxygen accumulated in the atmosphere during photosynthesis. Both biological oxidation and photosynthesis serve the purpose of generating energy in the form of ATP. Biological oxidation involves a transport of electrons through a mitochondrial electron transport chain, which is in part similar to the photosynthetic electron transport discussed in Chapter 3. The present chapter will show that the machinery of mitochondrial electron transport is also assembled of three modules. The second complex has the same basic structure as the cytochrome-b_6/f complex of the chloroplasts. As in photosynthesis, the mitochondrial oxidative electron transport and ATP synthesis are coupled to each other via a proton gradient. The synthesis of ATP proceeds by an F-ATP synthase, which was described in Chapter 4.

5.1 Biological oxidation is preceded by a degradation of substrates to form bound hydrogen and CO_2

The overall reaction of biological oxidation is equivalent to a combustion of substrates. In contrast to technical combustion, however, biological oxidation proceeds in a sequence of partial reactions, which allows the utilization of the major part of the free energy for ATP synthesis.

The principle of biological oxidation was formulated in 1932 by the Nobel Prize winner Heinrich Wieland (Germany):

$$XH_2 + 1/2\,O_2 \rightarrow X + H_2O$$

First, hydrogen is removed from substrate XH_2 and afterwards oxidized to water. The oxidation of carbohydrates $[CH_2O]_n$ involves a degradation by reaction with water to form CO_2 and bound hydrogen [H], which is oxidized to water:

$$[CH_2O] + H_2O \rightarrow CO_2 + 4[H]$$

$$4[H] + O_2 \rightarrow 2H_2O$$

In 1934 Otto Warburg (Berlin, winner of the 1931 Nobel Prize in Medicine) showed that the transfer of bound hydrogen from substrates to the site of oxidation occurs in the form of **NADH**. From studies with homogenates from pigeon muscles, Hans Krebs formulated the **citrate cycle** (also called the Krebs cycle) in England in 1937, as a mechanism for substrate degradation yielding NADH for biological oxidation. In 1953 he was awarded the Nobel Prize in Medicine for this discovery. The operation of the citrate cycle will be discussed in detail in section 5.3.

5.2 Mitochondria are the sites of cell respiration

Light microscopic studies of many different cells revealed small granules, with an appearance similar to bacteria. In 1898 the botanist Carl Benda (Berlin) named these granules **mitochondria** ("threadlike bodies"). For a long time, however, the function of these mitochondria remained unclear.

As early as 1913, Otto Warburg realized that cell respiration involves the function of granular cell constituents. He succeeded in isolating a protein from yeast that he termed "**Atmungsferment**" (respiratory ferment), which catalyzes the oxidation by oxygen. He also showed that iron atoms are involved in this catalysis. In 1925 David Keilin from Cambridge (England) discovered the cytochromes and their participation in cell respiration. Using

a manual spectroscope, he identified the cytochromes-*a*, -a_3, -*b*, and -*c* (Fig. 3.24). In 1928 Otto Warburg showed that his "Atmungsferment" contained **cytochrome-a_3**. A further milestone in the clarification of cell respiration was reached in 1937, when Hermann Kalckar (USA) observed that the synthesis of ATP in aerobic systems depends on the consumption of oxygen. The interplay between cell respiration and ATP synthesis, termed **oxidative phosphorylation**, was now apparent. In 1948 Eugene Kennedy and Albert Lehninger (USA) showed that mitochondria contain the enzymes of the citrate cycle and oxidative phosphorylation. These findings demonstrated the function of the mitochondria as the **power station of the cell**.

Mitochondria form a separated metabolic compartment

Like plastids, mitochondria also form a separated metabolic compartment. The structure of the mitochondria is discussed in section 1.4. Figure 5.1 provides an overview of mitochondrial metabolism. The degradation of substrates to CO_2 and hydrogen (the latter bound to the transport metabolite NADH) takes place in the mitochondrial matrix. NADH thus formed diffuses through the matrix to the mitochondrial inner membrane and is oxidized there by the **respiratory chain**. The respiratory chain comprises

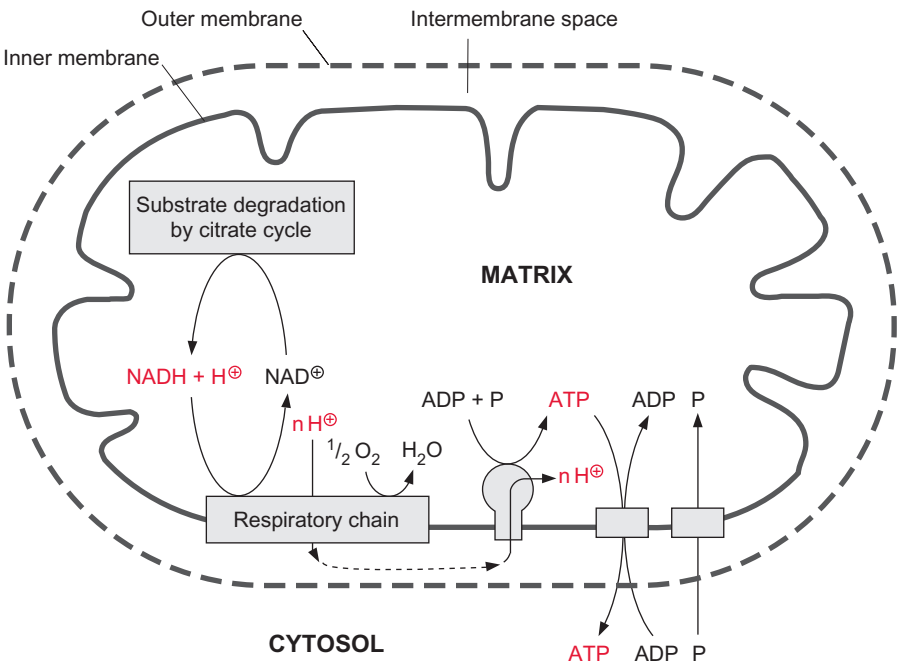

Figure 5.1 Schematic presentation of the mitochondrial energy metabolism.

a sequence of redox reactions by which electrons are transferred from NADH to oxygen. As in the photosynthetic electron transport, the mitochondrial electron transport by the respiratory chain releases free energy which is used to generate a proton gradient. This in turn drives the synthesis of ATP, which is exported from the mitochondria and provides the energy required for cellular metabolism. This process is universal and functions in the mitochondria of all eukaryotic cells.

5.3 Degradation of substrates applicable for biological oxidation takes place in the matrix compartment

Pyruvate, which is synthesized by the glycolytic catabolism of carbohydrates in the cytosol, is the starting compound for substrate degradation by the citrate cycle (Fig. 5.2). Pyruvate is first oxidized to acetate (in the form of acetyl coenzyme A), which is then completely degraded to CO_2 by the citrate cycle, yielding 10 reducing equivalents [H] to be oxidized by the respiratory chain to generate ATP. Figure 5.3 shows the reactions of the citrate cycle.

Pyruvate is oxidized by a multienzyme complex

Pyruvate oxidation is catalyzed by the **pyruvate dehydrogenase complex**, a multienzyme complex located in the mitochondrial matrix. It consists of three different catalytic subunits: **pyruvate dehydrogenase**, **dihydrolipoyl transacetylase**, and **dihydrolipoyl dehydrogenase** (Fig. 5.4). The pyruvate dehydrogenase subunit contains **thiamine pyrophosphate** (**TPP**, Fig. 5.5A) as the prosthetic group. The reactive group of TPP is the thiazole ring. Due to the presence of a positively charged N atom, the thiazole ring contains an acidic C-atom. After dissociation of a proton, a carbanion is formed,

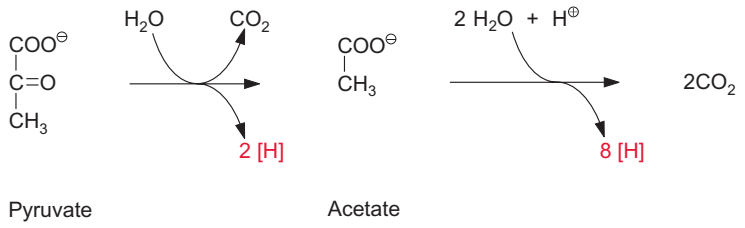

Figure 5.2 Overall reaction of the oxidation of pyruvate by mitochondria. The acetate is formed as acetyl coenzyme A. [H] represents bound hydrogen in NADH and $FADH_2$, respectively.

which binds to the carbonyl group of the pyruvate. The positively charged N atom of the thiazole ring enhances the decarboxylation of the bound pyruvate and hydroxethyl-TPP is formed (Fig. 5.4). The hydroxethyl group is now transferred to lipoic acid.

