8. CELLULAR RESPIRATION AND LIGHT-DEPENDENT PHASE OF PHOTOSYNTHESIS

8.1 Proton-motive force as the dominant element of bioenergetics

As we have shown in the previous chapter, the transfer of **active hydrogen** ([H]) into the **respiratory chain** belongs to the dominant characteristics of the catabolism of aerobic chemoorganotrophs. The hydrogen donors are various substrates which are dehydrogenated and whose hydrogen atoms are transferred to the relevant cofactors; afterwards these cofactors undergo re-oxidation by passing the hydrogen atoms by means of the respiratory chain to the **final electron acceptor** which, in the case of aerobic organisms, is molecular oxygen. The primary carrier of [H] is NADH; this coenzyme is present mainly in the mitochondrial **matrix** (mitosol, Fig. 8.1) and its re-oxidation takes place at the beginning of the respiratory chain.

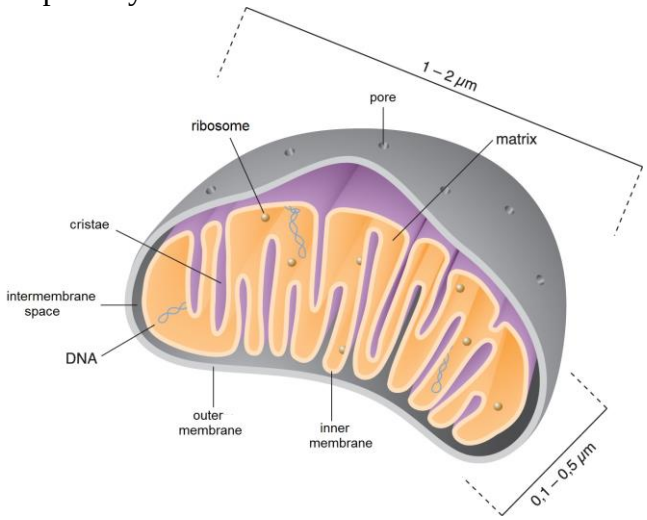


Fig. 8.1: **Mitochondria, a schematic representation**

Since the middle of the previous century it had been already known that exergonic aerobic reoxidation of reduced cofactors takes place, in eukaryotic organisms, in mitochondria and that it is accompanied by ATP synthesis. e.g.:

$$\mathrm{NADH + H^+ + \tfrac{1}{2}\, O_2} \longrightarrow \mathrm{NAD^+ + H_2O}$$
$$\mathrm{n\ (ADP + P_i)} \longrightarrow \mathrm{n\ (ATP + H_2O)} \qquad (8\text{-}1)$$

Two fundamental characteristics of this complex process, called **oxidative phosphorylation**, were also known:

a) Reducing equivalents (hydrogen atoms or electrons) are gradually transferred by a chain of oxidation-reduction systems that are lined up in the direction of growing standard redox potentials: from -0.32 V for system NAD^+/NADH up to +0.8 V for O_2/H_2O. This highly organized system of carriers is placed in a mitochondria's internal membrane and is called **respiratory chains**, sometimes more generally the **electron-carrier system** or **electron-transporting chain**.

b) The value *n* in equation (8-1) can attain the value of 3. If hydrogen enters the respiratory chain by a reducing reduction agent other than NADH, the value of *n* is lower.

8/1 The chemical theory of oxidative phosphorylation

The manner in which exergonic oxidation-reduction reactions interconnect with the endergonic synthesis of ATP remained at the level of hypotheses for the longest time. It was believed that in the "battery" of the oxidoreductases forming respiratory chains there are places where ADP phosphorylation actually takes place. Some of the oxidoreductases of the respiratory chain should therefore have the capability of storing energy they gained by the transfer of electrons or hydrogen from the donor to the acceptor, directly into the macroergic compound of ATP. This picture is in no way counterintuitive; it would be the interconnection of the exergonic redox reaction with the endergonic ATP synthesis. However, this hypothesis was never experimentally proven primarily because, in spite of a great effort, the "directly phosphorylating" oxidoreduction systems in mitochondrial membranes have never been found. /MK/

Currently it is the **chemiosmotic theory** that is generally accepted. Awarded the Nobel prize in 1978, it was introduced in 1961 by the English biochemist Peter D. Mitchell. It appears, in fact, that the oxidation-reduction activities of the respiratory chain are not directly connected with ADP phosphorylation (Fig. 8.2). The energy that is acquired through the oxidation of reduced cofactors is first utilized for the active transport of protons (hydrogen ions) from the mitochondrial matrix across an internal membrane into intermembrane space (Fig. 8.1.) It is not yet known with certainty how many protons are transferred through the membrane at the cost of a single NADH molecule oxidation; the newer data speak of 12 hydrogen ions. In the second, independently occurring step, protons are passively transported back into the mitochondrial matrix; this exergonic process provides energy to the endergonic ATP synthesis, which is mediated by an enzyme called ATP synthase.

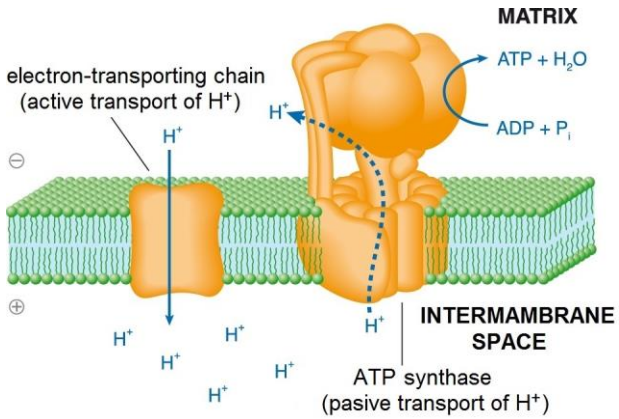


Fig.8.2: **Schematic depiction of the origin of the proton-motive force and its coupling with ATP synthesis in mitochondria**

8/2 Mitochondria → eukaryotic organisms

As with many other cases while explaining the origin of proton-motive force, it is important to distinguish what type of organism is being considered. Keep in mind that the very mentioning

of mitochondria clearly shows that we are focusing on eukaryotic organisms since prokaryotic cells do not contain organelles! /MK/

The active transport of H^+ ions leads to a substantial increase of **hydronium ion concentration** on the external side of the mitochondrial membrane and thus the pH value here drops. Simultaneously, the space is enriched by a positive charge whereby there originates a measurable difference in electric potentials (the **membrane potential** $\Delta\varphi$.) An actively working mitochondria maintains the difference of about one pH unit and a membrane potential of around -0.15 V. It is understandable that H^+ ions have a great tendency to transfer back into the matrix. They thus have a certain potential energy and during the transition through the membrane they can perform useful work. For this special kind of electroosmotic energy a term **proton-motive force** (PMF for short) was coined. A physical chemist would most likely rather speak of a difference in electrochemical potentials of hydrogen ions that would be directly equal to the change of Gibbs energy connected to the return transfer of H^+ ions into a matrix:

$$\Delta G = F\,\Delta\varphi - 2{,}303RT\log\frac{a_{\mathrm{H^+,ext}}}{a_{\mathrm{H^+,int}}} \qquad \text{(8-2)}$$

where F is Faraday constant, R the universal gas constant, T the thermodynamic temperature and $a_{H^+,ext}$ and $a_{H^+,int}$ are activities of hydronium ions outside and inside the space discussed. If we divide the equation by F, we shall get the known definitional relation for PMF:

$$\mathrm{PMF} = \Delta\varphi - \frac{2{,}303RT}{F}\Delta\mathrm{pH} \qquad \text{(8-3)}$$

The dimension of PMF is, clearly, the volt (or millivolt).

8/3 Sign convention

Because the external space of the compartment carries a positive charge (in relation to the internal space), the membrane potential is negative (section 5.9) Because the second term on the right side of the equation (8-3) is positive, the sign placed in front of it must be minus, for the bigger this term, the more negative ΔG will be and the energy yield from the transport of protons back into the mitochondria will be higher. */MK/*

8/4 Chemiosmotic theory and the pumped storage hydroelectric plant

Mittchell's chemiosmotic theory explaining the conversion of the proton electrochemical gradient into chemical energy, often found difficult to understand, is easily explained by analogy to the working of a pumped storage hydroelectric plant. The basis of such a hydroelectric plant is a dammed lake that serves as a surge tank and a higher placed 'pond'. These two storage reservoirs are connected by a pipe (indicated on the map as a purple line) at the bottom end of which a reverse turbine is located. During the "top down" flow of water, this setup can be utilized for the production of electric energy, but with the reverse water flow and electric use it can also pump water from the lower to the upper reservoir. The whole mechanism is used for the accumulation of energy at night, when there is a surplus of electric energy in the network (and is therefore cheaper), by pumping water from the lower to the upper reservoir while during the daytime energy peaks the water is released back so that with the help of the turbine it can make expensive and, at that point, needed electricity.

