Oxidative phosphorylation

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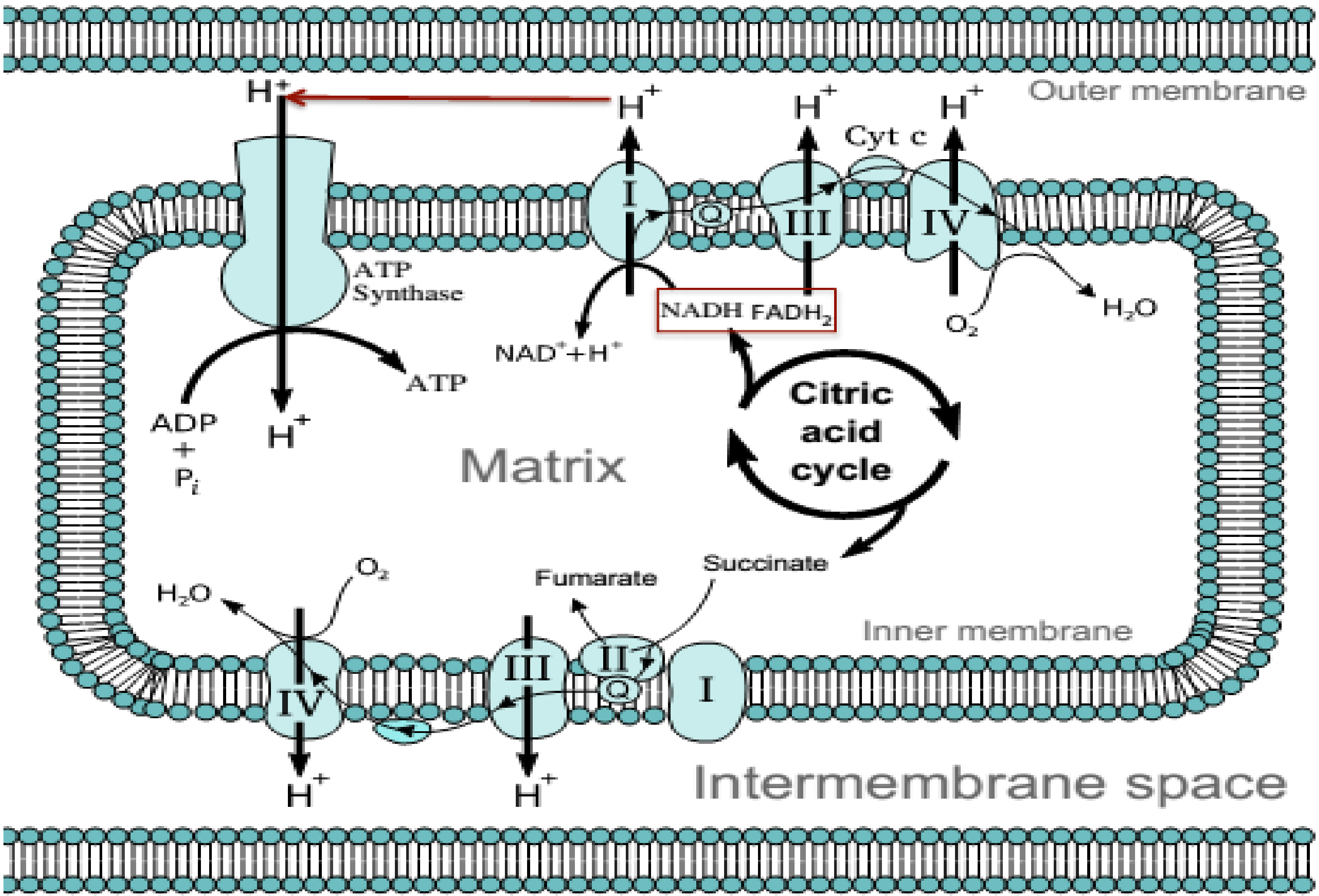
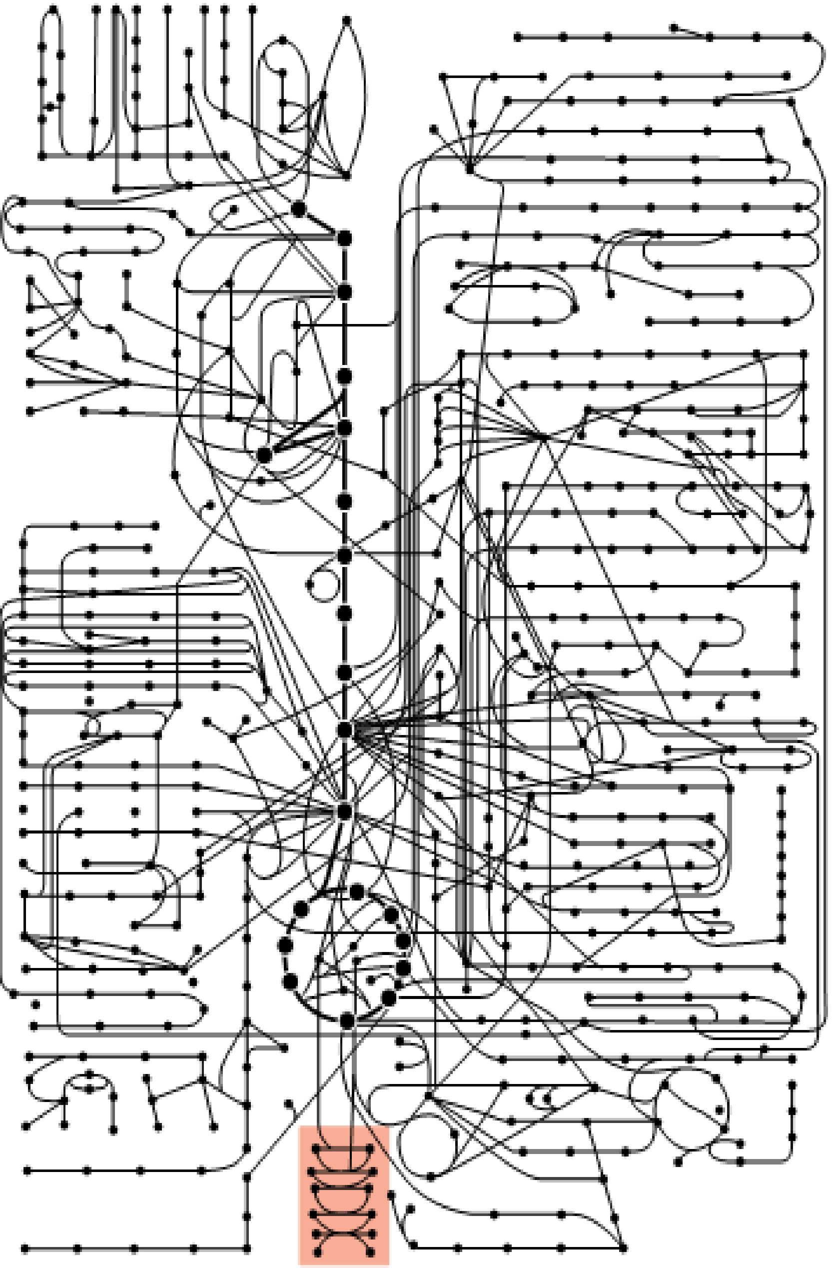
outline

- **Introduction**
- **Mitochondrial Structure and Function**
- **Energy Generation**
- **Electron Transport**
- **Oxidative Phosphorylation**
- **Shuttling Electron Carriers into the Mitochondrion**
- **respiratory control**
- **summary**

1. TCA cycle is coupled to electron transport and oxidative phosphorylation





Electron Transport and
Oxidative Phosphorylation

Oxidative phosphorylation（**氧化磷酸化**） is the most common energy-yielding metabolism in **aerobic**（好氧） organisms，in which the energy of oxidation drives the synthesis of **ATP**.

