Oxidative phosphorylation

Biochemistry I

Lecture 11

2007 (J.S.)

Living organisms require a **continual input of free energy** for three major purposes:

– the performance of **mechanical work** in cellular movements,

– the **active transport of molecules and ions** across membranes (osmotic work),

– the **synthesis of macromolecules and other biomolecules** from simple precursors (chemical work).

The reaction, which is used to drive endergonic events, is very oft the hydrolysis of ATP.

ATP is a high-energy compound that serves as the "universal currency" of free energy in biological systems through coupling with endergonic reactions.

There are two processes, in which ATP is synthesized from ADP and inorganic phosphate:

– **oxidative phosphorylation** coupled to electron transport along the terminal respiratory chain, and
– **phosphorylation of ADP on the substrate level**.

Phosphorylations on the substrate level occur only in **few reactions**, in which the formation of nucleoside triphosphate is driven by the free energy of hydrolysis of a soluble energy-rich compound.
Examples of that type of phosphorylation are hydrolysis of 1,3-bisphosphoglycerate and phosphoenolpyruvate in the glycolytic pathway, and hydrolysis of succinyl-S-CoA in the citrate cycle.

Oxidative phosphorylation

is located in the inner mitochondrial membrane and accounts for more than 90 % of ATP generated in animals.

It consists of **two separate but coupled processes**:

– the terminal respiratory chain that oxidizes substrates (NADH, succinate, glycerol 3-phosphate, etc.) by dioxygen and produces water, and
– the synthesis of ATP driven by a proton motive force, which is created by the terminal respiratory (electron transporting) chain.

Nutrients rich in hydrogen
(reduced forms of carbon compounds)

dehydrogenations ↓

Reducing equivalents NADH + H^+ →
or **substrates** for FAD-dehydrogenases

TERMINAL RESPIRATORY CHAIN
transfers electrons to O_2 (dioxygen)
that is reduced to two O^{2-} (oxide anions)
accepting H^+ and giving water. This
process results in the **pumping of protons out of the mitochondrial matrix**

↓

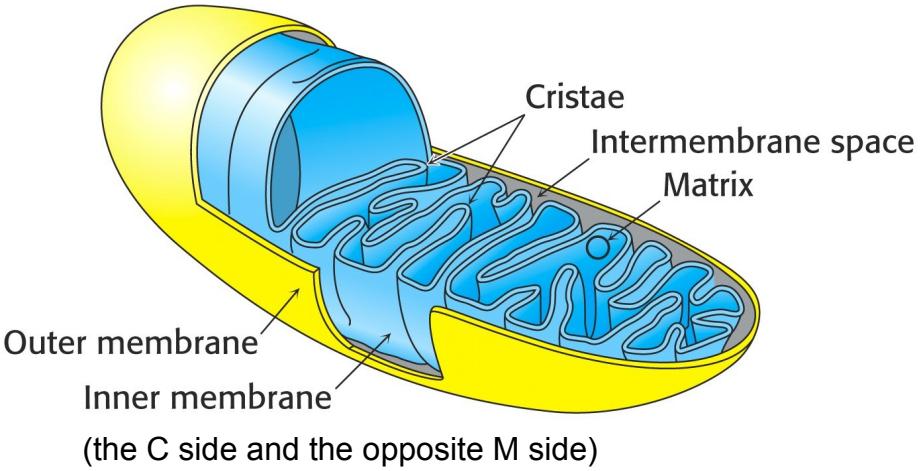
PROTON MOTIVE FORCE
the uneven distribution of H^+ across the membrane
(pH gradient and transmembrane electric potential)

↓

ENDERGONIC ATP SYNTHESIS from ADP and P_i
is driven by the proton motive force:
the enzyme **ATP SYNTHASE**, which enables
the **re-entry of protons into the matrix** simultaneously,
catalyzes the phosphorylation of ADP to ATP.

A proton motive force represents the link between oxidation and phosphorylation.

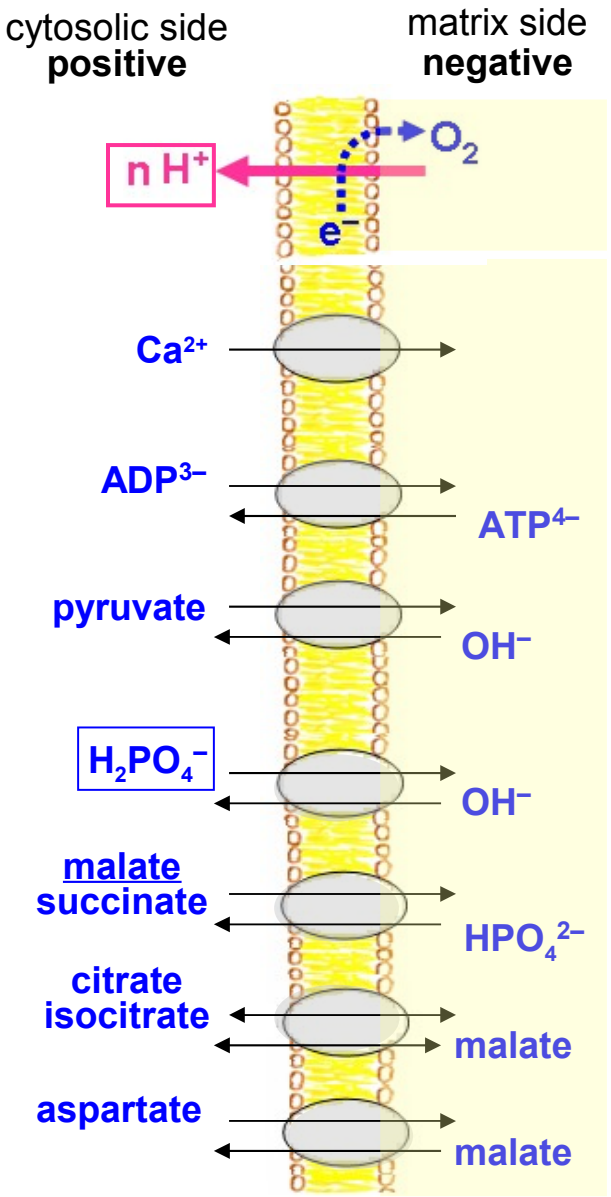
Mitochondrial metabolite transport



The outer membrane is quite permeable for small molecules and ions – it contains many copies of **mitochondrial porin** (voltage-dependent anion channel, VDAC).

The inner membrane is intrinsically impermeable to nearly all ions and polar molecules, but there are many **specific transporters** which shuttles **metabolites** (e.g. pyruvate, malate, citrate, ATP) and **protons** (terminal respiratory chain and ATP synthase) across the membrane.

Transport through the inner mitochondrial membrane – examples:



Free diffusion of O_2, CO_2, H_2O, NH_3

Primary active H^+ transport forms the proton motive force (the primary gradient)

Secondary active transports driven by a H^+ gradient and dissipating it:

ATP/ADP translocase

pyruvate transporter

phosphate permease
– forms a (secondary) phosphate gradient

dicarboxylate carrier

tricarboxylate carrier

the malate shuttle for NADH + H^+

The substrates that supply electrons to the terminal respiratory chain

1 NADH + H^+, which is reoxidized to NAD^+ by the **complex I** of the chain;
2 substrates for flavin dehydrogenases (components of the **complex II**), the electron transport system obtains electrons from $FADH_2$.

1 NADH + H^+

In matrix of mitochondria, NADH + H^+ is the product of many reactions catalyzed by NAD^+-linked dehydrogenases, e.g.,

oxidative decarboxylation of pyruvate and other α-ketoacids,
the second dehydrogenation (of 3-hydroxyacyl-CoAs) in β-oxidation of FA,
dehydrogenation of isocitrate and malate in the citrate cycle, and
deamination of glutamate by glutamate dehydrogenase.

In cytosol, NADH + H^+ is also the product of dehydrogenations, e.g.,

dehydrogenation of 1,3-bisphosphoglycerate to 3-phosphoglycerate, and
dehydrogenation of lactate to pyruvate.