Lipoic acid is the prosthetic group of the dihydrolipoyl transacetylase subunit. It is covalently bound by its carboxyl group to a lysine residue of the enzyme protein via an amide bond (Fig. 5.5B). The lipoic acid residue is attached to the protein by a long carbon chain and therefore able to react with the various reaction sites of the multienzyme complex. Lipoic acid is equipped with two S atoms linked by a disulfide bond. When the hydroxyethyl residue is transferred to the lipoic acid residue, lipoic acid is

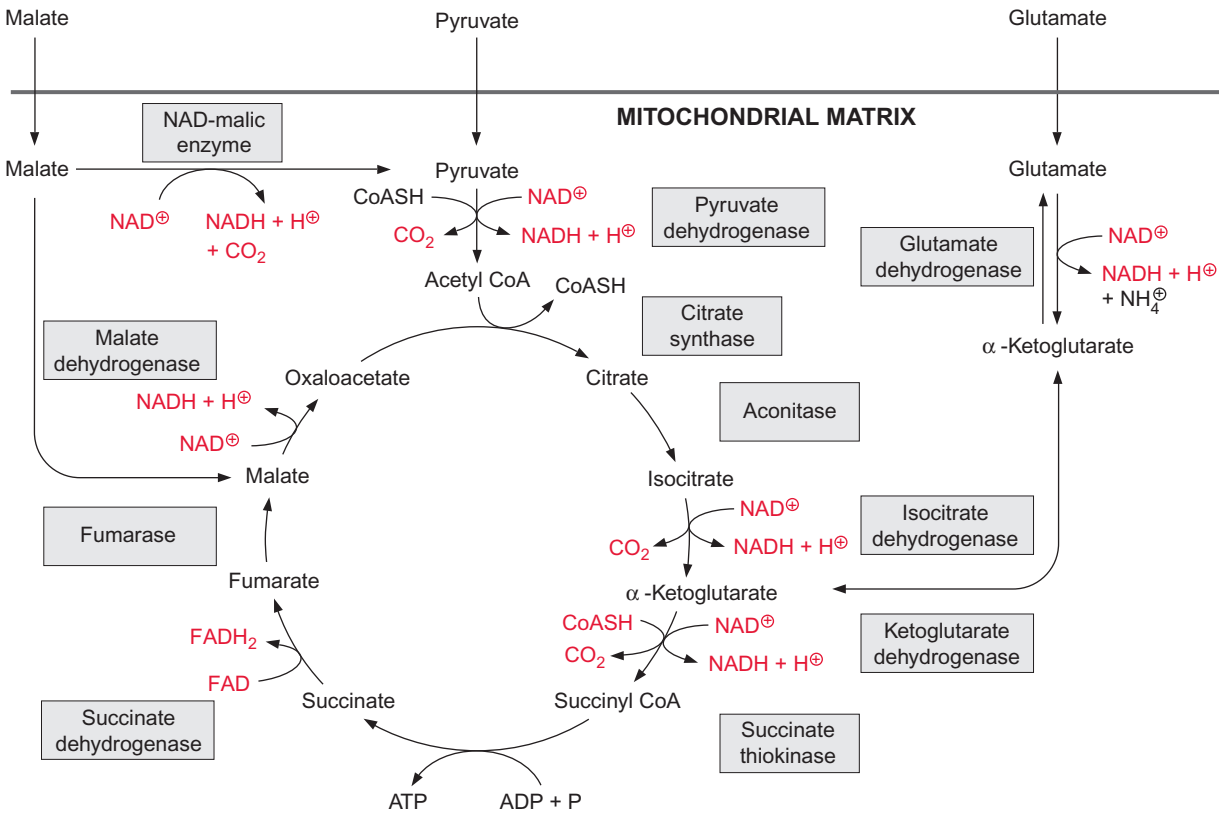

Figure 5.3 Schematic presentation of the citrate cycle. The enzymes are all localized in the mitochondrial matrix, with the exception of succinate dehydrogenase, which is located in the inner mitochondrial membrane. NAD-malic enzyme in the mitochondrial matrix allows plant mitochondria to also oxidize malate via the citrate cycle when no pyruvate is delivered by glycolysis. Glutamate dehydrogenase enables mitochondria to oxidize glutamate.

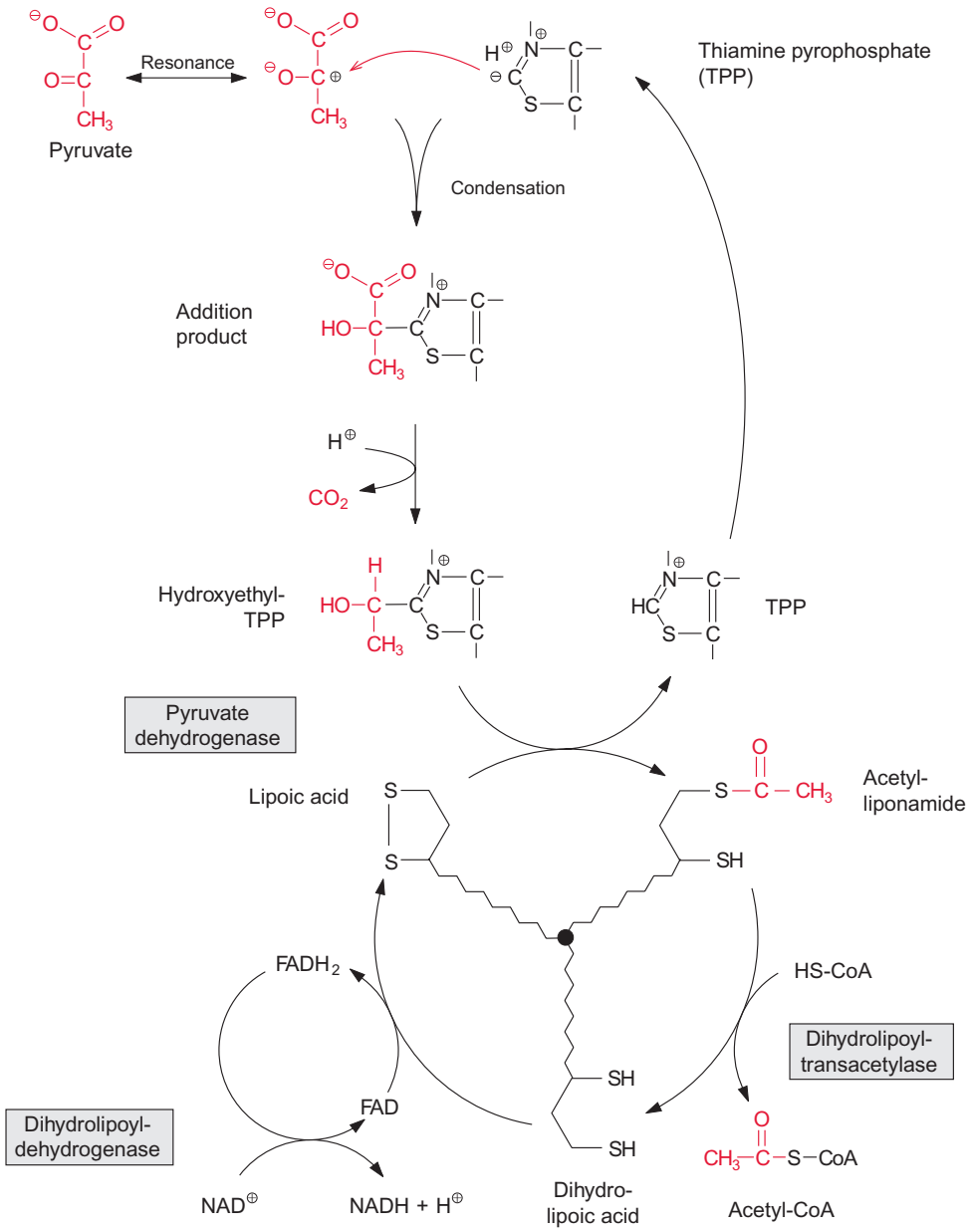

Figure 5.4 Oxidation of pyruvate by the pyruvate dehydrogenase complex, consisting of the subunits pyruvate dehydrogenase (with the prosthetic group thiamine pyrophosphate), dihydrolipoyl transacetylase (prosthetic group lipoic acid), and dihydrolipoyl dehydrogenase (prosthetic group FAD). The reactions of the cycle are described in the text.

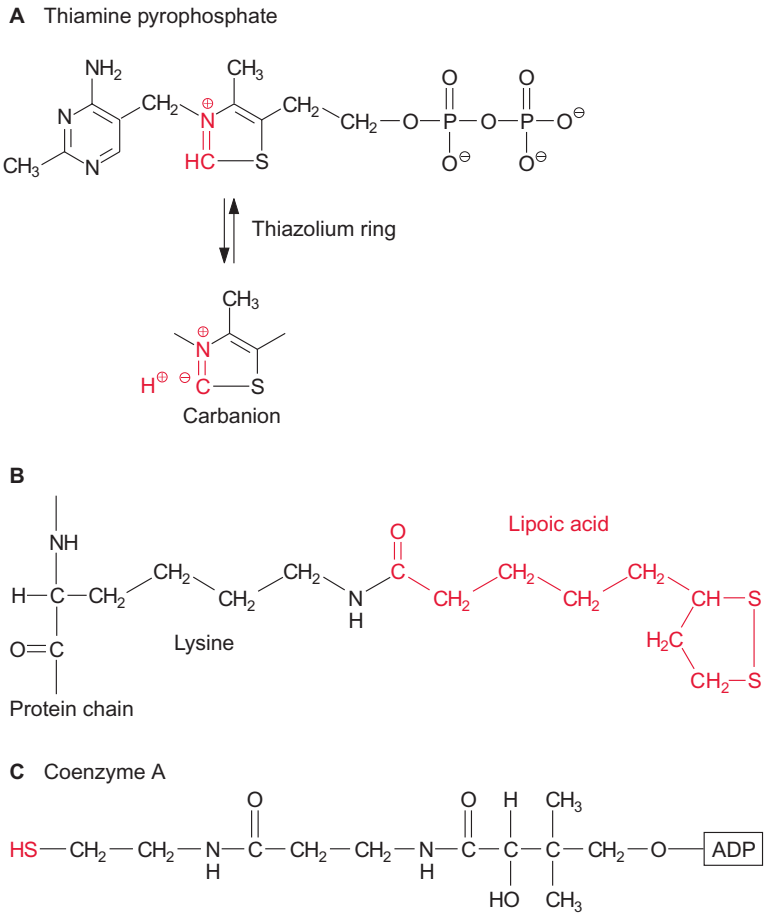

Figure 5.5 Reaction partners of pyruvate oxidation: A. Thiamine pyrophosphate; B. Lipoic amide; C. Coenzyme A.

reduced to dihydrolipoic acid and the hydroxyethyl residue is oxidized to an acetyl residue. The latter is now attached to the dihydrolipoic acid by a thioester bond. Thioester bonds are rich in energy, and store the energy released during oxidation of the carbonyl group. The acetyl residue is then transferred by dihydrolipoyl transacetylase to the sulfhydryl group of coenzyme A (Fig. 5.5C) to synthesize acetyl coenzyme A. **Acetyl CoA**—also called active acetic acid—was discovered by Feodor Lynen from Munich (1984 Nobel Prize in Medicine). Dihydrolipoic acid is reoxidized to lipoic acid by dihydrolipoyl dehydrogenase and NAD^+ is reduced to NADH via **FAD** (see Fig. 5.16). A pyruvate dehydrogenase complex is also present in the chloroplasts and its function in lipid biosynthesis will be discussed in section 15.3.