The system of inner mitochondrial membranes functions quite analogically. The electron-transporting system, at the cost of redox reactions energy, actively pumps protons against their electrochemical potential (simply "energetically up hill") so that, subsequently, these particles could flow via the proton ATP synthase channel back and thereby start up the rotor of the catalytic part of this enzyme and thus produce ATP. It remains to be added that the system works only when the turbine is not lying "upside down," for which we use the term "correct vector placement".

A well known pumped storage hydroelectric plant has been working, since the 1930´s, about 30 km south of Prague (Czech Republic) near the village of Štěchovice. It can pump water from the river Vltava up to the artificial pond Homole (see the violet line on the enclosed map). A few more facts about the Štěchovice pumping power plant: the maximum electric output is 48 MW, the effectiveness of energy storage 75 %, the elevation difference 220 m, the maximum water throughput 24 $m^3 s^{-1}$, and the volume of the Homole reservoir 467 000 m^3. */Jan Borovanský, MK/*

The source of energy for the formation of PMF need not be the oxidation of reduced cofactors, as is the case in mitochondria of eukaryotes or in respiring chemotrophic prokaryotes where the complete process takes place on the cell membrane. The identical form of energy originates in the chloroplasts by the transformation of light energy during the light-dependent phase of photosynthesis through the analogical mechanisms as in mitochondria (para 8.4.2). Bacteriorhodopsin from *Halobacterium halobium* belongs to the well studied membrane systems actively transporting protons. Thanks to its prosthetic retinal group, this rather simple membrane

protein absorbs light energy and uses it directly for the creation of the proton gradient (mentioned in sect. 5.9). The chlorophyll reaction center in photosynthetic purple bacteria functions in a similar manner. In all these cases PMF is used for ATP synthesis during which the membrane enzyme, similar to the mitochondrial ATP synthase, is employed.

It is important to keep in mind that the energy of PMF components, i.e. the electrical ($\Delta\varphi$) and the concentration (ΔpH), are independent of each other. Table 8.1 shows that while on the mitochondrial membrane both components of PMF are important, the membrane potential of the chloroplast thylakoid membrane (sect. 8.4) is practically zero and the PMF exists here only thanks to the enormous difference in the concentration of hydrogen protons.

Table 8.1: Components of the proton motive force on the mitochondrial internal membrane and on the thylakoid membrane

Membrane	**$-\Delta\varphi$ (V)**	**ΔpH**
mitochondria	0,15 až 0,20	$\approx$ 0,75
thylakoid	$\approx$ 0	$\approx$ 3,5

The ATP synthesis in mitochondria (**oxidative phosphorylation**) as well as in thylakoid chloroplasts (**photo-phosphorylation**) therefore have a common dominating sign: the primary form of energy (chemical from reduced cofactors or light absorbed by molecules of the relevant pigments) is first transformed to a specific form of energy (PMF), which is then utilized for the synthesis of ATP. This action is tied to biological membranes. In the following text we shall use the term **membrane phosphorylation**, which suitably accentuates the common nature and factual course of energy storage into ATP in the processes of cell respiration and photosynthesis.

8/5 The origin of proton-motive force on the membrane of fermenting cells

PMF, as a form of potential energy, is inextricably needed for all cells and not only for the synthesis of ATP, as we shall soon see. But how do fermenting cells, the dominating sign of which is precisely the fact that they do not acquire ATP through membrane phosphorylation (sect. 7.1, sect 7.5, sect. 10.5, and sect. 10.6), get it? Because the primary form of their energy is chemical, stored in ATP, they can make use of it in a reverse process to how respiring organisms achieve it: through the form of the primary active transport with the help of H^+-ATPase that translocates protons through the cell membrane and so creates PMF for further use. /MK/

The crossing of protons in the direction of the membrane electrochemical potential gradient (in the direction of PMF decline) is an exergonic process. The released energy can be utilized in different ways that we will now outline:

- **For ATP synthesis**. As we have already stated, this process is one of the dominant steps in the transformation of energy in biological systems. All the ATP produced by photosynthesizing cells is synthesized through the process of membrane phosphorylation just like the vast majority ATP synthesized by chemotrophic respiring cells! For example, during the complete oxidative degradation of glucose (glycolysis → citrate cycle →

respiratory chain) approximately 90 % of ATP molecules originates through membrane phosphorylation and only the remaining 10 % through substrate phosphorylation.

EC 3.6.3.14
ATP-phosphohydrolase (H^+-transporting)
newly EC 7.1.2.2

All membranes in which membrane phosphorylation takes place contain an enzyme of analogical structure. It is called **ATP synthase** and is formed by two non-covalently connected parts: a transmembrane channel (Fo) that enables passive transport of protons and an oligomeric head (F_1) catalyzing the actual reaction ADP + P_i → ATP + H_2O (Fig. 8.3) Whereas channel Fo and the head F_1 occupy a stable position in relation to the membrane, the stalk (subunit γ) turns where each full turn corresponds to the synthesis of three ATP molecules in consequence of conformational changes in α and β subunits forced by the rotational movement of the subunit γ. And it is just this movement of subunit γ, strikingly reminiscent of the Francis turbine, that is evoked by the exergonic passive transport of protons.

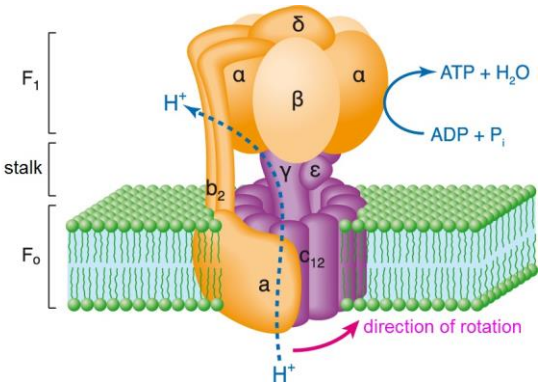


Fig. 8.3: **The structure of the mitochondrial ATP synthase**

8/6 How to name ATP synthase without embarrassment?

The terminological chaos relating to ATP synthase is astounding. Let us here review three most commonly used names of this enzyme and look at their weaknesses:

ATP synthase: As we have already shown in sect. 4.6, the concept of synthase continues to be linked to lyase; our enzyme, however, was for many years classified as hydrolase, but in 2018 it became translocase (class 7).

F_oF_1-ATPasa: The letter *o* got into the index thanks to the fact that the membrane channel can be blocked by the antibiotic oligomycin. But try to repeatedly say *f o f one ATPase* and, for sure, you'll have the impression that you've lost your mind.

F_0F_1-ATPasa (that is *"ef zero ef one ATPasa*"): This designation originated from the earlier one when users couldn't believe that in an index a letter and a number can be next to each other. But it is wrong, the zero really has no place there.

As you see, the choice of a name for this enzyme is a selection from poor variants. After a diffident discussion we chose the first one for this textbook; to defend this decision is however not easy. */MK, OV, RH/*

- **For the secondary active transport** (sect. 5.9.2). A lot of molecules or ions are transported with the participation of protons via symport or antiport: in bacteria nutrients are transported by symport (glucose, lactose, succinate, amino acids, etc.) while sodium ions are secreted by

antiport. For mitochondria, symport of H^+ ions with pyruvate (with the help of pyruvate translocase) or phosphates (posphate carrier protein) is typical.

8/7 What is a "proton translocation?"