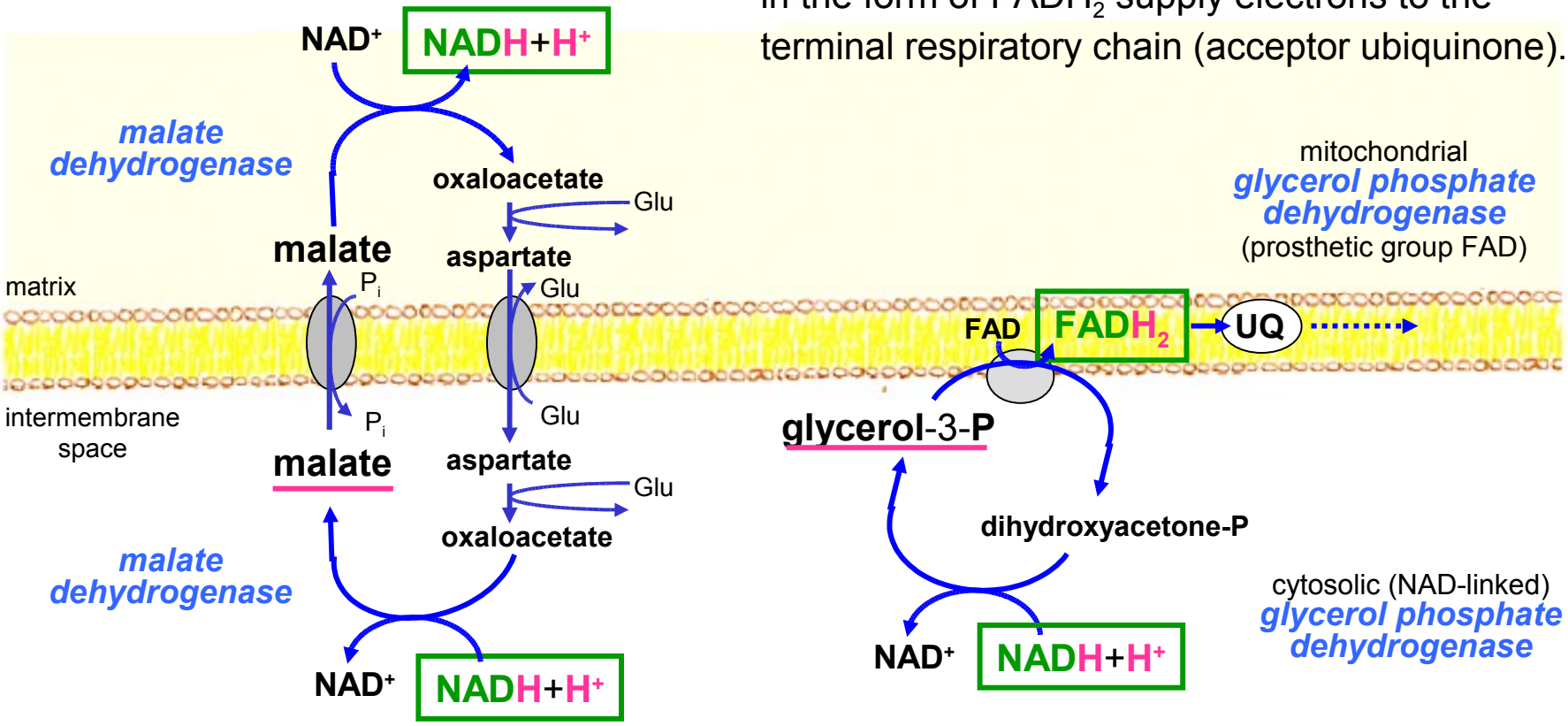
Because the inner mitochondrial membrane is impermeable to molecules of NADH, the transfer of reducing equivalents from cytosol across the mitochondrial membrane is mediated by means of **redox shuttles**:

Transport of reducing equivalents from cytoplasm into mitochondria by redox shuttles:

The malate shuttle
(is universal)

The glycerophosphate shuttle
(of minor importance in most human tissues)

Without entering the matrix, reducing equivalents in the form of $FADH_2$ supply electrons to the terminal respiratory chain (acceptor ubiquinone).



2 Substrates for flavin dehydrogenases of the complex II

(the reduced prosthetic groups $FADH_2$ supply electrons to ubiquinone)

In mitochondrial matrix, such substrates are predominantly

fatty acyl-CoAs – ***acyl-CoA dehydrogenase(s)*** catalyze the first dehydrogenation of the β-oxidation pathway, which introduces a 2,3-*trans*-double bound into aliphatic chain of fatty acyl-CoAs, and

succinate – ***succinate dehydrogenase*** transforms succinate into fumarate in the course of the citrate cycle.

In cytosol formed

glycerol 3-phosphate is reoxidized in the intermembrane space by ***glycerolphosphate dehydrogenase*** of the inner mitochondrial membrane to dihydroxyacetone phosphate (the glycerolphosphate shuttle of reducing equivalents).

The mitochondrial terminal respiratory chain

The chain reoxidizes NADH+H^+ or $FADH_2$ by transporting electrons to the terminal acceptor O_2, which is reduced to form water.

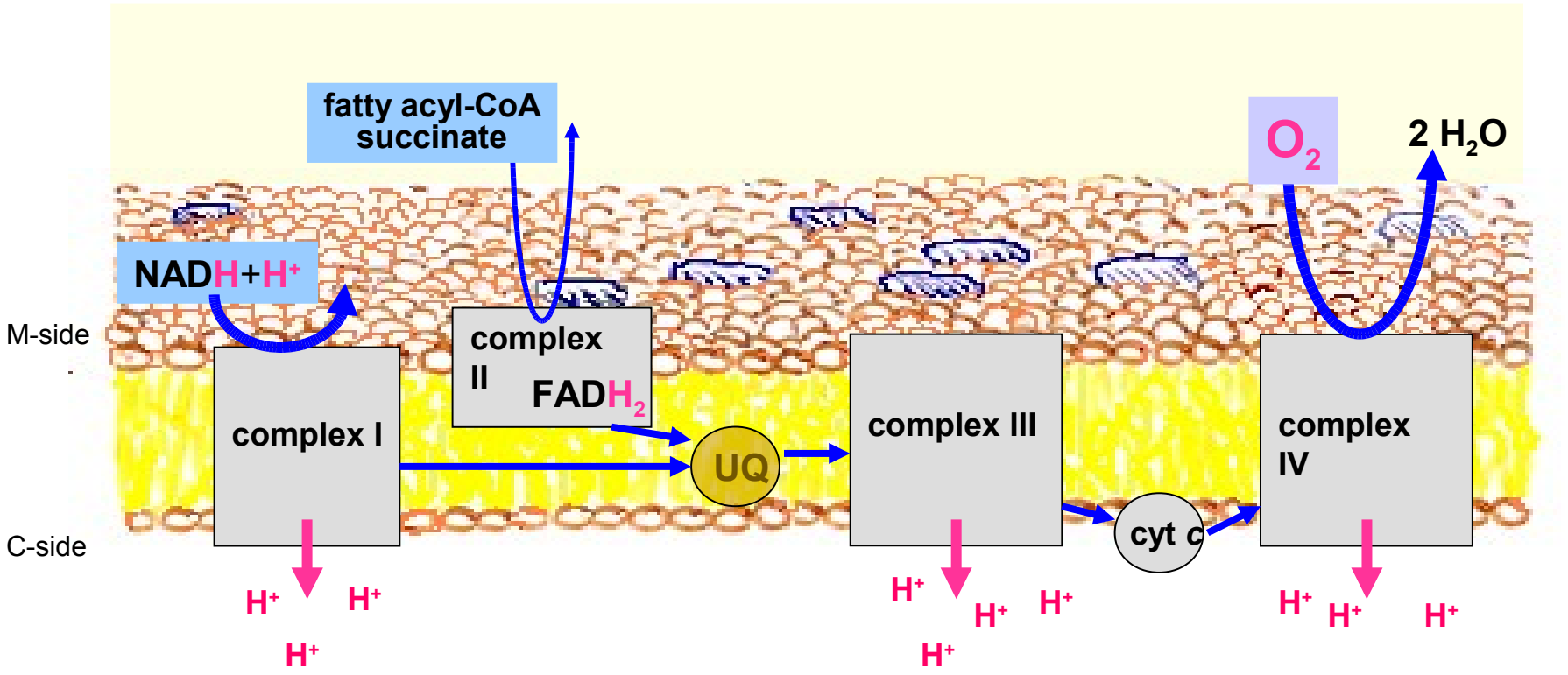
The free energy of oxidation of NADH or $FADH_2$ is utilized for pumping protons to the outside of the inner mitochondrial membrane.

The **proton gradient** across the inner mitochondrial membrane represents the **proton motive force.**

The proton motive force couples the terminal respiratory chain with the phosphorylation of ADP. It is a source of free energy for ATP synthesis that is driven by re-entry of protons into the matrix through an ATP synthase complex.