Acetate is completely oxidized in the citrate cycle

Acetyl coenzyme A enters the citrate cycle and condenses with oxaloacetate to citrate (Fig. 5.6). This reaction is catalyzed by the enzyme **citrate synthase**. The energy of the thioester group promotes the removal of a proton of the acetyl residue, and the carbanion thus formed binds to the carbonyl carbon of oxaloacetate. Subsequent release of CoA-SH makes the reaction irreversible. The enzyme **aconitase** (Fig. 5.7) catalyzes the reversible isomerization of citrate to isocitrate. In this reaction, first water is released, and the *cis*-aconitate thus formed remains bound to the enzyme and is then isomerized to isocitrate by the addition of water. In addition to the mitochondrial aconitase, there is also an isoenzyme of aconitase in the cytosol of plant cells.

Oxidation of isocitrate to α-ketoglutarate by **NAD isocitrate dehydrogenase** (Fig. 5.8) results in the formation of NADH. Oxalosuccinate is formed as intermediate, tightly bound to the enzyme to be decarboxylated to **α-ketoglutarate** (also termed 2-oxo-glutarate). This **oxidative decarboxylation** is an irreversible reaction. Besides the NAD-isocitrate dehydrogenase, mitochondria also contain an NADP-isocitrate dehydrogenase.

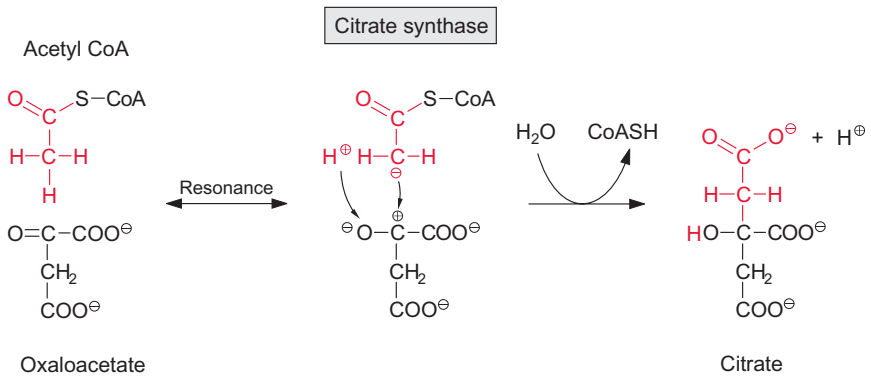

Figure 5.6 Condensation of acetyl CoA with oxaloacetate to synthesize citrate catalyzed by citrate synthase.

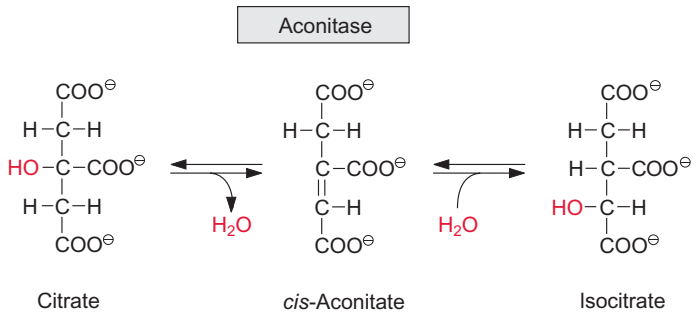

Figure 5.7 Isomerization of citrate to isocitrate catalyzed by aconitase.

NADP-isocitrate dehydrogenases also occur in the chloroplast stroma and in the cytosol. The function of the latter enzyme will be discussed in section 10.4.

Oxidation of α-ketoglutarate to succinyl-CoA (Fig. 5.8) is catalyzed by the **α-ketoglutarate dehydrogenase multienzyme complex**. This complex contains thiamine pyrophosphate, lipoic acid, and FAD, analogously to the pyruvate dehydrogenase multienzyme complex which catalyzes the reaction of pyruvate to acetyl CoA.

The thioester bond of the succinyl CoA is rich in energy. In the **succinate thiokinase** reaction, the free energy released upon the hydrolysis of this thioester is utilized to form ATP (Fig. 5.9). It may be noted that in animal metabolism the mitochondrial succinate thiokinase reaction yields GTP. The succinate formed is oxidized by **succinate dehydrogenase** to synthesize fumarate. Succinate dehydrogenase is the only enzyme of the citrate cycle that is not located in the matrix, but in the mitochondrial inner membrane, with its succinate binding site accessible from the matrix (section 5.5). Reducing equivalents ($FADH_2$) derived from succinate oxidation are transferred to ubiquinone. Catalyzed by **fumarase**, water reacts by *trans*-addition with the C-C double bond of fumarate to form L-malate. This is a reversible reaction (Fig. 5.9). Oxidation of malate by **malate dehydrogenase**, yielding oxaloacetate and NADH, is the final step in the citrate cycle (Fig. 5.9). The reaction equilibrium of this reversible reaction favors strongly the educt malate.

$$\frac{[\text{NADH}] \cdot [\text{oxaloacetate}]}{[\text{NAD}^+] \cdot [\text{malate}]} = 2.8 \cdot 10^{-5} (\text{pH } 7)$$

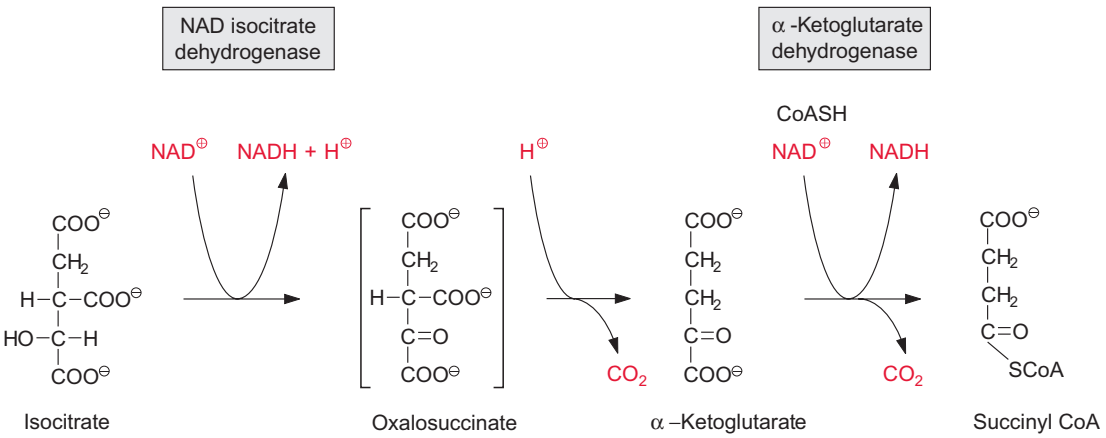

Figure 5.8 Oxidation of isocitrate to synthesize succinyl CoA catalyzed by NAD isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.

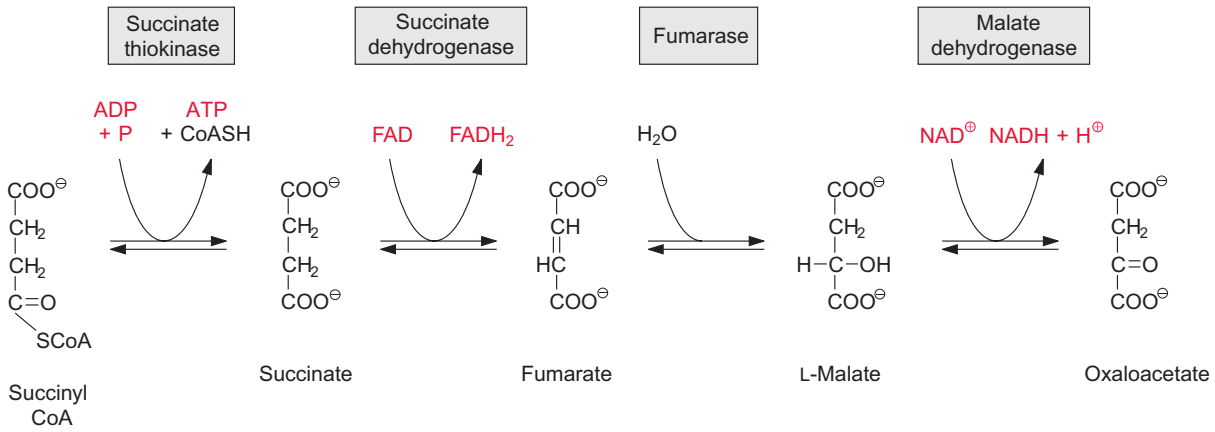

Figure 5.9 Conversion of succinyl CoA to oxaloacetate catalyzed by succinate thiokinase, succinate dehydrogenase, fumarase, and malate dehydrogenase.

Due to this equilibrium reaction, it is essential for an efficient operation of the citrate cycle that the citrate synthase reaction is irreversible. In this way oxaloacetate can be withdrawn from the malate dehydrogenase equilibrium to further support the reactions of the citrate cycle. Isoenzymes of malate dehydrogenase also occur outside the mitochondria. Both the cytosol and the peroxisomal matrix contain NAD-malate dehydrogenases, while an NADP-malate dehydrogenase is present only in the chloroplast stroma. These enzymes will be discussed in Chapter 7.