Experimentally it is difficult to distinguish the movement of ions OH^- from the directionally opposite H^+ movement. That is why, in this context, many authors use, instead of proton transport, the term **proton translocation** which also subsumes the eventual transport of OH^- in the opposite direction. */MK/*

- **For the flagella movement in bacteria**. The rotation of flagella of some bacteria is controlled directly by the exergonic movement of protons at the cost to their electrochemical potential. For example, the structure of this 'rotation motor,' strongly reminiscent of the Francis water turbine, has been studied in detail in *E.coli*. The mechanical nature of the engine is supported by the following observations:

  (i) The speed of the flagella rotation in an intensively metabolizing cell is indirectly proportional to the environment's viscosity. It indicates that with a constant PMF the torque moment of the motor remains constant.

  (ii) The flagella are calm in starving cells the PMF of which is zero.

  (iii) The flagella rotation of starving cells can be controlled by an artificial pH gradient or by artificial membrane potential; the rotational speed is then directly proportional to the created PMF. From this it was calculated that for a single flagella turn approximately 1000 protons must pass through the motor.

  (iv) By changing the polarity of an artificially induced PMF one can change the direction of the flagella's movement.

- **For the production of heat in brown fat tissue.** As noted in sect. 7.5, in justifiable cases PMF is utilized for the direct production of heat. In brown fat tissue, whose cells contain a large amount of mitochondria, the specialized, carefully controlled channels (formed by the protein called thermogenin) are incorporated into the inner mitochondrial membrane. They can release protons back into the matrix without other useful work being performed. The total PMF energy is thus converted to heat. This mechanism is referred to as "non shivering thermogenesis" and it is utilized, for example, by hibernating animals (see the image of a skinny bear awakening from winter sleep) or human newborns.

- **For the production of NADPH**. For reducing, usually anabolic processes NADPH is often used as a reducing agent (para. 4.5.2) It is often spoken of as the **reduction equivalent** and a variety of reactions supply the cells with this molecule. The following reaction

$$NADP^+ + NADH \rightarrow NADPH + NAD^+$$

is reversible in a solution and has an equilibrium constant equal to 1, since the standard redox potentials of both systems are identical. However, for reasons of intracellular concentrations, it is not realizable because $[NADP^+] << [NADPH]$ but $[NADH] << [NAD^+]$. In bacterial and mitochondrial membranes the enzyme named pyridine nucleotide transhydrogenase (EC 1.6.1.2) is present; it utilizes PMF energy for the balance shift such that the resulting NADPH concentration can be as much as 500 times higher than the concentration of NADH.

8/8 The truths about proton-motive force

Let us review our story about PMF by means of not entirely trivial propositions.

a) PMF is a form of energy that has a decisive role in the phosphorylation of ADP in mitochondria and even in chloroplasts, i.e. thylakoids.
b) PMF applies itself also during the synthesis of ATP in bacteria with anaerobic respiration (sect. 8.3)
c) PMF consists of two mutually independent components: membrane potential and pH differential.
d) PMF can be utilized for ATP synthesis, but also for several other important endergonic membrane processes. /*MK*/

8/9 Sodium-motive force?

In conclusion of the section about proton-motive force we will allow ourselves a short detour. For extremely alkalophilic microorganisms living in high pH environments (cyanobacteria even up to pH 13) it would be very disadvantageous to transport protons from a cell since they would never reach a state where, in the extracellular space, their electrochemical potential would be greater than in cytosol. That is why all the above described mechanisms are "arranged" differently: instead of protons they transport sodium ions from the cell and, if needed, they release them back into the cytosol through cleverly arranged channels. With a bit of linguistic dare we could therefore, in their case, speak of a "sodium-motive force," expressible as the transmembrane electrochemical potential difference of sodium ions. /*MK/*

8/1 QUESTION

It is know that 2,4-dinitrophenol increases the permeability of the inner mitochondrial membrane for hydrogen ions.

a) In what way will this substance influence the process of oxidative phosphorylation?
b) If we inject a rat with 2,4-dinitrophenol, its body temperature will increase rapidly. Explain why.

8.2 Respiratory chain of aerobic chemoorganotrophs

Let us now describe in more detail the structure and function of the respiratory chain localized in the mitochondrial inner membrane of the aerobic chemoorganotrophic eukaryotes. We had already introduced the summary equation of this process in the preceding text (eq. 8-1). However, the process brakes down into three partial redox reactions that can be described with three partial equations (we call on the reader to stoichiometrically adjust and add up these equations so as it gain a key part of the (8-1) equation!):

$$NADH + H^+ + CoQ \xrightarrow{\text{C I}} NAD^+ + CoQH_2 \qquad (8\text{-}4)$$

$$CoQH_2 + 2\ \text{cyt-c}(Fe^{III}) \xrightarrow{\text{C III}} CoQ + 2\ \text{cyt-c}(Fe^{II}) + 2H^+ \qquad (8\text{-}5)$$

$$4\ \text{cyt-}c(Fe^{II}) + 4\ H^+ + O_2 \xrightarrow{\text{C IV}} 4\ \text{cyt-}c(Fe^{III}) + 2\ H_2O \qquad (8\text{-}6)$$

where the symbols C I, C III and C IV mark individual **enzyme complexes** integrated within the membrane (in the following text they are called **Complexes**). CoQ is a **coenzyme Q** (also called ubiquinone) and cyt-*c* is a heme protein **cytochrome-*c***. The Complexes here play the role of oxireductases in the full sense of that term. However, compared to "common" enzymes of this class, they are capable of utilizing energy provided by the exergonic redox reaction, which they catalyze, for the active transport of hydrogen protons. Coenzyme Q and cytochrome-*c* function as **mobile carriers**; in individual reactions that are catalyzed by the Complexes these carriers are reduced (they accept hydrogen atoms or only electrons) so as to transfer them further (see the previous chemical equations.) A true "chain" transfer of electrons (i.e. hydrogen atoms) thus takes place in sequence NADH → CoQ → cyt-c → O_2. The overall scheme of the process is depicted schematically in Fig. 8.4 and we will now look at it more closely.

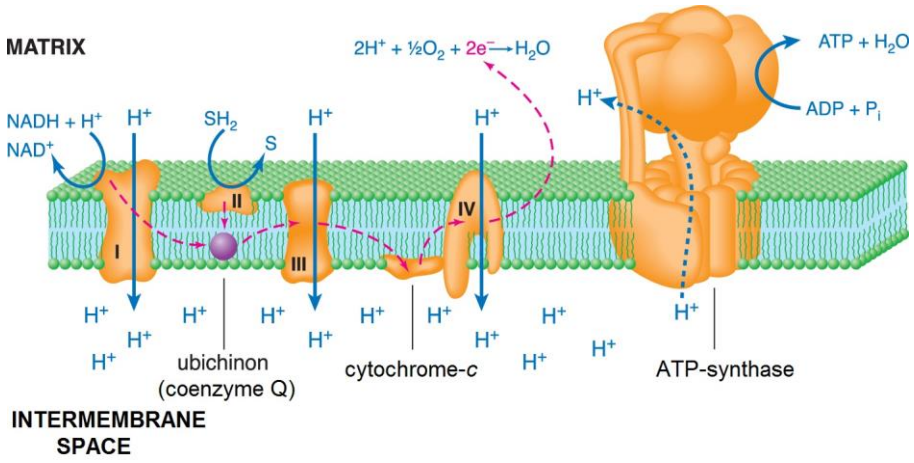


Fig. 8.4: Scheme of the mitochondrial respiratory chain (detailed description in text)

Nicotinamide adenine dinucleotide (formula in para. 4.5.2) is one of the most important coenzymes of oxireductases. Its main task in a metabolism is to receive hydrogen atoms (i.e. electrons) in dehydrogenation catabolic reactions (the reduction of NAD^+ to NADH). A range of such substrates exists and in the relevant chapters we will see that it can, primarily, be glyceraldehyde-3-phosphate in glycolysis, isocitrate, 2-oxoglutarate or malate in citrate cycle, pyruvate during its oxidative decarboxylation or the 3-hydroxyacyl-SCoA in β-oxidation of fatty acids. With the exception of glyceraldehyde-3-phosphate dehydrogenation, all these reactions take place in the mitochondrial matrix (in eukaryotes) and it is into this compartment that the active site of the Complex I intervenes. NADH submits its electrons (hydrogen atoms) to the Complex I, thus re-oxidizing to NAD^+ which can enter the next oxidation-reduction cycle (oxidize another substrate molecule and transfer the gained electrons to C I). The task of the **Complex I**, considered

to be the **main entry** into the respiratory chain is, therefore, to re-oxidize NADH and, inversely, reduce the mobile carrier coenzyme Q (eq. 8-4 and Tab. 8.2). Like all the others, Complex I also contains non-peptide parts (prosthetic groups and metal ions.)