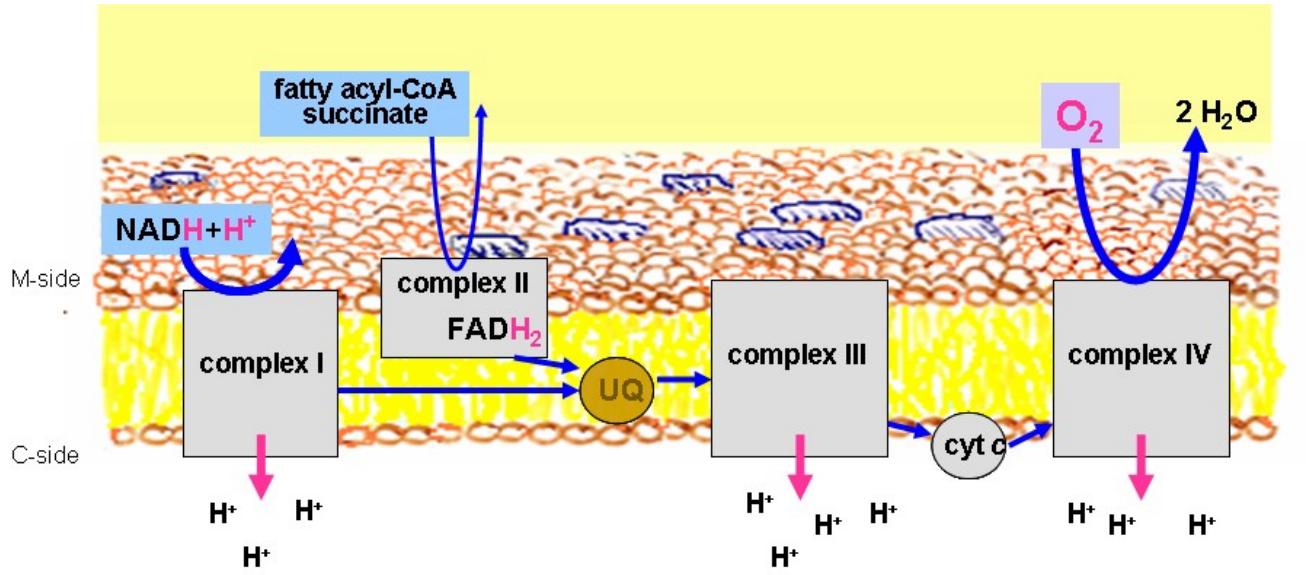
(The chemiosmotic hypothesis was mentioned primarily by Peter Mitchell in 1961.)

The respiratory chain consists of **four large protein complexes** and **ubiquinone and cytochrome *c***, two small, independent transporters:



Complexes I, III and IV catalyze active, electrogenic H^+ transport.

Enzymes that transfer electrons from $FADH_2$ to ubiquinone (**complex II**) do not transport protons.



Complex I **NADH dehydrogenase** (NADH:ubiquinone oxidoreductase)

Complex II succinate dehydrogenase,
acyl-CoA dehydrogenase, and
glycerolphosphate dehydrogenase

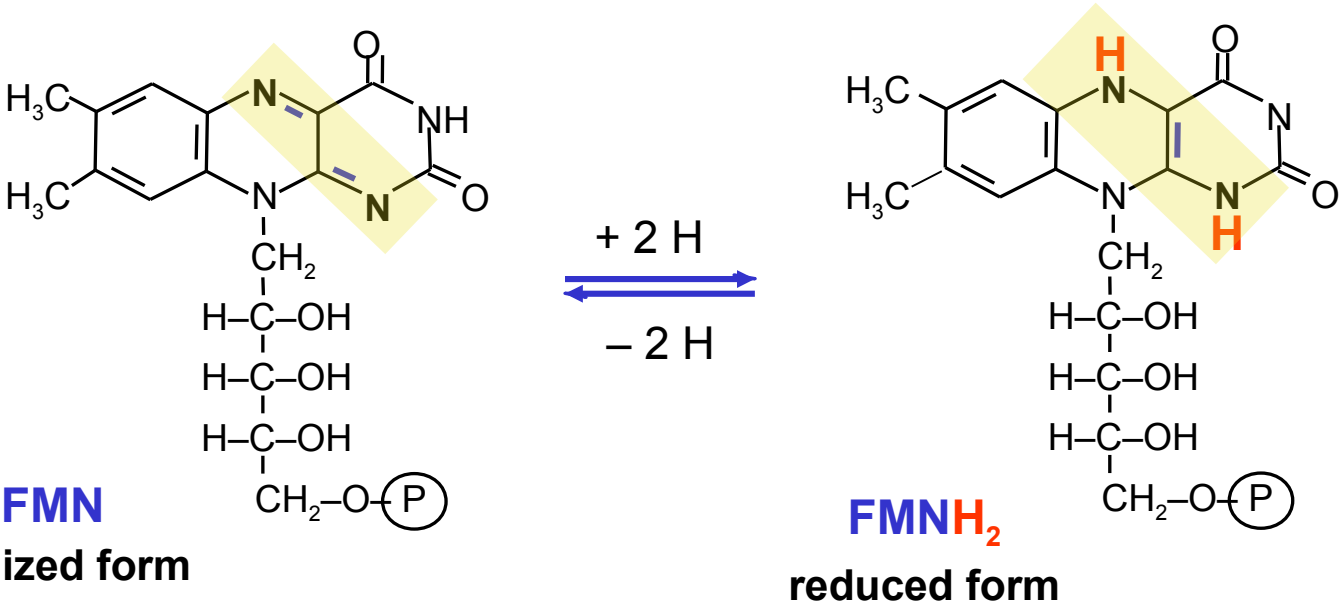
Complex III **cyt *c* reductase** (ubiquinone:cyt *c* oxidoreductase)

Complex IV **cytochrome *c* oxidase** (cyt c : O_2 oxidoreductase)

Individual redox components in the electron transport chain

Flavoproteins

contain flavin prosthetic group either as **flavin mononucleotide** (FMN, component of complex I) or as **flavin adenine dinucleotide** (FAD, dehydrogenases - components of complex II):

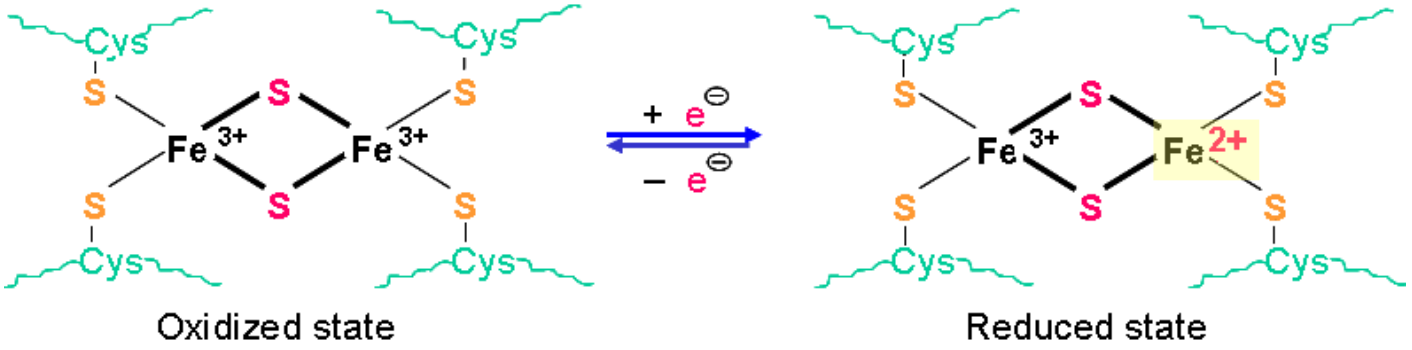


Coenzyme FMN (as well as FAD) transfers **two atoms of hydrogen**.

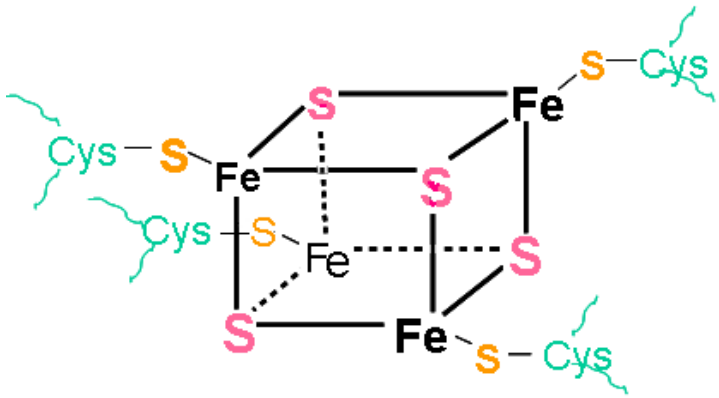
Iron-sulphur proteins (**FeS**-proteins, non-haem iron proteins)

Despite the different number of iron atoms present,
each cluster accepts or donates only **one electron.**

Fe_2S_2 cluster

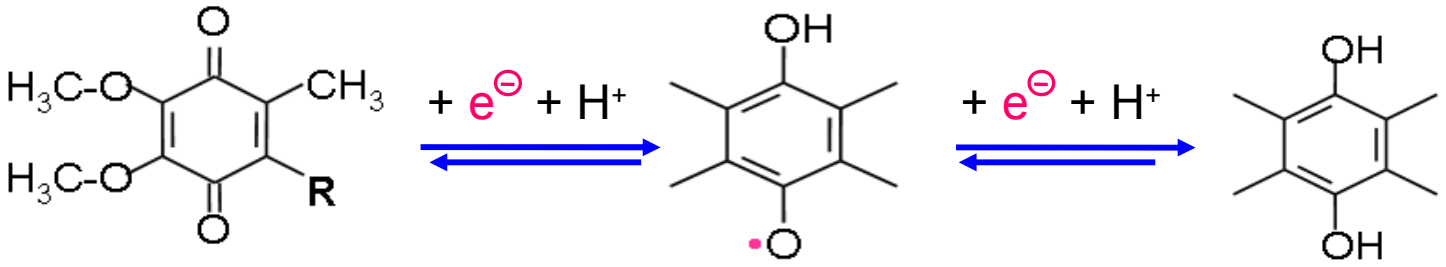


Fe_4S_4 cluster



Ubiquinone (coenzyme Q) acts as a free hydrogen transporter.

