



AULAS DE BIOQUÍMICA

METABOLISMO DOS GLÚCIDOS (cont.)

Fosforilação oxidativa. Cadeia mitocondrial de transporte de electrões. Localização celular, potencial redox, transferência de electrões e variações de energia. Sequência de reacções. Funcionamento dos complexos supramoleculares I, II, III e IV. Movimento de H⁺ (bombas de protões). Teoria quimiosmótica. Complexo enzimático ATP sintase. Fosforilação oxidativa. As proteínas desacopladoras (UCP). A termogenina e a termogénese nas células gordas do tecido castanho dos mamíferos. As vias alternativas de transporte de electrões dos mitocôndrios vegetais (fundamentos). Rendimento energético global do catabolismo da glucose em condições de aerobiose ou de anaerobiose. Síntese de glúcidos na fotossíntese (fundamentos). Reacções fotoquímicas e reacções de assimilação do dióxido de carbono. Fotofosforilação. Ciclo de Calvin.

Material de estudo: diapositivos das aulas e bibliografia recomendada.



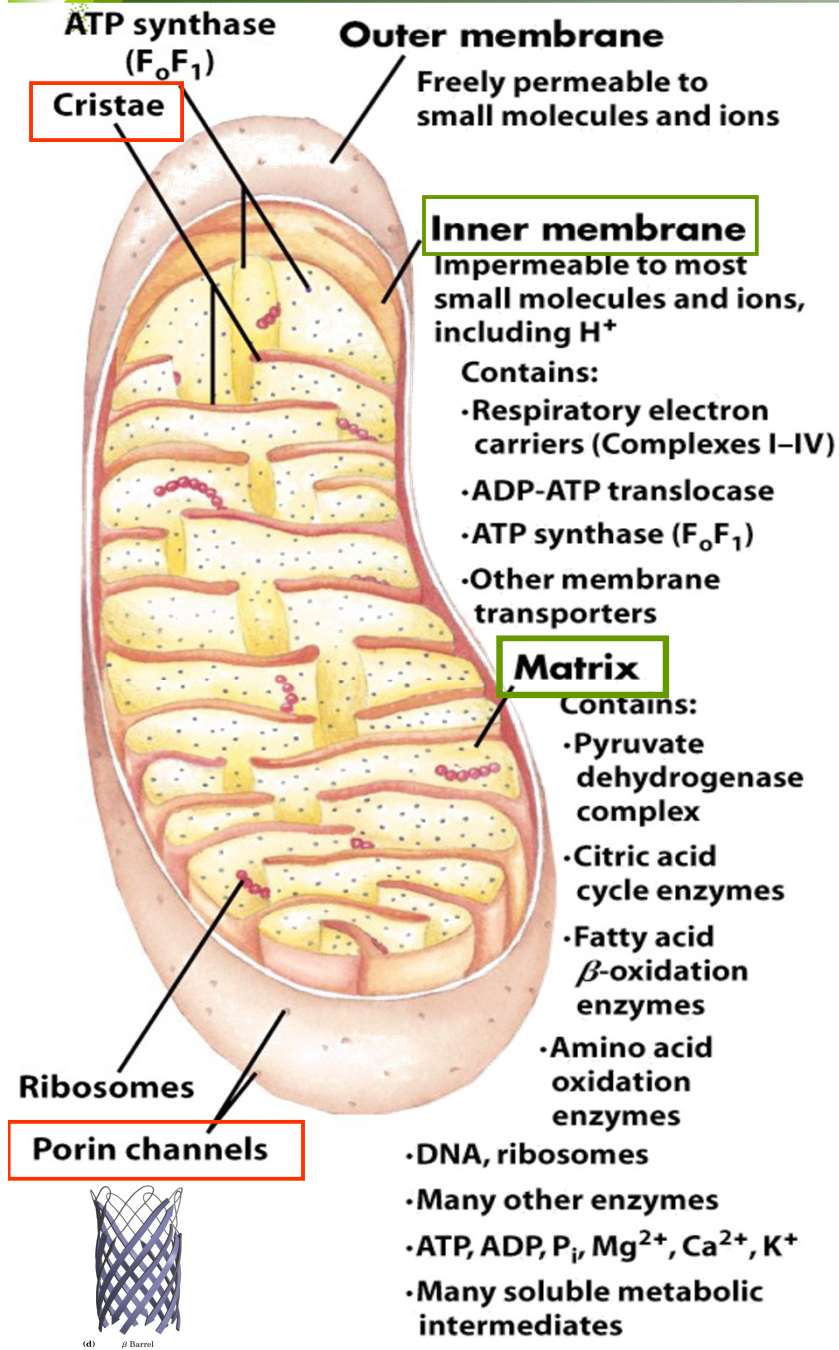
Oxidative phosphorylation	Photophosphorylation
degradation of carbohydrates, fats, and amino acids converge at this final stage of cellular respiration drives the synthesis of ATP	photosynthetic organisms capture the energy of sunlight and harness it to make ATP
occurs in mitochondria	occurs in chloroplasts
<i>reduction</i> of O ₂ to H ₂ O with electrons donated by NADH and FADH ₂	<i>oxidation</i> of H ₂ O to O ₂ , with NADP ⁺ as ultimate electron acceptor
occurs equally well in light or darkness	dependent on the energy of light



Electron-Transfer Reactions in Mitochondria

- Electrons Are Funneled to Universal Electron Acceptors
- Electrons Pass through a Series of Membrane-Bound Carriers
- Electron Carriers Function in Multienzyme Complexes
- The Energy of Electron Transfer Is Efficiently Conserved in a Proton Gradient
- Plant Mitochondria Have Alternative Mechanisms for Oxidizing NADH

Biochemical anatomy of a mitochondrion



- The convolutions (**cristae**) of the inner membrane provide a very large surface area.
- The inner membrane of a single liver mitochondrion may have more than **10,000** sets of **electron-transfer systems** (respiratory chains) and **ATP synthase** molecules, distributed over the membrane surface.
- Heart mitochondria, which have more profuse cristae and thus a much larger area of inner membrane, contain more than **three** times as many sets of electron-transfer systems as liver mitochondria.
- The mitochondrial pool of coenzymes and intermediates is functionally separate from the cytosolic pool.



Mitochondrion:

- like Gram-negative bacteria, have two membranes
- The outer mitochondrial membrane is readily permeable to small molecules (M_r 5,000) and ions, which move freely through transmembrane channels formed by a family of integral membrane proteins called porins.
- The inner membrane is impermeable to most small molecules and ions, including protons (H); the only species that cross this membrane do so through specific transporters. The inner membrane bears the components of the respiratory chain and the ATP synthase.
- Outer membrane contains porins (< 5000).
- Matrix pyruvate deHase complex, TCA enzymes, and fatty acid β -oxidation pathway, and amino acids oxidation



Electrons are funneled to universal electron acceptors

- Oxidative phosphorylation begins with the entry of electrons into the respiratory chain.
- Most of these electrons arise from the action of dehydrogenases that collect electrons from catabolic pathways and funnel them into universal electron acceptors
 - nicotinamide nucleotides (NAD or NADP)
 - flavin nucleotides (FMN or FAD)
- **Nicotinamide nucleotide–linked dehydrogenases**
- **Flavoproteins**

Nicotinamide nucleotide (NAD or NADP)-linked dehydrogenases

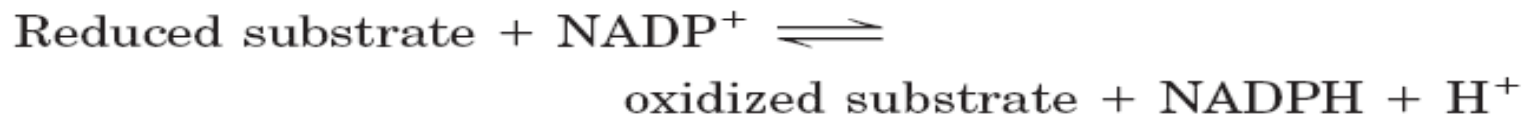
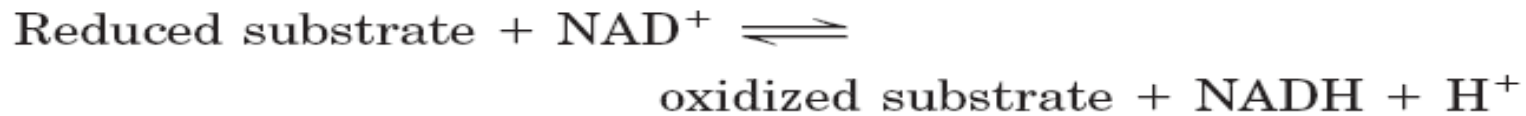


TABLE 19-1 Some Important Reactions Catalyzed by NAD(P)H-Linked Dehydrogenases

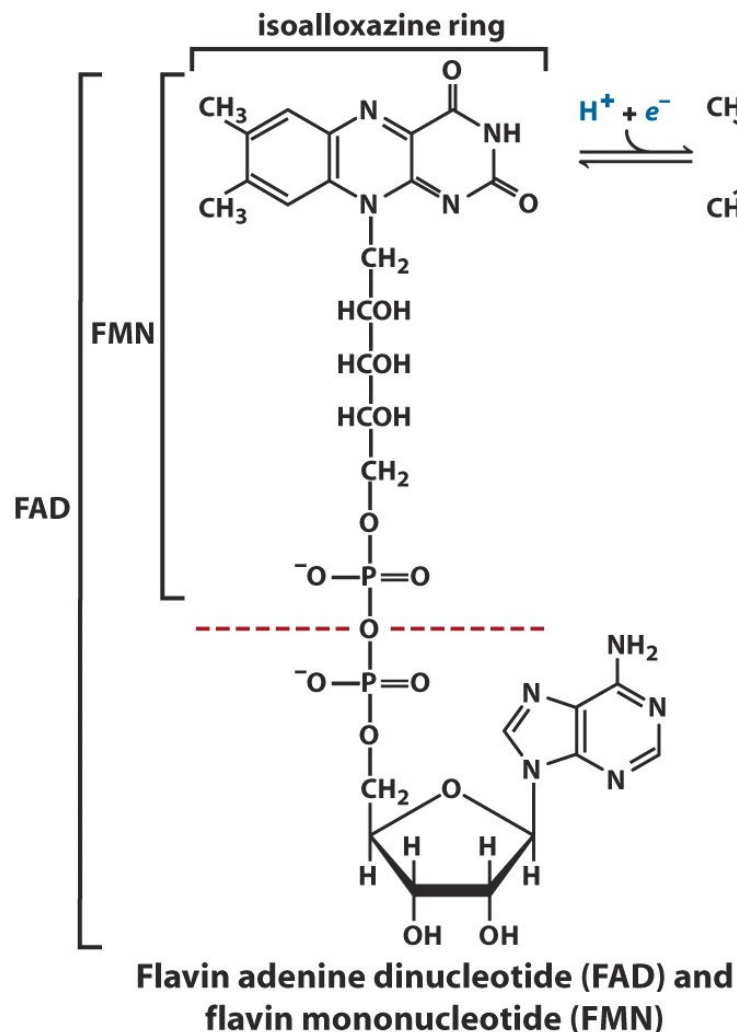
<i>Reaction[†]</i>	<i>Location[†]</i>
NAD-linked	
$\alpha\text{-Ketoglutarate} + \text{CoA} + \text{NAD}^+ \rightleftharpoons \text{succinyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$	M
$\text{L-Malate} + \text{NAD}^+ \rightleftharpoons \text{oxaloacetate} + \text{NADH} + \text{H}^+$	M and C
$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightleftharpoons \text{acetyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$	M
$\text{Glyceraldehyde 3-phosphate} + \text{P}_i + \text{NAD}^+ \rightleftharpoons \text{1,3-bisphosphoglycerate} + \text{NADH} + \text{H}^+$	C
$\text{Lactate} + \text{NAD}^+ \rightleftharpoons \text{pyruvate} + \text{NADH} + \text{H}^+$	C
$\beta\text{-Hydroxyacyl-CoA} + \text{NAD}^+ \rightleftharpoons \beta\text{-ketoacyl-CoA} + \text{NADH} + \text{H}^+$	M
NADP-linked	
$\text{Glucose 6-phosphate} + \text{NADP}^+ \rightleftharpoons \text{6-phosphogluconate} + \text{NADPH} + \text{H}^+$	C
NAD- or NADP-linked	
$\text{L-Glutamate} + \text{H}_2\text{O} + \text{NAD(P)}^+ \rightleftharpoons \alpha\text{-ketoglutarate} + \text{NH}_4^+ + \text{NAD(P)H}$	M
$\text{Isocitrate} + \text{NAD(P)}^+ \rightleftharpoons \alpha\text{-ketoglutarate} + \text{CO}_2 + \text{NAD(P)H} + \text{H}^+$	M and C



Electrons are funneled to universal electron acceptors: **NAD** and **FAD**

- Most deHase that act in catabolism are specific for NAD^+ as electron acceptor
- NAD-linked deHase remove two hydrogne atoms from substrates, one of these is transferred as a **hydride inon ($:\text{H}^-$) to NAD^+** ; the other is releases as **H^+ in medium**.
- NADH and NADPH are water-soluble electron carriers that associate reversibly with deHase. NADH carries electrons from catabolic reactions to their point of entry into the ETC, NADPH supplies electrons to anabolic reactions.
- Flavoproteins (FMN or FAD) accept either one electron or two.

Flavin nucleotides: FMN or FAD



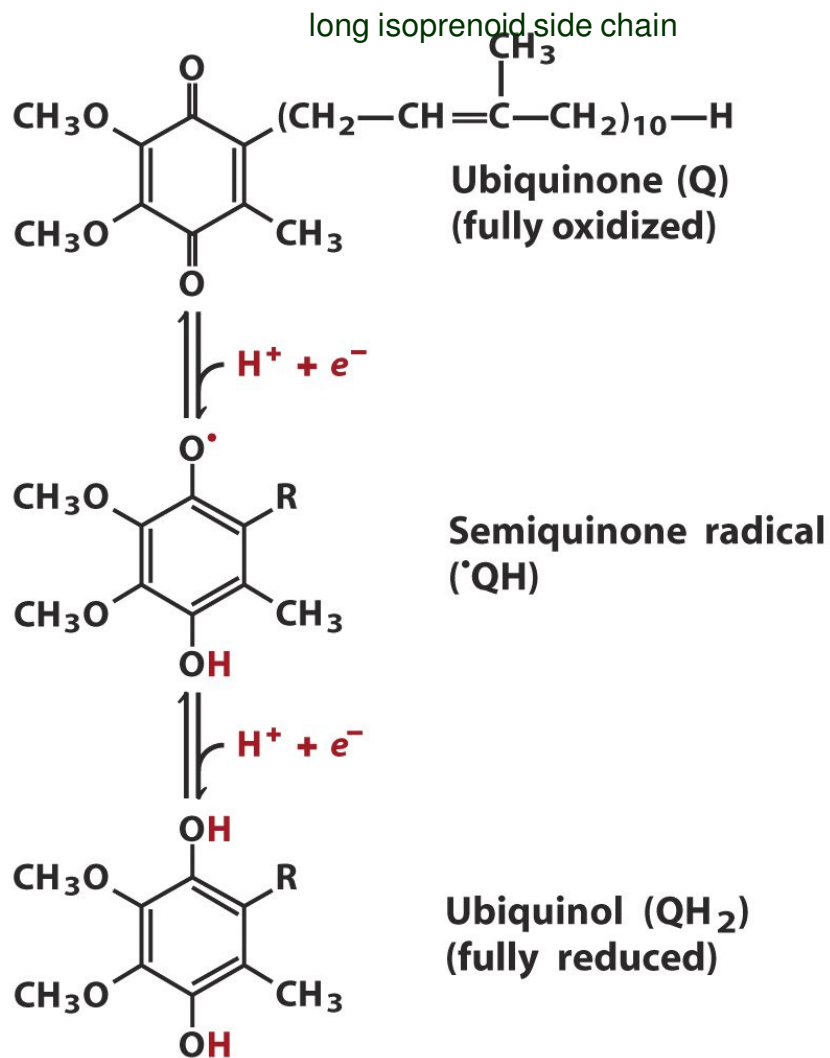
- Flavoproteins (FMN or FAD) accept either one electron (yielding the semiquinone form) or two (yielding FADH₂ or FMNH₂).
- Participate in either one- or two-electron transfers, they can serve as intermediates between reactions in which two electrons are **donated** (as in dehydrogenations) and those in which only one electron is **accepted**.



Electrons Pass through a Series of Membrane-Bound Carriers: Uniquinone (Q or coenzyme Q)

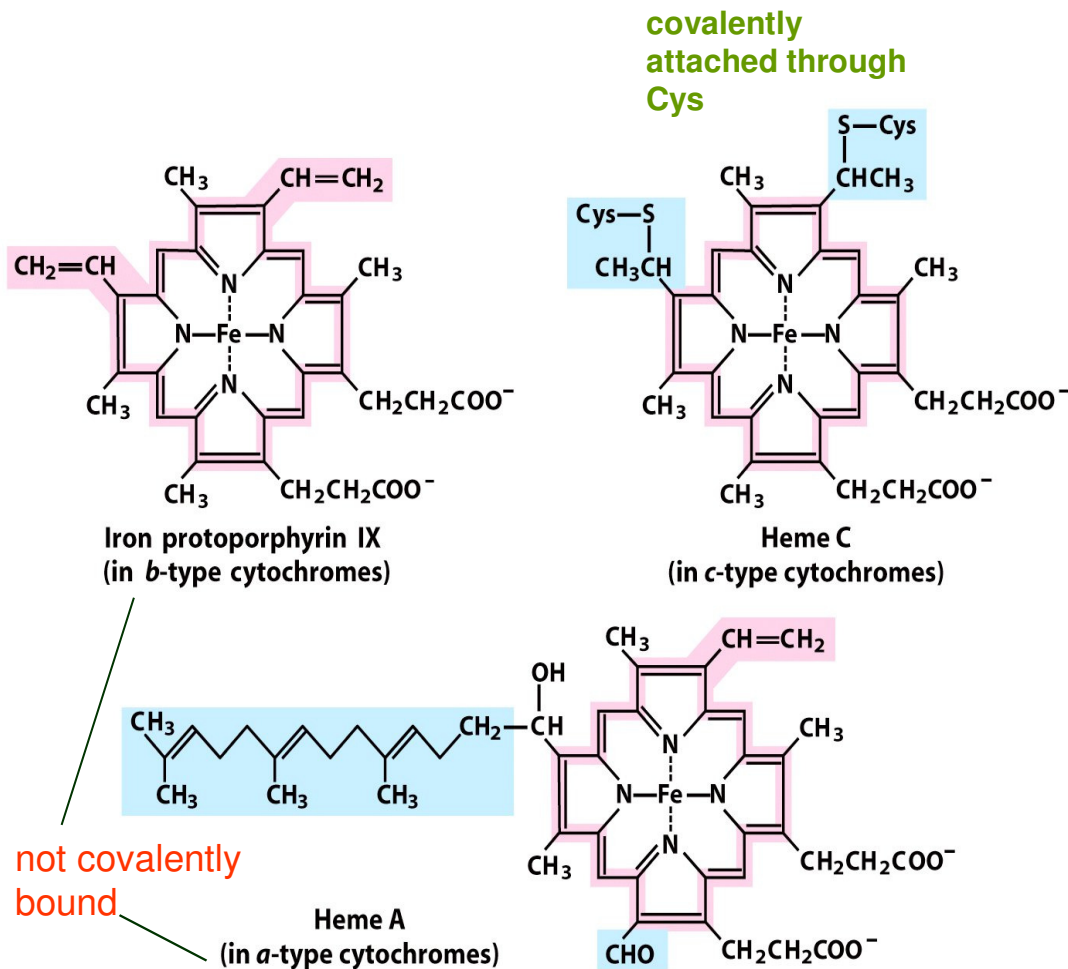
- Three types of electron transfers occur in oxidative phosphorylation:
 1. direct transfer of electrons, as in the reduction of Fe^{3+} to Fe^{2+} ;
 2. transfer as a hydrogen atom ($\text{H}^+ + e^-$); and
 3. transfer as a hydride ion ($:\text{H}^-$), which bears two electrons.
- The term **reducing equivalent** is used to designate a single electron equivalent transferred in an oxidation-reduction reaction.
- Complete reduction of ubiquinone requires **two electrons and two protons**, and occurs in two steps through the semiquinone radical intermediate.
- Q is **lipid-soluble** benzoquinone with a long isoprenoid side chain---freely diffusible within the lipid bilayer of the **inner mitochondrial membrane** and can shuttle reducing equivalents between other, less mobile electron carriers in the membrane, plays a central role in coupling electron flow to proton movement