Table 8.2 The basic characteristics of mitochondrial respiratory chain complexes

Complex	Catalyzed reaction	Systematic name	EC (newly)	Other names	Non-peptide parts (prosthetic groups)	Active transport of protons
I	8-4	NADH:ubichinon oxidoreductase	1.6.5.3 (7.1.1.2)	ubichinon reductase	flavinmononucleotide, non-heme iron	+
II	8-7	succinate: ubichinon oxidoreductase and others	1.3.5.1	succinate dehydrogenase and others, „side entrances“	flavin adenine dinucleotide	–
III	8-5	ubichinol: ferricytochrome-*c* oxidoreductase	1.10.2.2 (7.1.1.8)	cytochrome-*c* reductase	heme, non-heme iron	+
IV	8-6	ferrocytochrome-*c*: oxygene oxidoreductase	1.9.3.1 (up to now no new EC number)	cytochrome-*c* oxidase	heme, copper ions	+

The first mobile carrier of the respiratory chain, **coenzyme Q** (also called **ubichinon**, Fig. 8.5a), is 1,4-benzoquinone with an isoprenoid side chain; this molecule of a hydrophobic nature is easily soluble in the non-polar environment of the biological membrane. It transfers two hydrogen atoms (a reduction of the quinone to a hydroquinone structure); it can also occur in semiquinone form and transfer one hydrogen atom.

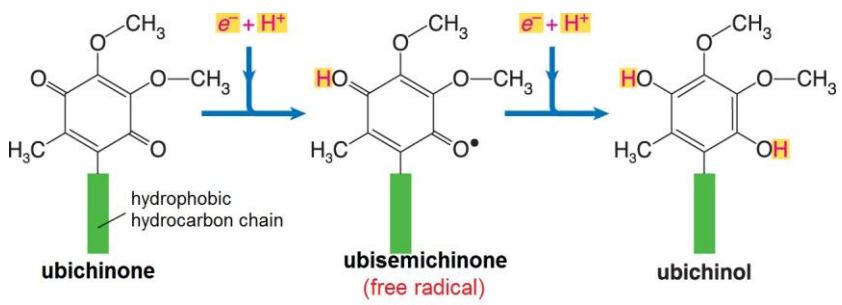
The reduced ubiquinon transfers hydrogen atoms to **Complex III** which catalyzes its reoxidation in accordance with the equation 8.5. With the participation of its heme structures and “non heme” iron ions, electrons are transferred to the next mobile carrier, this time of a protein nature. It is a small hemoprotein, **cytochrome-*c***, which moves around predominantly within the intermembrane mitochondrial space. It takes over an electron from the prosthetic groups of Complex III, whereby its iron ion Fe^{3+} is reduced to Fe^{2+} (Fig. 8.5b).

ANSWER

8/1 a) It reduces the effectiveness of membrane phosphorylation because at least part of H^+ transfers back into the mitochondrial matrix without performing any useful work (e.g. to enable the ATP synthesis).

b) The above mentioned H^+ translocation is a type of passive transport where the total released energy is changed to heat.

a)



b)

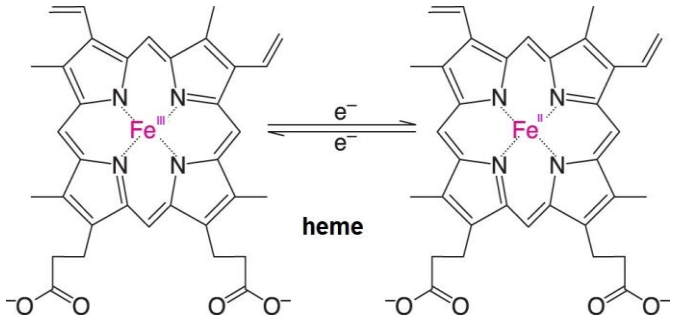


Fig 8.5: (a) Gradual reduction of ubichinone, (b) redoxreaction of heme

At the end of the chain, cytochrome-*c* transfers an electron to **Complex IV**, which gradually receives the electrons from the four molecules of this mobile carrier and, by means of its metal ions (heme Fe^{3+}, Cu^{2+}), it transfers them to an oxygen molecule in accordance with the summary equation (8-6). This results in two molecules of water. The fundamental significance of the respiratory chain for the energetics of an aerobic metabolism is also confirmed by the known toxicity of cyanides; namely, CN^- inhibits Complex IV and thereby blocks the whole pathway.

8/10 Other entries of oxygen into metabolism

Except for Complex IV, molecular oxygen enters the metabolism of aerobic organisms also by means of other reactions catalyzed by oxidoreductases from the oxidase and oxygenase groups (sec. 4.6); these reactions are however quantitatively much less important than the cytochrome-*c* oxidase reaction. */MK/*

QUESTION

8/2 Characterize individual components of the mitochondrial respiratory chain by filling out the following table. Specify the sequence in the respiratory chain with the numbers 1-5. For the molecular type, mark as L the low-molecular-weight substances and H as the high-molecular-weight ones. In the column Component characteristics, mark enzymes as E, carrier as C (use Ce^- for electron carriers and CH for hydrogen carriers).

Component	Numerical order in respiratory chain	Molecular type	Component characteristics
cytochrome-*c*			
ubiquinone reductase			
cytochrome-*c* oxidase			
ubiquinone			
cytochrom-*c* reduktase			

The question what is the molecular basis of the so called **side entry** into the respiratory chain, sometimes referred to as **Complex II** (Tab. 8.2.), is a frequent source of quandary. One often gets the impression from biochemistry textbooks, that this somewhat mysterious formation receives two hydrogen atoms from $FADH_2$ and transfers them directly to the respiratory chain or, more precisely, to the coenzyme Q molecule. Above all, it is important to take in that $FADH_2$, as opposed to NADH, does not exist in the liquid environment of the cell as such. As a prosthetic group it is always bound to the peptide chain of an enzyme. The side entry should, according to this view, reoxidize all these soluble enzymes, which is hard to imagine given the known substrate specificity of enzymes. However, the situation is somewhat different. Those FAD-dependent dehydrogenases (e.g., succinate dehydrogenase from citrate cycle or acyl-CoA dehydrogenase from β-oxidation of fatty acids) are parts of the enzyme complexes embedded in the inner mitochondrial membrane and are reoxidized by ubiquinone; each such membrane FAD-dependent dehydrogenase can thus be proud of being called the "side entry into the respiratory chain." Let us assign the general symbol SH_2 to the reduced substrate; the catalyzed reaction will then have the form:

$$SH_2 + CoQ \xrightarrow{\text{C II}} S + CoQH_2 \qquad (8\text{-}7)$$

It is important to keep in mind that, in contrast to the Complexes I, III and IV, Complex II is not capable of utilizing the energy of the redox reaction (which it catalyses) for the active transport of protons. The entry of hydrogen atoms into the respiratory chain this way is therefore less energetically advantageous than the "main" entrance, i.e., through the Complex I.

From the overall scheme of the respiratory chain and the ATP biosynthesis in the mitochondria (Fig. 8.4) it does not follow how many molecules of ATP are gained at the cost of a single NADH molecule reoxidation (parameter *n* in eq. 8-1). The data in the literature and textbooks differ in this parameter which is not surprising. As we have already shown, the proton-motive force is formed at the cost of energy of reoxidation NADH but is not utilized for the ATP synthesis only. Usually it is stated that the oxidation of one NADH molecule enables the synthesis of 2.5 to 3 ATP molecules and that the entry of two hydrogen atoms (the oxidation of one molecule of a reduced SH_2 substrate) by means of the Complex II corresponds to the formation of 1.5 to 2 ATP molecules. In calculations, traditionally, the values of 3 ATP / 1 NADH and 2 ATP / 1 SH_2 are counted on, most likely because natural numbers are so charming and close to the human heart.

8.3 Anaerobic respiration

In the previous paragraph we described "respiratory chain of aerobic chemoorganotrophs." Is this wording not a pleonasm, a superfluous accumulation of semantically close concepts? Had we not, as children, learned in biology that breathing (more nobly respiration) is the intake of oxygen and expeling of carbon dioxide? Can anaerobic organisms actually respire? In this section we shall explain that in biochemistry the concept of respiration has a somewhat different meaning and the term introduced in section 8.3 is not nonesensical.