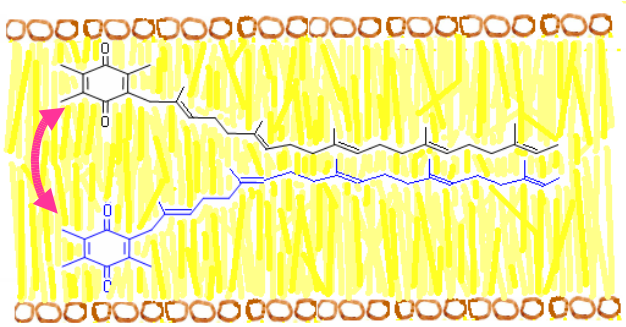
It accepts stepwise **two electrons** (one from the complex I or II and the second from the cytochrome *b*) **and two protons** (from the mitochondrial matrix), so that it is fully reduced to ubiquinol:



ubiquin**one**, **Q** — semiquinone, •QH — ubiquin**ol**, QH_2

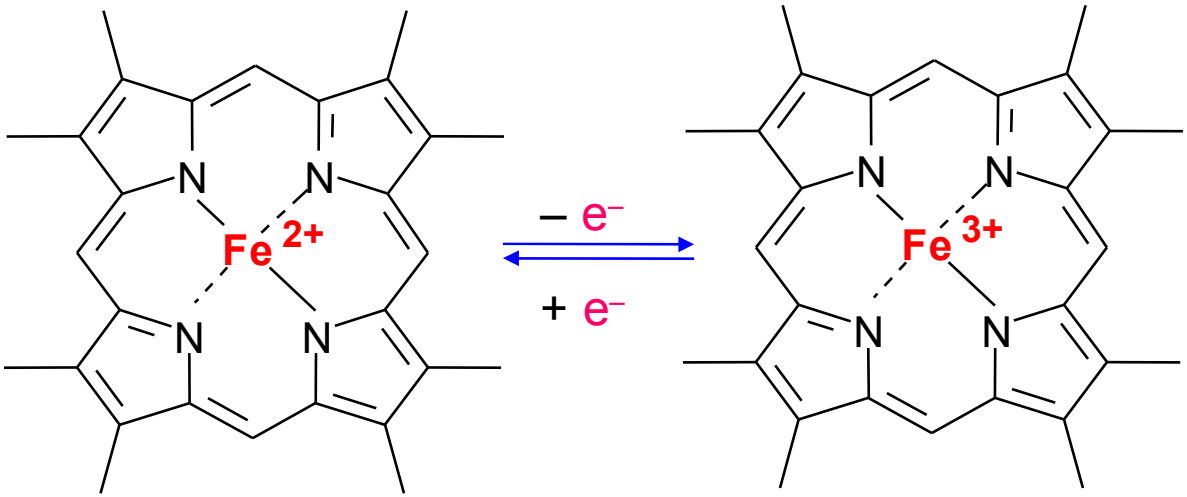
R = $-(CH_2-CH=\underset{\displaystyle CH_3}{\underset{|}{C}}-CH_2)_{10}-H$

The extremely lipophilic polyisoprenoid chain is anchored within the lipidic dilayer. The ring of ubiquinone or ubiquinol (not the ring of semiquinone) can move from the membrane matrix side to the cytosolic side and is able to translocate electrons and protons.



Cytochromes

are **haem-containing proteins**, which are **one-electron carriers** due to reversible oxidation of the iron atom:



Mammalian cytochromes are of three types – ***a***, ***b***, and ***c***. They differ in the substituents attached to the porphin ring. All these types of cytochromes occur in the mitochondrial respiratory chain.

Cytochromes type ***b*** (including cytochromes class P-450) occur also in membranes of endoplasmic reticulum and the outer mitochondrial membrane.

Some differences in cytochrome structures

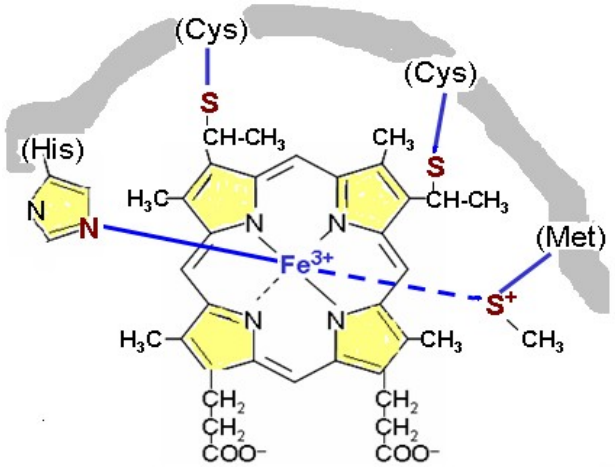
Cytochrome *c*

M_r 12 000, the central Fe ion is attached by coordination to N-atom of His_{18} and to S-atom of Met_{80};
two vinyl groups bind covalently S-atoms of Cys_{14} and Cys_{17}.

The haem is dived deeply in the protein terciary structure so that it is **unable to bind dioxygen, carbon monoxide or CN^- ion.**

Cyt *c* is water-soluble, peripheral protein that moves on the outer side of the inner mitochondrial membrane.

Haem of cytochrome *c*



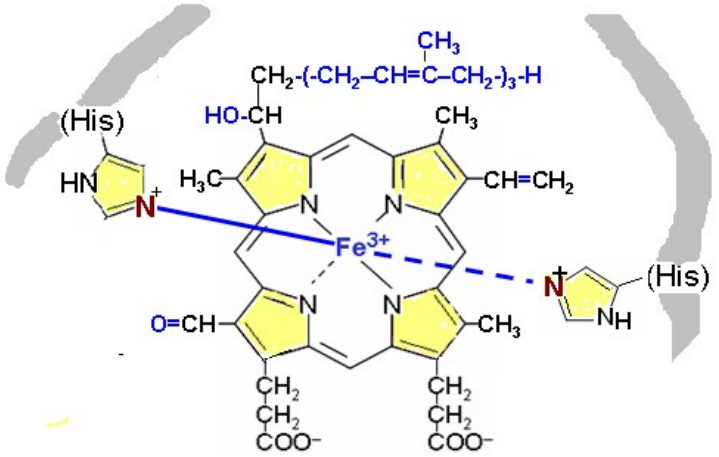
Cytochrome *aa*$_3$

M_r ~ 170 000, the central Fe ion is attached by coordination to two histidyl residues;
one of substituents is a hydrophobic isoprenoid chain, another one is oxidized to formyl group.

The haem *a* is the accepts an electrons from the copper centre A (two atoms Cu_A).

Its function is inhibited by **carbon monoxide, CN^-, HS^-, and N_3^- anions.**

Haem *a* of cytochrome *aa*$_3$



Redox components of the respiratory complexes

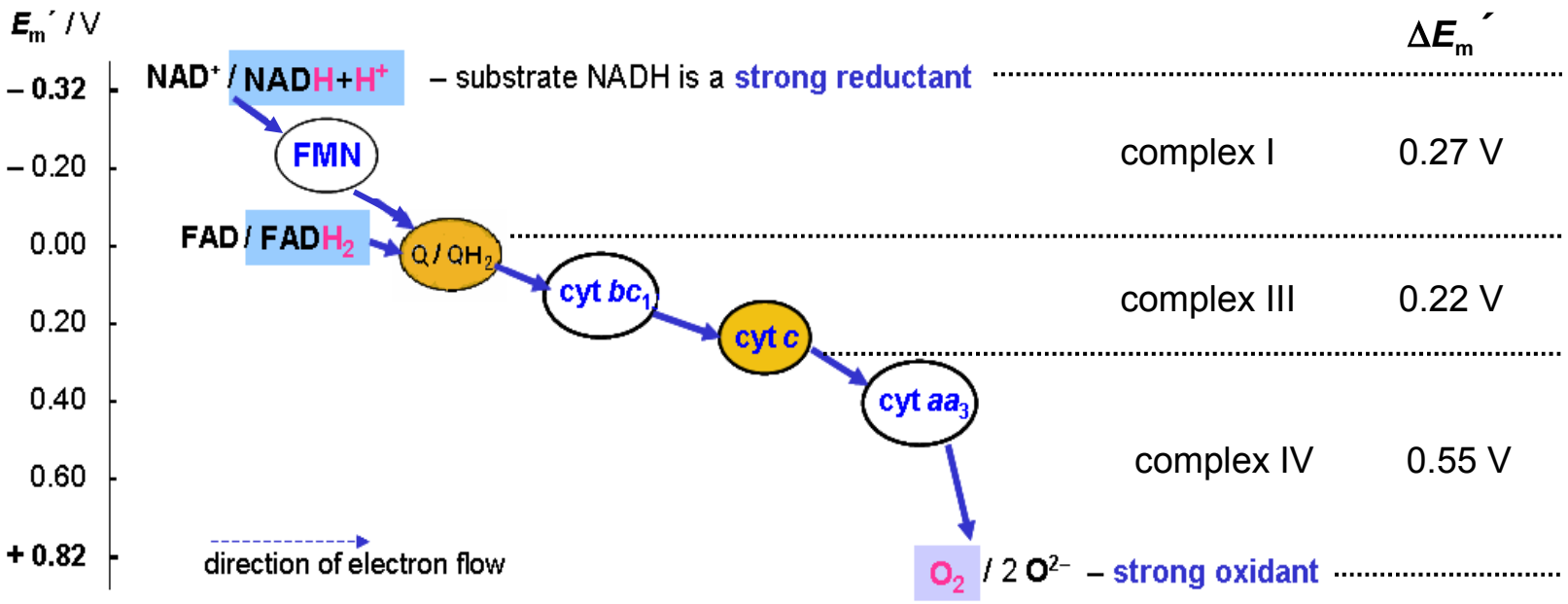
Complex I – **NADH dehydrogenase** consists of more then 30 subunits:
one subunit with a prosthetic group **FMN** accepts 2 H-atoms from NADH to give $FMNH_2$ and two electrons transfer to FeS-proteins;
a large number of **FeS-proteins** (clusters both Fe_2S_2 and Fe_4S_4) transfer 2 electrons one after another to two molecules •QH in the lipidic core at the matrix side of the membrane.