A loss of intermediates of the citrate cycle is replenished by anaplerotic reactions

The citrate cycle can proceed only when the oxaloacetate required as acceptor for the acetyl residue is fully regenerated. Section 10.4 describes how citrate and α-ketoglutarate are withdrawn from the citrate cycle to synthesize the carbon skeletons of amino acids in the course of nitrate assimilation. It is necessary, therefore, to replenish the loss of citrate cycle intermediates by **anaplerotic reactions**. In contrast to mitochondria from animal tissues, plant mitochondria are able to transport oxaloacetate into the chloroplasts via a specific translocator of the inner membrane (section 5.8). Therefore, the citrate cycle can be replenished by the uptake of oxaloacetate, which has been synthesized by phosphoenolpyruvate carboxylase in the cytosol (section 8.2). Oxaloacetate can also be delivered by oxidation of malate in the mitochondria. Malate is stored in the vacuole (sections 1.2, 8.2, and 8.5)

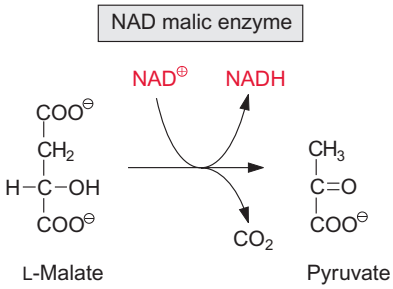

Figure 5.10 Oxidative decarboxylation of malate to synthesize pyruvate catalyzed by NAD-malic enzyme.

and is an important substrate for mitochondrial respiration. A special feature of plant mitochondria is that malate is oxidized in the matrix via **NAD-malic enzyme** to pyruvate with the reduction of NAD^+ and the release of CO_2 (Fig. 5.10). Thus interplay of malate dehydrogenase and NAD-malic enzyme allows citrate to be formed from malate without the operation of the complete citrate cycle (Fig. 5.3). It may be noted that an **NADP-dependent malic enzyme** is present in the chloroplasts, especially in C_4 plants (section 8.4).

Another important substrate of mitochondrial oxidation is **glutamate**, which is one of the main products of nitrate assimilation (section 10.1) and, besides sucrose, the most highly concentrated organic compound in the cytosol of many plant cells. Glutamate oxidation, accompanied by formation of NADH, is catalyzed by **glutamate dehydrogenase** located in the mitochondrial matrix (Fig. 5.11). This enzyme also reacts with $NADP^+$. NADP-glutamate dehydrogenase activity is also present in plastids, although its function is yet not understood.

Glycine is the main substrate of respiration in the mitochondria from mesophyll cells of illuminated leaves. The oxidation of glycine as a partial reaction of the photorespiratory pathway will be discussed in section 7.1.

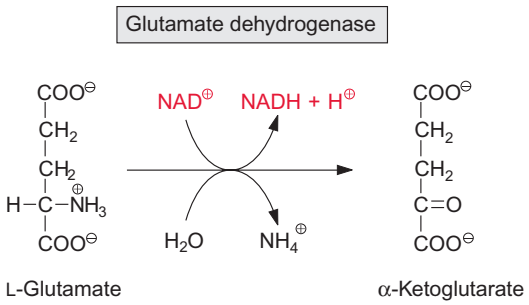

Figure 5.11 Oxidation of glutamate catalyzed by glutamate dehydrogenase.

5.4 How much energy can be gained by the oxidation of NADH?

How much energy is released during mitochondrial respiration or, to be more exact, how large is the difference in free energy in the mitochondrial redox processes? To answer this question the differences of the potentials of the redox pairs are calculated by the Nernst equation:

$$E = E^{0'} + \frac{RT}{nF} \ln \frac{\text{oxidized substance}}{\text{reduced substance}} \tag{5.1}$$

where $E^{0'}$ = standard potential at pH 7, 25°C; R (gas constant) = 8.31 J/K·mol; $T = 298$ K; n is the number of electrons transferred; and F (Faraday constant) = 96,485 A s/mol.

The standard potential for the redox pair NAD^+/NADH is:

$$E^{0'} = -0.320 \text{ V}$$

Under certain metabolic conditions, an NAD^+/NADH ratio was found to be 3 in mitochondria from leaves. The introduction of this value into equation 5.1 yields:

$$E_{NAD^+/NADH} = -0.320 + \frac{RT}{2F} \ln 3 = -0.306 \text{ V} \tag{5.2}$$

The standard potential for the redox pair H_2O/O_2 is:

$$E^{0'} = +0.815 \text{ V} \qquad ([H_2O] \text{ in water } 55 \text{ mol/L})$$

To evaluate the actual potential of $[O_2]$ the partial pressure of the oxygen in the air is introduced:

$$E_{H_2O/O_2} = 0.815 + \frac{RT}{2F} \ln \sqrt{p}\ O_2 \tag{5.3}$$

The partial pressure of the oxygen in the air (pO_2) is 0.2. Introducing this value into equation 5.3 yields:

$$E_{H_2O/O_2} = 0.805 \text{ V}$$

The difference of the potentials amounts to:

$$\Delta E = E_{H_2O/O_2} - E_{NAD^+/NADH} = +1.11\,V \tag{5.4}$$

The free energy (ΔG) is related to ΔE as follows:

$$\Delta G = -nF\Delta E \tag{5.5}$$

Two electrons are transferred in the reaction. The introduction of ΔE into equation 5.5 shows that the change of free energy during the oxidation of NADH by the respiratory chain amounts to:

$$\Delta G = -214\,kJ/mol$$

How much energy is required for the formation of ATP? It has been calculated in section 4.1 that the synthesis of ATP under the metabolic conditions in the chloroplasts requires a change of free energy of $\Delta G \approx +50\,kJ/mol$. This value also applies approximately for the ATP which mitochondria provide for the cytosol.

The calculated free energy released by the oxidation of NADH would therefore be sufficient to generate four molecules of ATP, but in fact the amount of ATP synthesized by NADH oxidation *in vivo* is much lower (section 5.6).

5.5 The mitochondrial respiratory chain shares common features with the photosynthetic electron transport chain

The photosynthesis of cyanobacteria led to the accumulation of oxygen in the early atmosphere, which was the basis for the oxidative metabolism of mitochondria. Many cyanobacteria can satisfy their ATP demand both by photosynthesis and by oxidative metabolism. Cyanobacteria contain a photosynthetic electron transport chain that consists of three modules (complexes), namely, photosystem II, the cyt-b_6/f complex, and photosystem I (Chapter 3, Fig. 5.12). These complexes are located in the inner membrane of cyanobacteria, where the enzymes of the respiratory electron transport chain are also localized. This respiratory chain consists of three modules: an **NADH**

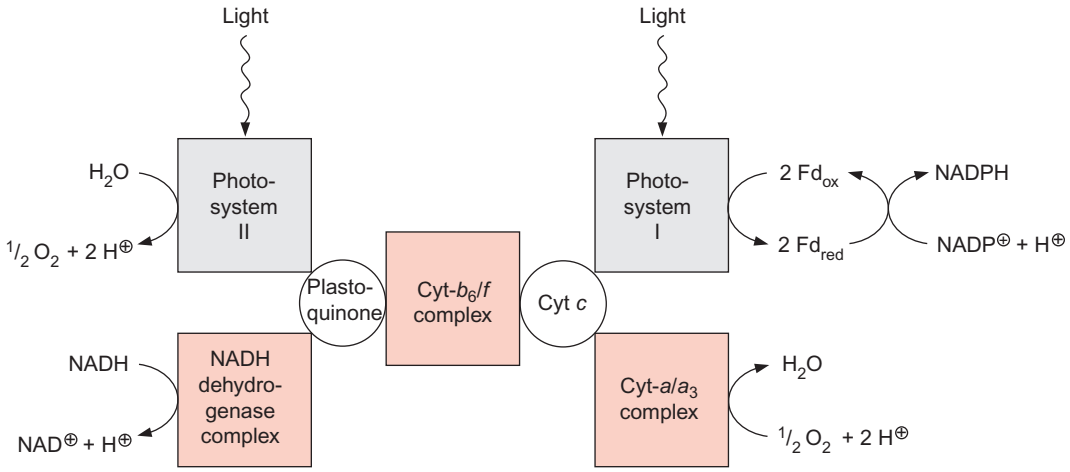

Figure 5.12 Schematic presentation of photosynthetic and oxidative electron transport in cyanobacteria. In both electron transport chains the cytochrome-b_6/f complex functions as the central complex.

dehydrogenase complex, catalyzing the oxidation of NADH; the same **cyt-b_6/f complex** that is also part of the photosynthetic electron transport chain; and a **cyt-a/a_3 complex**, by which oxygen is reduced to water. Plastoquinone feeds the electrons into the cyt-b_6/f complex not only in photosynthesis (section 3.7), but also in the respiratory chain of the cyanobacteria. Likewise, cytochrome-*c* mediates the electron transport from the cyt-b_6/f complex to photosystem I as well as to the cyt-a/a_3 complex. The relationship between photosynthetic and oxidative electron transport in cyanobacteria is obvious; both electron transport chains possess the same module as the middle of the reaction sequence, the cyt-b_6/f complex. Section 3.7 described how the cyt-b_6/f complex released the energy during electron transport to build up a proton gradient. The function of the cyt-b_6/f complex in respiration and photosynthesis shows that the principle of energy conservation in photosynthetic and oxidative electron transport is the same.

The mitochondrial respiratory chain is analogous to the respiratory chain of cyanobacteria (Fig. 5.13), but with ubiquinone instead of plastoquinone as redox carrier and slightly different cytochromes. The mitochondria contain a related cyt-b/c_1 complex instead of a cyt-b_6/f complex, but both cyt-*c* and cyt-*f* contain heme-*c*.

Figure 5.13 shows succinate dehydrogenase, another electron acceptor of the mitochondrial respiratory chain. This enzyme (historically termed complex II) catalyzes the oxidation of succinate to fumarate, a step of the citrate cycle (Fig. 5.9).

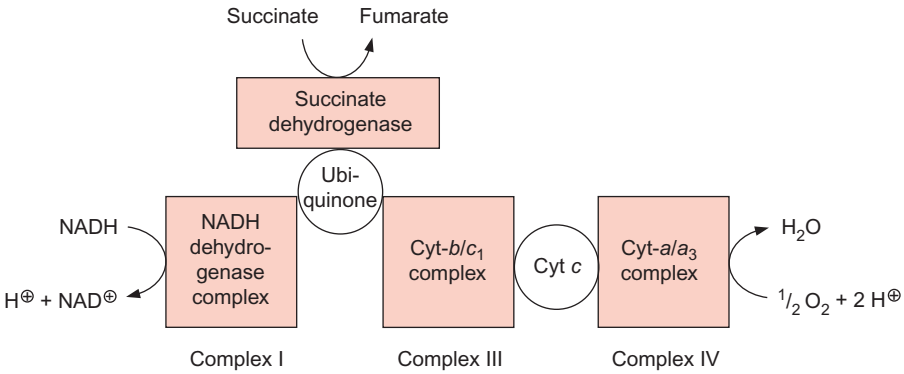

Figure 5.13 Schematic presentation of the mitochondrial electron transport. The respiratory chain consists of four complexes; the central cyt-b/c_1 complex corresponds to the cyt-b_6/f complex of cyanobacteria and chloroplasts.