Ubiquinone (Q or coenzyme Q)



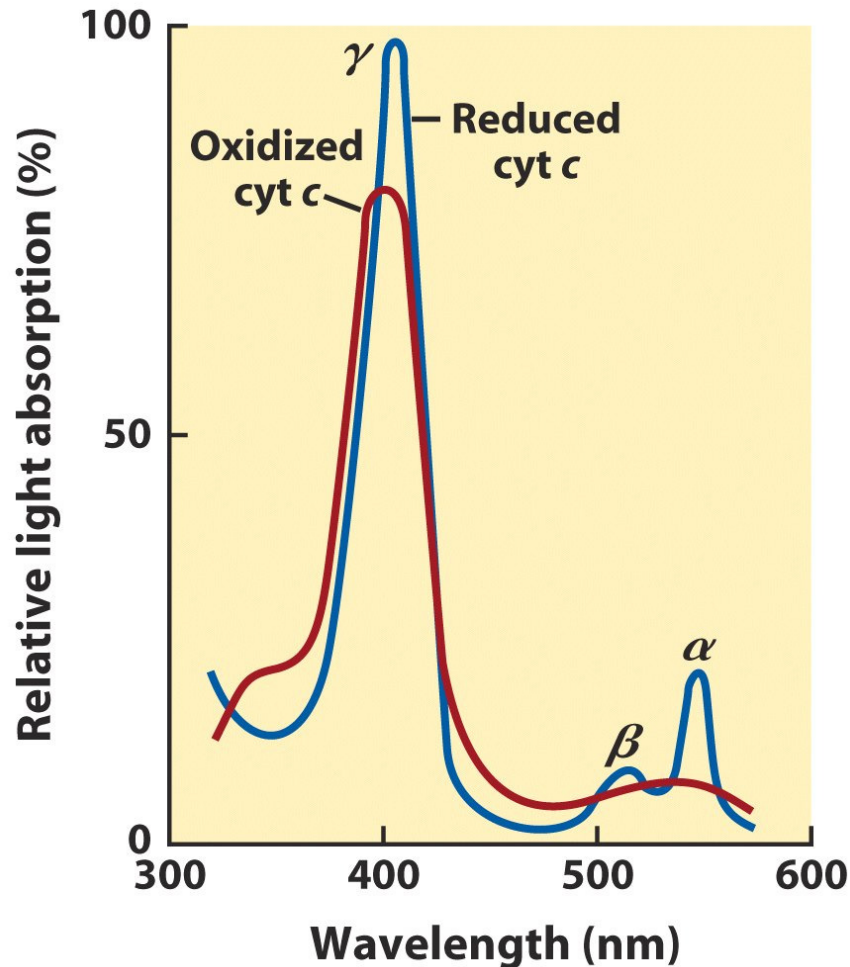
- Complete reduction of **ubiquinone** requires two electrons and two protons, and occurs in two steps through the **semiquinone** radical intermediate.
- a lipid-soluble benzoquinone with a long isoprenoid side chain.
- act at the junction between a two-electron donor and a one-electron acceptor.
- **plastoquinone** (in plant chloroplasts) and **menaquinone** (in bacteria)

Prosthetic groups of cytochromes



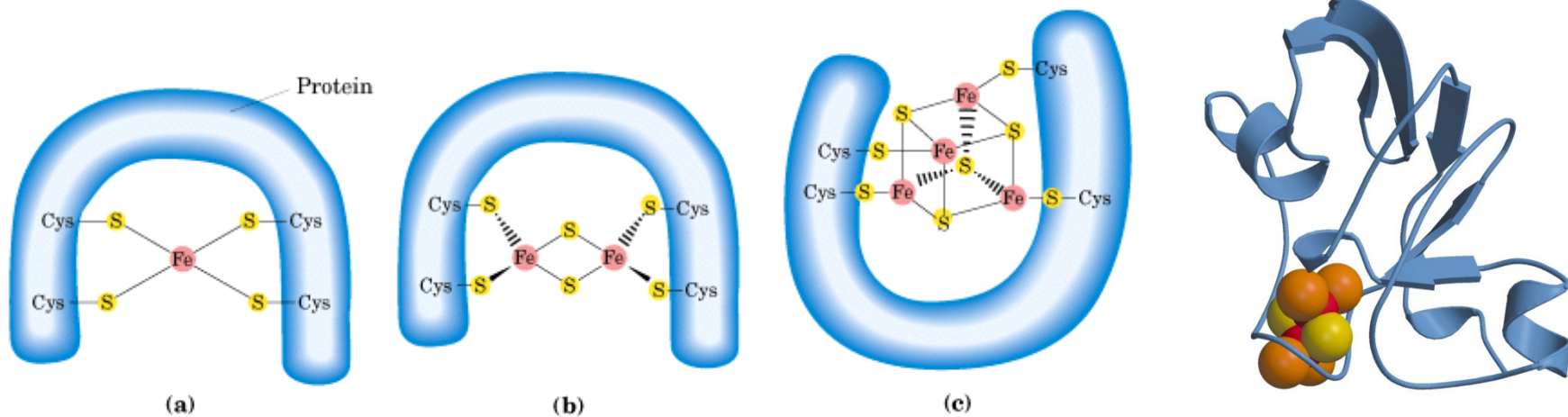
- Each group consists of four five-membered, nitrogen-containing rings in a cyclic structure called a **porphyrin**.
- 4 Ns are coordinated with a central **Fe** ion.
- The heme cofactors of *a* and *b* cytochromes are tightly, but **not covalently**; the hemes of *c*-type cytochromes are covalently attached through Cys residues.
- The cytochromes of type *a* and *b* and some of type *c* are **integral** proteins of the inner mitochondrial membrane.
- One striking exception is the **cytochrome c** of mitochondria, a soluble protein that associates through electrostatic interactions with the outer surface of the inner membrane.

Absorption spectra of Cytochrome c



- Mitochondria contain three classes of cytochromes, designated *a*, *b*, and *c*, which are distinguished by differences in their **light-absorption spectra**. Each type of cytochrome in its **reduced (Fe^{2+}) state** has three absorption bands in the visible range.

Iron-sulfur (Fe-S) proteins/centers



- the iron is in association with **inorganic sulfur** atoms or with the sulfur atoms of **Cys residues** in the protein, or both. These iron-sulfur (Fe-S) centers range from simple structures with a single Fe atom coordinated to four Cys -SH groups to more complex Fe-S centers with two or four Fe atoms; (a) single Fe, (b) 2Fe-2S, or (c) 4Fe-4S centers.
- Rieske iron-sulfur protein (**His not Cys**)
- Participate in **one-electron transfers** in which one iron atom of the iron-sulfur cluster is oxidized or reduced.
- At least 8 Fe-S proteins in ETC

Electrons tend to flow from carriers of lower E'° to higher E'°

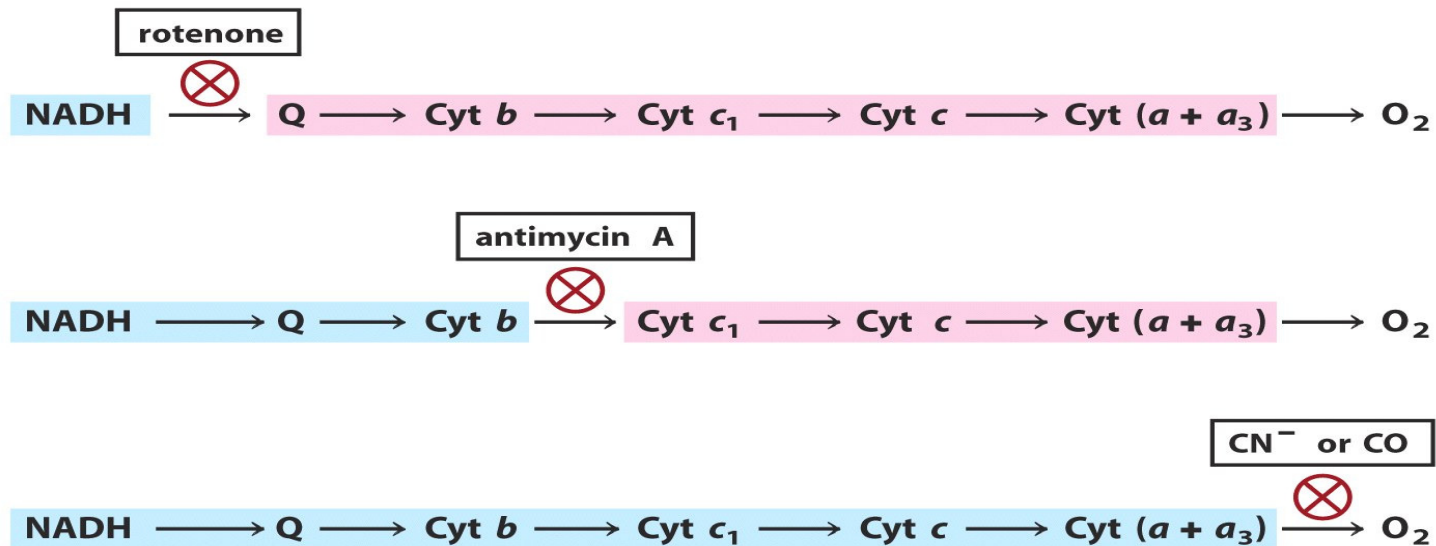
TABLE 19-2 Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

Redox reaction (half-reaction)	E'° (V)
$2\text{H}^+ + 2\text{e}^- \longrightarrow \text{H}_2$	-0.414
$\text{NAD}^+ + \text{H}^+ + 2\text{e}^- \longrightarrow \text{NADH}$	-0.320
$\text{NADP}^+ + \text{H}^+ + 2\text{e}^- \longrightarrow \text{NADPH}$	-0.324
$\text{NADH dehydrogenase (FMN)} + 2\text{H}^+ + 2\text{e}^- \longrightarrow \text{NADH dehydrogenase (FMNH}_2)$	-0.30
$\text{Ubiquinone} + 2\text{H}^+ + 2\text{e}^- \longrightarrow \text{ubiquinol}$	0.045
$\text{Cytochrome } b (\text{Fe}^{3+}) + \text{e}^- \longrightarrow \text{cytochrome } b (\text{Fe}^{2+})$	0.077
$\text{Cytochrome } c_1 (\text{Fe}^{3+}) + \text{e}^- \longrightarrow \text{cytochrome } c_1 (\text{Fe}^{2+})$	0.22
$\text{Cytochrome } c (\text{Fe}^{3+}) + \text{e}^- \longrightarrow \text{cytochrome } c (\text{Fe}^{2+})$	0.254
$\text{Cytochrome } a (\text{Fe}^{3+}) + \text{e}^- \longrightarrow \text{cytochrome } a (\text{Fe}^{2+})$	0.29
$\text{Cytochrome } a_3 (\text{Fe}^{3+}) + \text{e}^- \longrightarrow \text{cytochrome } a_3 (\text{Fe}^{2+})$	0.35
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \longrightarrow \text{H}_2\text{O}$	0.8166

NADH \rightarrow Q \rightarrow cytochrome *b* \rightarrow cytochrome *c*₁ \rightarrow cytochrome *c* \rightarrow cytochrome *a* \rightarrow cytochrome *a*₃ \rightarrow O₂

- **Standard reduction potentials:** the carriers to function in order of increasing reduction potential, because electrons tend to flow spontaneously from carriers of lower E'° to carriers of higher E'°
- however, that the order of standard reduction potentials is not necessarily the same as the order of *actual* reduction potentials under cellular conditions, which depend on the concentration of reduced and oxidized forms

Method for determining the sequence of electron carriers- inhibitors of electron transfer



- A second method for determining the sequence of electron carriers involves reducing the entire chain of carriers experimentally by providing an electron source but no electron acceptor (no O₂).
- When O₂ is suddenly introduced into the system, The carrier **nearest O₂** (at the end of the chain) gives up its electrons first, the second carrier from the end is oxidized next, and so on.
- The actual order depends on concentration of reduced and oxidized forms.
- In the presence of O₂ and an electron donor, carriers that function before the inhibited step become fully reduced, and those that function after this step are completely oxidized.

Separation of functional complexes of the respiratory chain

- The outer mitochondrial membrane is first removed by treatment with the **detergent digitonin**.
- Fragments of inner membrane are then obtained by **osmotic rupture** of the mitochondria, and the fragments are gently dissolved in a second detergent.
- The resulting mixture of inner membrane proteins is resolved by **ion-exchange chromatography** into different complexes (I through IV) of the respiratory chain, each with its unique protein composition
- **Complexes I and II** catalyze electron transfer to **ubiquinone** from two different electron donors: NADH (Complex I) and succinate (Complex II).
- **Complex III** carries electrons from reduced ubiquinone to **cytochrome c**,
- **Complex IV** completes the sequence by transferring electrons from cytochrome c to **O₂**
- **Complex V: ATP synthase**.

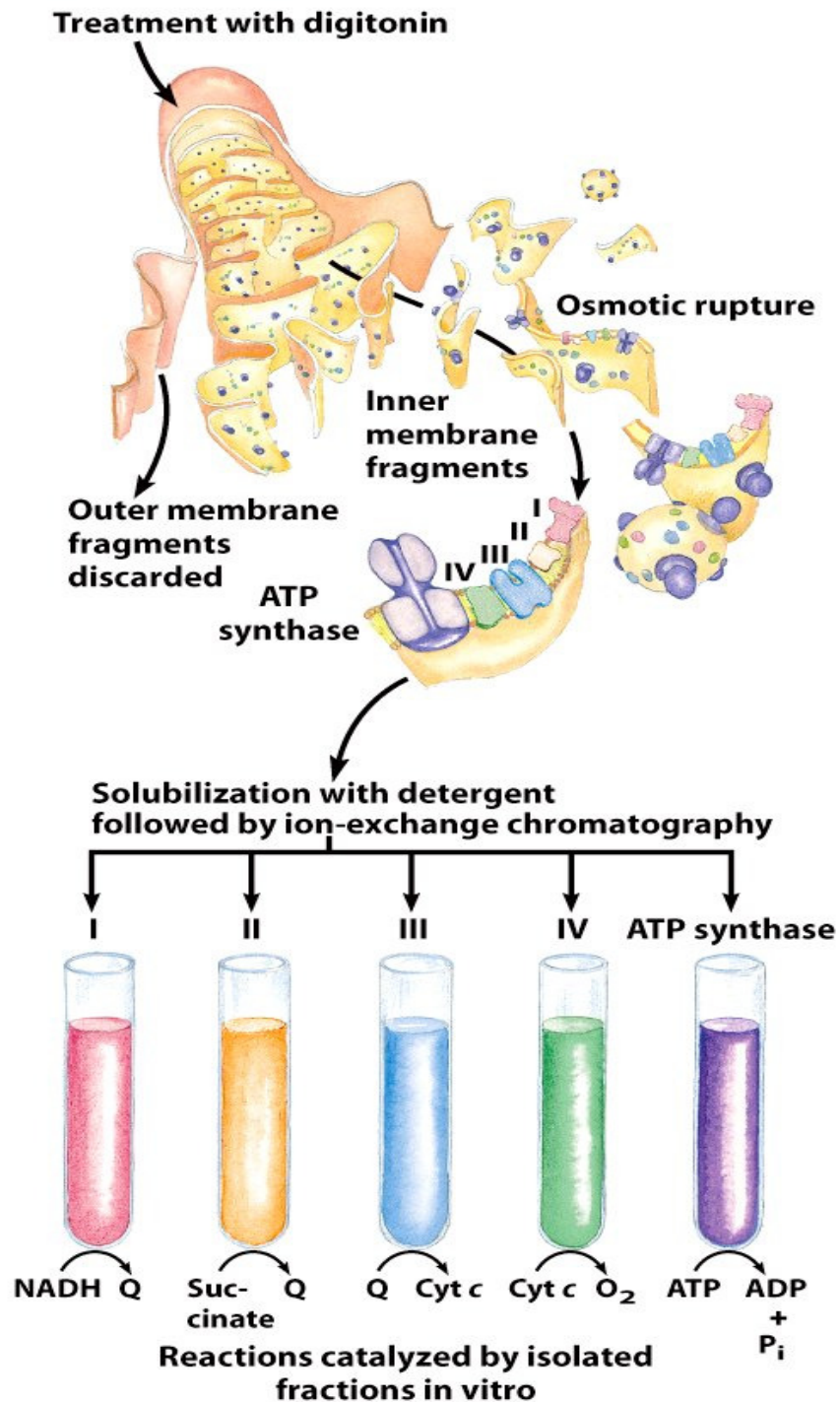


TABLE 19-3 The Protein Components of the Mitochondrial Electron-Transfer Chain

<i>Enzyme complex/protein</i>	<i>Mass (kDa)</i>	<i>Number of subunits*</i>	<i>Prosthetic group(s)</i>
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone cytochrome c oxidoreductase	250	11	Hemes, Fe-S
Cytochrome c [†]	13	1	Heme
IV Cytochrome oxidase	160	13 (3-4)	Hemes; Cu _A , Cu _B

*Numbers of subunits in the bacterial equivalents in parentheses.