Complex II – **succinate dehydrogenase** (and two other dehydrogenases have one **FAD** as prosthetic group, three **FeS-proteins** and one **cyt b_{560}** (the function of which is transfer of electrons to semiquinone •QH).

Complex III – Q : cyt *c* oxidoreductase (**cytochrome *c* reductase**) consists of 11 subunits; the most important are **cytochrome *b*** that contains two haems – **haem b_L** (L for low affinity) and haem b_H (high affinity), one **FeS-protein** (cluster Fe_2S_2, the Rieske centre), and **cytochrome c_1** that is firmly bound within the complex.

Complex IV – **cytochrome oxidase** that catalyzes the reduction of O_2 to water (previously called **cytochrome aa_3**) consists of 13 subunits; it contains two atoms of **copper** (**centre Cu_A**), **haem *a***, and a binuclear complex of **haem a_3** and the third atom of **copper** (**Cu_B**).

The components of the respiratory chain are arranged in the order of ascending midpoint electrode potentials E'_m:

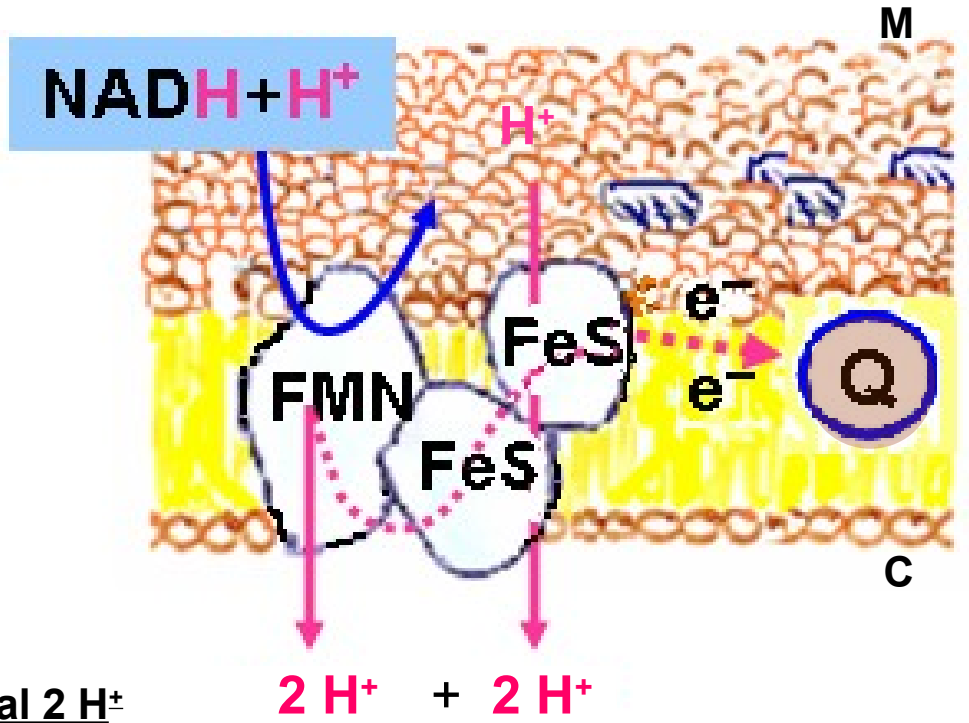


The **standard electrode potential difference** $\Delta E°'$ for the reduction of O_2 with NADH **1.14 V** drives the electron transport through the chain.
The corresponding **standard free energy** $\Delta G°' = -220$ **kJ mol**$^{-1}$.

This substantial release of free energy is used initially to form a proton gradient that is then used for the synthesis of ATP and the transport of metabolites across the inner mitochondrial membrane.

Complex I NADH dehydrogenase

(NADH : ubiquinone oxidoreductase)



$NADH+H^+ + FMN \rightarrow NAD^+ +$

$FMNH_2 \rightarrow FMN + 2\,H^+ + 2\,e^-$

Two protons are released from the outer side of the inner membrane and **additional 2 H^+** are transferred across the membrane.

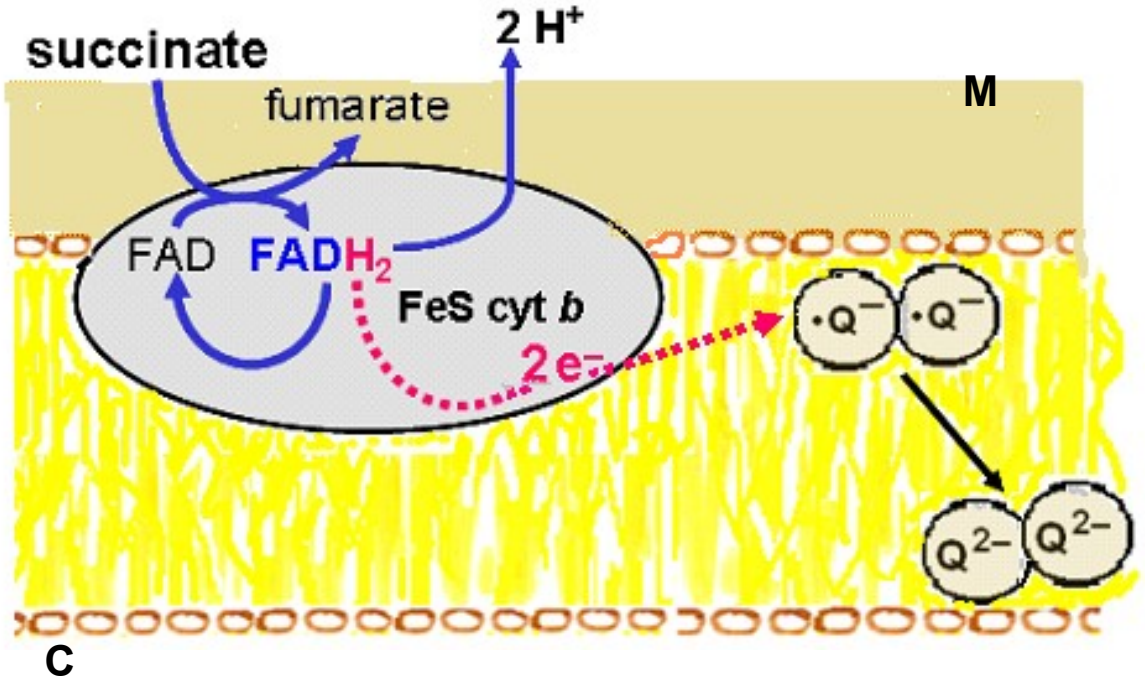
The mechanism is not yet clear.

Two electrons flow within the complex to Fe_4S_4 centres and then accepted one by one by the **semiquinone form** of **coenzyme Q** near the inner surface of the membrane.

Complex II

Three independent flavin dehydrogenases that act in a similar way.
Only one of them, **succinate dehydrogenase**, is mentioned here.

Succinate.DH is an enzyme that catalyzes one of the reactions of the citrate cycle. However, it is the only integral membrane protein – the other enzymes of the citrate cycle are soluble, dispersed in the matrix..