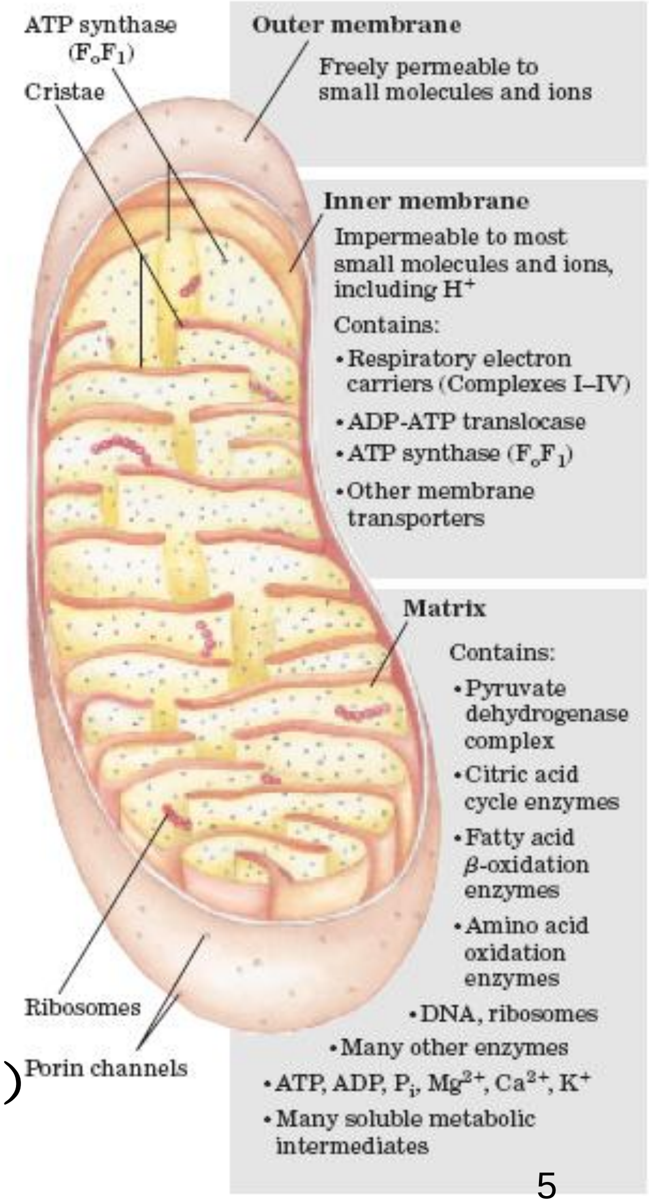
2. Mitochondrial Structure

- **outer membrane：** permeable to small molecules (M<5000) and ions（离子）-through porins（孔蛋白）.

- **inner membrane：** impermeable including protons (H)，respiratory chain and the ATP synthase

- **intermembrane space（膜间隙）**

- **Matrix**
  - **pyruvate oxidation (丙酮酸氧化）**
  - **fatty acid oxidation（脂肪酸氧化）**
  - **amino acid metabolism （尿素循环）**
  - **citric acid cycle （三羧酸循环）**