The most important role of molecular oxygen in an aerobic metabolism follows from the eq. (8-6): by means of the catalysis of cytochrome-*c* oxidase it accepts electrons and later protons and converts them to water. We say that oxygen plays a role of the **final acceptor of electrons in the respiratory chain**. Chemically, this can be expressed by saying that it functions as a terminal oxidizing agent. However, this role can also be fulfilled by other molecules or ions that the cell can take in from the external space, for example nitrate (NO_3^-) or sulphate (SO_4^{2-}) ions:

$$NADH + H^+ + NO_3^- \rightarrow NAD^+ + NO_2^- + H_2O$$

$$4\,(NADH + H^+) + H^+ + SO_4^{2-} \rightarrow 4\,NAD^+ + HS^- + 4\,H_2O$$

The disadvantage of these oxidizing agents, when compared to oxygen (eq.8-1) is a lower standard redox potential and thus lower energetic effectiveness of NADH reoxidation. On the other hand, "stoichiometric effectiveness" of Sulphur, when in the described reaction one atom of sulphur accepts eight electrons, is fascinating from a chemical point of view.

We call the described process **anaerobic respiration** (c.f., Tab. 7.1). It is primarily utilized by bacteria living in an anaerobic environment where the relevant oxidizing agents occur, i.e., primarily soil bacteria. Denitrification bacteria reduce nitrate ions to nitrite but sometimes even to forms of nitrogen with a lower oxidation number (sometimes even to ammonia). Similarly, sulphate reducing bacteria can reduce sulphate ions to sulphite, thiosulphate or sulphane. These processes contribute in important ways to the nitrogen and sulphur circulation in nature and enable chemoorganotrophic bacteria to fill the anaerobic niches in the biosphere (including, e.g. the intestinal tract of vertebrates).

8/11 The danger of anaerobic respiration for eukaryotes

Why then do we encounter anaerobic respiration in prokaryotes (bacteria) and not in eukaryotes? Because the products (the reduced electron acceptors) are toxic. Prokaryotes can afford to produce them for their respiratory chain that is localized on the cellular membrane and the toxic products do not enter the cell. But if the respiratory chain resided in the inner mitochondrial membrane, as is the case with eukaryotes, the cell would probably be poisoned quickly. */MK/*

8/3 QUESTION

List the most important characteristics of cells utilizing the fermentation type of metabolism.

8.4 The light dependent phase of photosynthesis - the top example of the complex cellular transformation of energy

The task of photosynthesis is to endergonically reduce carbon from carbon dioxide and build it into organic molecules, primarily into molecules of saccharides:

$$CO_2 + 4\,[H] + n\,(ATP + H_2O) \rightarrow \text{-CHOH-} + H_2O + n\,(ADP + P_i) \qquad (8\text{-}8)$$

The light-dependent phase of photosynthesis provides energy for this chemical reaction in the form of ATP and the reducing agent NADPH; in sum this process can be described by two simple equations:

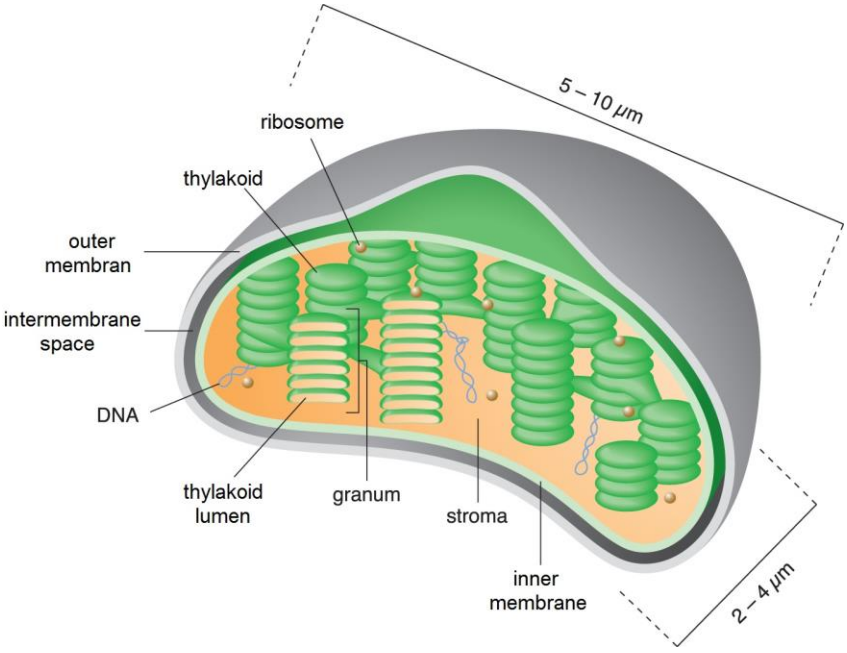
$$ADP + P_i \xrightarrow{h\nu} ATP + H_2O \qquad (8\text{-}9)$$

$$NADP^+ + H_2D \xrightarrow{h\nu} NADPH + H^+ + D \qquad (8\text{-}10)$$

where D is the hydrogen donor. Both chemical reactions are endergonic and for them to take place it is necessary to supply energy in the form of light quanta indicated as *hv*.

Photosynthesis in higher plants is localized in specialized organelles, **chloroplasts** (Fig. 8.6.). Similarly to mitochondria, they have two membranes on their surface. The inner space (stroma) contains an additional membrane system called **thylakoids**, in which the light phase of photosynthesis takes place (ATP and NADPH synthesis); they form stacked membrane regions called **grana** (sing. **granum**). Enzymes guaranteeing the reactions of the dark phase are localized in the chloroplast stroma: the fixation of aerial CO_2 and its incorporation into organic molecules, as well as subsequent metabolic processes. Like mitochondria, chloroplasts also include their own DNA and a proteosynthetic apparatus that is similar to the prokaryote one.

Fig. 8.6: The chloroplast of higher plants and green algae



We will discuss the **light-dependent phase of photosynthesis** (sometimes referred to as the **primary** phase) in three separate paragraphs describing the capture of the light quantum, the membrane phosphorylation of ADP, and the reduction of $NADP^+$. The **light-independent phase** (sometimes also called the **secondary** phase), i.e. the process of CO_2 incorporation into the monosaccharide molecule that does not directly require light (Calvin cycle), will be described in sect. 10.10 in the context of saccharide metabolism.

8/12 Mitochondria of green plant cells

It is worth emphasizing at this point that even photosynthesizing cells of plants have mitochondria. This is seemingly obvious but needs reminding since at times we encounter the faulty opinion that if cells have chloroplasts that produce ATP then they do not really need

mitochondria since they have the same function. The opposite is the case! By the way: How would green cells acquire the requisite amount of ATP at night? Furthermore, the plant mitochondrial genome is substantially bigger than in animals and is formed by a complex of linear and circular DNA molecules of varying sizes. /RH/

8.4.1 Photon capture

The absorption of visible or ultraviolet radiation converts molecules into a state with higher energy (into an electron excited state) that is always unstable and a molecule must, sooner or later, return to a ground state. Generally, it has several possibilities how to achieve this:

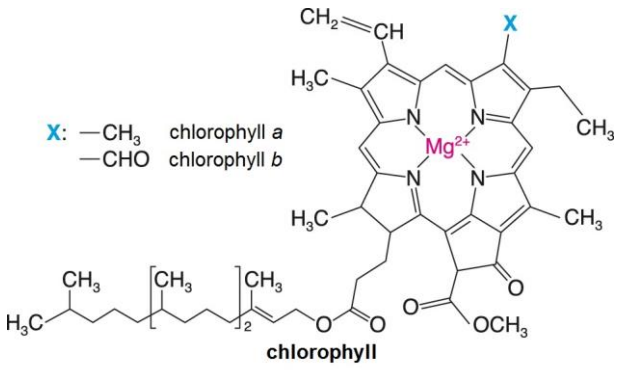
1. It can rid itself of energy by emission of photons (**fluorescence**, exceptionally even **phosphorescence**.)

2. It can return to its ground state by means of **non-radiative relaxation**, whereby energy is converted to heat in connection with a variety of intermolecular collisions.