[†]Cytochrome c is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

Complex I: NADH to Ubiquinone (NADH: ubiquinone oxidoreductase or NADH dehydrogenase)

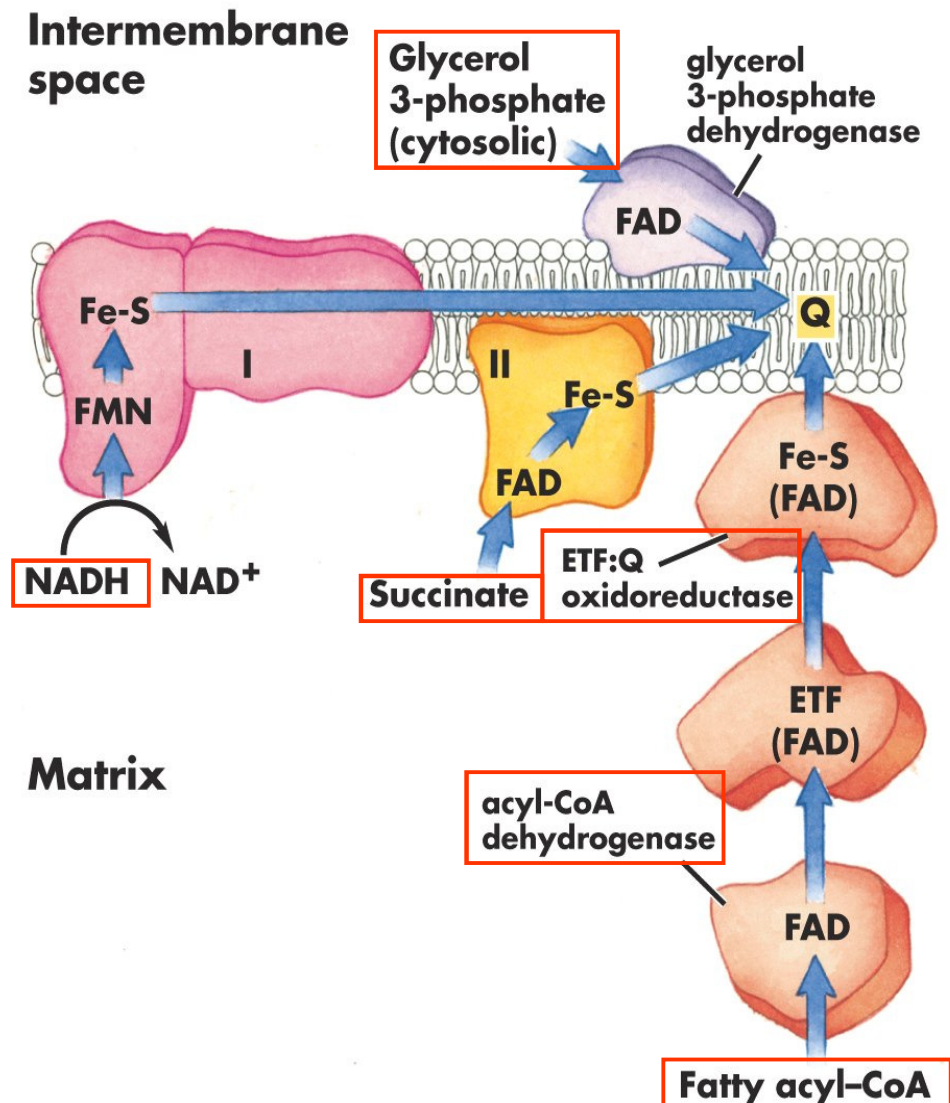
Complex II: Succinate to Ubiquinone (succinate dehydrogenase)

Complex III: Ubiquinone to Cytochrome c (cytochrome bc1 complex or ubiquinone:cytochrome c oxidoreductase,)

Complex IV: Cytochrome c to O₂ (cytochrome oxidase)

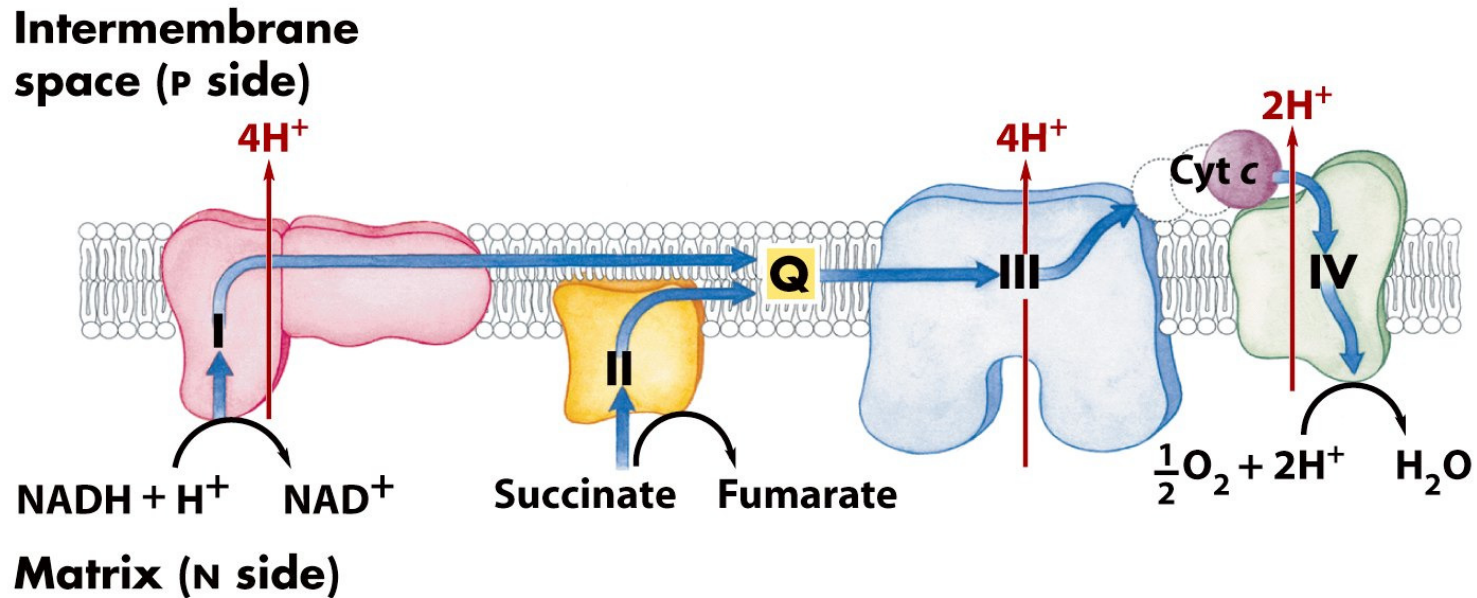
Complex V: ATP synthase

Path of electrons from NADH, succinate, fatty acyl-CoA and glycerol 3-phosphate to ubiquinone



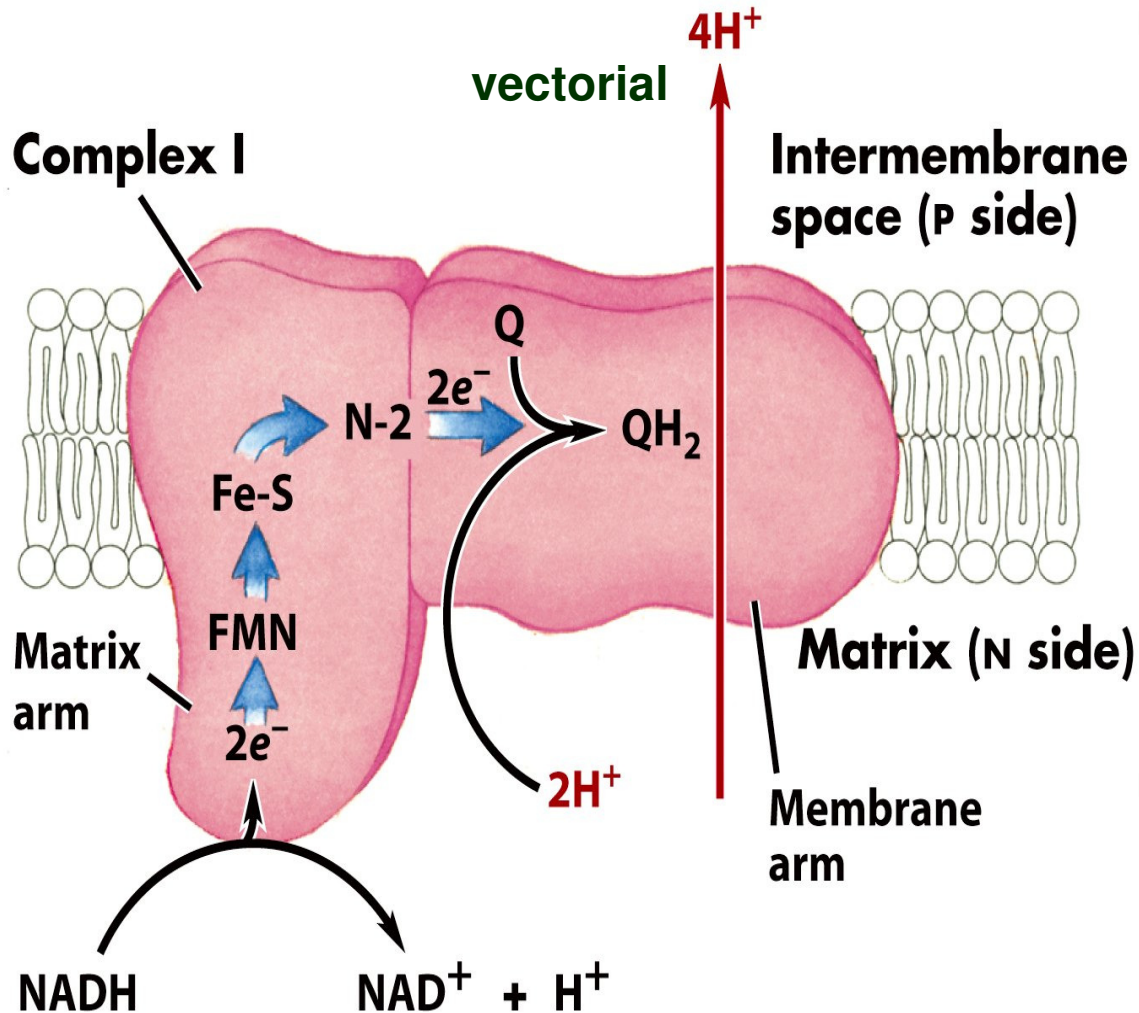
- Electrons from **NADH** pass through a flavoprotein to a series of iron-sulfur proteins (in **Complex I**) and then to Q.
- Electrons from **succinate** pass through a flavoprotein and several Fe-S centers (in **Complex II**) on the way to Q.
- **Glycerol 3-phosphate** donates electrons to a flavoprotein (glycerol 3-phosphate dehydrogenase) on the outer face of the inner mitochondrial membrane, from which they pass to Q.
- **Acyl-CoA dehydrogenase** (the first enzyme of β oxidation) transfers electrons from **fatty acyl-CoA** to electrontransferring flavoprotein (ETF), from which they pass to Q via ETF:ubiquinone oxidoreductase

The flow to electrons and protons through the four complexes of the respiratory chain



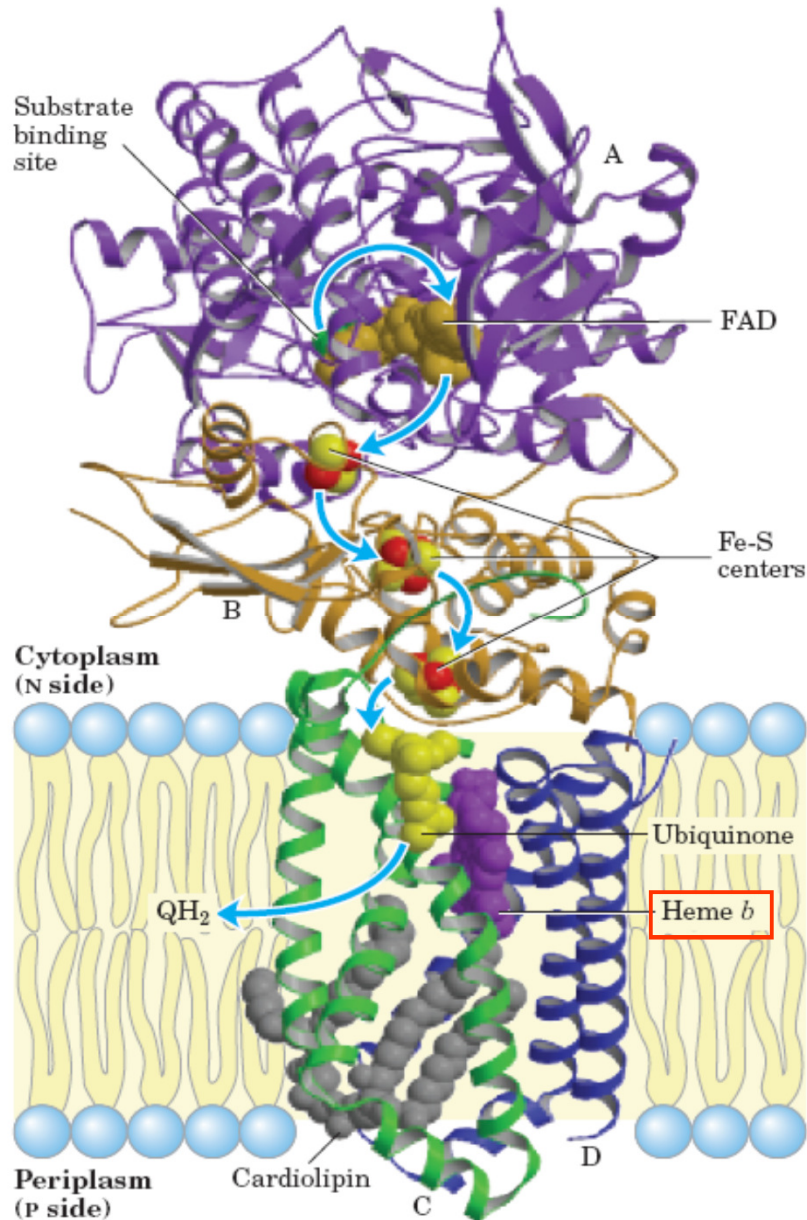
- Electrons reach Q through Complexes I and II. QH_2 serves as a mobile carrier of electrons and protons. It passes electrons to Complex III, which passes them to another mobile connecting link, cytochrome *c*. Complex IV then transfers electrons from reduced cytochrome *c* to O_2 .
- Electron flow through Complexes I, III, and IV is accompanied by proton flow from the matrix to the **intermembrane space**. electrons from oxidation of fatty acids can also enter the respiratory chain through Q.
- Much of this energy is used to pump protons out of the matrix. For **each pair of electrons** transferred to O_2 , four protons are pumped out by Complex I, four by Complex III, and two by Complex IV.

Complex I - NADH: ubiquinone oxidoreductase



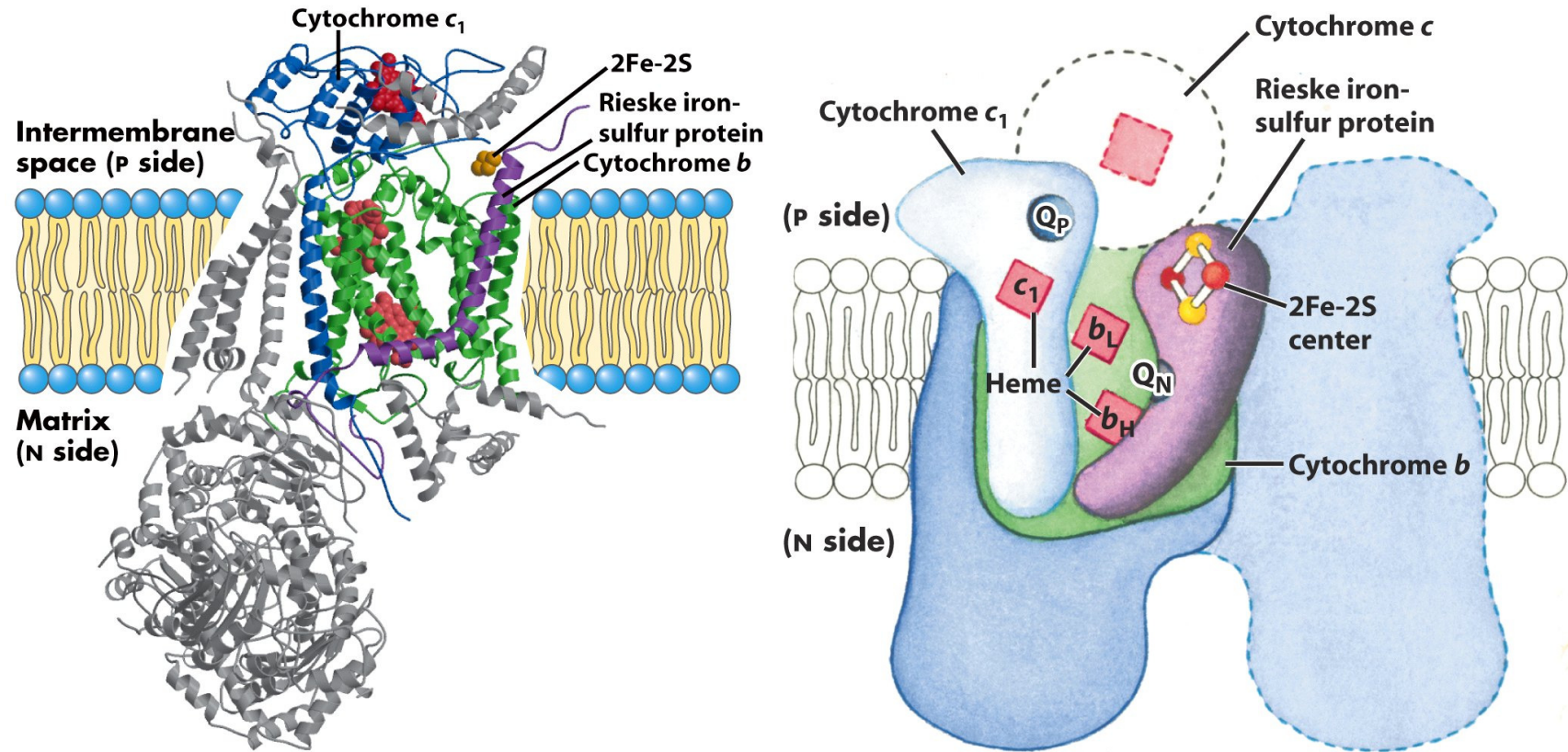
- Complex I catalyzes the transfer of a hydride ion from NADH to FMN, from which two electrons pass through a series of Fe-S centers to the iron sulfur protein N-2 in the matrix arm of the complex. Electron transfer from N-2 to ubiquinone on the membrane arm forms QH₂, which diffuses into the lipid bilayer.
- This electron transfer also drives the expulsion from the matrix of **four protons per pair of electrons**.
- Blocked by amytal and rotenone.

Complex II: succinate dehydrogenase - Succinate to Ubiquinone



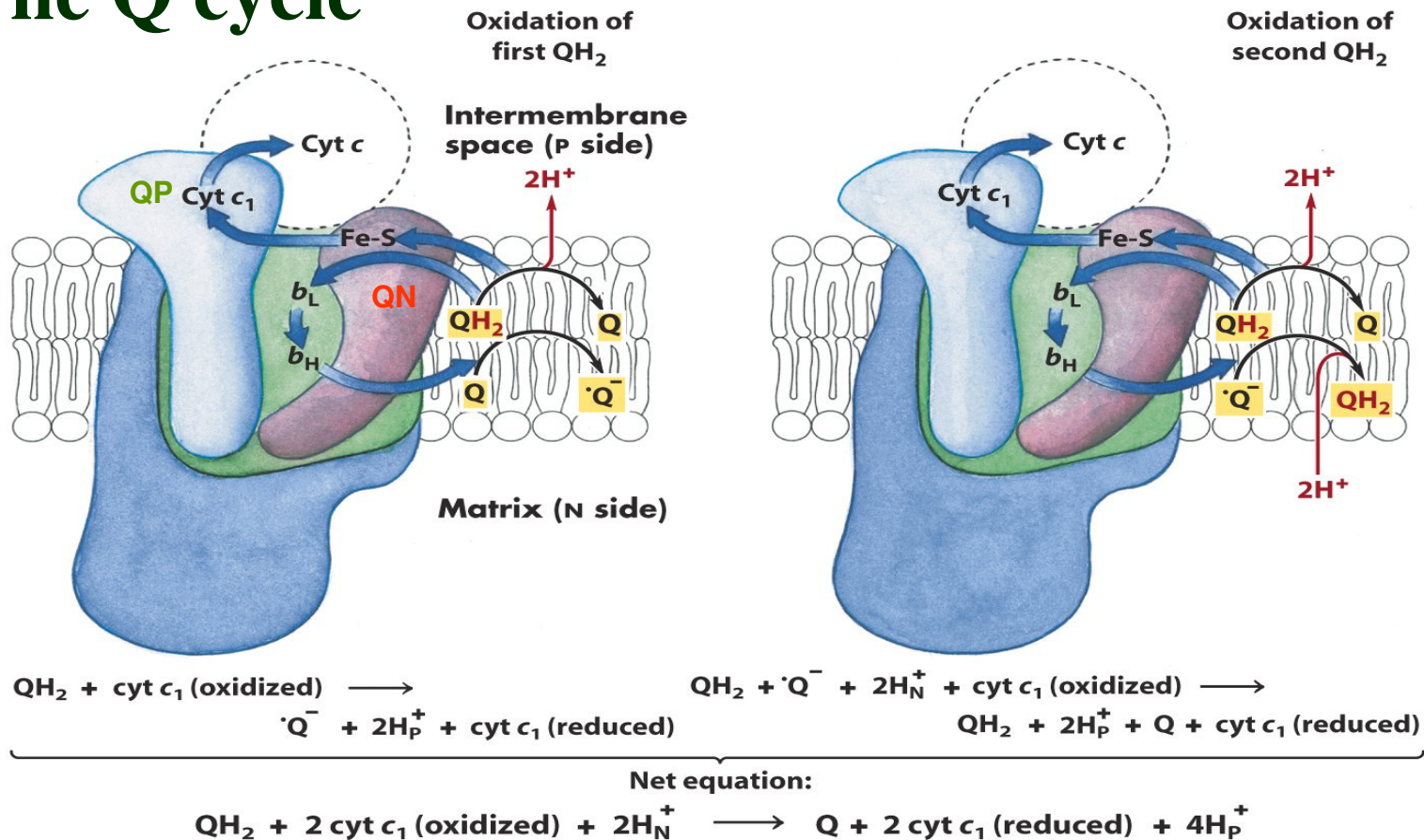
- **succinate dehydrogenase**, the only membrane-bound enzyme in the citric acid cycle.
- Electrons move from succinate to FAD, then through the three Fe-S centers to ubiquinone. The heme *b* is not on the main path of electron transfer but protects against the formation of **reactive oxygen species (ROS)** hydrogen peroxide (H₂O₂) and the **superoxide radical (O₂⁻)** that go astray.
- Humans with point mutations in Complex II subunits near heme *b* or the quinone-binding site suffer from hereditary paraganglioma 副神經節瘤. This inherited condition is characterized by benign tumors of the head and neck, commonly in the carotid body 頸動脈體, an organ that senses O₂ levels in the blood.