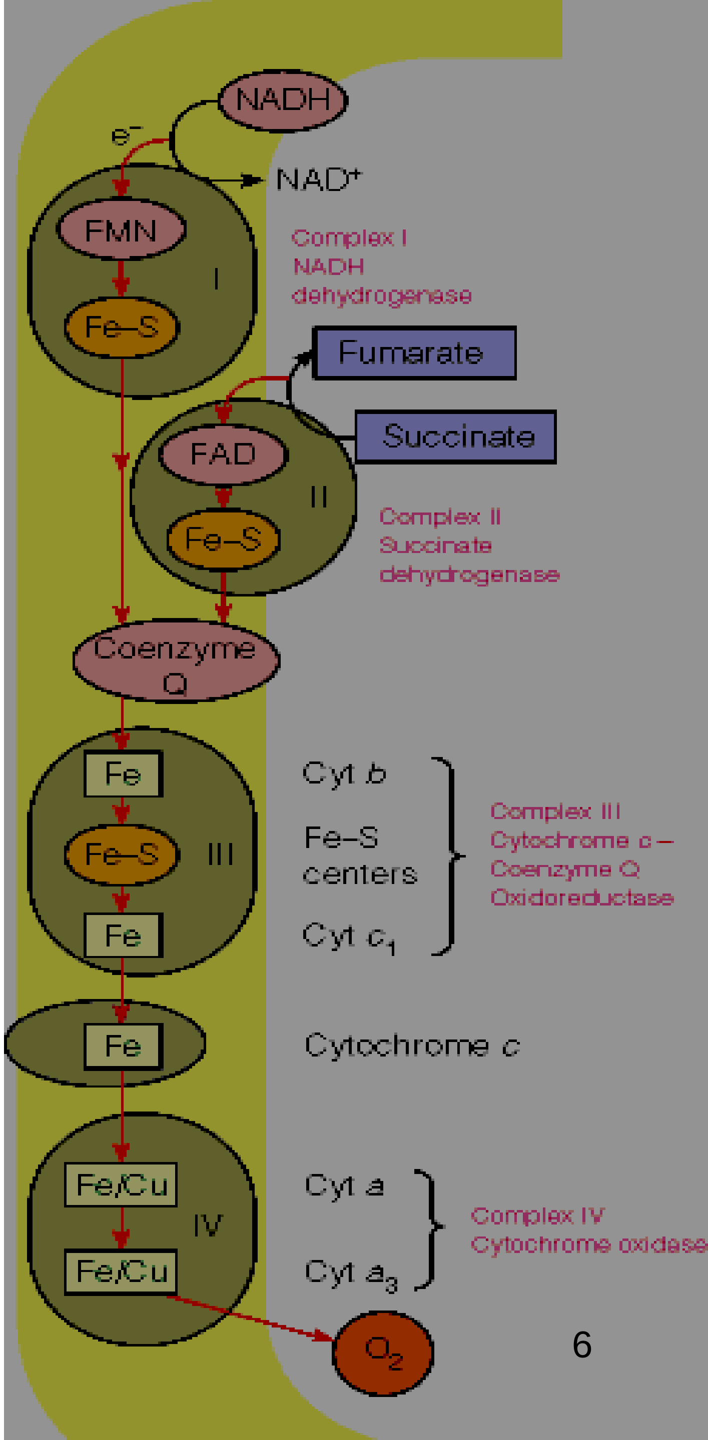


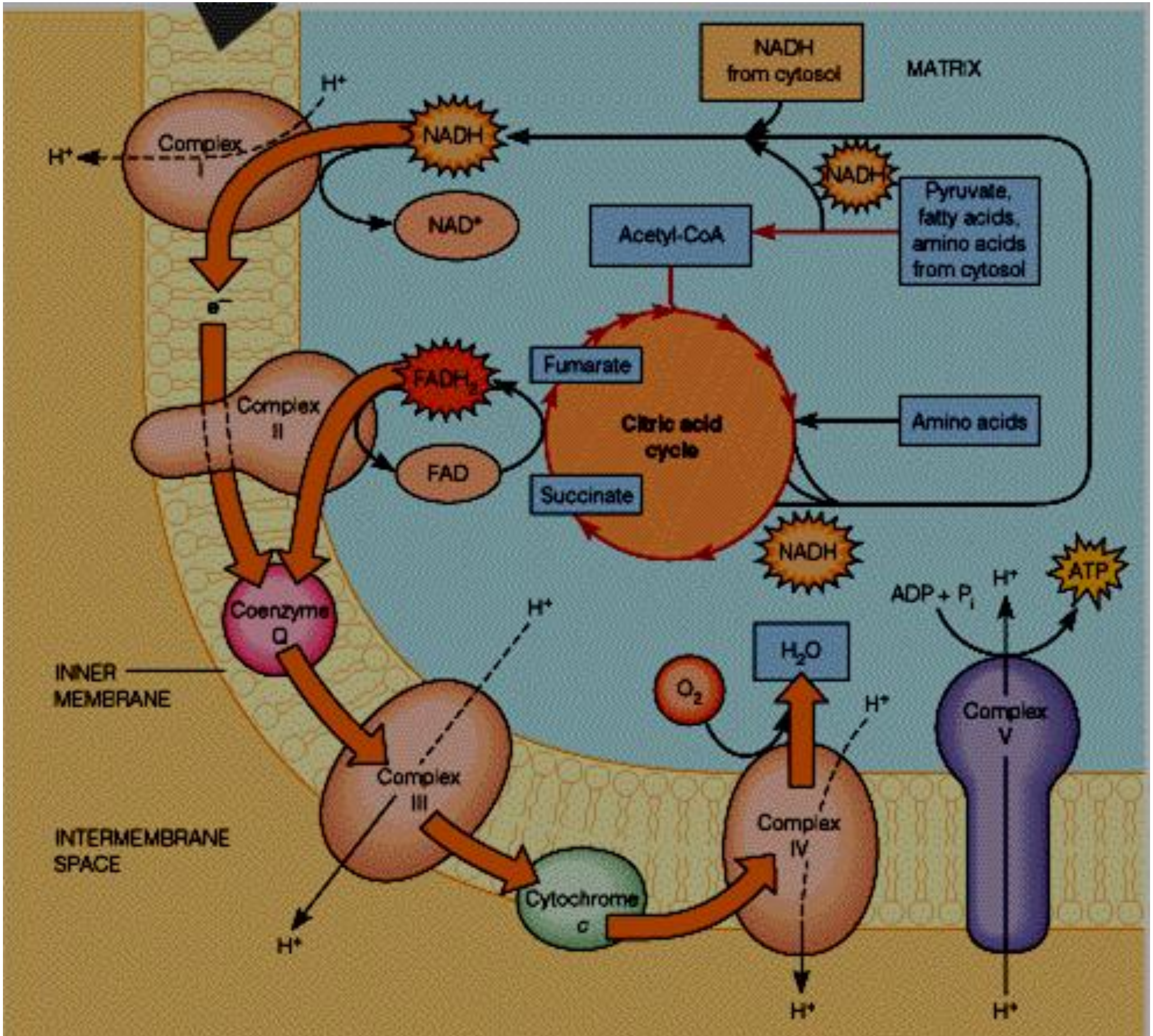
Two electron chains

protein electron carriers-- in the inner membrane

- **four complexes I, II, III, IV**
- **Smaller carriers：**

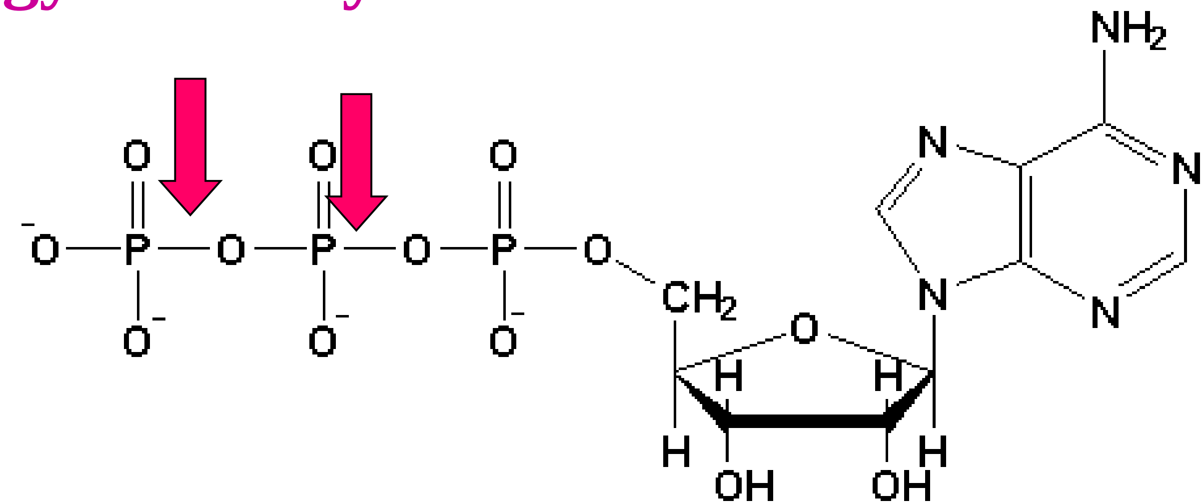
  coenzyme Q，cytochrome C




NADH from cytosol
MATRIX
H+
Complex I
H+
NADH
NAD+
NADH
Pyruvate, fatty acids, amino acids from cytosol
Acetyl-CoA
e−
FADH2
Fumarate
Complex II
Citric acid cycle
Amino acids
FAD
Succinate
NADH
ATP
H+
ADP + Pi
H+
Coenzyme Q
H2O
O2
H+
INNER MEMBRANE
Complex V
Complex III
Complex IV
INTERMEMBRANE SPACE
Cytochrome c
H+
H+
H+