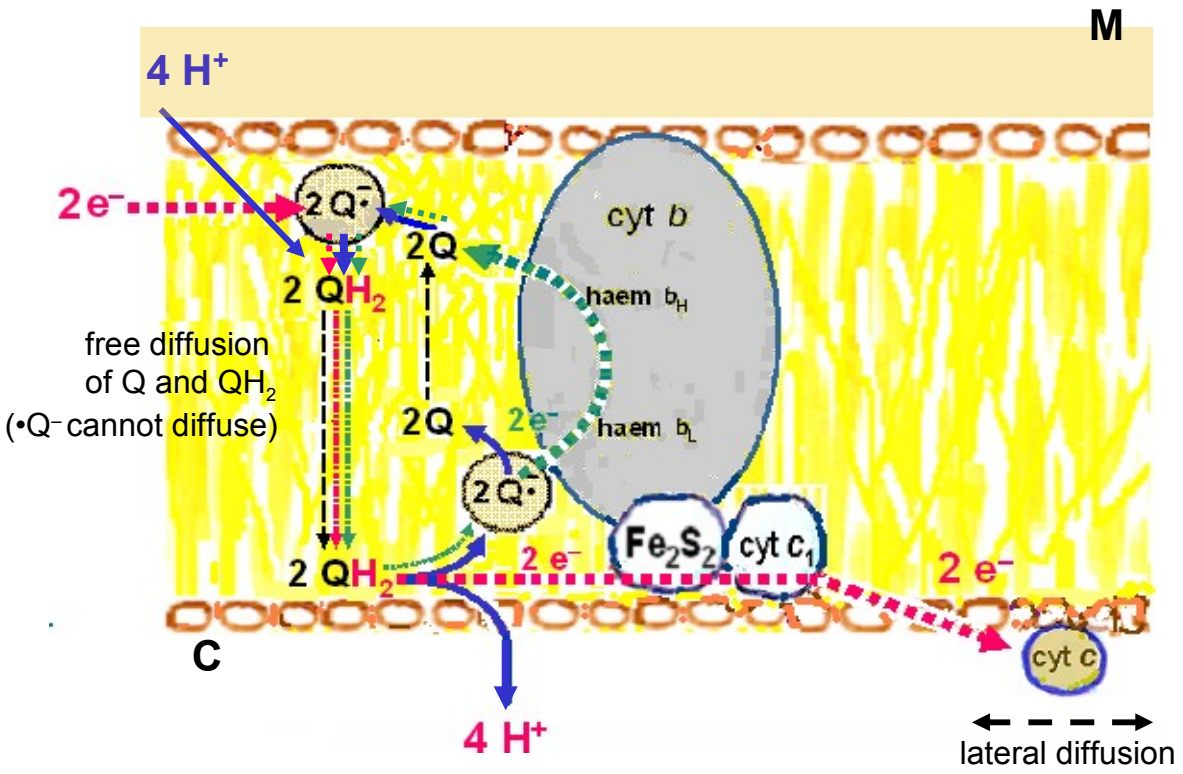


The enzymes that transfer electrons from $FADH_2$ to Q (complex II), in contrast to complex I, **do not transport protons across the inner membrane**. Consequently, less proton gradient (and less ATP) is formed from the oxidation of $FADH_2$ by dioxygen than from NADH.

Complex III Ubiquinone : cyt *c* oxidoreductase

(cytochrome *c* reductase, bc_1 complex)

The acceptor of electrons, supplied by the complex I or II, is the half-reduced (**semiquinone**) form of ubiquinone located at the matrix side of the membrane. The anion Q^{2-} binds four protons from the matrix and QH_2 moves within the lipidic layer. At the C-side, **one e⁻** from QH_2 is transferred by the Rieske Fe_2S_2 protein to cyt c_1 and cyt *c*, **the second e⁻** (from $\bullet Q^-$) by haem b_L of the cyt *b*. The mobile oxidized Q, at the matrix side, is reduced again to $\bullet Q^-$ (**Q-cycle**). **Four protons** are released into the intermembrane space.



Two electrons, which pass one by one from complex I or II to cyt *c,* due to the Q-cycle and cyt *b* (representing a twofold loop), translocate **four protons** across the membrane.

Cytochrome *c*

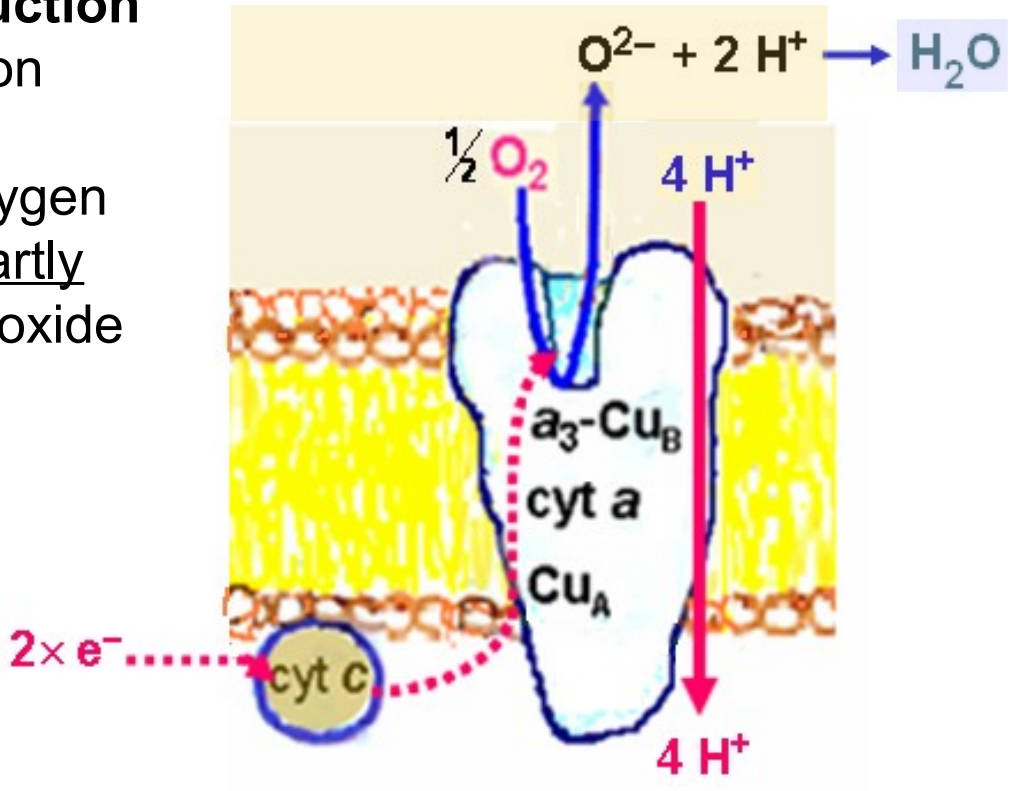
is soluble peripheral membrane haemoprotein, that is bound to the outside surface of the inner mitochondrial membrane through weak electrostatic interactions, so that it can move along the outside surface.

Cytochrome *c* transfers electrons from cyt c_1 of complex III to complex IV.

Complex IV Cytochrome *c* oxidase

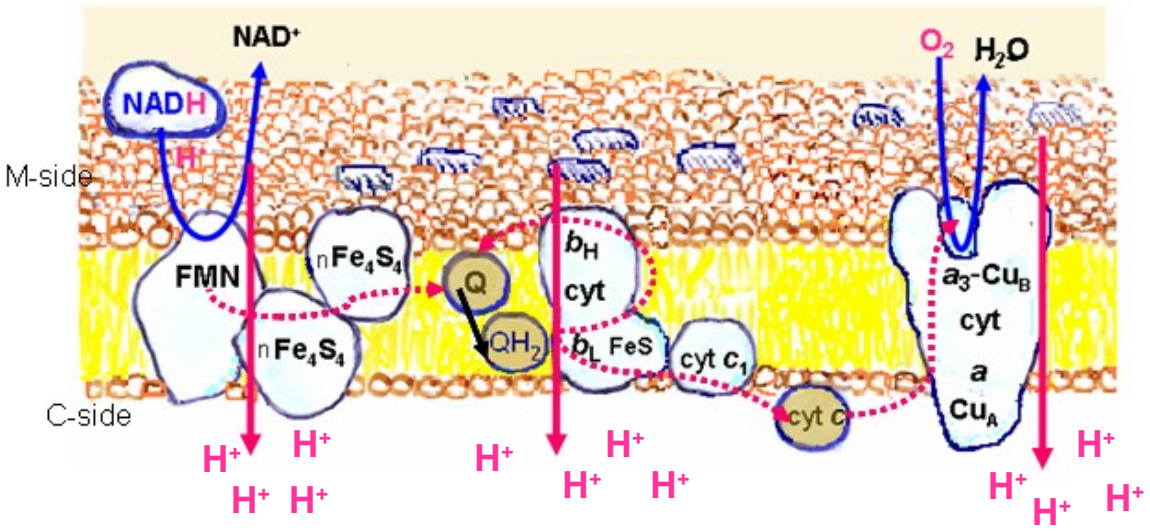
(cytochrome *c* : O_2 oxidoreductase. cytochrome aa_3)

catalyzes the **four-electron reduction of dioxygen**. It is the only electron carrier in which the Fe^{2+} of haem can directly reduce molecular oxygen to water without the release of partly reduced intermediates (no superoxide or peroxide anions are formed).