3. Through **resonance energy transfer** it can transfer to the excited state another molecule that absorbs light at the same or close wavelength and is in an appropriate geometrical position in relation to the excited molecule.

4. In an excited state molecules tend to be highly reactive and are easily subject to different **chemical changes**; these processes are the subject of **photochemistry**. A molecule can thus lower its energy in reacting with another molecule whereby the products of a reaction are, from the perspective of quantum mechanics, in a ground state.

Some form of **chlorophyll**, the common structure of which is similar to the structure of heme, is the most important pigment absorbing light in the membranes of photosynthetic bacteria and in the chloroplasts of algae and higher plants. However, the atom of metal bound in the tetrapyrrol ring by coordinate bonds is, in this case, magnesium (Mg^{2+}) and a part of the molecule is also a long aliphatic chain. Chlorophylls *a* and *b* are primarily found in the chloroplasts of higher plants. The molecules of chlorophyll are always part of proteins and should we continue to use this term in the text we will always mean a chlorophyll containing protein.



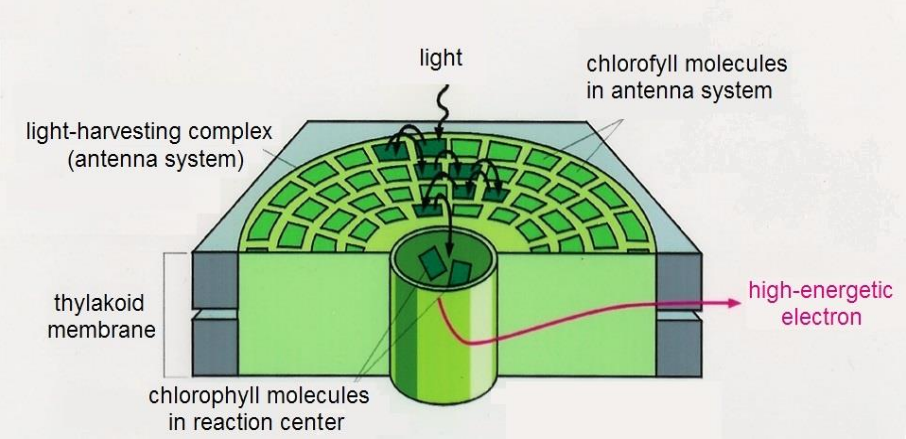
ANSWERS

8/2

COMPONENT	Numerical order in respiratory chain	Molecular type	Component´s characteristics
cytochrome-*c*	4	V	Pe^{-}

NADH-Q reductase	1	V	E
cytochrome-*c* oxidase	5	V	E
ubichinon	2	N	PH
cytochrome reductase	3	V	E

8/3 Cells active in an anaerobic chemoorganotrophic regime that do not receive any oxidizing or reducing agents from the external environment (they do not utilize the respiratory chain) and thus acquire all ATP through substrate phosphorylation.



Although all molecules of chlorophyll are capable of absorbing light, only a small number (in chloroplasts approximately every three hundredth molecule) is part of a **reaction center** where it has photochemical functions. The other chlorophyll molecules form a sort of **antennae system** also called the **light harvesting complex** (LHC). If one molecule of this system absorbs a photon, it can hand over its energy by means of the resonance energy transfer mechanism to a neighboring molecule (mechanism **3**, see above.) The energy absorbed by any chlorophyll molecule then travels via the antennae system for as long as it takes to reach the reaction center; the life expectancy of energy travelling this way is matter of nanoseconds. This travel of energetic quanta is very effective primarily because only a small amount of them is lost to fluorescence (mechanism **1**) or nonradiative relaxation (mechanism **2**). It provides evidence that chlorophyll molecules are perfectly spatially oriented in the membrane structures of chloroplasts.

Chloroplast chlorophylls absorb light in two regions: blue light in the range of 400-450 nm and red light at 670-710 nm; bacterial chlorophyll has these maxima shifted upward to higher wavelengths. For its activities (photochemical reaction, see further) chloroplasts are unable to effectively utilize the yellow-green spectrum range where, however, the intensity of sun radiation is the highest. The very fact that leaves are green proves that chlorophylls absorb the least in this range of the spectrum. Other chloroplast pigments have their absorption maximums in this part of the spectrum, primarily carotenoids. Resonance transfer of energy from carotenoids to chlorophylls is possible; however, as for its range and importance the literature does not provide straight answers.

8/13 The beauty of an autumn forest

In the fall trees dampen their metabolic activity. Mostly they limit photosynthesis by degrading chlorophyll due to which the yellow and red pigments become apparent in leaves. Not a very romantic explanation for the beauty of the autumn's leafy forest! */OV, MK/*

8.4.2 ATP synthesis by way of cyclic membrane phosphorylation

A reader who reviewed sections 8.1 and 8.2 with care must suspect that attaching a third phosphate to the ADP molecule, as suggested by eq. (8-9), is conditional on the existence of a sufficient proton-motive force and the activity of the membrane enzyme ATP synthase. Such an enzyme is always present in photosynthesizing membranes and all that needs answering is the question of how PMF is generated here.

Cyclic photophosphorylation in the cytoplasmic membrane of photosynthesizing bacteria, e.g., *Rhodopseudomonas viridis* (Fig. 8.7.), is a very illustrative, well described, and a relatively simple example of the transformation of light energy into the form of PMF and its subsequent deposit into the ATP structure. It contains, in its reaction center, next to four bacteriochlorophyll molecules (at the maximum long-wave absorption of 870nm) as well as two molecules of bacteriopheophytin pigment and four heme molecules. After photon absorption (more precisely, after the transfer of the energy quantum from the antenna into the reaction center) the bacteriochlorophyll (further designated as P_{870}) will switch to the excited state of P^*_{870}, while its standard redox potential becomes approximately 1 V more negative and this oxidizing agent will become a reducing agent. It will thus comply with its momentary reduction "passion" and transfer an electron to the oxidated form of the quinone carrier Q (a ubiquinone analogue). It itself passes into the cation-radical form in the ground state P^+_{870} (the above mentioned mechanism **4**). Processes, characterized by the following equations, thus took place**:**

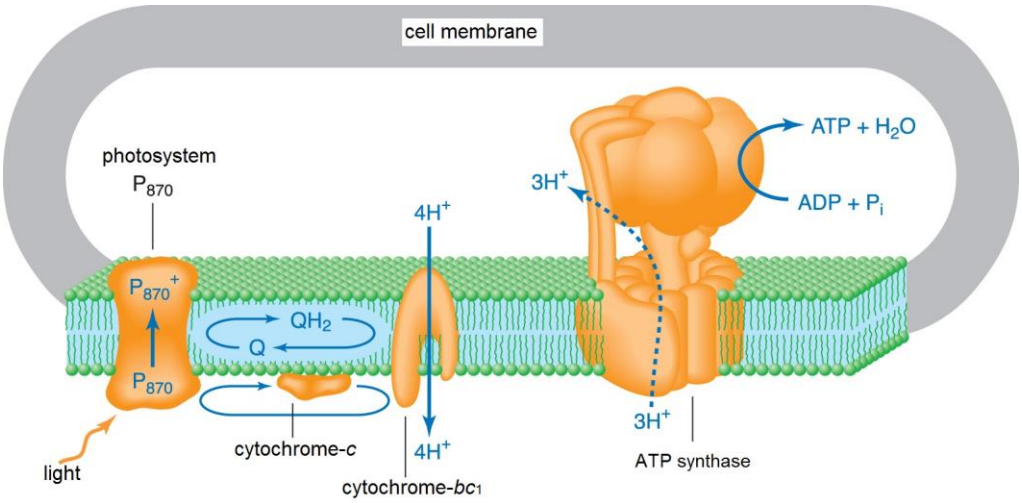


Fig. 8.7: A simplified schema of cyclical photophosphorylation in the bacteria *Rhodopseudomonas viridis*

$$2\ P_{870} + 2\ h\nu \rightarrow 2\ P_{870}^* \qquad (8\text{-}11)$$

$$2\ P^*_{870} + Q \rightarrow 2\ P_{870}^{+} + Q^{2-} \qquad (8\text{-}12)$$

The anion Q^{2-} binds two protons from the inner space (i.e. from cytoplasm):

$$Q^{2-} + 2\ H^{+} \rightarrow QH_2 \qquad (8\text{-}13)$$

The reduced form of the quinone carrier QH_2 then freely diffuses through the membrane and consequently transfers its electrons to the mobile carrier cytochrome-*c* (Fe^{III}). Cytochrome -bc_1 that catalyzes this redox reaction

$$QH_2 + 2\ \text{cyt-}c(Fe^{III}) \rightarrow Q + 2\ H^{+} + 2\ \text{cyt-}c(Fe^{II}) \qquad (8\text{-}14)$$

actively transports protons (most likely four) from the cytoplasm into the extracellular space similarly to the way Complexes of the mitochondrial respiratory chain do it.