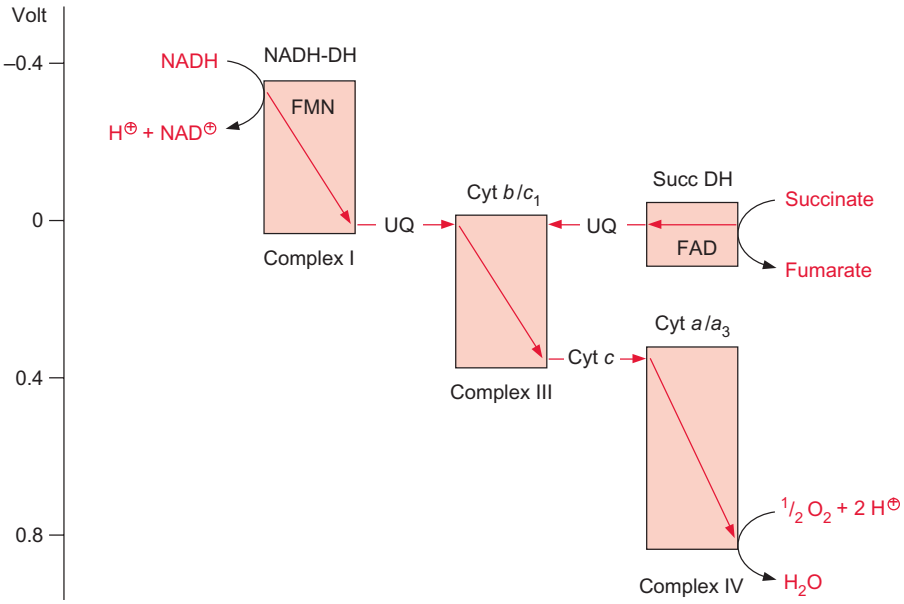

Figure 5.14 Schematic presentation of the complexes of the respiratory chain arranged according to their redox potentials.

The complexes of the mitochondrial respiratory chain

The subdivision of the respiratory chain into several complexes goes back to the work of Youssef Hatefi (USA, 1962), who succeeded in isolating four different complexes, which he termed complexes I–IV, while working with beef heart mitochondria. In the complexes I, III, and IV, the electron transport is accompanied by a decrease in the redox potential (Fig. 5.14); the energy thus released creates a proton gradient.

The **NADH dehydrogenase complex** (complex I) (Fig. 5.15) feeds the respiratory chain with the electrons from NADH formed from the degradation of substrates in the matrix. The electrons are transferred to ubiquinone via a flavin adenine mononucleotide (**FMN**) and several iron-sulfur centers.

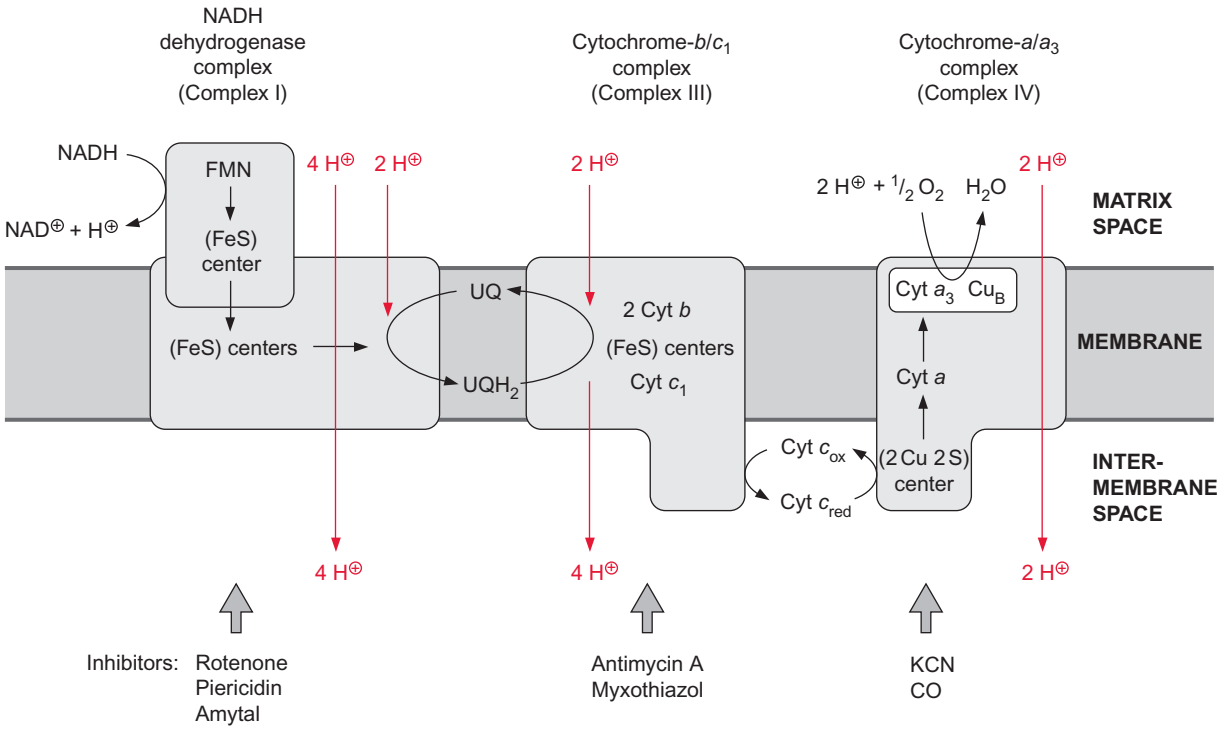

Figure 5.15 Schematic presentation of the location of complexes I, III, and IV in the mitochondrial respiratory chain. Positions of proton transfer are indicated. Antibiotics inhibit the membrane complexes.

Complex I has the most complicated structure of all the mitochondrial electron transport complexes. It consists of more than 40 different subunits (of which, depending on the organism, seven to nine are encoded in the mitochondria). Part of the complex is embedded in the membrane (**membrane part**) and a **peripheral part** protrudes into the matrix space. The peripheral part provides the binding site for NADH and comprises FMN (Fig. 5.16) and at least three Fe-S-centers (Fig. 3.26). The membrane part contains another Fe-S-center, as well as the binding site for ubiquinone. The electron transport can be inhibited by a variety of poisons deriving from plants and bacteria, such as **rotenone** (which protects plants from being eaten by animals); the antibiotic **piericidin A**; and **amytal**, a barbiturate. The electron transport catalyzed by complex I is reversible. It is therefore possible for electrons to be transferred from ubiquinone to NAD^+, driven by the proton motive force of the proton gradient. In this way the NADH dehydrogenase complex can provide purple bacteria with NADH (see Fig. 3.1).

In plants the **succinate dehydrogenase** (complex II) (Fig. 5.9) consists of seven subunits comprising a flavin adenine nucleotide (FAD, Fig. 5.16) as

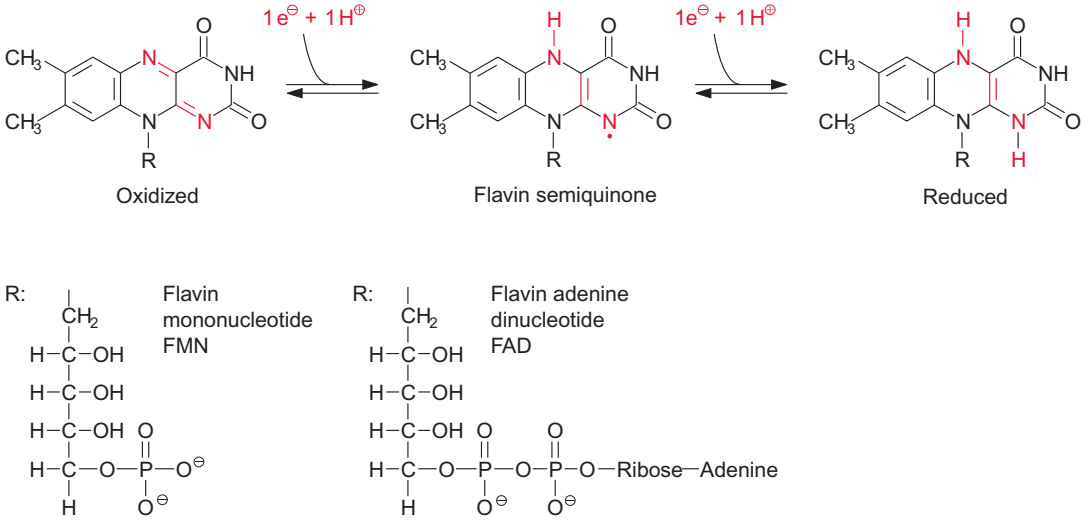

Figure 5.16 Structures of reduced and oxidized FMN and FAD.

the electron acceptor; several Fe-S-centers (Fig. 3.26) as redox carriers; and one cytochrome-*b*, of which the function is not known. Electron transport by succinate dehydrogenase to ubiquinone proceeds with no major decrease in the redox potential, so no energy is gained in the electron transport from succinate to ubiquinone.

Ubiquinone reduced by the NADH dehydrogenase complex or succinate dehydrogenase is oxidized by the **cyt-*b*/*c*$_1$ complex** (complex III) (Fig. 5.15). In mitochondria this complex consists of 11 subunits, only one of which (the cyt-*b* subunit) is encoded in the mitochondria. The cyt-*b*/*c*$_1$ complex is very similar in structure and function to the cyt-b_6/*f* complex of chloroplasts (section 3.7). Electrons are transferred by the cyt-*b*/*c*$_1$ complex to cyt-*c*, which is bound to the outer surface of the inner membrane. Several antibiotics, such as **antimycin A** and **myxothiazol**, inhibit the electron transport by the cyt-*b*/*c*$_1$ complex.