3. Energy Generation

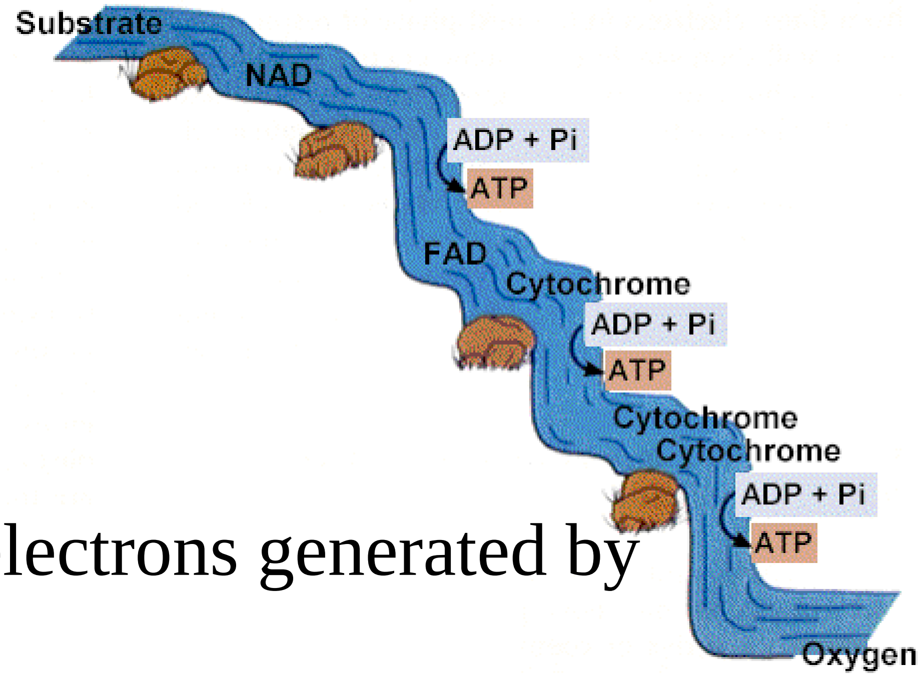
ATP --- free energy currency



Adenosine triphosphate (ATP)

- substrate-level phosphorylations （底物水平磷酸化）
- oxidative phosphorylation（氧化磷酸化，线粒体）
- photosynthetic phosphorylation （光合磷酸化）

4. Electron Transport

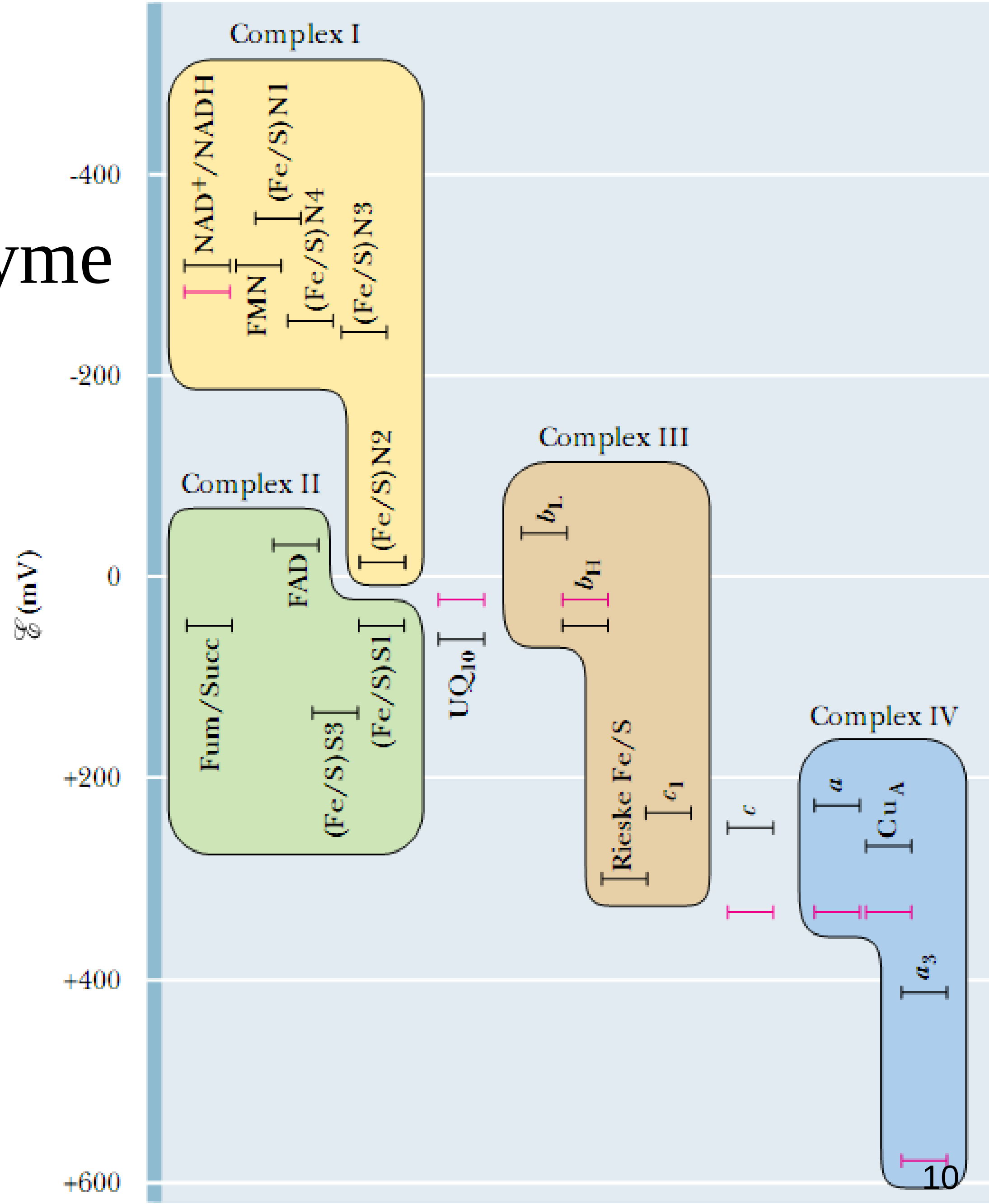


● Electron transport system

- The place in the cell where electrons generated by oxidation are transferred.

● Passage of the electrons through the **system** generates **potential energy** that is used to make **ATP** in **oxidative phosphorylation（氧化磷酸化）**

4.1 some electron carriers in multienzyme complexs



Complex I (or NADH Dehydrogenase)

- NADH is generated by numerous dehydrogenases.