The cycle is then closed as the reduced cytochrome-*c*(Fe^{II}) reaches the reaction center complex and transfers its electrons to the cation-radical P_{870}^{+}:

$$\text{cyt-}c(Fe^{II}) + P_{870}^{+} \rightarrow \text{cyt-}c(Fe^{III}) + P_{870} \qquad (8\text{-}14)$$

Let us look once again at the above equations and give them some thought from the point of view energy. It is obvious that all of them are exergonic. In sum, all that is taking place here is the transformation of energy according to the equation $h\nu \rightarrow$ active transport H^{+}; such is truly the overall result of the described cycle.

8/14 Cyclic photophosphorylation - an emotional explication

In reviewing the role that P_{870} has in the reaction center during the process described in Fig. 8.7., we find that bacteriochlorophyll P_{870} has a relatively high redoxpotential and so it "very much dislikes" loosing an electron. If, however, it is excited by a photon, its redox potential drops and P^{*}_{870} becomes a strong reducing agent, happy to let go of an electron and thanks to its reduction strength it can force it on the quinone carrier Q, whilst also losing its excitation energy, and finds itself in its ground state in the form P_{870}^{+}. What it "wanted so badly" happened to it: as an oxidizing agent it is in an oxidized state and desires an electron a lot. The latter will be provided, after much to do, by the reduced cytochrome-*c*. Generally speaking, the most important trick of chlorophyll photosynthesis is precisely the change of chlorophyll's redox potential in the reaction center induced by the absorption of light. */MK/*

The cyclic way of ATP synthesis, whether in a bacteria or a thylakoid, has a logic similar to the mitochondrial ATP synthesis: the primary form of energy (light during photosynthesis, energy from redox reactions in mitochondria) is first transformed into energy of PMF which is further, entirely analogically (with the use of ATP synthase), stored into an ATP molecule. Catalyzing the reaction (8-14), cytochrome-bc_1 has a truly fundamental role in this process. It could be added to the group of Complexes described in section 8.2: it is a membrane oxidoreductase that is catalyzing an exergonic reaction whose energy is exploited towards an active transport and thus develops PMF (c.f. Tab. 8.2) This process is very advantageous. It is not dependent on other chemical reactants for, as we saw, it is merely a matter of energy conversion. It can transpire without coupling up with any kind of other processes; the production of ATP by these means can therefore very quickly react to other energy needs of a cell. In the cells of some photosynthesizing bacteria this cycle is the only photosynthetic process.

In the thylakoids of **higher plants** there exist two **photosystems** that differ in the maximum of their absorption spectrum: photosystem I (also PS I or P_{700}) has the maximum absorption of light radiation at 700 nm, whereas photosystem II (PS II, P_{680}) at 680 nm. For the cyclic phosphorylation only photosystem I is utilized. In a manner quite analogical to activities described here in detail for bacteria, an electron is released from the excited molecule of chlorophyll, it then moves through the system of redox carriers (among which there is also the actively transporting enzyme complex, here identified as cytochrome-*b*6*f*) and returns to the chlorophyll molecule, at this time already in the electron ground state. The low-molecular **plastoquinone** (the analog to ubiquinone in the respiratory chain) and the copper containing protein, called **plastocyanin** (a cytochrome-*c* analog) are the mobile carriers here. In the overall balance it is an action where hydrogen protons, thanks to the energy acquired through the absorption of radiation, are actively pumped into the thylakoid. From here they are passively transported through the membrane back into the chloroplast stroma while the energy of this process is utilized by an enzyme analogical to the mitochondrial ATP synthase.

EC 1.10.9.1
plastochinol:oxidized plastocyanin oxidoreducstase
newly EC 7.1.1.6

An interesting circumstance that distinguishes the cyclic ATP synthesis in chloroplasts from a similar process in mitochondria remains to be emphasized: whereas the respiratory chain "pumps" protons from the mitochondrial matrix into the inter-membrane space (that is "out"), in thylakoids the protons are pumped into its cavity where the pH is lowered by up to three units (Tab. 8.1) without disturbance to the ion equilibrium (the membrane potential is insignificant here).

8.4.3 The synthesis of the reducing agent NADPH

The equation (8-10) describes in formal terms the manner in which, during photosynthesis, NADPH is formed. This reducing agent is needed necessarily in the subsequent biochemical reactions whose role is to fix carbon dioxide (sec. 10.10). However, it is important to realize that NADPH is a very strong reducing agent (at pH 7, the standard redox potential of the $NADP^+$/NADPH equals to -0.32 V) and the $NADP^+$ reduction must therefore be complicated and energy demanding.

A molecule of chlorophyll in the reaction center once again enters the game here. As we have already seen, it can function as a reducing as well as oxidizing agent, depending on whether it is in an excited or ground state. First, we will lean in our explication on the photochemical reduction $NADP^+$ in the chloroplasts of higher plants and then, later on, show what variants have developed in some lower organisms.

The initial step of $NADP^+$ reduction (Fig. 8.8) can be expressed with the equation (8-11) in which, obviously, the relevant photosystem must act, the P_{700} in the case of higher plants. It gains energy from light and transitions to the excited stage P^*_{700} whereby it lowers its redox potential and becomes a strong reducing agent. It is therefore able (and "joyfully" willing) to transfer an electron to a molecule which has a more positive redox potential, in this case (with several intermediary steps) to the membrane protein ferredoxin (containing non-heme iron ions) which thereby transfers from its Fe^{III} form to Fe^{II}. P^*_{700} changes to a cation-radical (in ground state) P^+_{700}. The reduced

ferredoxin is a strong enough reducing agent so that it can transfer ("force") an electron to the $NADP^+$ coenzyme:

$$2\ \text{ferredoxin-Fe}^{II} + NADP^+ + H^+ \rightarrow 2\ \text{ferredoxin-Fe}^{III} + NADPH \qquad (8\text{-}16)$$

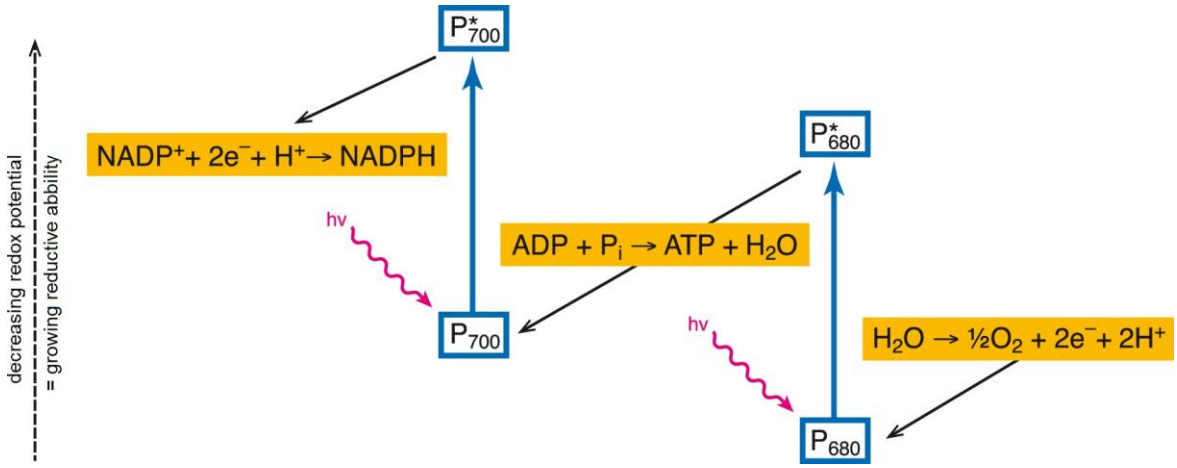


Fig. 8.8: A schematic representation of electrons path from water to NADPH during oxygenic photosynthesis The black arrows indicate the intermolecular enzyme redox reactions, the blue ones indicate the light initiated changes of redox potentials of both photosystems.