Complex III: cytochrome *bc*1 complex



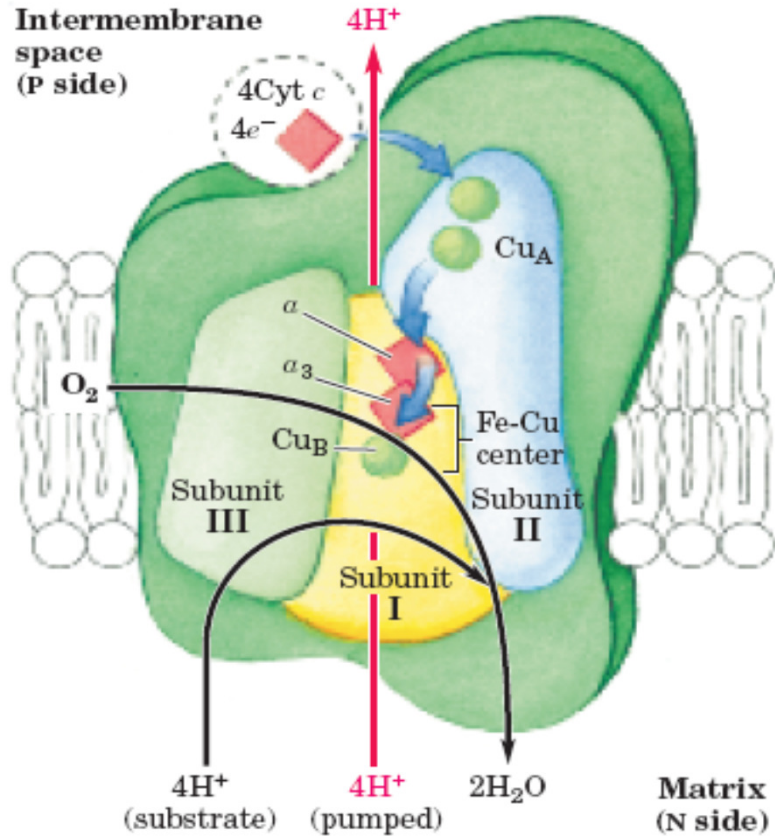
- The complex is a dimer of identical monomers, each with 11 different subunits.
- three subunits: cytochrome b (green) with its two hemes (bH and bL, light red); the Rieske iron-sulfur protein (purple) with its 2Fe-2S centers (yellow); and cytochrome c1 (blue) with its heme (red)
- The dimeric functional unit.
- Blocked by antimycin.

The Q cycle



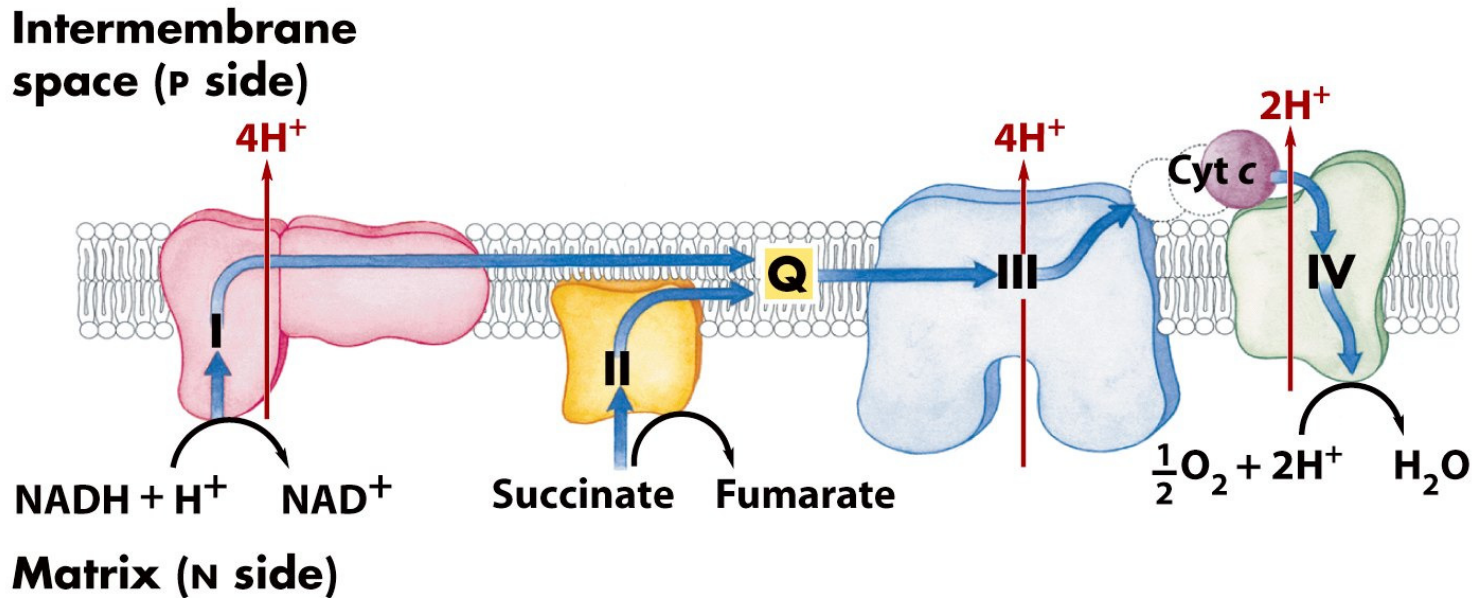
- The dimeric functional unit. Cytochrome *c*1 and the Rieske iron-sulfur protein project from the P surface and can interact with cytochrome *c* in the intermembrane space. The complex has two distinct binding sites for ubiquinone, QN and QP,
- The **Q cycle**: On the P side of the membrane, two molecules of QH₂ are oxidized to Q near the P side, releasing two protons per Q (four protons in all) into the intermembrane space. Each QH₂ donates one electron (via the Rieske Fe-S center) to cytochrome *c*1, and one electron (via cytochrome *b*) to a molecule of Q near the N side, reducing it in two steps to QH₂. This reduction also uses two protons per Q, which are taken up from the matrix.

Complex IV: cytochrome oxidase



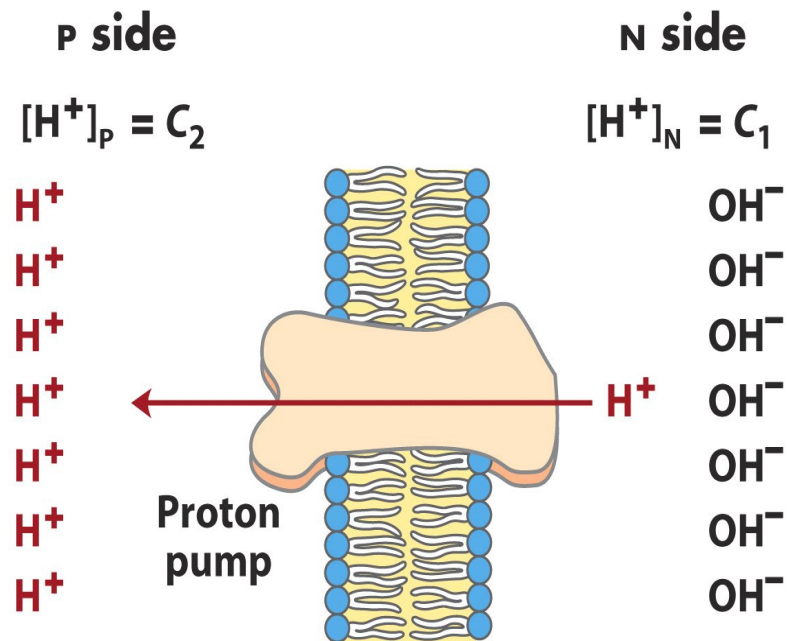
- the **final step** of the respiratory chain, carries electrons from cytochrome *c* to molecular oxygen, reducing it to H₂O.
- The three proteins critical to electron flow are subunits **I**, **II**, and **III**. The larger green structure includes the other ten proteins in the complex.
- Electron transfer through Complex IV begins when two molecules of reduced cytochrome *c* (top) each donate an electron to the binuclear center **CuA**. From here electrons pass through heme *a* to the **Fe-Cu center** (cytochrome *a*₃ and CuB).
- Oxygen now binds to heme *a*₃ and is reduced to its peroxy derivative (O₂²⁻) by two electrons from the Fe-Cu center. Delivery of two more electrons from cytochrome *c* (making four electrons in all) converts the O₂ to two molecules of water, with consumption of four “substrate” protons from the matrix. At the same time, four more protons are pumped from the matrix.
- Blocked by cyanide anion (CN⁻), CO, and sodium azide.

The flow to electrons and protons through the four complexes of the respiratory chain



- Electrons reach Q through Complexes I and II. QH₂ serves as a mobile carrier of electrons and protons. It passes electrons to Complex III, which passes them to another mobile connecting link, cytochrome *c*. Complex IV then transfers electrons from reduced cytochrome *c* to O₂.
- Electron flow through Complexes I, III, and IV is accompanied by proton flow from the matrix to the **intermembrane space**. electrons from oxidation of fatty acids can also enter the respiratory chain through Q.
- Much of this energy is used to pump protons out of the matrix. For **each pair of electrons** transferred to O₂, four protons are pumped out by Complex I, four by Complex III, and two by Complex IV.

Proton-motive force (pmf)

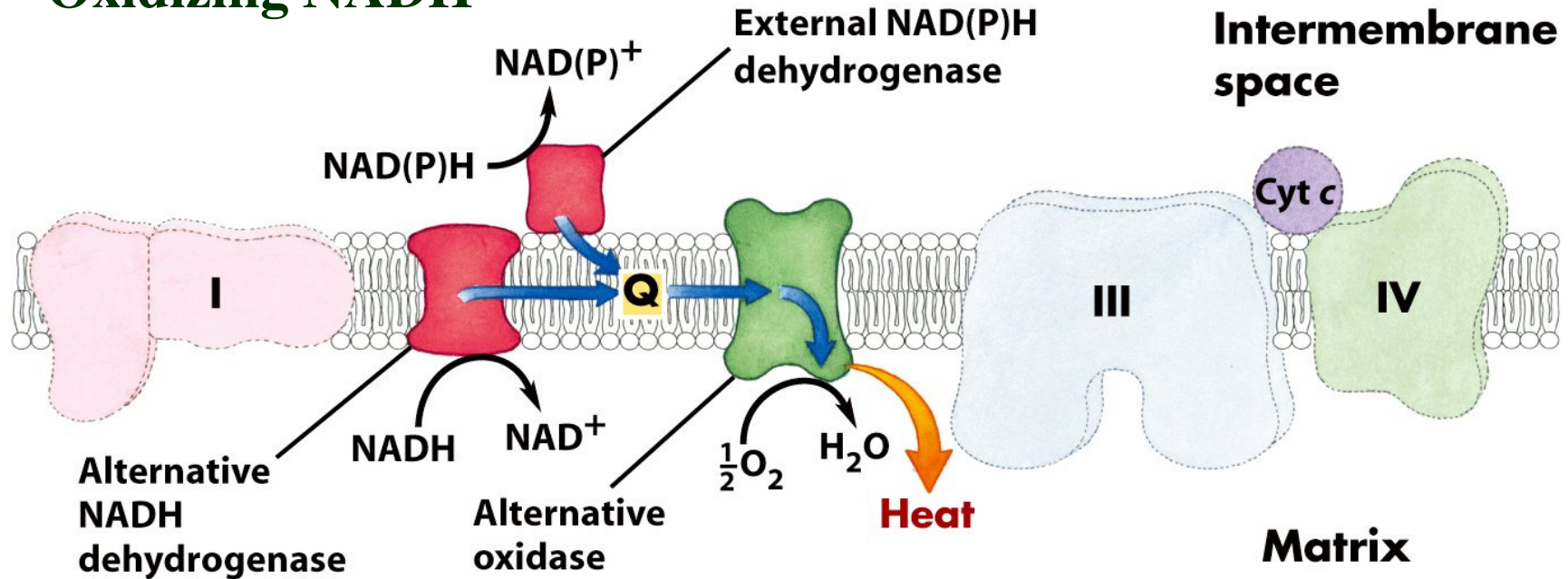


$$\Delta G = RT \ln (C_2/C_1) + Z \mathcal{F} \Delta \psi$$

$$= 2.3RT \Delta pH + \mathcal{F} \Delta \psi$$

- The energy of electron transfer is efficiently conserved in a **proton gradient**
- The chemical potential energy due to the difference in **concentration of H^+** , and **electrical potential energy** that results from the separation of charge
- The inner mitochondrial membrane separates two compartments of different $[H^+]$, resulting in differences in chemical concentration (ΔpH) and charge distribution ($\Delta \psi$) across the membrane.
- The net effect is the proton-motive force (ΔG).

Plant Mitochondria Have Alternative Mechanisms for Oxidizing NADH



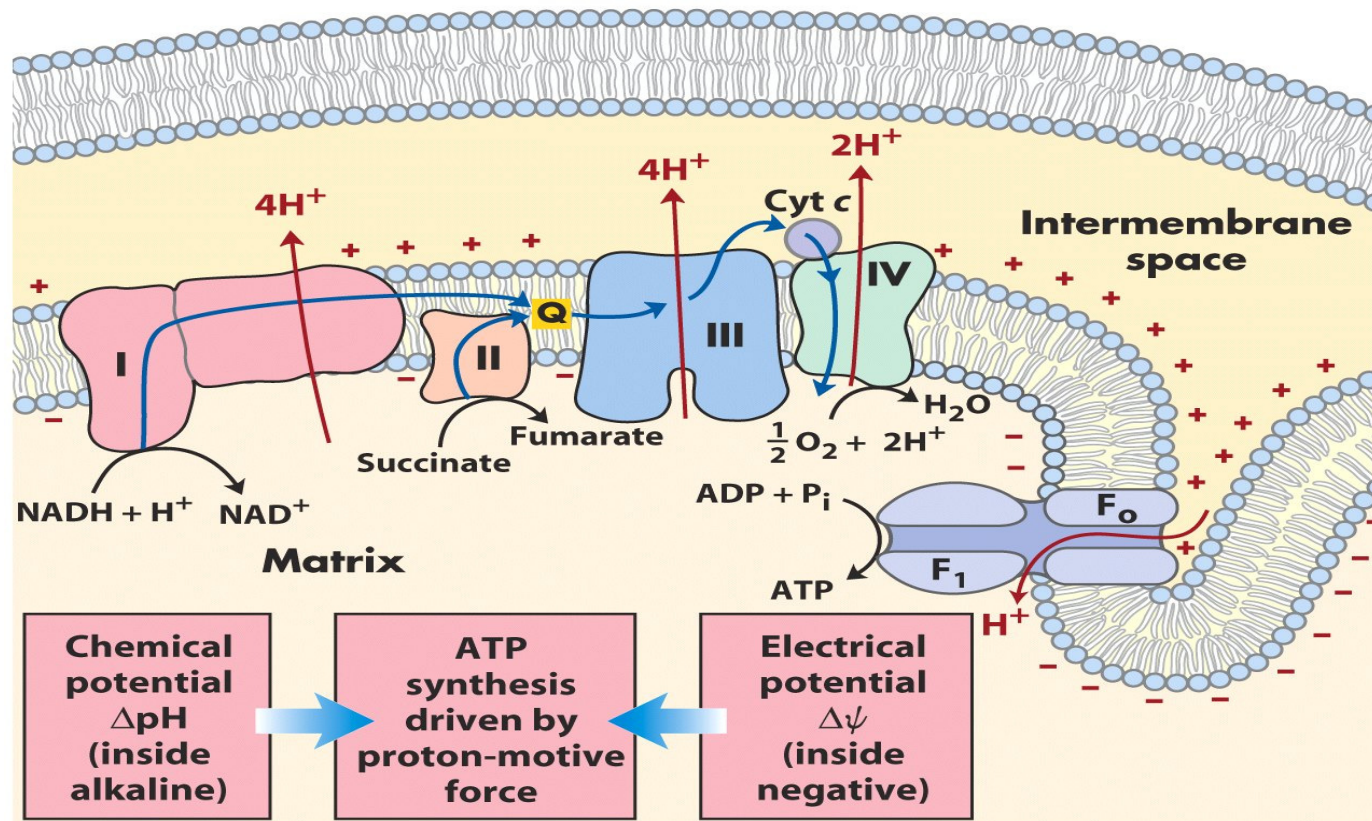
- Electron carriers of the inner membrane of plant mitochondria. Electrons can flow through Complexes I, III, and IV, as in animal mitochondria, or through plant-specific alternative carriers by the paths shown with blue arrows.
- In this process the energy in NADH is dissipated as **heat**, which can sometimes be of value to the plant.
- **Cyanide-resistant NADH** oxidation is therefore the hallmark of this unique plant electron-transfer pathway.