- NADH is reoxidized to NAD^+ by **complex I**

- NADH dehydrogenase contains flavin mononucleotide (**FMN**)

$$NADH + H^+ + FMN \Leftarrow\Rightarrow NAD^+ + \mathbf{FMNH_2}$$

- Complex I contains about 46 different polypeptide chains

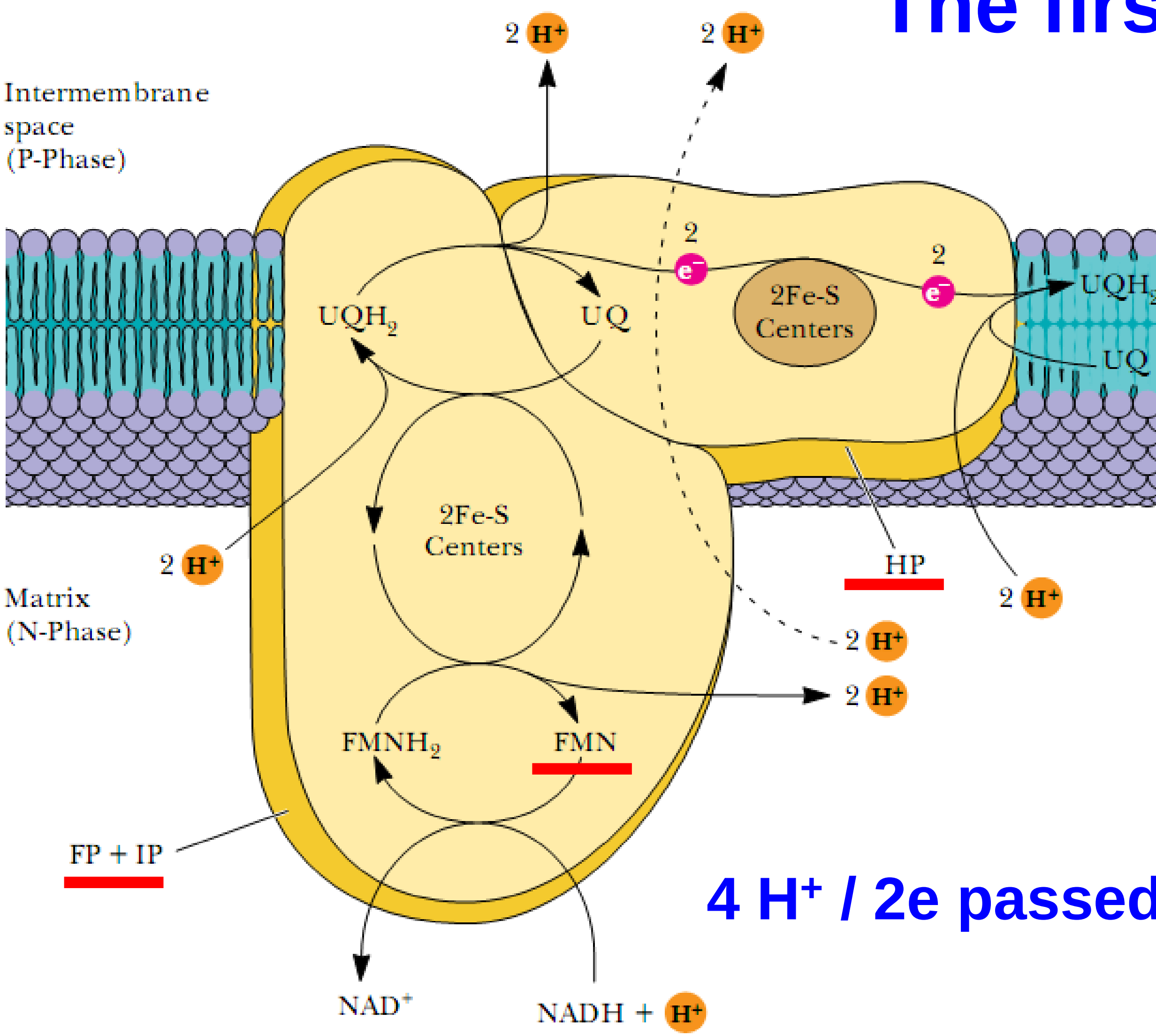
TABLE 19–1 Some Important Reactions Catalyzed by NAD(P)H-Linked Dehydrogenases

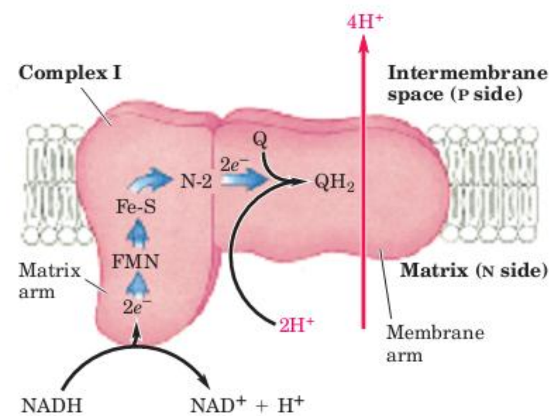
Reaction[*]	*Location*[†]
NAD-linked	
α-Ketoglutarate + CoA + NAD^+ $\rightleftharpoons$ succinyl-CoA + CO_2 + NADH + H^+	M
L-Malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+	M and C
Pyruvate + CoA + NAD^+ $\rightleftharpoons$ acetyl-CoA + CO_2 + NADH + H^+	M
Glyceraldehyde 3-phosphate + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	C
Lactate + NAD^+ $\rightleftharpoons$ pyruvate + NADH + H^+	C
β-Hydroxyacyl-CoA + NAD^+ $\rightleftharpoons$ β-ketoacyl-CoA + NADH + H^+	M
NADP-linked	
Glucose 6-phosphate + $NADP^+$ $\rightleftharpoons$ 6-phosphogluconate + NADPH + H^+	C
NAD- or NADP-linked	
L-Glutamate + H_2O + $NAD(P)^+$ $\rightleftharpoons$ α-ketoglutarate + NH_4^+ + NAD(P)H	M
Isocitrate + $NAD(P)^+$ $\rightleftharpoons$ α-ketoglutarate + CO_2 + NAD(P)H + H^+	M and C

[*]These reactions and their enzymes are discussed in Chapters 14 through 18.

[†]M designates mitochondria; C, cytosol.

The first proton pump





4 H+ / 2e passed from NADH to UQ

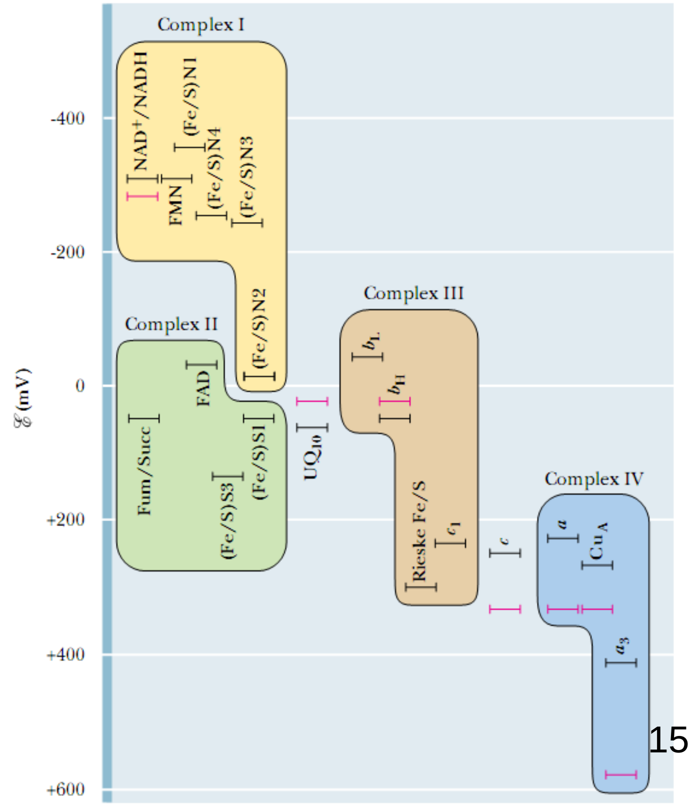
Three protein complexes have been isolated, including the **flavoprotein (FP), iron–sulfur protein (IP),** and **hydrophobic protein (HP).** FP contains three peptides (of mass 51, 24, and 10 kD) and bound FMN and has 2 Fe-S centers (a 2Fe-2S center and a 4Fe-4S center). IP contains six peptides and at least 3 Fe-S centers. HP contains at least seven peptides and one Fe-S center.