The situation with P^+_{700} becomes, at this point, complicated for its electron is definitively lost in the world of biochemical transformations (compare it with the cyclic pathway when an electron returned back again to it after a lengthy trip). It must therefore acquire it from a reducing agent that has a sufficiently negative redox potential.

Some bacteria solved this problem with a process termed **anoxygenic photosynthesis**. They have but one type of reaction center and in addition to the above described cyclic PMF formation they can, in a non-cyclic process, transfer electrons to $NAD(P)^+$. Molecular hydrogen or organic molecules, such as succinate, can be the source of hydrogen (electrons) for them; in the most frequent case of purple sulphur bacteria, sulphane is the reducing agent:

$$2\ P_{700}{}^+ + H_2S \rightarrow 2\ P_{700} + 2\ H^+ + S \qquad (8\text{-}17)$$

In connection with the cyclic membrane phosphorylation, purple sulphur bacteria thus gain all that is needed for an autotroph existence: ATP, NAD(P)H as well as CO_2 and additional inorganic nutrients from the environment.

We interrupted the explanation of the mechanisms of $NADP^+$ reduction in higher plants at the point when electrons from P_{700} were utilized for the reduction of $NADP^+$ and it finds itself in the form $P_{700}{}^+$ (Fig. 8.8). It is here that the second photosystem P_{680} (also called photosystem II) enters the game; in the excited state it is a stronger reducing agent than P_{700}. After the absorption of the light quantum by the photosystem P_{680}, the exergonic reaction can take place:

$$P_{700}{}^+ + P_{680}{}^* \rightarrow P_{700} + P_{680}{}^+ \qquad (8\text{-}18)$$

In words: The light excited photosystem P_{680}* transfers an electron to the cation-radical P_{700}^+ while it moves to the ground state and becomes a cation-radical P_{680}^+.

This summary reaction is formally correct as it is outlined here. In reality, however, the electron transfer between the two photosystems is enabled by several redox systems. Considering that we are dealing with a spontaneous exergonic process, the energy released can be utilized for the active transport of protons, i.e., deposited in PMF form. Here in fact electrons are actively transported by the above mentioned Complex cytochrome-*b*$_6$*f* that guarantees this process also for cyclic phosphorylation. In addition to the NADPH synthesis, the described action is thus also utilized in ATP synthesis, however this time in a non-cyclic manner. We speak of **non-cyclic photophosphorylation**.

It could appear that equation (8-18) doesn't solve anything: one unstable cation-radical (P_{700}^+) was replaced by another (P_{680}^+). However, we are here approaching one of the "magical tricks" of photosynthesis: because P_{680}^+ is a stronger oxidizing agent than oxygen, and water is of course always available in chloroplasts, the whole sequence can be concluded by the famous **water photolysis**, sometimes also introduced as the **Hill reaction**:

$$2\ P_{680}^+ + H_2O \rightarrow 2\ P_{680} + 2\ H^+ + ½\ O_2 \qquad (8\text{-}20)$$

From a plant's perspective oxygen is a **waste product** - yet, the reaction (8-20) is the **only source of atmospheric oxygen**.

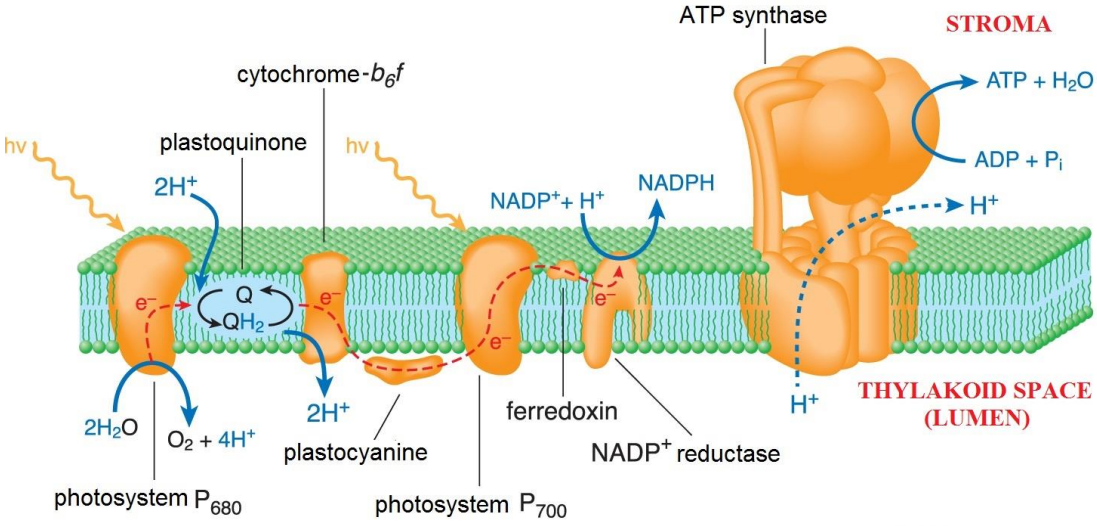


Fig 8.9: Complete scheme of the non-cyclic light-dependent phase of photosynthesis (for a detailed description see text)

We can therefore summarize our explication as follows (Fig. 8.8 and 8.9): In using two photosystems, water (i.e. O^{2-} in water molecule) is used as a reducing agent for the synthesis of NADPH and the residual energy is utilized for the synthesis of ATP. This whole membrane apparatus is "driven" by light energy where for the reduction of one $NADP^+$ molecule it is necessary to absorb two light quanta in both photosystems.

QUESTIONS

8/4 If we adjust the pH of a thylakoid suspension to the value of 4 and after a certain amount of time we sharply increase pH to 8, ATP will be synthesized in this suspension after adding ADP and P_i. Explain this phenomenon.

8/5 Indicate whether the following claims are true or not (if not, explain):

a) Plants are green because chlorophyll pigments absorb and most effectively utilize green light.

b) Photosynthesizing organisms producing O_2 have photosystems I and II, whereas other photosynthesizing organisms have only photosystem I.

c) In all phototrophic organisms the photosynthetizing apparatus is localized in the chloroplasts.

d) Oxygen released during the photolysis of water is incorporated into glucose.

8/6 What primary physico-chemical condition would have to be met so that the oxygenic photosynthesis could be realized by a single photosystem?

8/7 Write the summary equation of oxygenic photosynthesis in which the "reduction equivalent" of NADPH originates. In the equation indicate the stable chemical particles that occur in chloroplast!

8/8 Which of the following statement regarding cyclic photophosphorylation are correct? Refute the incorrect formulations.

a) Does not take part in the generation of NADPH.

b) Takes part in the generation of the proton-motive force through cytochrome-b_6f complex.

c) Does not take part in the generation of O_2.

d) Utilizes electrons produced by the photosystem II.

e) Its primary "task" is to enable ATP synthesis.

ANSWERS

8/4 Thylakoids placed in an acid solution will attain, after time, an equalization of the pH value outside and inside the thylacoids vesicles. If we then increase the pH of the external solution we will artificially create a proton-motive force that attempts to expurgate protons from the vesicles. It is a situation similar to the natural state in chloroplasts. No wonder then that the chloroplast ATP synthase begins to synthesize ATP from ADP and P_i that are both present.

8/5 a) NO: they are green precisely because chlorophyll pigments do not absorb green light (they "capture" blue and red from the white light while the green "remains").

b) YES.

c) NO: photosynthesizing bacteria (prokaryotes) do not, of course, have chloroplasts (nor other organelles).

d) NO (only the designer of a test in a state of total intellectual depletion can come up with such nonsense!)

8/6 Photosystem (here P_x) would, as a result of excitation, need to change its redox potential quite dramatically since, in the form radical-cation (P_x^+), it would have to be a stronger oxidizing agent than oxygen, and in the excited forms (P_x^*) a stronger reducing agent than NADPH.

8/7 $2\ H_2O + 2\ NADP^+ \rightarrow O_2 + 2\ (NADPH + H^+)$

8/8 The statements a), b), c) and e) are correct, d) is incorrect. Cyclic photophosphorylation does not need the cooperation of photosystem II for its "only task" (of creating a proton-motive force.)