ATP Synthesis

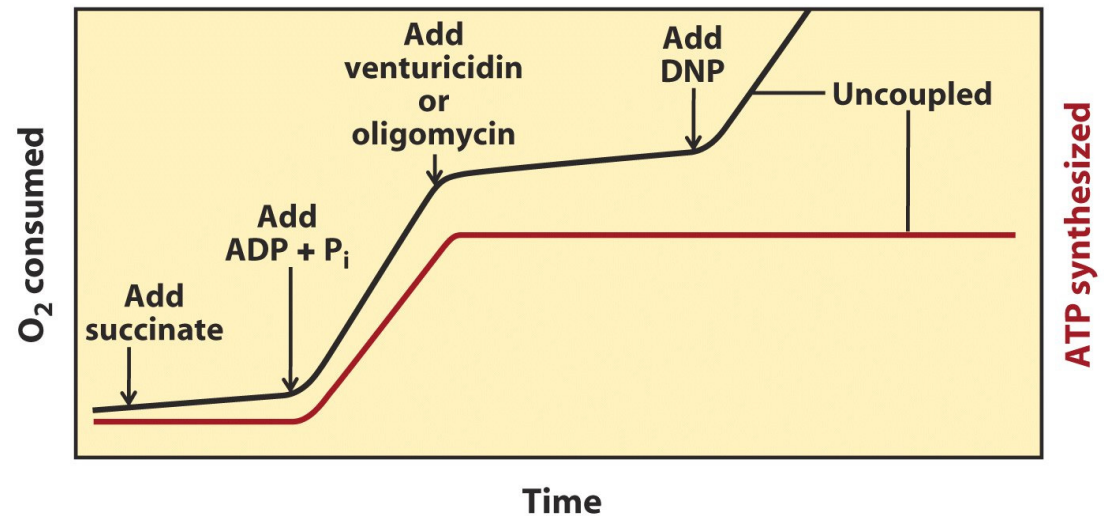
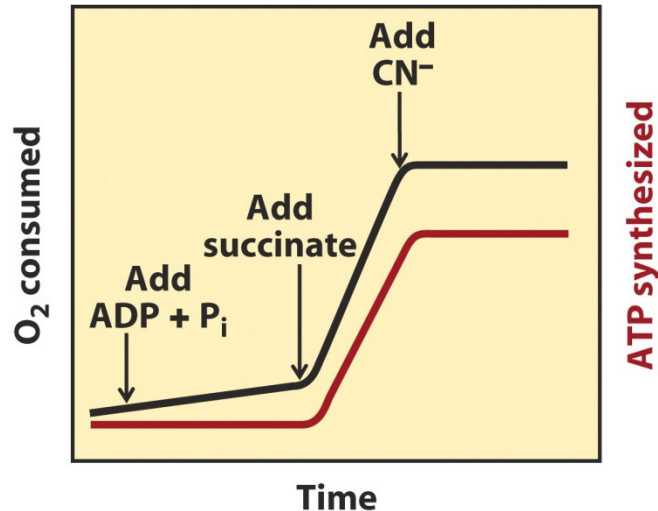
- ATP Synthase Has Two Functional Domains, Fo and F1
- ATP Is Stabilized Relative to ADP on the Surface of F1
- The Proton Gradient Drives the Release of ATP from the Enzyme Surface
- Each β Subunit of ATP Synthase Can Assume Three Different Conformations
- Rotational Catalysis Is Key to the Binding-Change Mechanism for ATP Synthesis
- Chemiosmotic Coupling Allows Nonintegral Stoichiometries of O₂ Consumption and ATP Synthesis
- The Proton-Motive Force Energizes Active Transport
- Shuttle Systems Indirectly Convey Cytosolic NADH into Mitochondria for Oxidation

Chemiosmotic model



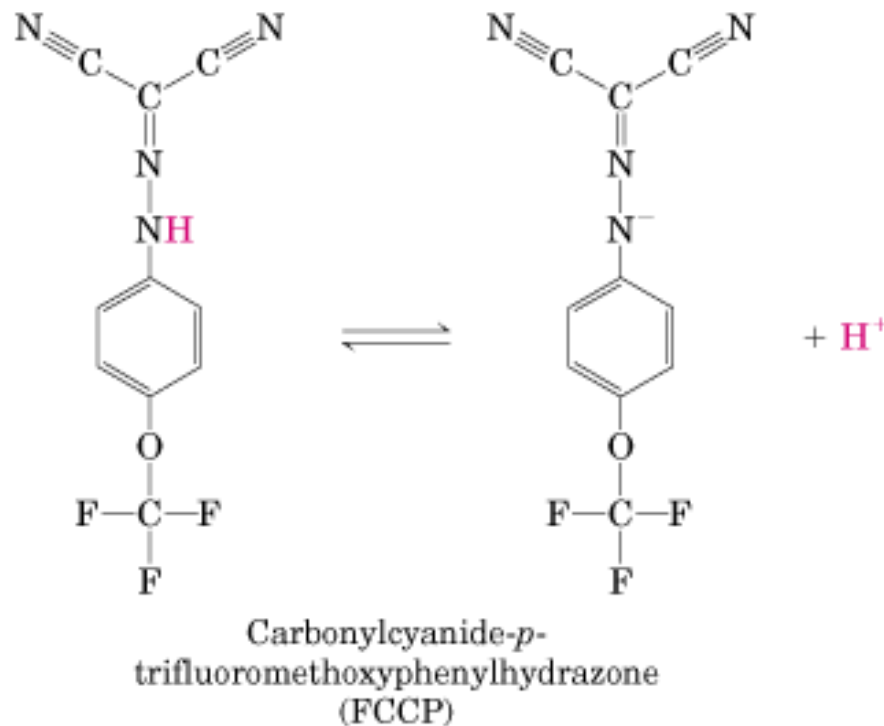
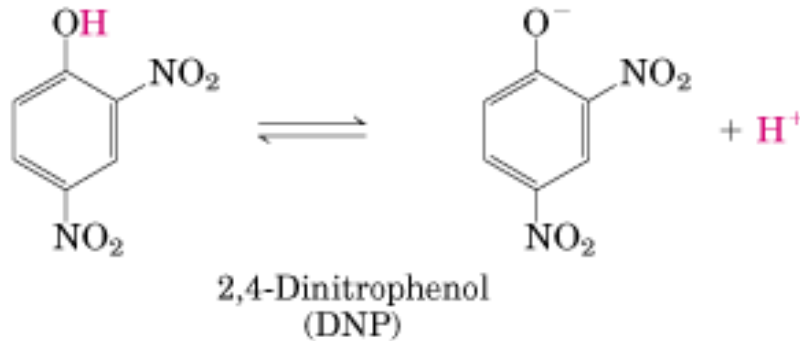
- electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner membrane.
- Electron flow is accompanied by proton transfer across the membrane, producing both a chemical gradient (ΔpH) and an electrical gradient ($\Delta\psi$).
- The inner mitochondrial membrane is impermeable to protons; protons can reenter the matrix only through **proton-specific channels (F_o)**.
- The proton-motive force that drives protons back into the matrix provides the energy for **ATP synthesis**, catalyzed by the **F₁ complex** associated with F_o.

Coupling of electron transfer and ATP synthesis



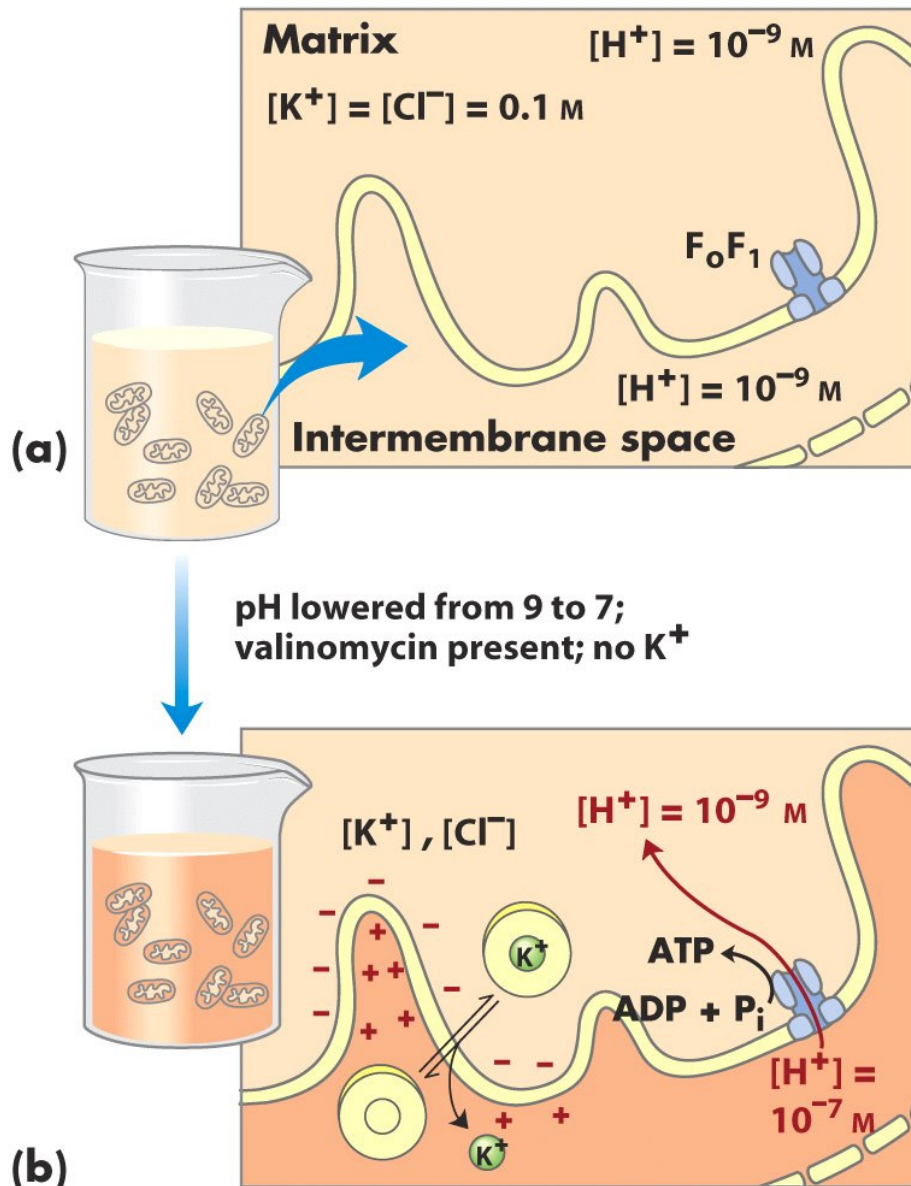
- Chemiosmotic theory readily explains the dependence of electron transfer on ATP synthesis in mitochondria.
- Addition of ADP and P_i alone results in little or no increase in either respiration (O₂ consumption; black) or **ATP synthesis** (red). When **succinate** is added, respiration begins immediately and ATP is synthesized. Addition of **cyanide (CN)**, which blocks electron transfer between cytochrome oxidase and O₂, inhibits both respiration and **ATP synthesis**.
- Mitochondria provided with succinate respire and synthesize ATP only when ADP and P_i are added. Subsequent addition of **venturicidin** or **oligomycin**, inhibitors of ATP synthase, blocks both ATP synthesis and respiration.
- **Dinitrophenol (DNP)** is an uncoupler, allowing respiration to continue without **ATP synthesis**.

Chemical uncouplers



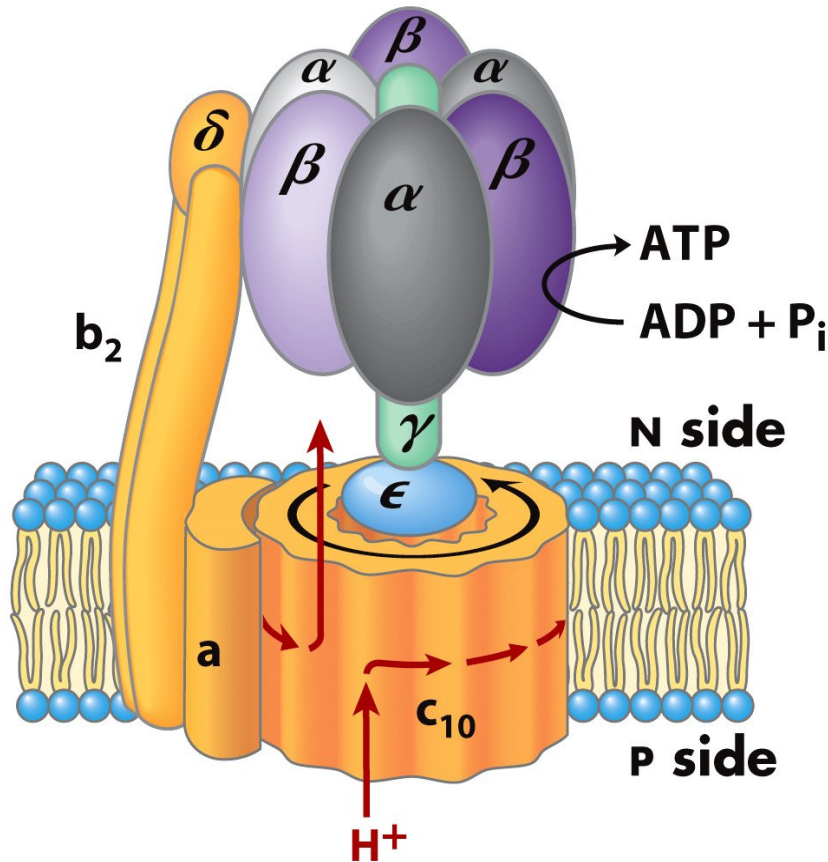
- Both DNP and FCCP have a dissociable proton and are very hydrophobic.
- They carry protons across the inner mitochondrial membrane, dissipating the **proton gradient**.

Evidence for the role of a proton gradient in ATP synthesis



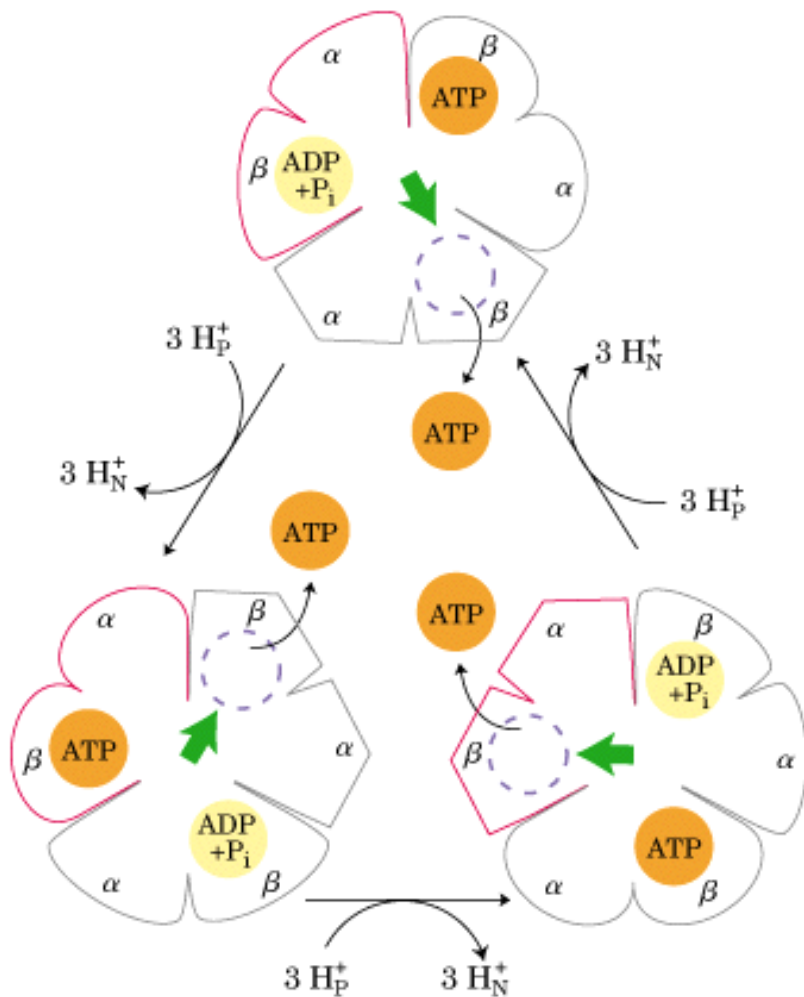
- An artificially imposed **electrochemical gradient** can drive ATP synthesis in the absence of an oxidizable substrate as electron donor.
- (a) isolated mitochondria are first incubated in a **pH 9** buffer containing 0.1 M KCl. Slow leakage of buffer and KCl into the mitochondria eventually brings the matrix into equilibrium with the surrounding medium. No oxidizable substrates are present.
- (b) Mitochondria are now separated from the pH 9 buffer and resuspended in **pH 7** buffer containing **valinomycin** (K^+ ionophore) but no KCl. The change in buffer creates a difference of two pH units across the inner mitochondrial membrane. The outward flow of K, carried (by valinomycin) down its concentration gradient without a counterion, creates a charge imbalance across the membrane (matrix negative).
- The sum of the chemical potential provided by the **pH difference** and the **electrical potential** provided by the separation of charges is a proton motive force large enough to support ATP synthesis in the absence of an oxidizable substrate.

Mitochondrial ATP synthase complex (FoF₁ complex)



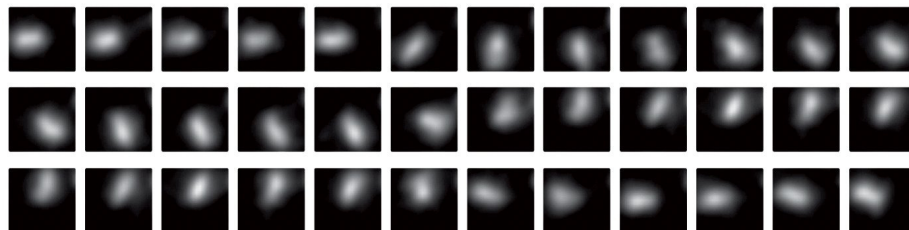
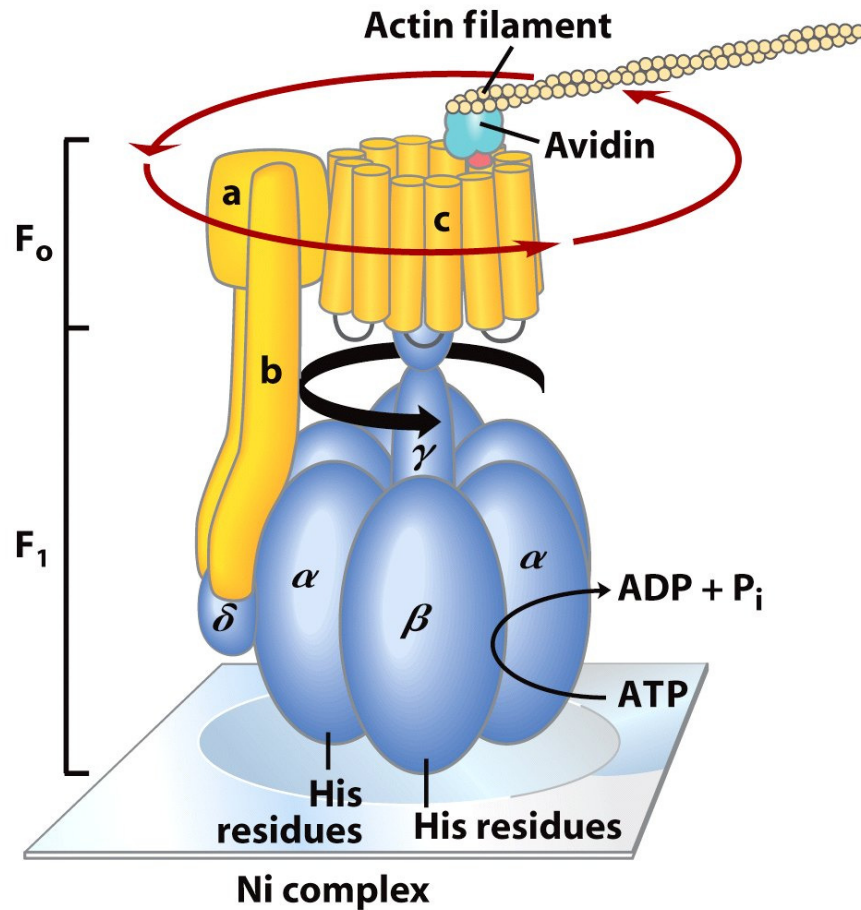
- The two b subunits of F_o associate firmly with the α and β subunits of F₁, holding them fixed relative to the membrane.
- In F_o, the membrane-embedded cylinder of c subunits is attached to the shaft made up of F₁ subunits and .
- As protons flow through the membrane from the P side to the N side through F_o, the cylinder and shaft rotate, and the subunits of F₁ change conformation as the subunit associates with each in turn.