Complex I (or NADH Dehydrogenase)

Accepts e^- from NADH	NADH can only participate in 2 e^- transfer reactions
2 cofactors	1 FMN
	6-7 Fe-S centers
$2e^-$ transferred	4 protons pumped

Complex II (succinate-Coenzyme Q reductase)

- is not in the path traveled by electrons from Complex I
- Both complexes I & II donate electrons to **coenzyme Q**
- contains iron-sulfur proteins, participate in electron transfer



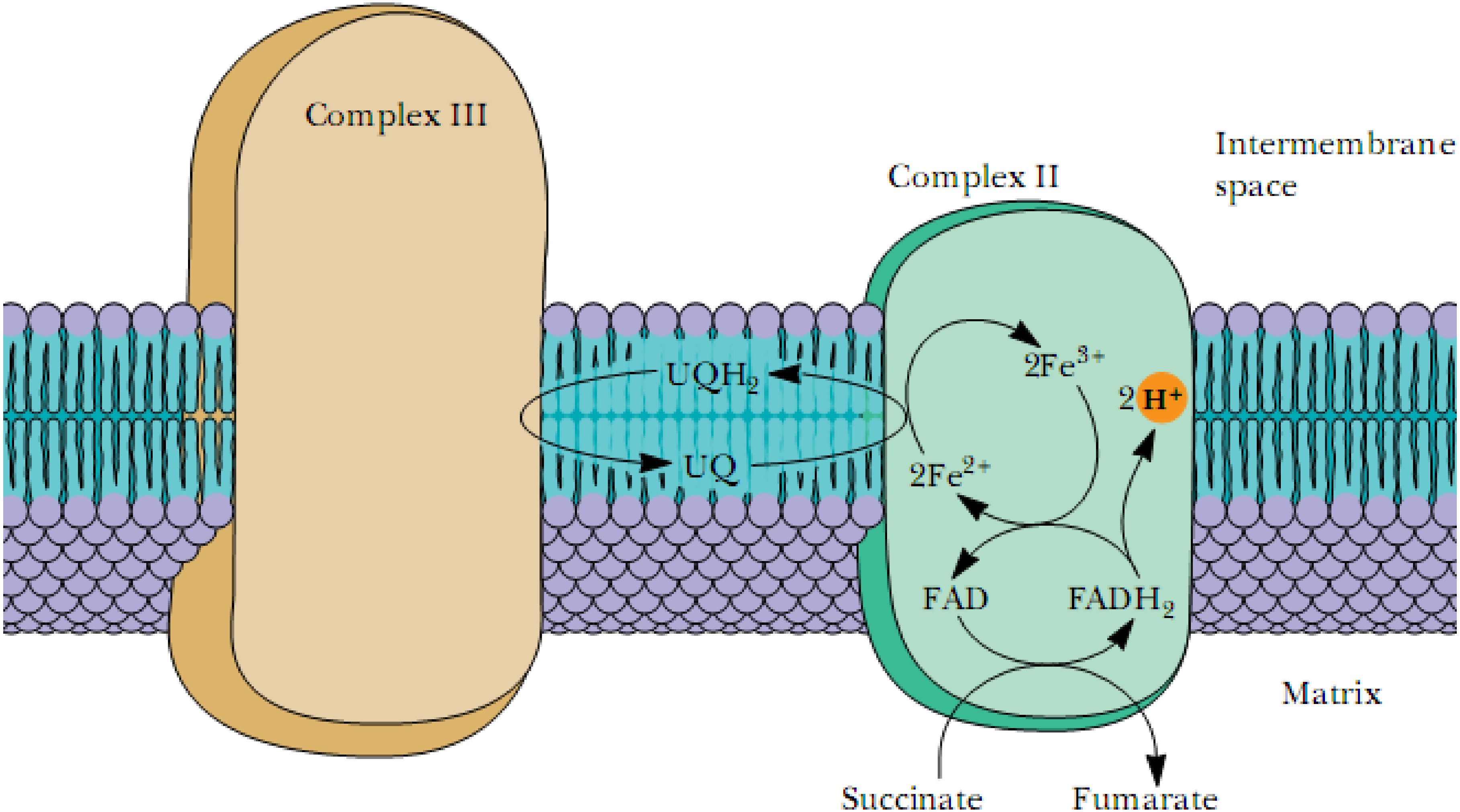
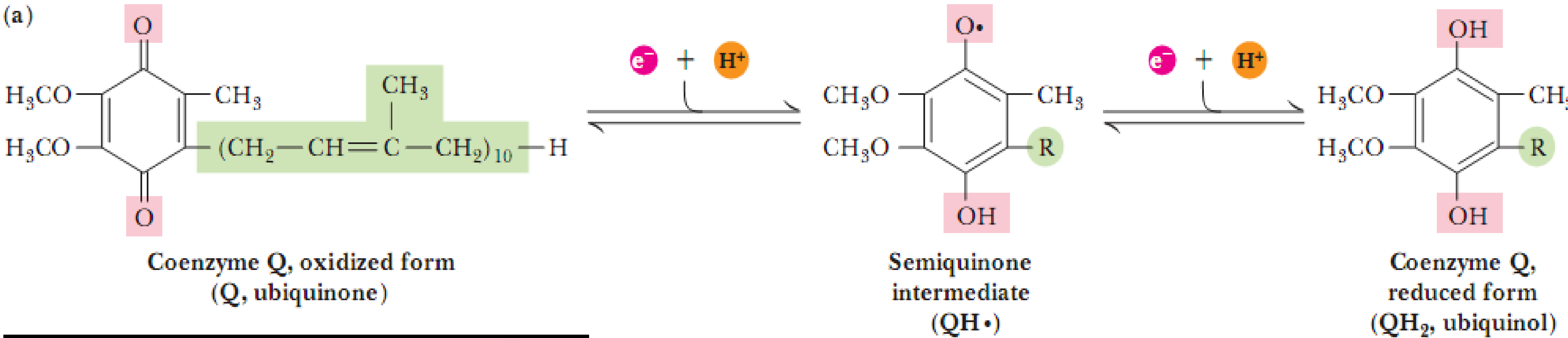
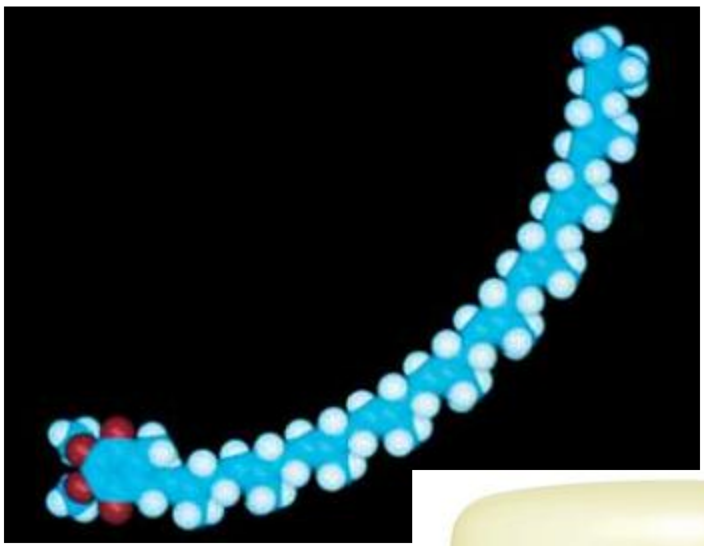