Due to its positive charge, reduced cyt-*c* diffuses along the negatively charged surface of the inner membrane to the **cyt-*a*/*a*$_3$ complex** (Fig. 5.15), also termed **complex IV** or **cytochrome oxidase**. The cyt-*a*/*a*$_3$ complex contains 13 different subunits, three of which are encoded in the mitochondria. The three-dimensional structures of the beef heart mitochondrial and *Paracoccus denitrificans* cyt-*a*/*a*$_3$ complex have been resolved by X-ray crystallography. The complex has a large hydrophilic region that protrudes into the intermembrane space and provides the binding site for cyt-*c*. During the oxidation of cyt-*c* the electrons are transferred to a **copper sulfur cluster** containing two Cu atoms called Cu$_A$. These two Cu atoms are linked by two S-atoms of cysteine side chains (Fig. 5.17). This copper-sulfur cluster

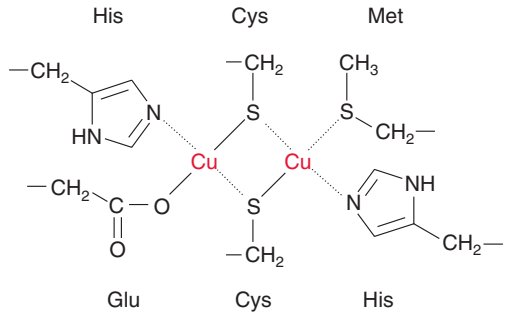

Figure 5.17 A copper-sulfur cluster of the cytochrome-a/a_3 complex. Cu_A, a Cu^{2+}- and a Cu^{+}-ion are linked by two cysteine residues, two histidines, one glutamate and one methionine to the protein. Cu_A probably transfers one electron.

probably takes up one electron and transfers it via cyt-*a* to a **binuclear center**, consisting of cyt-a_3 and a Cu atom (Cu_B), bound to histidine. This binuclear center functions as a redox unit in which the Fe atom of the cyt-a_3, together with Cu_B, take up two electrons.

$$[Fe^{+++} \cdot Cu_B^{++}] + 2e^- \rightarrow [Fe^{++} \cdot Cu_B^{+}]$$

In contrast to cyt-*a* and the other cytochromes of the respiratory chain, the sixth coordination position of cyt-a_3 of the heme Fe atom is not saturated by an amino acid of the protein (Fig. 5.18). This free coordination position as well as Cu_B are the binding site for the oxygen molecule, which is reduced to water by the uptake of four electrons:

$$O_2 + 4\,e^- \rightarrow 2\,O^{2-} + 4\,H^+ \rightarrow 2\,H_2O$$

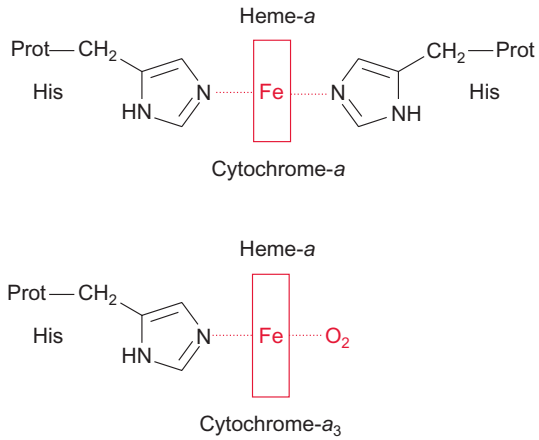

Figure 5.18 Axial ligands of the Fe atoms in the heme groups of cytochrome-*a* and -a_3. Of the six coordinative bonds of the Fe atom in the heme, four are saturated by the N atoms present in the planar tetrapyrrole ring. Whereas in cytochrome-*a* the two remaining coordination positions of the central Fe atom bind to two histidine residues of the protein, positioned at either side vertically to the plane of the tetrapyrrole, in cytochrome-a_3 one of these coordination positions is free and functions as binding site for the O_2 molecule.

Furthermore, Cu_B probably has an important function in electron-driven proton transport, which is discussed in the next section. Instead of O_2, also CO and CN^- can be very tightly bound to the free coordination position of the Cyt-a_3, and efficiently inhibit the respiration. Therefore, both carbon monoxide and prussic acid (HCN) are very potent poisons.

5.6 Electron transport of the respiratory chain is coupled to the synthesis of ATP via proton transport

The electron transport of the respiratory chain is coupled to the formation of ATP. This is illustrated in the experiment of Figure 5.19, in which the velocity of respiration in a mitochondrial suspension was determined by measuring the decrease of the oxygen concentration in the suspension medium. The addition of a substrate alone (e.g., malate) causes only a minor increase in respiration. The subsequent addition of a limited amount of ADP results in a considerable acceleration of respiration. After some time, however, respiration returns to the lower rate prior to the addition of ADP,

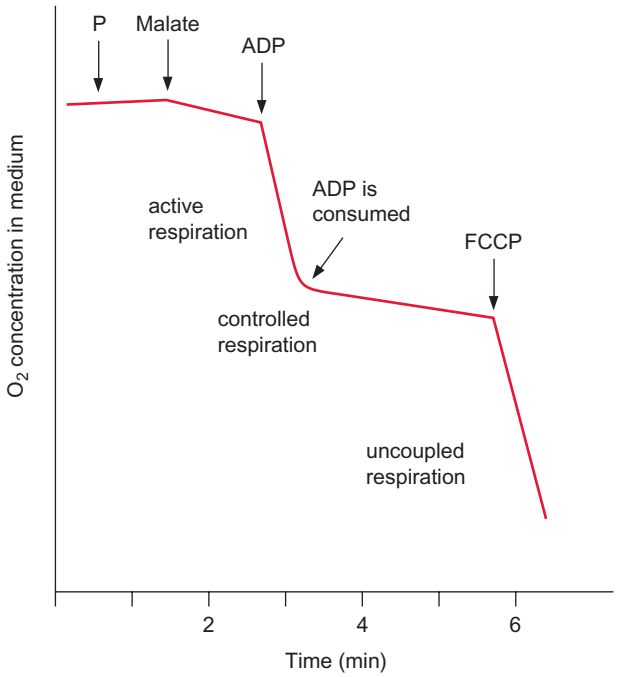

Figure 5.19 Registration of oxygen consumption by isolated mitochondria. Phosphate and malate are added one after the other to mitochondria suspended in a buffered osmotic solution. Addition of ADP results in a high rate of respiration. The subsequent decrease of oxygen consumption indicates that the conversion of the added ADP into ATP is completed. Upon the addition of an uncoupler (e.g., FCCP), a high respiration rate is attained without ADP: respiration is now uncoupled from ATP synthesis.

as the ADP has been completely converted to ATP. Respiration in the presence of ADP is called **active respiration**, whereas that after ADP is consumed is called **controlled respiration**. As the ADP added to the mitochondria is completely converted to ATP, the amount of ATP formed with the oxidation of a certain substrate can be determined from the ratio of ADP added to oxygen consumed (**ADP/O**). An ADP/O of about 2.5 is determined for substrates oxidized in the mitochondria via the formation of NADH (e.g., malate), and of about 1.6 for succinate, from which the redox equivalents are directly transferred via FADH to ubiquinone. The problem of ATP stoichiometry of respiration will be discussed at the end of this section.

Like photosynthetic electron transport (Chapter 4), the electron transport of the respiratory chain is accompanied by the generation of a proton

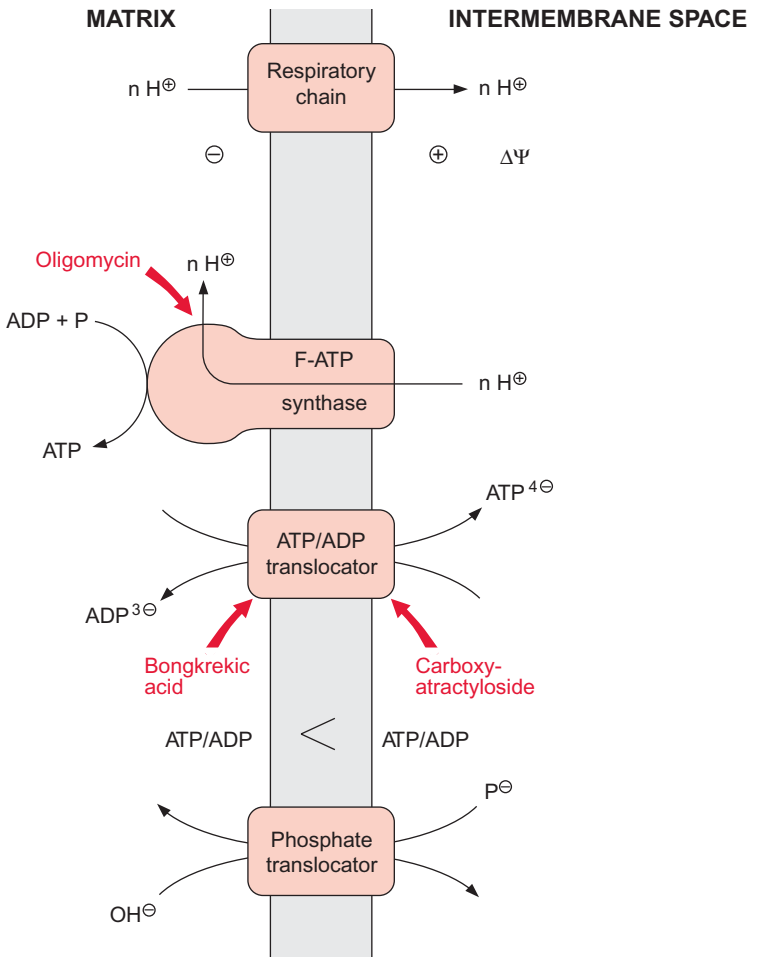

Figure 5.20 ATP synthesis by mitochondria requires an uptake of phosphate by the phosphate translocator in counter-exchange for OH^- ions, and an electrogenic exchange of ATP for ADP, as catalyzed by the ATP/ADP translocator. Due to the membrane potential generated by electron transport of the respiratory chain, ADP is preferentially transported inward and ATP outward. As a result of this the ATP/ADP ratio in the cytosol is higher than in the mitochondrial matrix. ATP/ADP transport is inhibited by carboxyactractyloside (binding from the intermembrane space) and bongkrekic acid (binding from the matrix side). F_1 part of the F-ATP synthase of the mitochondria is inhibited by oligomycin.

motive force (Fig. 5.15), which in turn drives the synthesis of ATP (Fig. 5.20). Therefore substances such as FCCP (Fig. 4.2) function as uncouplers of mitochondrial as well as photosynthetic electron transport. Figure 5.19 shows that the addition of the uncoupler FCCP results in a high stimulation of respiration. As discussed in section 4.2, the uncoupling function of the FCCP is due to a short circuit of protons across a membrane, resulting in the elimination of the proton gradient. The respiration is then uncoupled from ATP synthesis and the energy set free during electron transport is dissipated as heat.