Rotational Catalysis Is Key to the Binding-Change Mechanism for ATP Synthesis



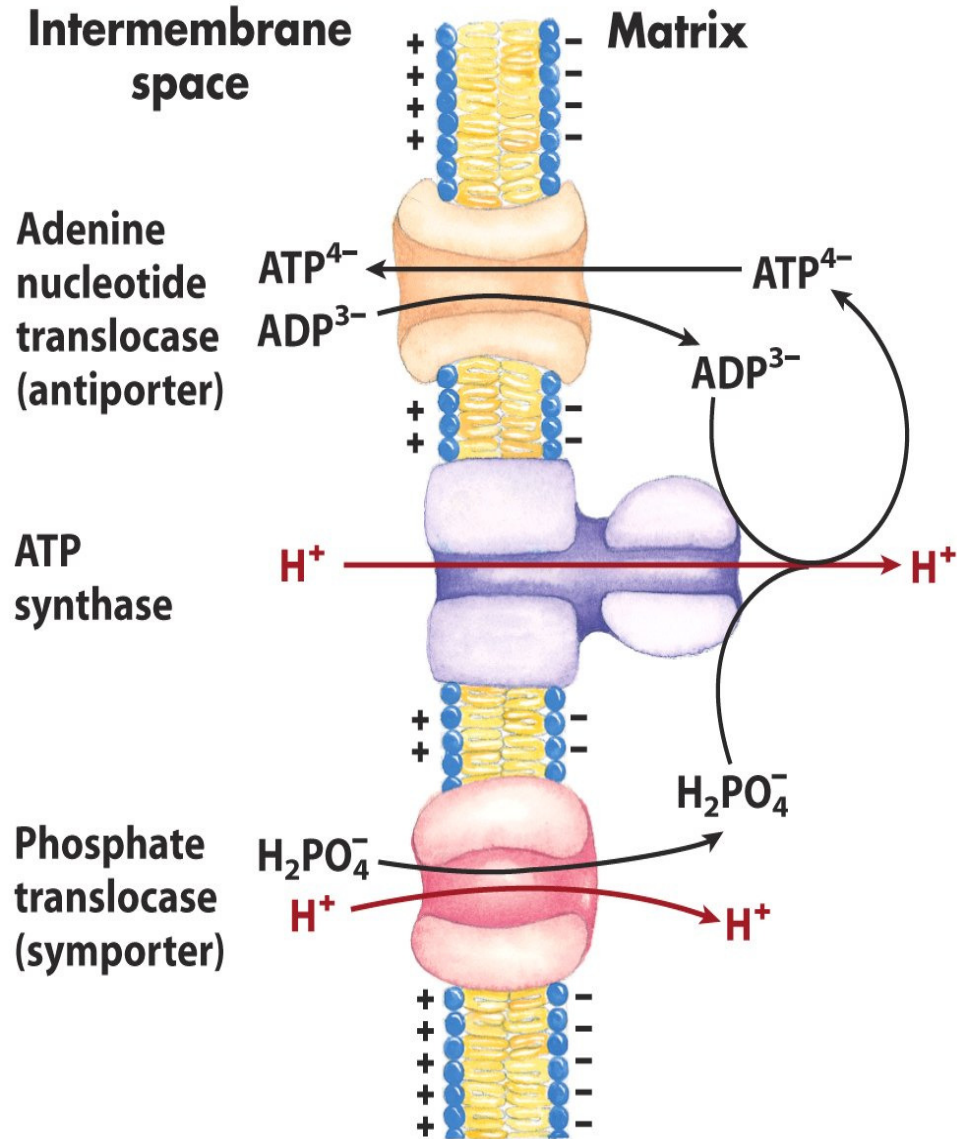
- The F1 complex has **three** nonequivalent adenine nucleotide-binding sites, one for each pair of α and β subunits.
- At any given moment, one of these sites is in the **β -ATP** conformation (which binds ATP tightly), a second is in the **β -ADP** (loose-binding) conformation, and a third is in the **β -empty** (very-loose-binding) conformation.
- The proton-motive force causes rotation of the central shaft—the **γ subunit**, which comes into contact with each subunit pair in succession. This produces a cooperative conformational change in which the β -ATP site is converted to the β -empty conformation, and ATP dissociates; the β -ADP site is converted to the β -ATP conformation, which promotes condensation of bound ADP + Pi to form ATP; and the -empty site becomes a β -ADP site, which loosely binds ADP + Pi entering from the solvent.
- This model, based on experimental findings, requires that at least two of the three catalytic sites alternate in activity; ATP cannot be released from one site unless and until ADP and Pi are bound at the other.

Rotation of Fo and γ -subunit



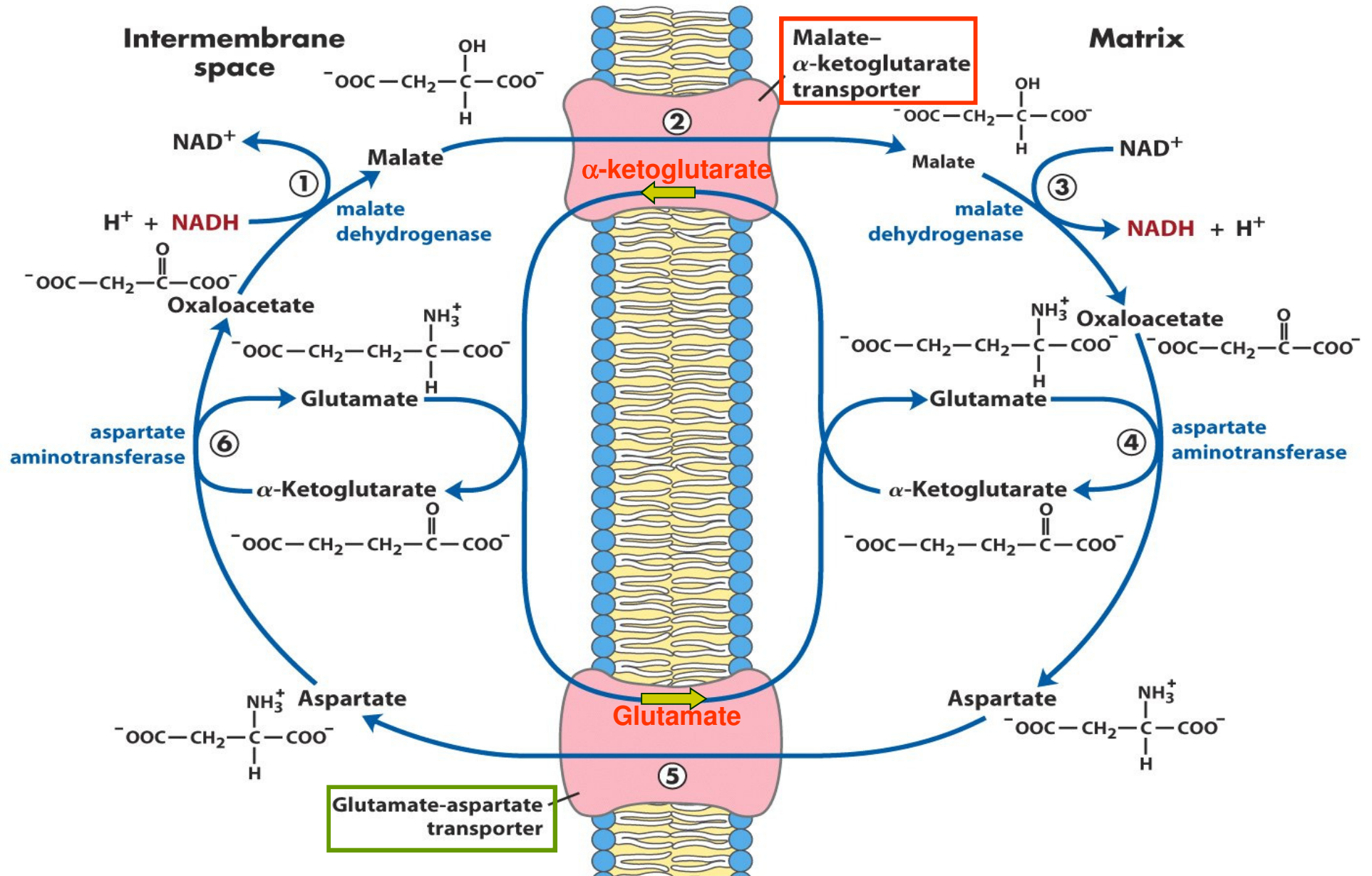
- F1 genetically engineered to contain a run of His residues adheres tightly to a microscope slide coated with a Ni complex; **biotin** is covalently attached to a c subunit of Fo. The protein **avidin**, which binds biotin very tightly, is covalently attached to long filaments of **actin labeled with a fluorescent probe**. Biotin-avidin binding now attaches the actin filaments to the c subunit.
- When ATP is provided as substrate for the ATPase activity of F1, the labeled filament is seen to rotate continuously in one direction, proving that the Fo cylinder of c subunits rotates.
- In another experiment, a fluorescent actin filament was attached directly to the subunit. The series of fluorescence micrographs shows the position of the actin filament at intervals of 133 ms.
- Note that as the filament rotates, it makes a discrete jump about every eleventh frame. Presumably the cylinder and shaft move as one unit.

Adenine nucleotide and phosphate translocases

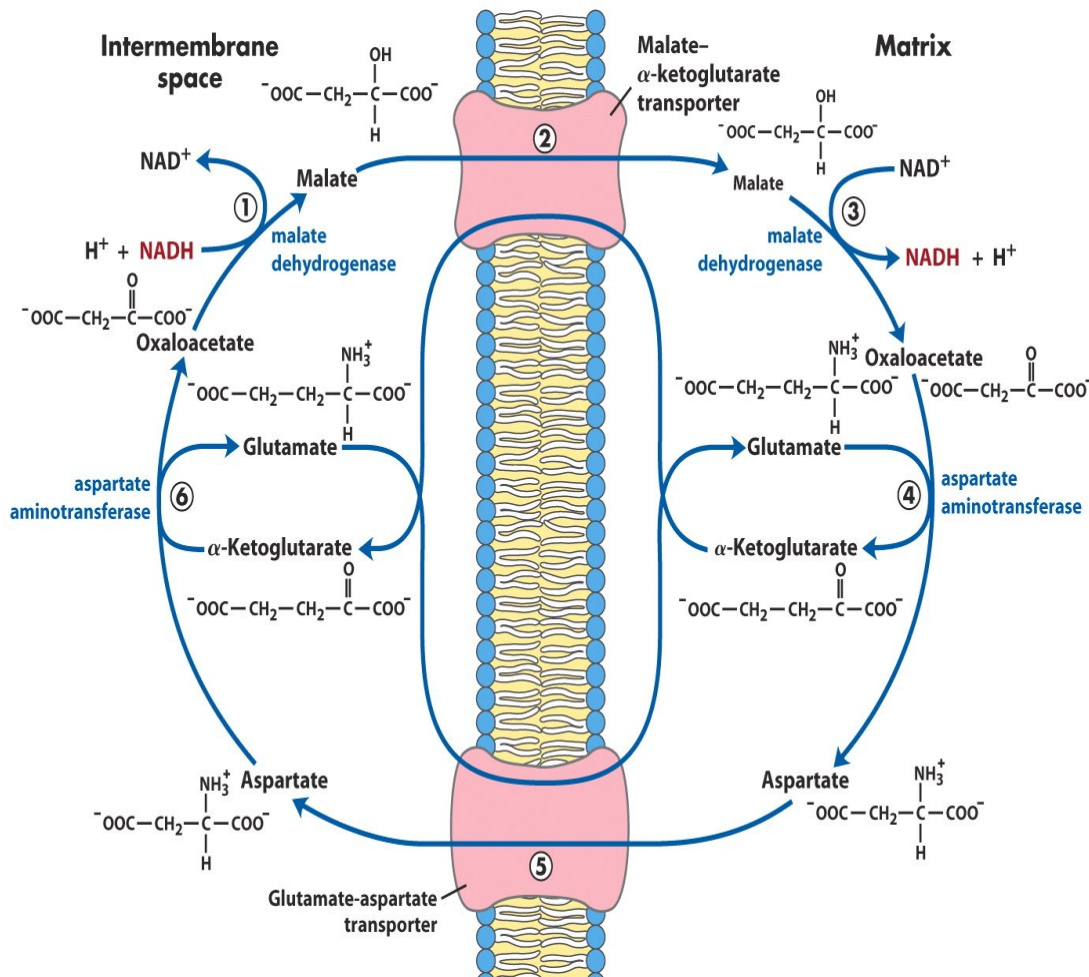


- **ATP synthasome**
- Transport systems of the inner mitochondrial membrane carry ADP and Pi into the matrix and newly synthesized ATP into the cytosol.
- **Adenine nucleotide translocase** is an antiporter; the same protein moves ADP into the matrix and ATP out. The effect of replacing ATP^{4-} with ADP^{3-} is the net efflux of **one negative charge**, which is favored by the charge difference across the inner membrane (outside positive). At pH 7, Pi is present as both HPO_4^{2-} and H_2PO_4^- ;
- **Phosphate translocase** is specific for H_2PO_4^- . There is no net flow of charge during symport of H_2PO_4^- and H^+ , but the relatively low proton concentration in the matrix favors the inward movement of H^+ .
- **Proton-motive force** (pmf) is responsible both for providing the energy for ATP synthesis and for transporting substrates (ADP and Pi) in and product (ATP) out of the mitochondrial matrix.

Malate-Aspartate Shuttle (liver, kidney, heart)

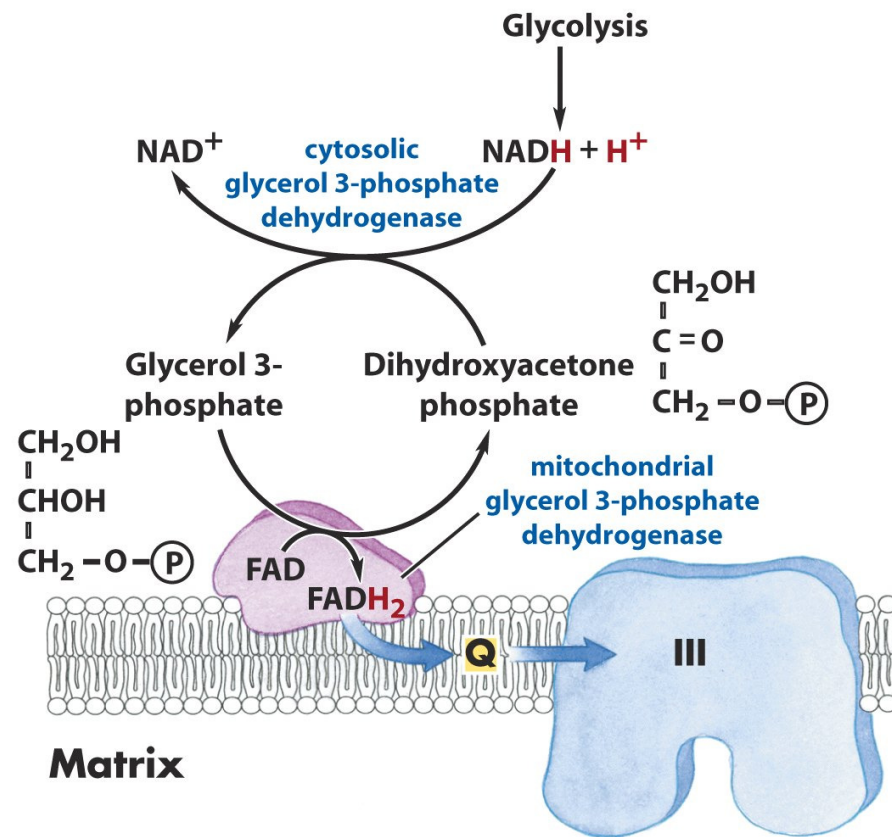


Malate-Aspartate Shuttle (liver, kidney, heart)



1. NADH in the cytosol (intermembrane space) passes two reducing equivalents to oxaloacetate, producing malate.
 2. Malate crosses the inner membrane via the **malate- α -ketoglutarate transporter**.
 3. In the matrix, malate passes two reducing equivalents to NAD^+ , and the resulting NADH is oxidized by the respiratory chain.
 4. The oxaloacetate formed from malate cannot pass directly into the cytosol. It is first transaminated to aspartate,
 5. Aspartate can leave via the **glutamate-aspartate transporter**.
 6. Oxaloacetate is regenerated in the cytosol, completing the cycle.
- NADH can pass electrons directly to the respiratory chain of complex I. About **2.5 molecules of ATP** are generated as this pair of electrons passes to O_2 .

Glycerol 3-phosphate shuttle (Skeletal muscle and brain)



- **Skeletal muscle** and **brain** use a different NADH shuttle, the **glycerol 3-phosphate shuttle**.
- It differs from the malate-aspartate shuttle in that it delivers the reducing equivalents from NADH to **ubiquinone** and thus into **Complex III**, not Complex I providing only enough energy to synthesize **1.5 ATP molecules** per pair of electrons.
- not involve membrane transport systems.

Inhibitors of electron transport and oxidative phosphorylation

TABLE 19-4 Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

<i>Type of interference</i>	<i>Compound*</i>	<i>Target/mode of action</i>
Inhibition of electron transfer	Cyanide	Inhibit cytochrome oxidase Complex IV
	<u>Carbon monoxide</u>	
	<u>Antimycin A</u>	Blocks electron transfer from cytochrome <i>b</i> to cytochrome <i>c</i> ₁ Complex III
	Myxothiazol	
	Rotenone	
	Amytal	Prevent electron transfer from Fe-S center to ubiquinone Complex I or III
	<u>Piericidin A</u>	
	DCMU	Competes with Q _B for binding site in PSII
Inhibition of ATP synthase	<u>Aurovertin</u>	Inhibits F ₁
	Oligomycin	
	<u>Venturicidin</u>	
	DCCD	Blocks proton flow through F _o and CF _o
Uncoupling of phosphorylation from electron transfer	FCCP	Hydrophobic proton carriers
	<u>DNP</u>	
	<u>Valinomycin</u>	K ⁺ ionophore
	Thermogenin	In brown fat, forms proton-conducting pores in inner mitochondrial membrane
Inhibition of ATP-ADP exchange	Atractyloside	Inhibits adenine nucleotide translocase

*DCMU is 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCCD, dicyclohexylcarbodiimide; FCCP, cyanide-*p*-trifluoromethoxyphenylhydrazone; DNP, 2,4-dinitrophenol.



Uncouplers: Stimulate e- transport: short circuit proton gradient: Block ATP production

- Carry protons from cytosolic surface of the membrane and then carry them back to matrix.
- Proton ionophores: Lipid soluble substance with dissociable proton, eg., 2,4 dinitrophenol (DNP) and FCCP.
- Thermogenin: uncoupler protein: Generates heat using proton gradient.

Heat generation by uncoupled mitochondria

Brown fat thermogenesis

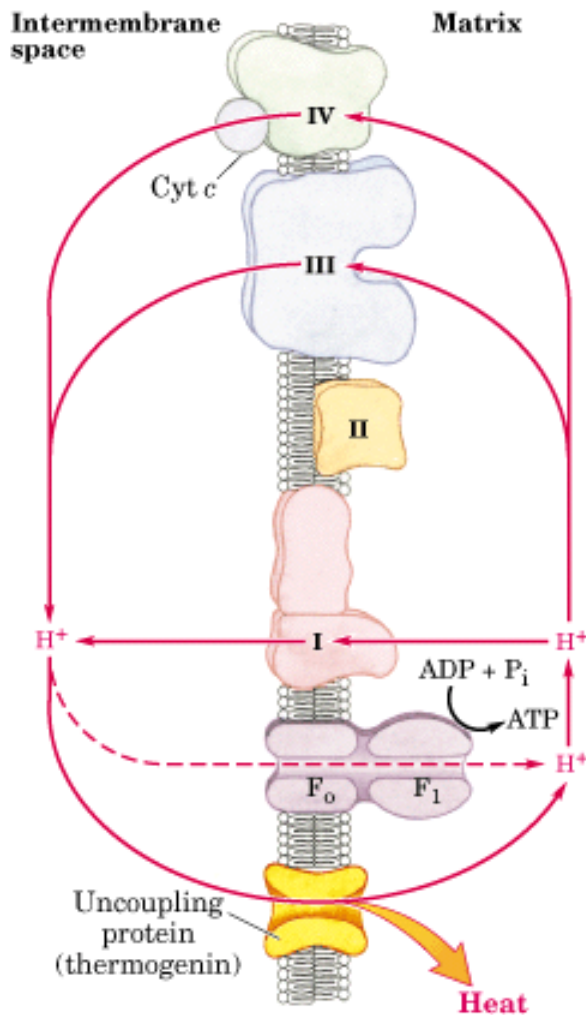
Brown fat, rich in mitochondria and cytochromes

Thorax near shoulders
Hibernating animals

Protein thermogenin is an “uncoupler” (uncoupling protein)

Creates a H^+ pore in inner mitochondrial membrane

- Produces heat instead of ATP





Regulation of Oxidative Phosphorylation

- Oxidative Phosphorylation Is Regulated by Cellular Energy Needs
- An Inhibitory Protein Prevents ATP Hydrolysis during Ischemia
- Uncoupled Mitochondria in Brown Fat Produce Heat
- ATP-Producing Pathways Are Coordinately Regulated



TABLE 19–5 ATP Yield from Complete Oxidation of Glucose

<i>Process</i>	<i>Direct product</i>	<i>Final ATP</i>
Glycolysis	2 NADH (cytosolic) 2 ATP	3 or 5* 2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix) 2 FADH ₂ 2 ATP or 2 GTP	15 3 2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into the mitochondrion.

TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO₂ and H₂O

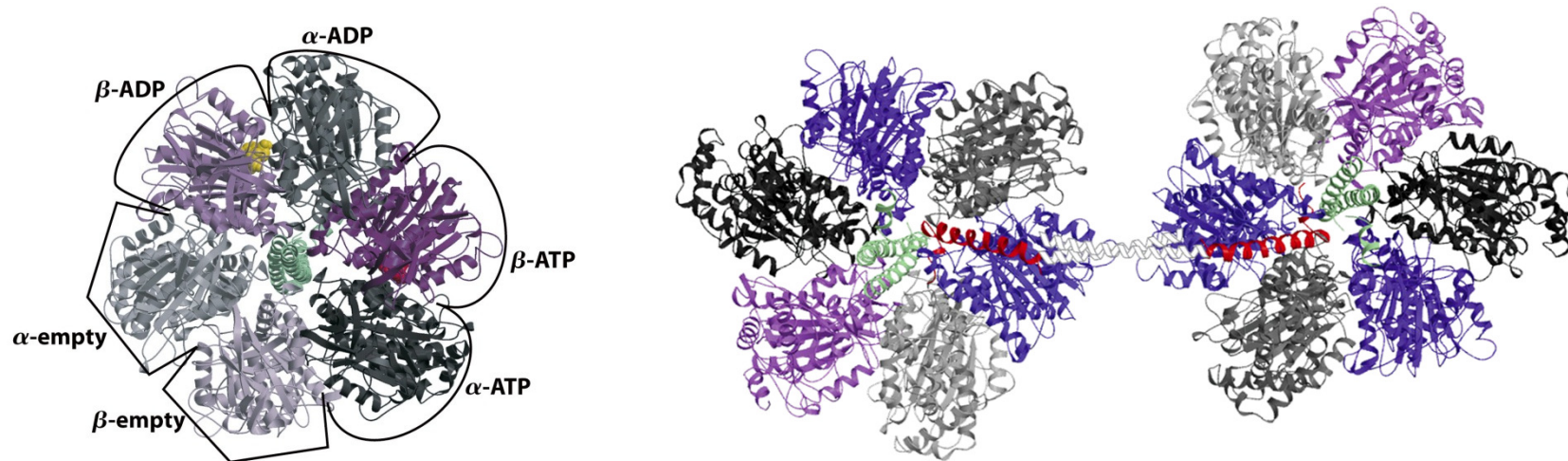
<i>Enzyme catalyzing the oxidation step</i>	<i>Number of NADH or FADH₂ formed</i>	<i>Number of ATP ultimately formed*</i>
Acyl-CoA dehydrogenase	7 FADH ₂	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8 [†]
Succinate dehydrogenase	8 FADH ₂	12
Malate dehydrogenase	8 NADH	20
Total		108



Oxidative Phosphorylation Is Regulated by Cellular Energy Needs

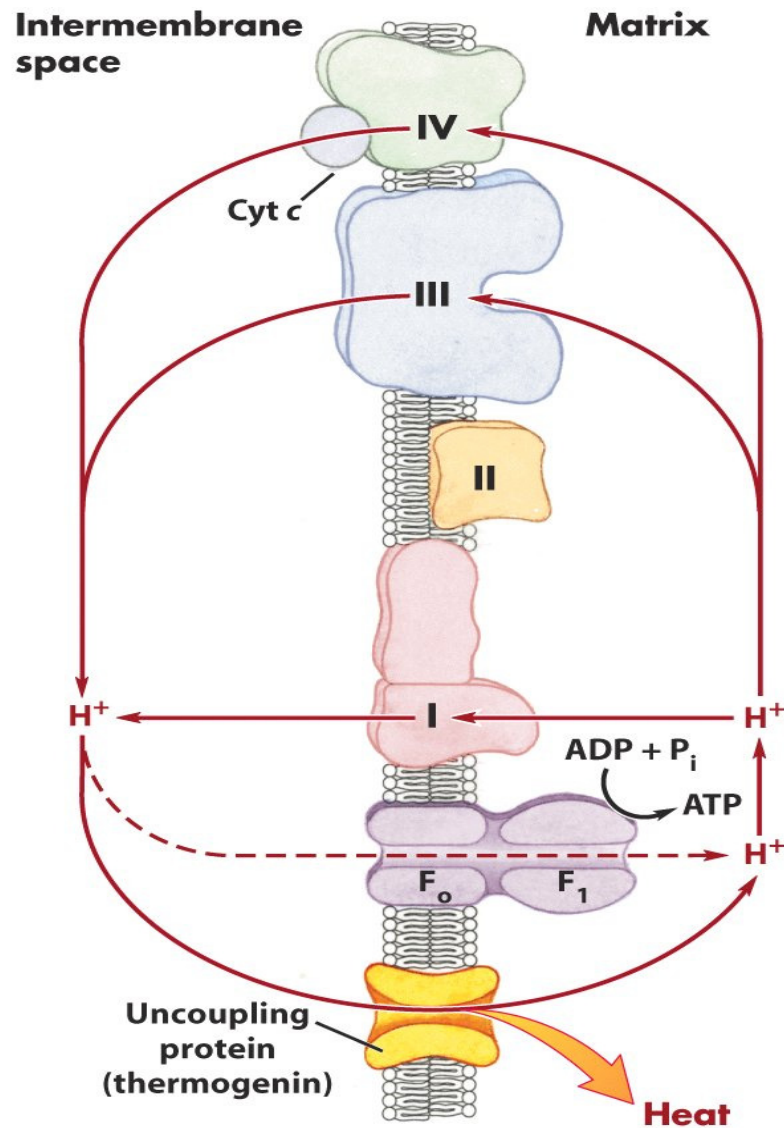
- The rate of respiration (O₂ consumption) is generally limited by the availability of ADP as a substrate for phosphorylation.
- In some animal tissues, the **acceptor control ratio**, the ratio of the maximal rate of ADP-induced O₂ consumption to the basal rate in the absence of ADP, is at least ten.
- the **mass-action ratio** of the ATP-ADP system ($[ATP]/([ADP][P_i])$).
 - some energy-requiring process (eg. Protein synthesis) increases, the rate of breakdown of ATP to ADP and P_i increases,
 - lowering the mass-action ratio.
 - With more ADP available for oxidative phosphorylation,
 - the rate of respiration increases, causing regeneration of ATP.

An Inhibitory Protein (IF₁) Prevents ATP Hydrolysis during Ischemia



- When a cell is **ischemic** (deprived of oxygen), as in a heart attack or stroke, electron transfer to oxygen ceases, and so does the pumping of protons. The proton-motive force soon collapses.
- the ATP synthase operate in reverse, hydrolyzing ATP to pump protons outward [ATP]↓
- protein inhibitor, **IF1**, a small (84 amino acids), binds to two ATP synthase and inhibit their ATPase activity
- IF1 is inhibitory only in its **dimeric form**, which is favored at pH lower than 6.5. In a cell starved for oxygen, the main source of ATP becomes glycolysis, and the pyruvic or lactic acid thus formed lowers the pH in the cytosol and the mitochondrial matrix. This favors IF1 dimerization, leading to inhibition of the ATPase ⇔
- In **aerobic metabolism** resumes, production of pyruvic acid slows, the pH of the cytosol rises, the IF1 dimer is destabilized, and the inhibition of ATP synthase is lifted.

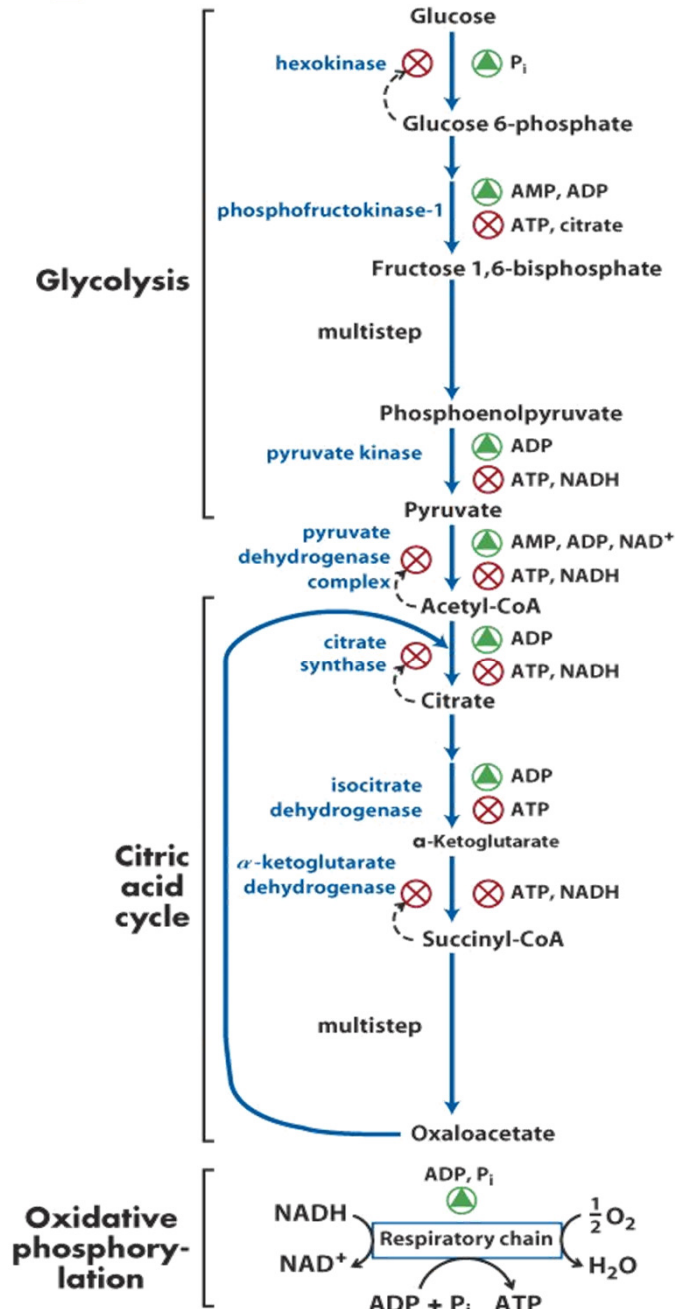
Heat generation by uncoupled mitochondria



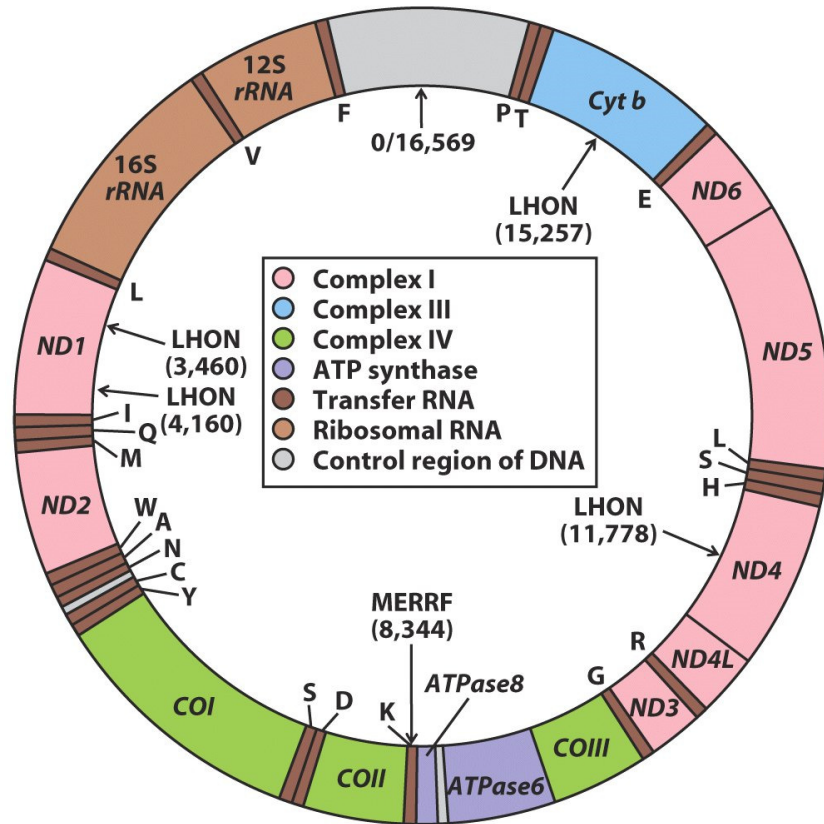
- Most newborn mammals, **brown fat** fuels oxidation serves not to produce ATP but to generate heat to keep the newborn **warm**.
- **Brown fat** with large numbers of **mitochondria** and thus large amounts of **cytochromes**, whose heme groups are strong absorbers of visible light.
- **Thermogenin (uncoupling protein)** short-circuiting of protons, the energy of oxidation is not conserved by ATP formation but is dissipated as **heat** – maintaining the body temperature of the newborn.
- Hibernating animals also depend on uncoupled mitochondria of brown fat to generate heat during their long dormancy

Regulation of The ATP-production pathways

- **acceptor control ratio:** the ratio of the maximal rate of ADP-induced O₂ consumption to the basal rate in the absence of ADP.
- ATP-producing pathways are coordinately regulated.
- Interlocking regulation of **glycolysis, pyruvate oxidation, the citric acid cycle, and oxidation phosphorylation** by the relative conc. of ATP, ADP, and AMP, and by NADH.
- All four pathway are accelerate when the use of ATP and the formation of ADP, AMP, and Pi increase.
- Interlocking of glycolysis and the citric acid cycle by citrate, which inhibits glycolysis, supplements the action of the adenine nucleotide system
- Increase **NADH** and **acetyl-CoA** inhibit the oxidation of pyruvate to acetyl-CoA,
- High **NADH/NAD⁺ ratios** inhibit the dehydrogenase reaction of TCA cycle.

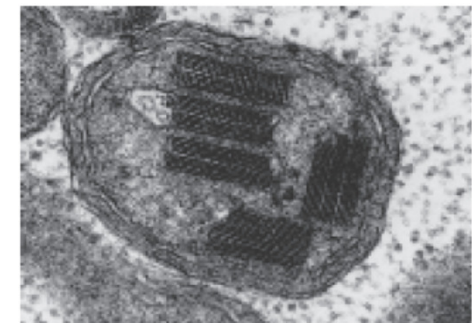


Mitochondrial genes and mutations



- Mitochondria disease--maternal transmitted —**LHON** (leber's hereditary optic, *ND4* gene of complex I Arg to His) —NADH to uniuquinone is defected not enough ATP for neurons.
- Single base change in cytochrome b of complex III---LHON .
- Myoclonic epilepsy and ragged red fiber disease (**MERRF**,)—tRNA (leucyl-tRNA)---protein defective-prarcryalline structure.
- The accumulation of mutations in mitochondrial DNA during a lifetime of exposure to DNA-damaging ---not enough ATP.

- human mitochondrial genome contains 37 genes (16,569 bp): 13 subunits of ETS proteins and 24 rRNA and tRNA gene ⇔ 900 mitochondrial proteins are encoded by nuclear - synthesized in cytosol then imported and assembled within the mitochondria

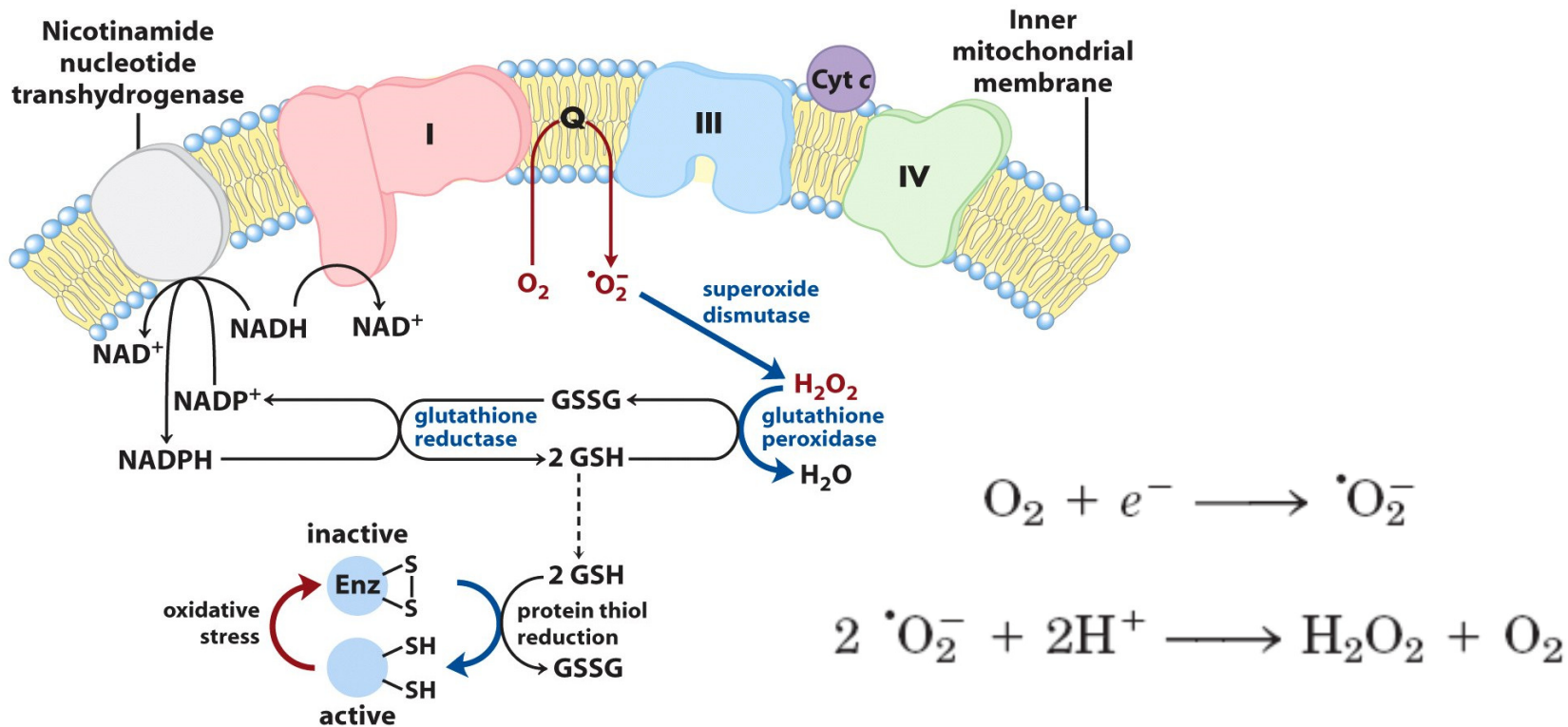




The Role of Mitochondria in Apoptosis and Oxidative Stress

- Apoptosis is a controlled process by which cells die for the good of the organism, while the organism conserves the molecular components (amino acids, nucleotides, and so forth) of the dead cells.
- Apoptosis - increase in the permeability of the outer mitochondrial membrane - escape of the cytochrome c - activates proteolytic enzymes (caspase 9) - protein degradation.
- steps in the ETC have the potential to produce highly reactive free radicals (0.1~4%): (1). from QH₂ to cytochrome b_L through Complex III, and (2). passage of electrons from Complex I to QH₂, - Q can pass an electron to O₂ in the reaction. antimycin A, an inhibitor of Complex III occupies the QN sites - block the Q cycle and prolonging the binding of Q to the QP site - increase formation of superoxide radical
- Reduced glutathione (GSH) donates electrons for the reduction of hydrogen peroxide (H₂O₂) and of oxidized Cys residues (-S-S-) in proteins - Glutathione reductase recycles oxidized glutathione to its reduced form, using electrons from the NADPH formed by nicotinamide nucleotide transhydrogenase or by the pentose phosphate pathway

The Role of Mitochondria in Apoptosis and Oxidative Stress



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Rendimento em ATP no STE:

- Na ausência de fluxo de electrões ainda se mantém a síntese de ATP
- Para a síntese de 1 ATP são necessários 4 H⁺
- O coeficiente PO (nº de moléculas de ATP formado por par de e⁻ transferido para o oxigénio) verificada é:

→ 2,5 ATP por reoxidação de 1 NADH — 1 NADH

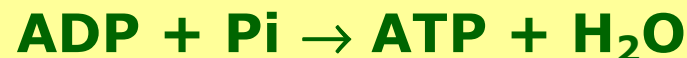
→ 1,5 ATP por reoxidação de 1 FADH₂ — 1 FADH₂


- Para que se verifiquem estes valores de coeficiente PO é necessário que:

→ na reoxidação de NADH ocorra a formação de 10 H⁺ ao longo do STE (nos complexos I, III, IV) 10/4=2,5 ATP

→ na reoxidação de FADH₂ ocorra a formação de 6 H⁺ ao longo do STE (nos complexos III, IV) 6/4=1,5 ATP