FIGURE 21.8 • A probable scheme for electron flow in Complex II. Oxidation of succinate occurs with reduction of [FAD]. Electrons are then passed to Fe-S centers and then to coenzyme Q (UQ). Proton transport does not occur in this complex.

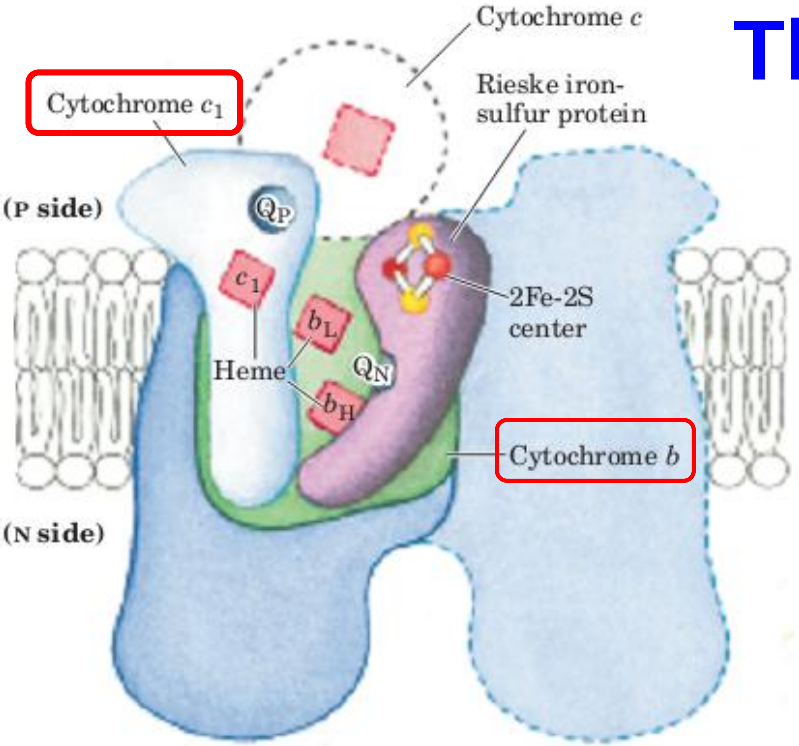


(a) The three oxidation states of coenzyme Q.



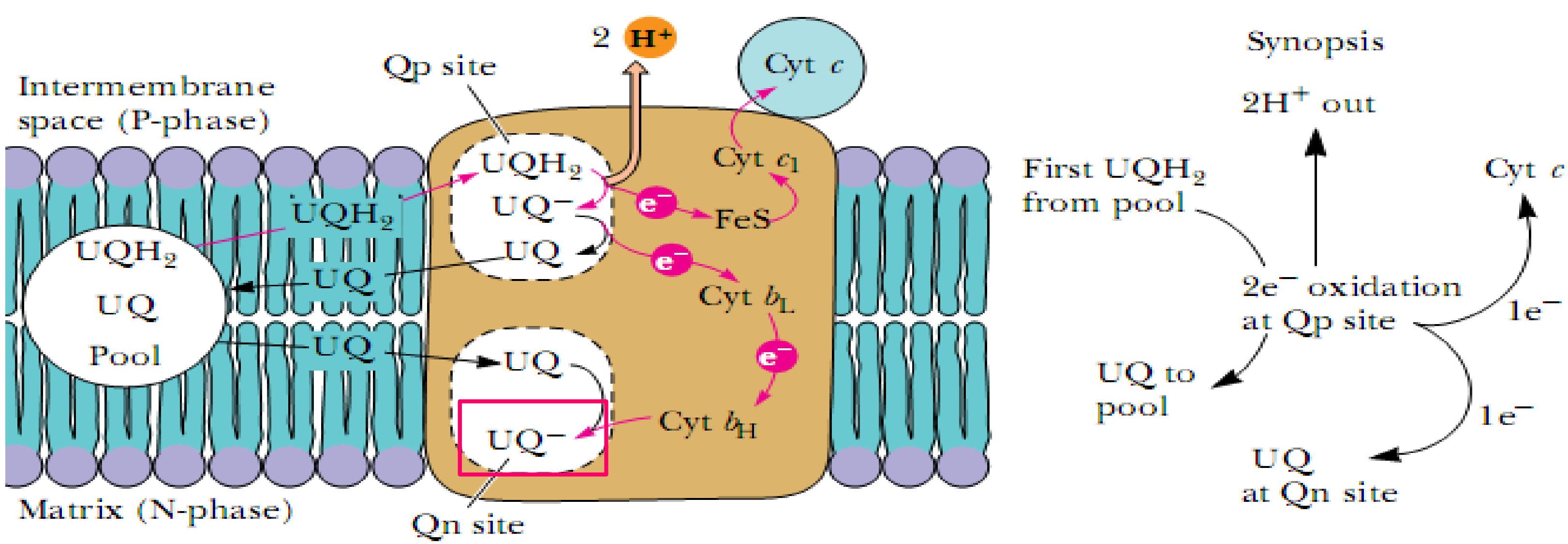
Complex III (or CoQ-cytochrome c reductase)

- **contains electron carrying proteins： cytochrome b, iron sulfur centers, and cytochrome C1.**