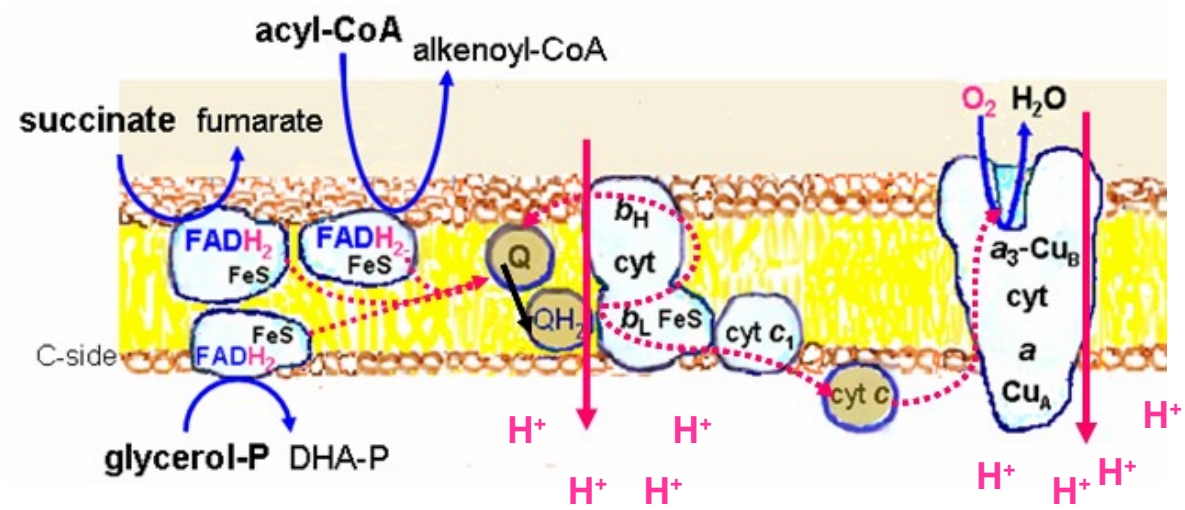


Complex IV consists of more than 13 subunits. The mechanism of proton pumping is not yet quite clear. Hypothetically, it is explained by the mechanism resembling the Bohr effect (haemoglobin releases protons after binding O_2).

NADH+H⁺ → complex I → complex III → complex IV → O_2



Complex II ($FADH_2$) → **complex III → complex IV → O_2**



Coupling of phosphorylation to terminal respiratory chain

Oxidative phosphorylation of ADP to ATP consists of two separate processes, which are efficiently coupled only in intact mitochondria. The **link between oxidation and phosphorylation is a proton motive force.**

Translocation of protons across the inner mitochondrial membrane results in formation of an **electrochemical gradient Δ μ_H+**

(the **free energy change Δ*G*** for translocation of *n* protons from inside to outside of the membrane). It consists of <u>two components</u>:

– the difference in **chemical potential of protons**,

$$\Delta G = RT \ln ([H^+]_{outside} / [H^+]_{inside}) = 2.3\, RT\, (pH_{out} - pH_{in})$$

– the **electric potential** ($\Delta\Psi$, negative inside) that depends not only on protons, but also on concentrations of other cations and anions,

$$\Delta G = -nF\,\Delta\Psi .$$

The **proton motive force Δ *p*** is the quantity expressed in the term of potential (milivolts per mole of H^+ transferred): $\Delta p = -\Delta G / nF = \Delta\Psi + 60\ \Delta\ pH$.

Approximate data (coupled rat liver mitochondria):

$\Delta \mu_{H^+}$ = 22 – 27 kJ per 1 mol of H^+ transferred

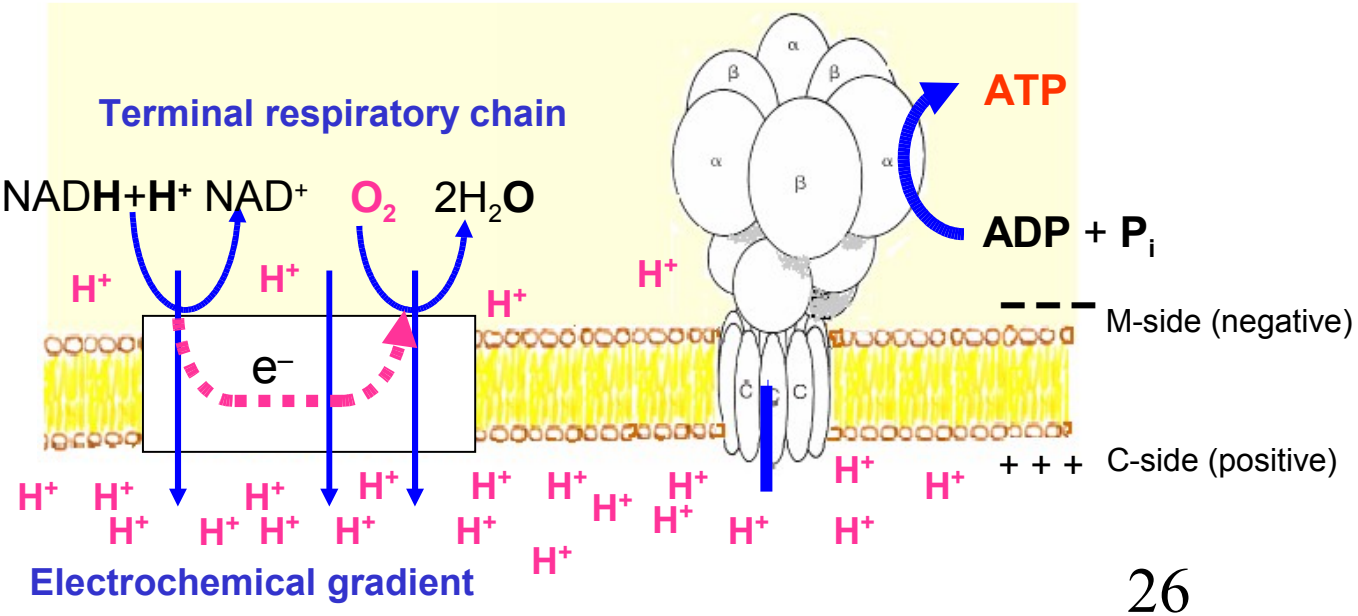
Δp = 213 – 280 mV ($\Delta\Psi$ = 140 – 220 mV ; Δ pH = 0.75 – 1.40)

A proton motive force represents the **energy available for ATP synthesis**, as well as for **other endergonic processes** (secondary active transports of ions across the membrane) or **production of heat** (dissipation of $\Delta\mu_{H}+$ by re-entry of protons through thermogenin in the brown adipose tissue).

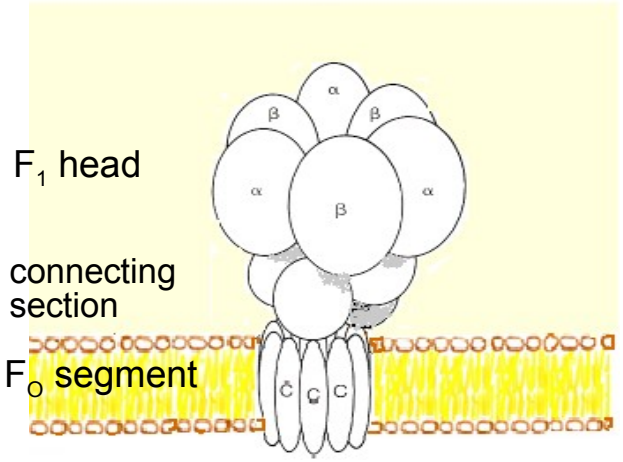
In intact mitochondria, free protons leakage back into the matrix is very slow, so that the proton gradient is efficiently maintained for ATP synthesis or exchange of protons for other ions.

Phosphorylation – ATP synthase

The phosphorylation of ADP is **driven by the flux of protons** back into the matrix along the electrochemical gradient **through ATP synthase.**



ATP synthase (synonyms: mitoch. H^+-ATPase, F_1F_O-ATPase, complex V)



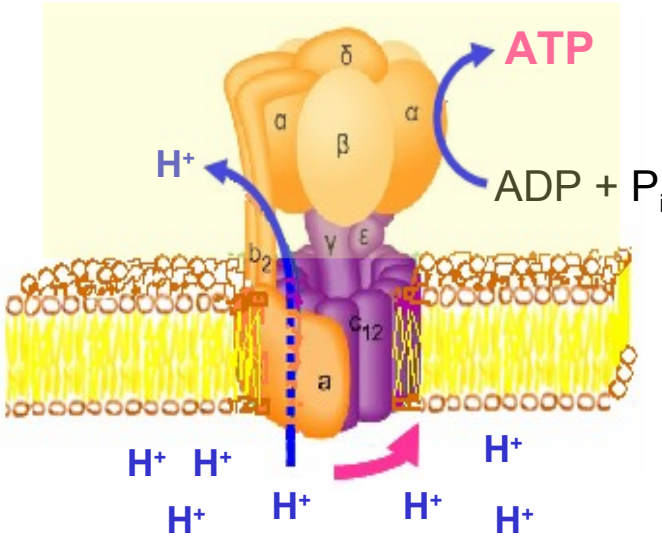
consists of three complexes:

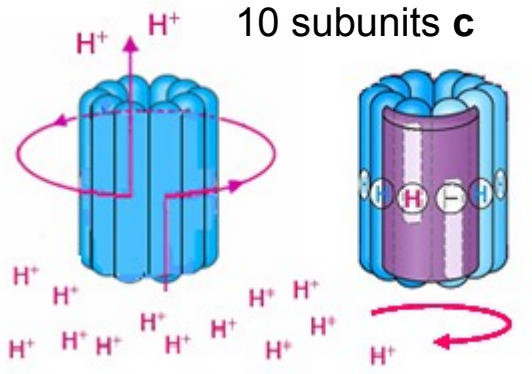
F_1 complex projecting into the matrix, 5 subunit types (α_3, β_3, rotating γ. δ, ε) that **catalyze ATP synthesis** in coupled system, or ATP hydrolysis in uncoupled mitochondria;

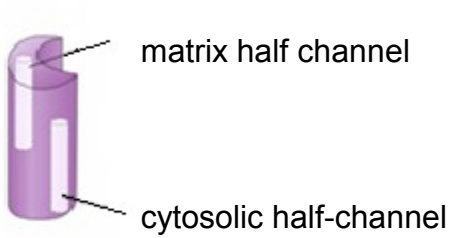
connecting section (formerly OSCP, oligomycin sensitivity conferring protein), and

F_O inner membrane component, 3 subunit types (**a**, **b_2**, **c_{10}**), that forms a rotating **proton channel**.