To match respiration to the energy demand of the cell, it is regulated by an overlapping of two different mechanisms. The classic mechanism of **respiratory control** is based on the fact that when the ATP/ADP ratio increases, the proton motive force also increases, which in turn causes a decrease of electron transport by the respiratory chain. Recently it was discovered that ATP also impedes the electron transport by binding to a subunit of cytochrome oxidase, which results in a decrease of its activity.

Mitochondrial proton transport results in the formation of a membrane potential

Mitochondria, in contrast to chloroplasts, have no closed thylakoid space to form a proton gradient. Instead, in mitochondrial electron transport, protons are transported from the matrix to the intermembrane space, which is, however, connected to the cytosol by pores (formed by porines (Fig. 1.30)). In chloroplasts the formation of a proton gradient of $\Delta pH = 2.5$ in the light results in a decrease of pH in the thylakoid lumen from about pH 7.5 to pH 5.0. If during mitochondrial oxidation such a strong acidification were to occur in the cytosol, it would have a grave effect on the activity of the cytosolic enzymes. In fact, during mitochondrial controlled respiration the ΔpH across the inner membrane is only about 0.2, and therefore mitochondrial proton transport leads primarily to the formation of a membrane potential ($\Delta\Psi \approx 200\,mV$). Mitochondria are unable to generate a larger proton gradient, as their inner membrane is impermeable for anions, such as chloride. As shown in Figure 4.1, a proton concentration gradient can be formed only when the charge of the transported protons is compensated by the diffusion of a counter anion.

Despite intensive research for more than 30 years our knowledge of the mechanism of coupling between mitochondrial electron transport and transport of protons is still incomplete. Four protons are probably taken up from the matrix side during the transport of two electrons from the NADH dehydrogenase complex to ubiquinone and released into the intermembrane space by the cyt-b/c_1 complex (Fig. 5.15). It is generally accepted that in

mitochondria the cyt-b/c_1 complex catalyzes a **Q-cycle** (Fig. 3.30) by which, when two electrons are transported, two additional protons are transported out of the matrix space into the intermembrane space. Finally, the cyt-a/a_3 complex transports two protons per two electrons. The three-dimensional structure of the cyt-a/a_3 complex indicates that the binuclear center from cytochrome-a_3 and Cu_B is involved in this proton transport. If these stoichiometries are correct, altogether 10 protons would be transported during the oxidation of NADH and only six during the oxidation of succinate.

Mitochondrial ATP synthesis serves the energy demand of the cytosol

The energy of the proton gradient is used in the mitochondria for ATP synthesis by an F-ATP synthase (Fig. 5.20), which has the same basic structure as the F-ATP synthase of chloroplasts (section 4.3). However, there are differences regarding the inhibition by **oligomycin**, an antibiotic from *Streptomyces*. Whereas the mitochondrial F-ATP synthase is very strongly inhibited by oligomycin, due to the presence of an oligomycin binding protein, the chloroplast enzyme is insensitive to this inhibitor. Although the mechanism of ATP synthesis appears to be identical for both ATP synthases, the proton stoichiometry in mitochondrial ATP synthesis has not been resolved unequivocally. Assuming that the rotor of the F-ATP synthase in mitochondria has 10 c-subunits, 3.3 protons would be required for the synthesis of 1 mol of ATP (according to the mechanism for ATP synthesis discussed in section 4.4). This rate corresponds more or less with previous independent investigations.

In contrast to chloroplasts, which synthesize ATP essentially for their own consumption, the ATP in mitochondria is synthesized mainly for export into the cytosol. This requires the uptake of ADP and phosphate from the cytosol into the mitochondria and vice versa the release of the synthesized ATP. The uptake of phosphate proceeds by the **phosphate translocator** in a counter-exchange for OH^- ions, whereas the uptake of ADP and the release of ATP are mediated by the **ATP/ADP translocator** (Fig. 5.20). The mitochondrial ATP/ADP translocator is inhibited by **carboxyatractyloside**, a glucoside from the thistle *Atractylis gumnifera*, and by **bongkrekic acid**, an antibiotic from the bacterium *Cocovenerans*, growing on coconuts. Both compounds are deadly poisons.

The ATP/ADP translocator catalyzes a strict **counter-exchange**; for each ATP or ADP transported out of the chloroplasts, an ADP or ATP is transported inward. Since the transported ATP contains one negative charge more than the ADP, the transport is electrogenic. Due to the membrane potential generated by the proton transport of the respiratory chain, there

is a preference for ADP to be taken up and ATP to be transported outward. As a result of this asymmetric transport of ADP and ATP the ATP/ADP ratio outside the mitochondria is much higher than in the matrix. In this way mitochondrial ATP synthesis maintains a high ATP/ADP ratio in the cytosol. With the exchange of ADP for ATP, one negative charge is transferred from the matrix to the outside, which requires the transport of a proton in the other direction to compensate this charge difference. This is why protons from the proton gradient are consumed not only for ATP synthesis as such, but also for export of the synthesized ATP from the mitochondria.

Let us return to the stoichiometry between the transported protons and the ATP formation during respiration. It is customary to speak of three coupling sites of the respiratory chain, which correspond to the complexes I, III, and IV. Textbooks often state that during NADH oxidation by the mitochondrial respiratory chain, one molecule of ATP is formed per coupling site, and as a result of this, the ADP/O quotient for oxidation of NADH amounts to three, and that for succinate to two. However, considerably lower values have been determined in experiments with isolated mitochondria. The attempt was made to explain this discrepancy by assuming that owing to a proton leakage of the membrane, the theoretical ADP/O values were not attained in the isolated mitochondria. It appears now that even in theory these whole numbers for ADP/O ratios are incorrect. Probably 10 protons are transported upon the oxidation of NADH. In the event that 3.3 protons are required for the synthesis of ATP and another one for its export from the mitochondria, the resulting ADP/O would be 2.3. With isolated mitochondria, values of about 2.5 have been obtained experimentally.

At the beginning of this chapter, the change in free energy during the oxidation of NADH was evaluated to be $-214\,kJ/mol$ and for the synthesis of ATP as about $+50\,kJ/mol$. An ADP/O of 2.3 for the respiration of NADH-dependent substrates indicates that about 54% of the free energy released during oxidation is used for the synthesis of ATP. However, these values must still be treated with caution.

5.7 Plant mitochondria have special metabolic functions

The function of the mitochondria as being the power station of the cell applies for all mitochondria, from unicellular organisms to animals and

plants. In plant cells which perform photosynthesis, the role of the mitochondria as a supplier of energy is not restricted to the dark phase; the mitochondria provide the cytosol with ATP also during photosynthesis.

Plant mitochondria fulfill additional functions. The mitochondrial matrix contains enzymes for the oxidation of glycine to serine, an important step in the photorespiratory pathway (section 7.1):

$$2\ \text{glycine} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{serine} + \text{NADH} + \text{CO}_2 + \text{NH}_4^+$$

The NADH generated from glycine oxidation is the main fuel for mitochondrial ATP synthesis during photosynthesis. Another important role of plant mitochondria is the conversion of oxaloacetate and pyruvate to form citrate, a precursor for the synthesis of α-ketoglutarate. This pathway is important for providing the carbon skeletons for amino acid synthesis during nitrate assimilation (Fig. 10.11).

Mitochondria can oxidize surplus NADH without forming ATP

In mitochondrial electron transport, the participation of flavins, ubisemiquinones, and other electron carriers leads to the formation of superoxide radicals, H_2O_2, and hydroxyl radicals (summarized as **ROS**, reactive oxygen species (section 3.9)) as by-products. These by-products cause severe cell damage. Since the formation of ROS is especially high, when the components of the respiratory chain are highly reduced, there is a necessity to avoid an overreduction of the respiratory chain. On the other hand, it is essential for a plant that glycine, formed in large quantities by the photorespiratory cycle (section 7.1), is converted by mitochondrial oxidation even when the cell does not require ATP. Plant mitochondria have several **overflow mechanisms**, which oxidize surplus NADH without synthesizing ATP in order to prevent an overreduction of the respiratory chain (Fig. 5.21). Among those are an alternative NADH-dehydrogenase, an alternative oxidase (Fig. 5.21) and uncoupling proteins. The **alternative NADH dehydrogenase**, located in the inner mitochondrial membrane, transfers electrons from NADH to ubiquinone, without coupling to proton transport. This pathway is not inhibited by rotenone. However, oxidation of NADH via this rotenone-insensitive pathway proceeds only when the NADH/NAD^+ ratio in the matrix is exceptionally high. In addition, the matrix side of the mitochondrial inner membrane contains an alternative NADPH dehydrogenase (not shown in Figure 5.21).