Reacção global da fosforilação oxidativa:



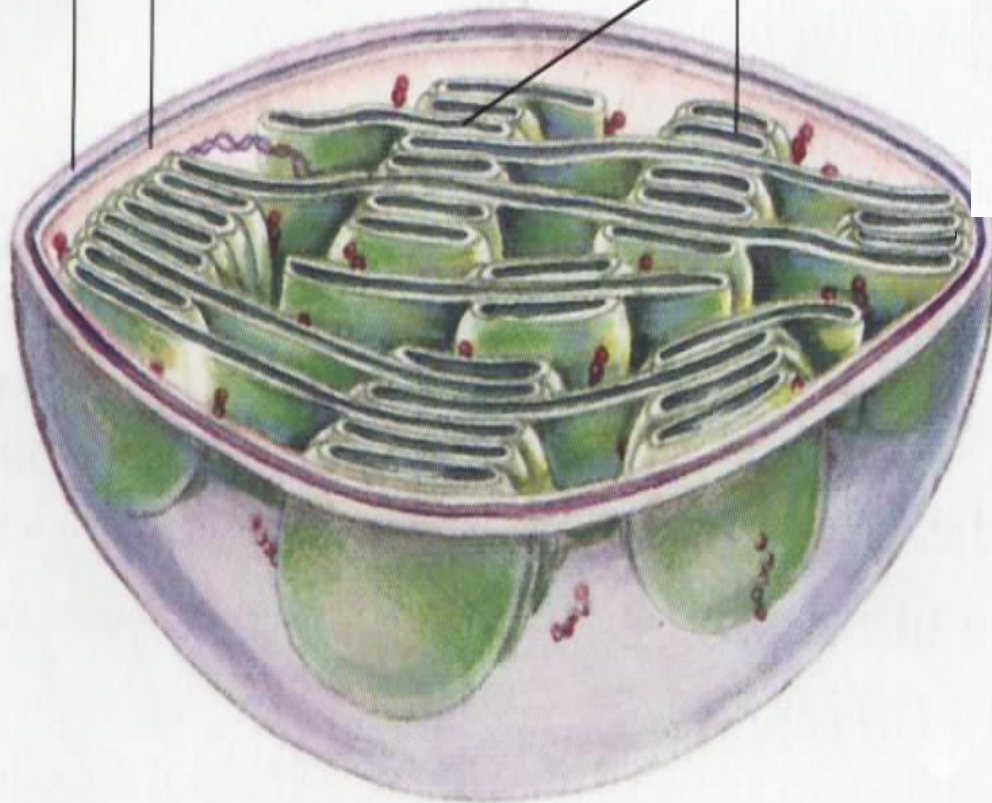


**Photosynthetic organisms generate
ATPs (and NADPH) via
photophosphorylation.**

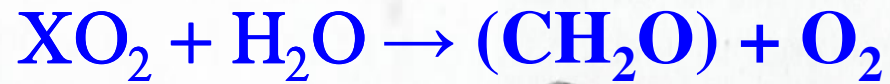
Outer membrane

Inner membrane

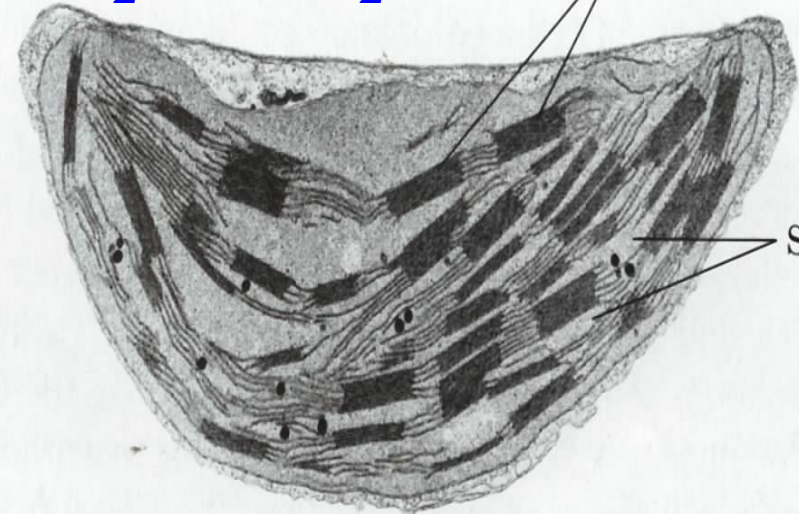
Thylakoids



(a)



Grana (thylakoids)



(b)

Photosynthesis occurs in chloroplasts, which have three membranes (*outer, inner, and thylakoid*) & three separate spaces (*intermembrane, stroma, and thylakoid spaces*)



Diferentes vias de formação de ATP:

- Fosforilação à custa do substrato (glicólise e C. Krebs)
- Fosforilação oxidativa (transferência de electrões pela cadeia respiratória das células aeróbias) em que o **OXIGÉNIO** é aceitador final de electrões – sistema de transporte electrónico - **STE** (*mitocôndrias*)
- Fase luminosa da fotossíntese (conversão da energia luminosa em energia química) – **fotofosforilação** (*cloroplastos*)



Síntese do ATP pela energia luminosa: Fotofosforilação (fundamentos).

-Ocorre nos cloroplastos



❖ Fase luminosa

- conversão da energia luminosa em energia química \Rightarrow formação de O_2 , NADPH e ATP.

- Fotofosforilação não-cíclica - utiliza os foto-sistemas I e II com formação de O_2 , NADPH e ATP.

- Fotofosforilação cíclica - utiliza o fotosistema I e apenas se produz ATP (utilizada por procariontas e alguns eucariontas)



❖ Fase escura

- síntese de compostos orgânicos, a partir da fixação de CO_2 e utilizando o ATP e NADPH formados na fase luminosa (Ciclo de Calvin)

**Fase escura:
síntese de glucose pelo
ciclo de Calvin**

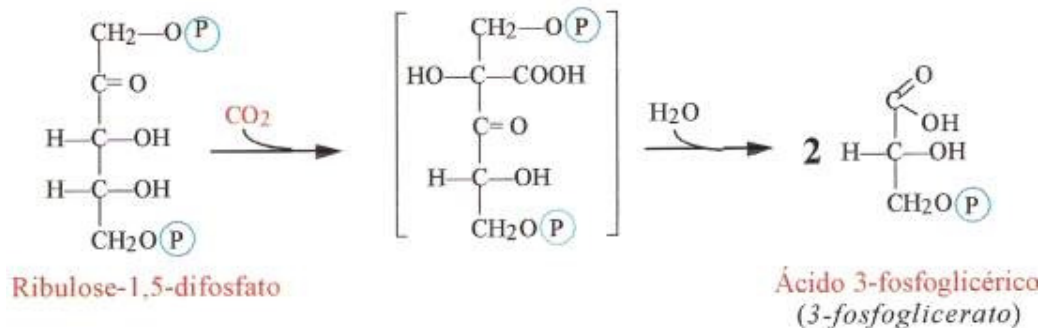
-A primeira reacção é a fixação do CO₂

-É uma reacção de síntese (redução) pelo que necessita fornecimento de energia na forma de **ATP e NADPH** (formados na fase luminosa)

Ciclo de Calvin

ciclo fotossintético de redução do CO₂ a glúcidos

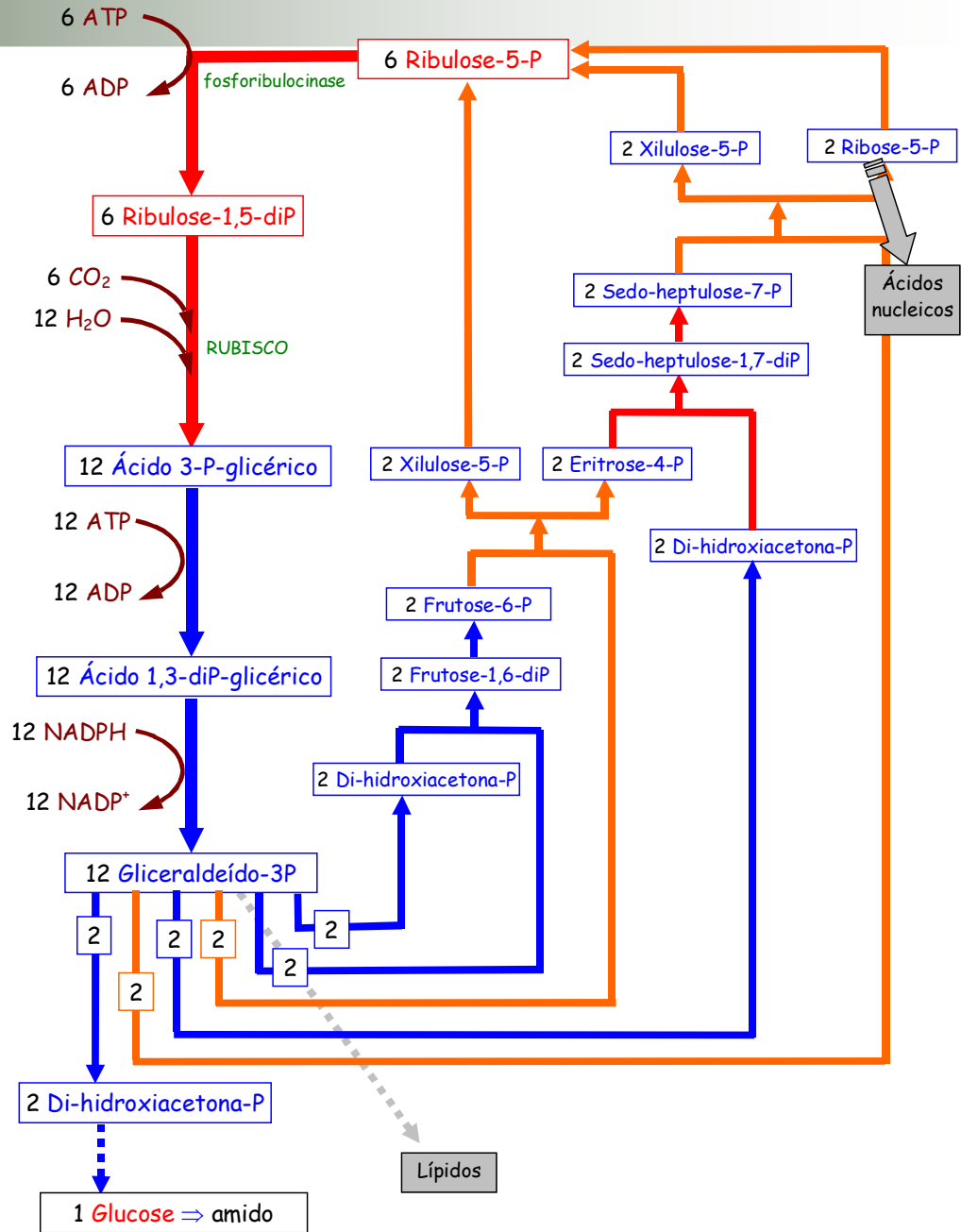
Fixação do CO₂ na ribulose-1,5-difosfato pela *ribulose-1,5-difosfato carboxilase*

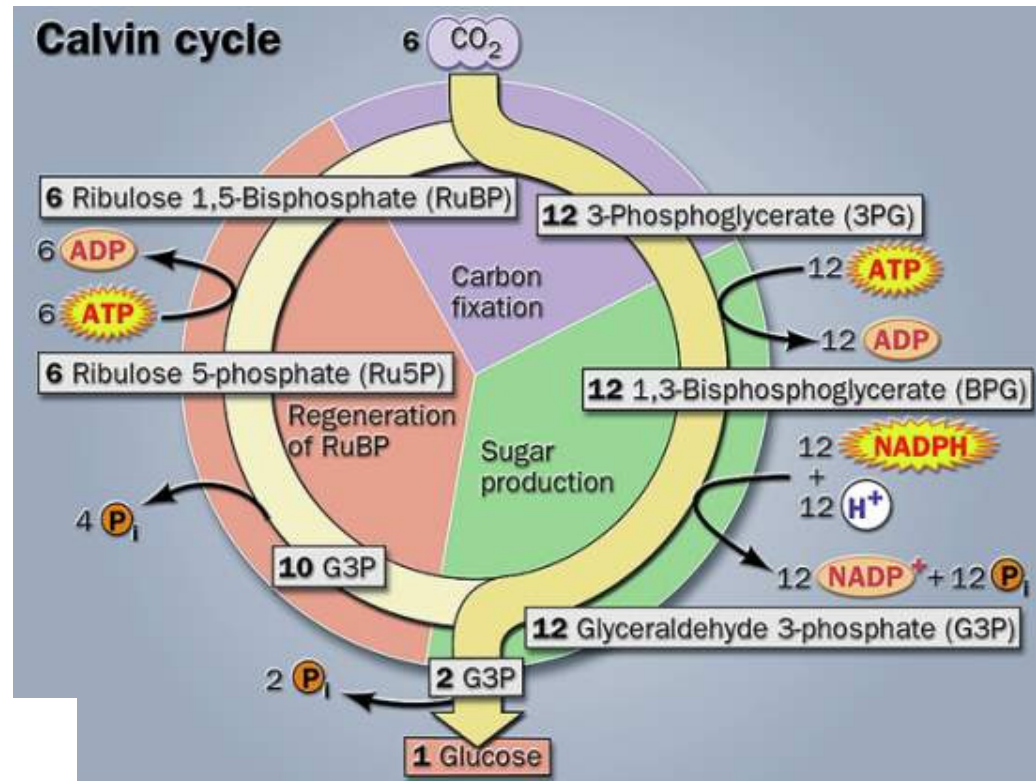


O ciclo prossegue por reacções inversas da **glicólise** e da **via das pentoses**

Ciclo de Calvin

Reacção de fixação do CO_2 catalisada pela enzima ribulose-1,5-difosfato-carboxilase (RUBISCO)





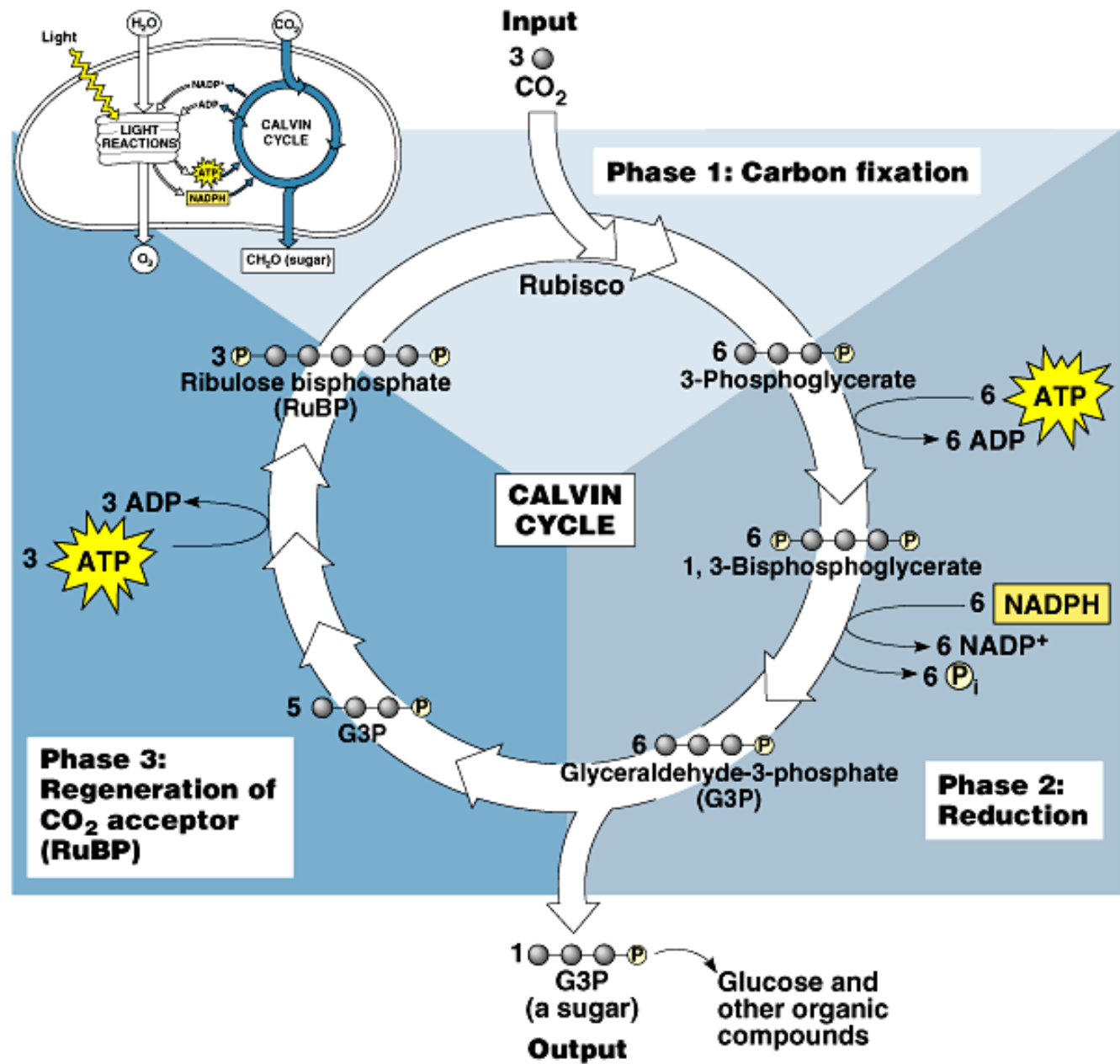
Reacções de regeneração da ribulose-1,5-
Utilização de:
6 ATP

Reacções de fixação do CO_2 e produção de trioses-fosfato
Utilização de:
12 ATP
12 NADPH

Reacção global:



Moléculas do Ciclo de Calvin



Origem do ATP e NADPH para a síntese de glúcidos

17.5 Photosynthetic Electron Transport 467

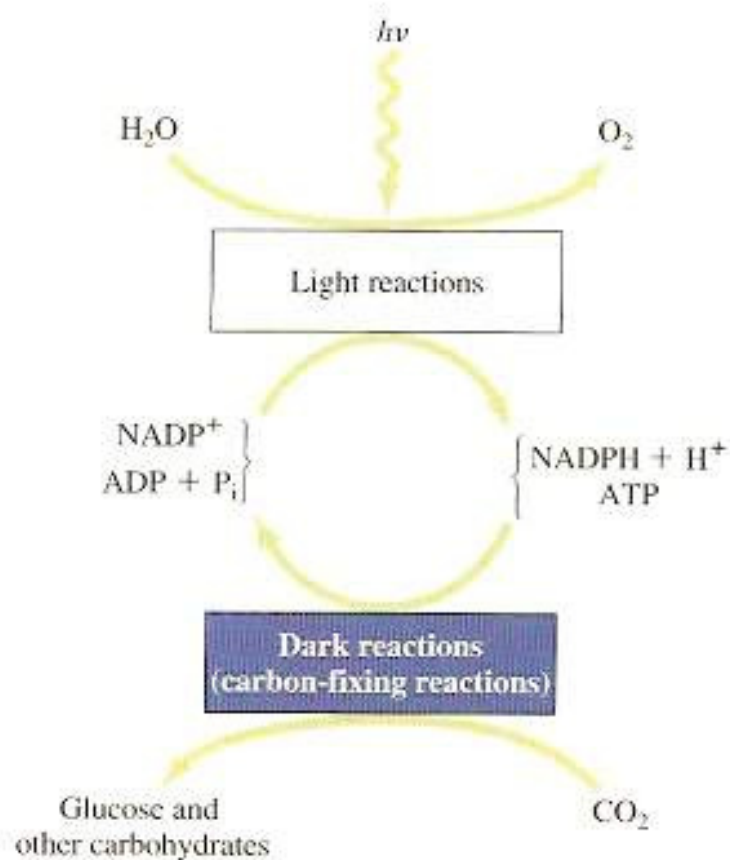


Figure 17.17

Two phases of photosynthesis linked together in a cycle. During the light reactions, light energy, shown as a photon, $h\nu$, is used to make NADPH and ATP. These products are used to provide energy for the synthesis of glucose and other "dark" reactions.