The proton flux drives rotation the γ-subunit. Due to conformational changes of α/β subunits the three nucleotide-binding sites alternate between three states.







Stoichiometry of the ATP synthesis is not exactly recognized.
It seems to be presumable that re-entry of four protons drives synthesis of 1 ATP.

P/O ratio is a **measure of the number of ATP molecules synthesized** (P_i or ADP consumed) **per one atom of oxygen reduced to water**.

Transfer of 2 e^- from **NADH** to O_2 results in **3 ATP**, from **$FADH_2$** only **2 ATP**.

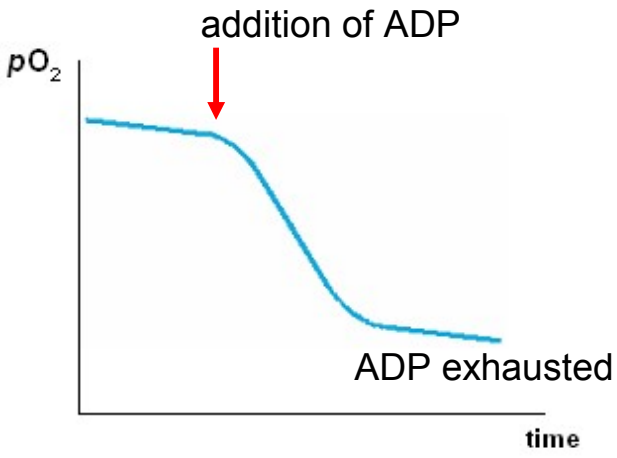
Control of the oxidative phosphorylation

Production of ATP is strictly coordinated so that

ATP is never produced more rapidly than necessary.

Synthesis of ATP depends on – supply of **substrates** (mainly NADH + H^+),
– supply of a sufficient amount of **dioxygen**, and
– the **energy output of the cell**; hydrolysis of ATP increases the **concentration of ADP** in the matrix, which activates ATP production.

This mechanism is called **respiratory control**.



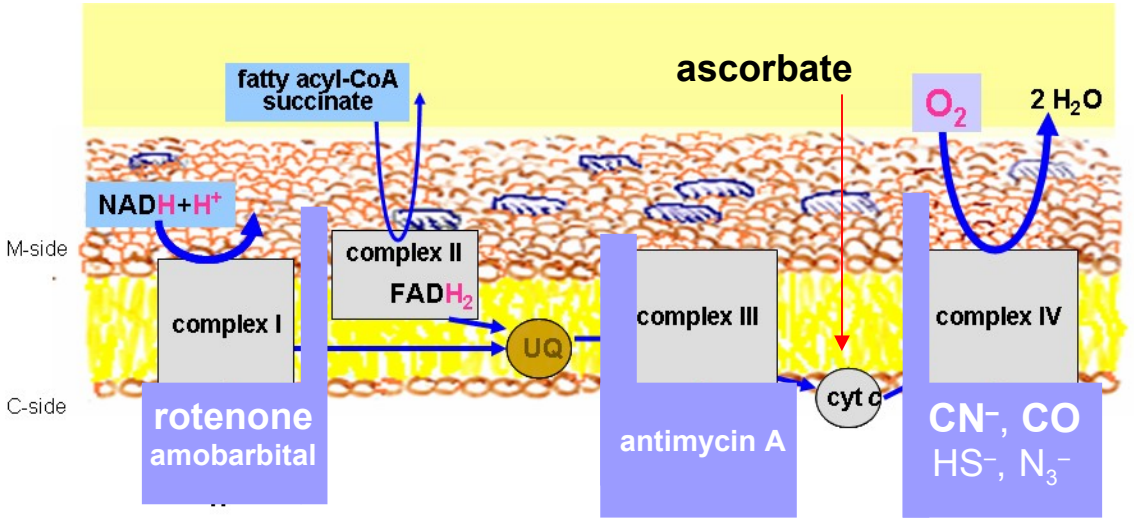
Effect of ADP on the uptake of oxygen by isolated mitochondria. studied in an isolated system with an oxygen electrode and a recording device. The rate of oxygen consumption increases after addition of ADP.

Inhibitors of the terminal respiratory chain

Complex I is blocked by an insecticide rotenone. A limited synthesis of ATP exists due to electrons donated to ubiquinone through complex II.

Complex III is inhibited by antimycin A – complexes I and II become reduced, complexes III and IV remain oxidized. Ascorbate restores respiration, because it can reduce cytochrome *c*.

Complex IV, the terminal complex, is effectively blocked by carbon monoxide, cyanide ion, and also by HS^- (sulfane intoxication) and azide ion N_3^-. Respiration is disabled, complexes preceding complex IV become reduced, inhibition of complex IV cannot be bypassed.



Cyanide poisoning

occurs after ingestion of alkali cyanides or inhalation of hydrogen cyanide. Bitter almonds or apricot kernels contain amygdalin, which can release HCN.

Cyanide ion, besides inhibition of cytochrome *c* oxidase, binds with high affinity onto methaemoglobin (haemin, Fe^{3+}).

The lethal dose LD_{50} of alkali cyanide is about 250 mg. Symptoms - dizziness, gasping for breath, cramps, and unconsciousness follow rapidly.

Antidotes may be effective, when applied without any delay:
Hydroxycobalamin (a semisynthetic compound) exhibits high affinity to CN^- ions, binds them in the form of harmless cyanocobalamin (B_{12}).
Sodium nitrite $NaNO_2$ or **amyl nitrite** oxidize haemoglobin (Fe^{II}) to methaemoglobin (Fe^{III}), which is not able to transport oxygen, but binds CN^- and may so prevent inhibition of cytochrome *c* oxidase.
Sodium thiosulfate $Na_2S_2O_3$, administered intravenously, can convert cyanide to the relatively harmless thiocyanate ion: $CN^- + S_2O_3^{2-} \rightarrow SCN^- + SO_3^{2-}$.

Carbon monoxide poisoning

CO binds primarily to haemoglobin (Fe^{II}) and inhibits oxygen transport, but it also blocks the respiratory chain by inhibiting cytochrome oxidase (complex IV).

Oxygenotherapy can improve blood oxygen transport, administered **methylene blue** serves as acceptor of electrons from complex III so that limited ATP synthesis can continue.

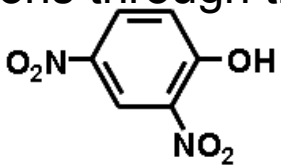
Uncoupling of the respiratory chain and phosphorylation

is the **wasteful oxidation of substrates without concomitant ATP synthesis:** protons are pumped across the membrane, but they re-enter the matrix using some other way than that represented by ATP synthase.

The free energy derived from oxidation of substrates appears as heat..

There are **four types** of artificial or natural uncouplers:

1 **"True" uncouplers** – compounds that transfer protons through the membrane.
A typical uncoupler is **2,4-dinitrophenol** (DNP):



DNP is very toxic, the lethal dose is about 1 g.

More than 80 years ago, the long-term application of small doses (2.5 mg/kg) was recommended as a "reliable" drug in patients seeking to lose weight. Its use has been banned, because hyperthermia and toxic side effect (with fatal results) were excessive.

2 **Ionophors** that do not disturb the chemical potential of protons, but diminish the electric potential $\Delta\Psi$ by enabling free re-entry of K^+ (e.g. valinomycin) or both K^+ and Na^+ (e.g. gramicidin A).

3 **Inhibitors of ATP synthase –** oligomycin.

4 **Inhibitors of ATP/ADP translocase** like unusual plant and mould toxins bongkrekic acid (irreversibly binds ADP onto the translocase) and atractylate (inhibits binding of ATP to the translocase). ATP synthase then lacks its substrate.

Thermogenin (uncoupler protein, UCP)**: a natural uncoupler**

is a inner mitochondrial membrane protein that transports protons back into the matrix, bypassing so ATP synthase.

It occurs in **brown adipose tissue** of newborn children and hibernating animals, which spend the winter in a dormant state.

The activity of UCP is stimulated by fatty acids.