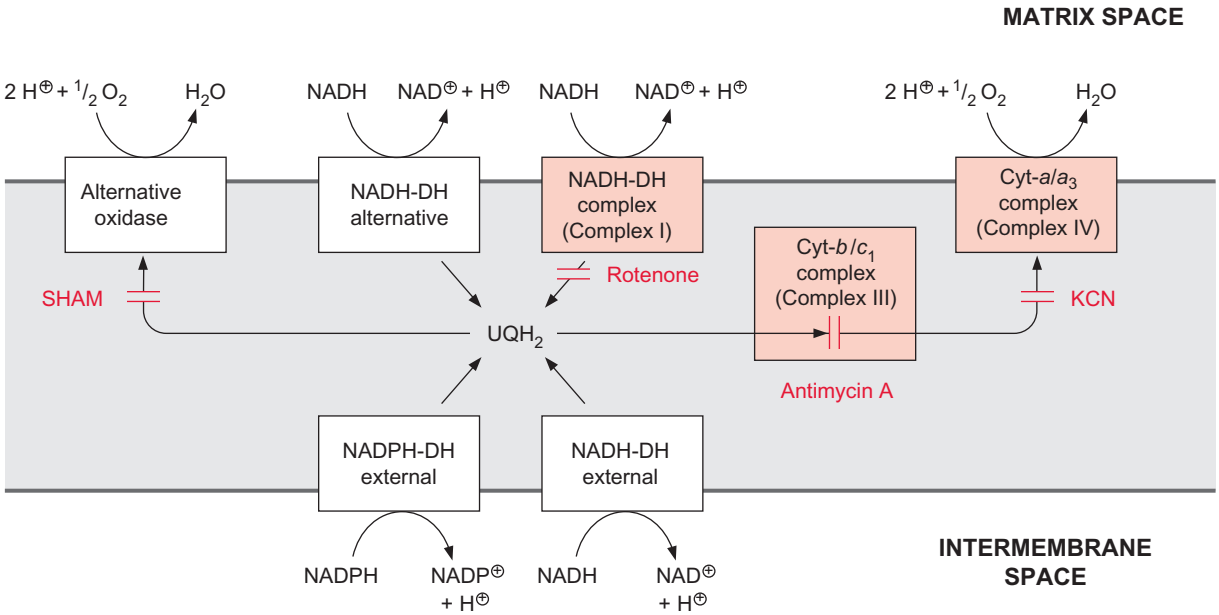

Figure 5.21 Besides the rotenone-sensitive NADH dehydrogenase (NADH DH) of the respiratory chain, there are other dehydrogenases that transfer electrons to ubiquinone without an accompanying proton transport. An alternative NADPH dehydrogenase exists that is directed to the matrix side (not shown). An alternative oxidase enables the oxidation of ubihydroquinone (UQH_2). This pathway is insensitive to the inhibitors antimycin A and KCN, but it is inhibited by salicylic hydroxamate (SHAM).

The **alternative oxidase** transfers electrons directly from ubiquinone to oxygen; this pathway is also not coupled to proton transport. The alternative oxidase is insensitive to **antimycin-A** and **KCN** (inhibitors of complex III and II, respectively), but is inhibited by salicylic hydroxamate (**SHAM**). Recent results show that the alternative oxidase is a membrane protein consisting of two identical subunits (each 36 kDa). From the amino acid sequence it can be predicted that each subunit possesses two transmembrane helices. The two subunits together form a **di-iron oxo-center** (like in the fatty acid desaturase, Fig. 15.16), which catalyzes the oxidation of ubiquinone by oxygen. Electron transport via the alternative oxidase can be understood as a short circuit. It occurs only when the mitochondrial ubiquinone pool is highly reduced. The alternative oxidase is activated by a high concentration of pyruvate which is a signal for an excess of metabolites.

Mitochondrial uncoupling proteins were first detected in animal tissues. These proteins are closely related to the mitochondrial ATP/ADP translocator. They build a channel in the inner mitochondrial membrane which is permeable to protons, resulting in the elimination of the membrane potential,

and therefore in the uncoupling of electron transport from ATP synthesis. Uncoupling proteins are widely distributed in eukaryotes; thus also in plants, where they are called **PUMP**s (plant uncoupling mitochondrial proteins). Their apparent function is the prevention of excessive increase of the mitochondrial membrane potential, in order to minimize the formation of reactive oxygen species (ROS).

When metabolites in the mitochondria are in excess, the interplay of the alternative NADH dehydrogenase, the alternative oxidase and the uncoupling proteins lead to their elimination by oxidation without accompanying ATP synthesis, and the oxidation energy is dissipated as heat. The capacity of the alternative oxidase in the mitochondria from different plant tissues is variable and also depends on the developmental state. Thus one observes a high expression of PUMPs in plants that have been subjected to a cold stress. An especially high alternative oxidase activity has been found in the spadix of the voodoo lily *Sauromatum guttatum*, which uses the alternative oxidase to heat up the spadix by which volatile amine compounds are emitted, which produce a nasty smell like carrion or dung. This strong stench attracts insects from far and wide. The formation of the alternative oxidase is synchronized in these spadices with the beginning of flowering.

NADH and NADPH from the cytosol can be oxidized by the respiratory chain of plant mitochondria

In contrast to mitochondria from animal tissues, plant mitochondria can also oxidize cytosolic NADH and in some cases cytosolic NADPH. Oxidation of this external NADH and NADPH proceeds via two specific dehydrogenases of the inner membrane, of which the substrate binding site is directed towards the intermembrane space. As in the case of succinate dehydrogenase, the electrons from external NADH and NADPH dehydrogenase are fed into the respiratory chain at the site of **ubiquinone**, and therefore this electron transport is not inhibited by rotenone. As oxidation of external NADH and NADPH (like the oxidation of succinate) does not involve a proton transport by complex I (Fig. 5.21), the oxidation of external pyridine nucleotides yields less ATP than the oxidation of NADH provided from the matrix. Oxidation by external NADH dehydrogenase proceeds only when the cytosolic NAD system is reduced excessively. Also, the external NADH dehydrogenase may be regarded as part of an overflow mechanism, which comes into action only when the NADH in the cytosol is overreduced. As discussed in section 3.10, in certain situations photosynthesis may produce a surplus of reducing power, which is hazardous for a cell. The plant cell has the capacity to eliminate excessive reducing power by making use of the uncoupling protein PUMP, the external NADH

dehydrogenase, the alternative dehydrogenase for internal NADH from the matrix, and the alternative oxidase mentioned earlier.

5.8 Compartmentation of mitochondrial metabolism requires specific membrane translocators

The mitochondrial inner membrane is impermeable for metabolites. Specific translocators enable a specific transport of metabolites between the mitochondrial matrix and the cytosol in a counter-exchange mode (Fig. 5.22).

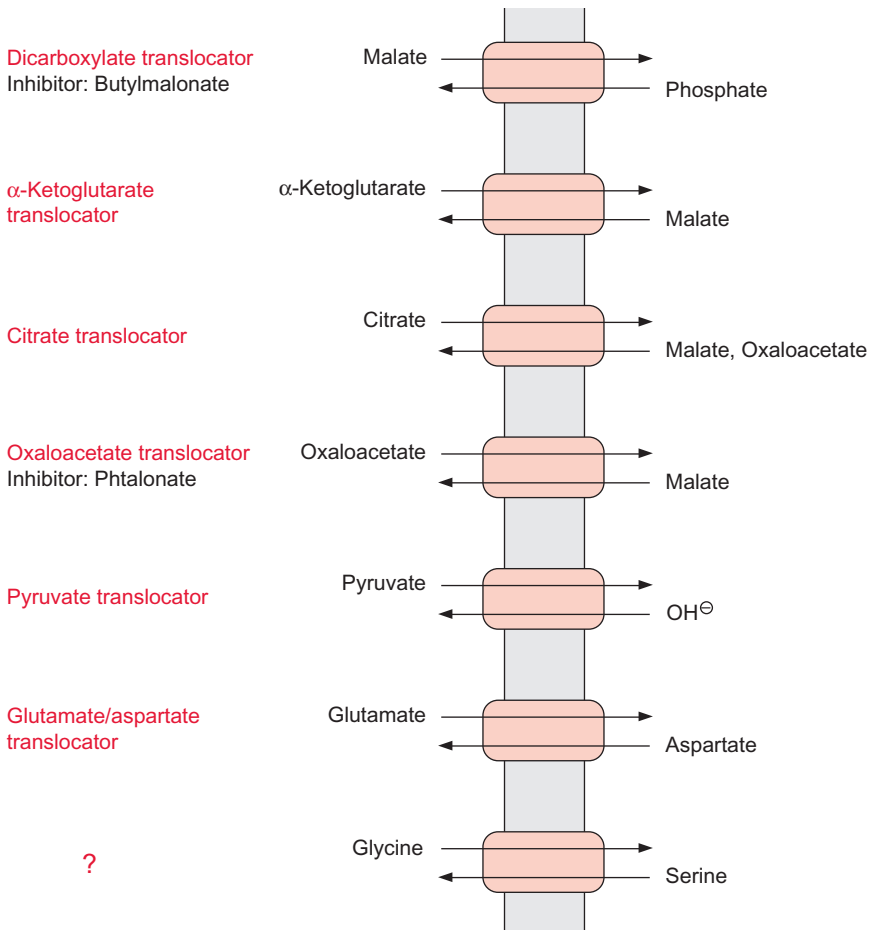

Figure 5.22 Important translocators of the inner mitochondrial membrane. The phosphate- and the ATP/ADP-translocator are shown in Figure 5.20.

The role of the ATP/ADP and the phosphate translocators (Fig. 5.20) has been discussed in section 5.6. Malate and succinate are transported into the mitochondria in counter-exchange for phosphate by a **dicarboxylate translocator**. This transport is inhibited by **butylmalonate**. α-Ketoglutarate, citrate, and oxaloacetate are transported in counter-exchange for malate. By these translocators, substrates can be fed into the citrate cycle. Glutamate is transported in counter-exchange for aspartate, and pyruvate in counter-exchange for OH^- ions. Although these translocators all occur in plant mitochondria, most of our present knowledge about them is based on studies with mitochondria from animal tissues. A comparison of the amino acid sequences known for the ATP/ADP, phosphate, citrate, and glutamate/aspartate translocators shows that they are homologous; the proteins of these translocators represent a family deriving from a common ancestor. As mentioned in section 1.9, all these translocators are composed of 2×6 transmembrane helices.

The malate-oxaloacetate translocator is a special component of plant mitochondria and has an important function in the malate-oxaloacetate cycle described in section 7.3. It also transports citrate and is involved in providing the carbon skeletons for nitrate assimilation (Fig. 10.11). The oxaloacetate translocator and, to a lesser extent, the α-ketoglutarate translocator are inhibited by the dicarboxylate **phthalonate**. The transport of glycine and serine, involved in the photorespiratory pathway (section 7.1), has not yet been characterized. Although final proof is still lacking, it is expected that this transport is mediated by one or two mitochondrial translocators.

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