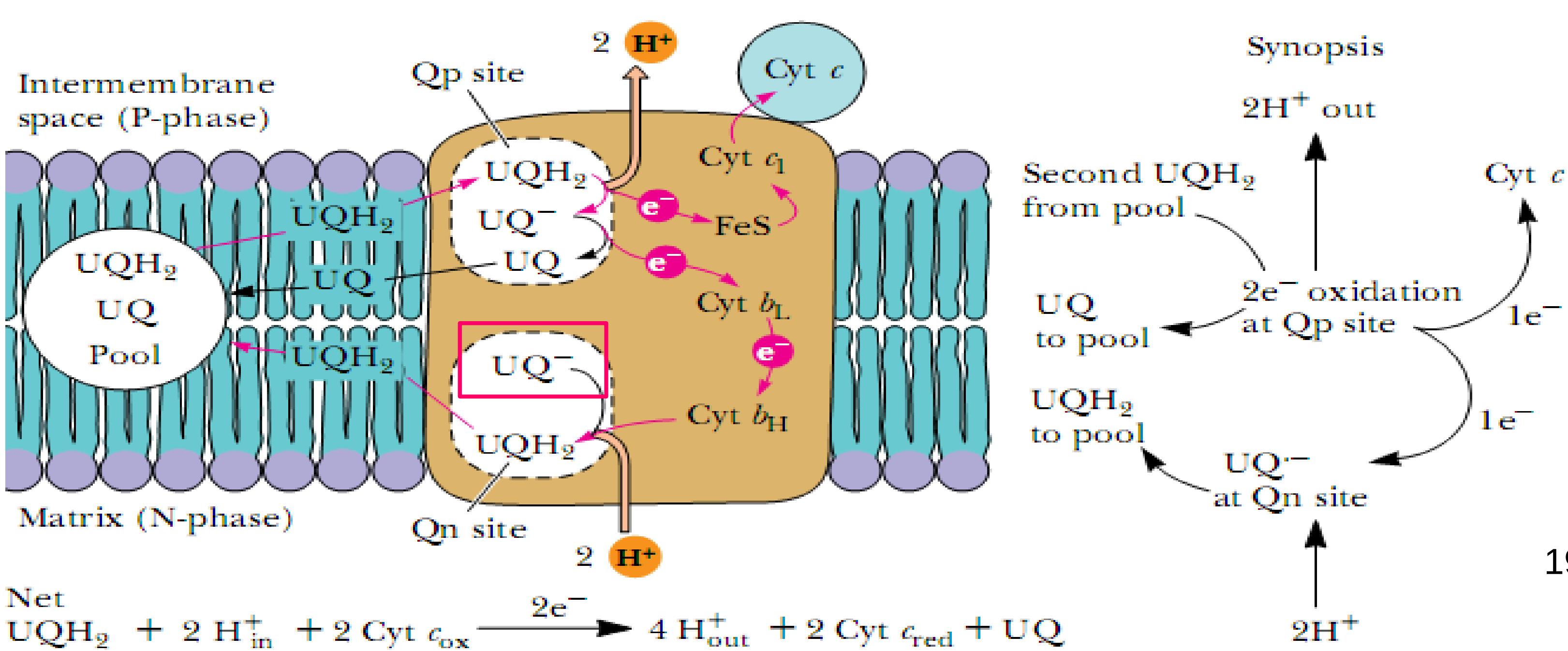


The second proton pump

(a) First half of Q cycle

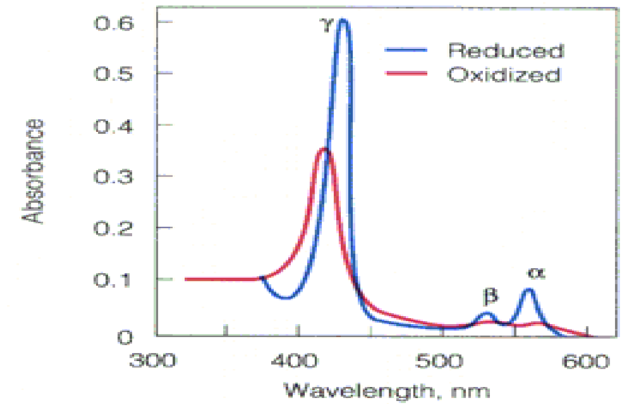


(b) Second half of Q cycle

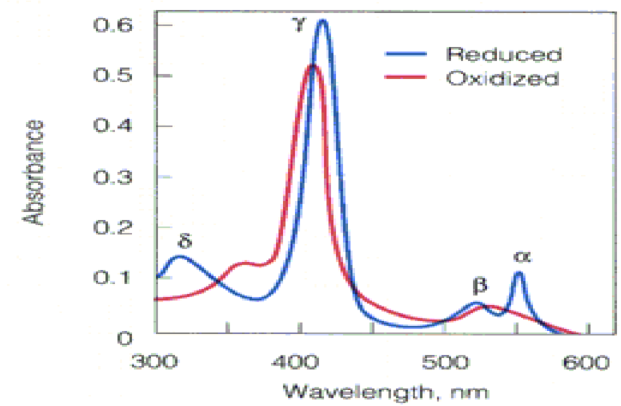


Cytochromes—are proteins, strong absorption of visible light（可见光）.

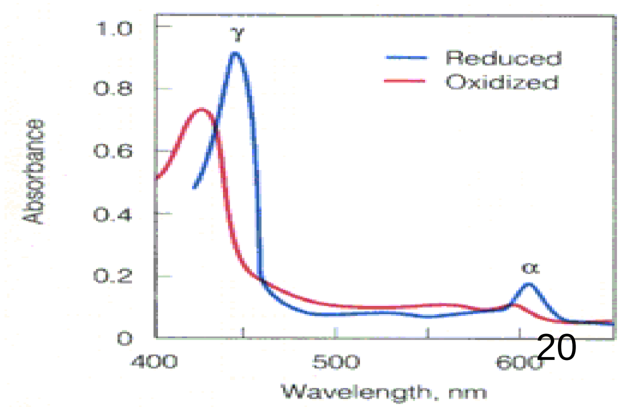
➢ **Each type of cytochrome in its reduced (Fe^{2+}) state has three absorption bands in the visible range.**



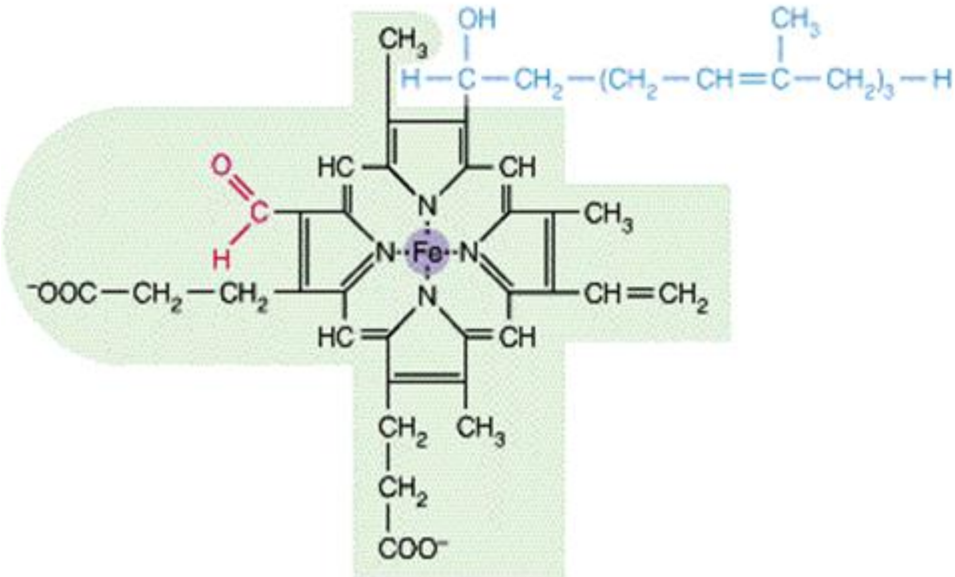
(a) Cytochrome *b*



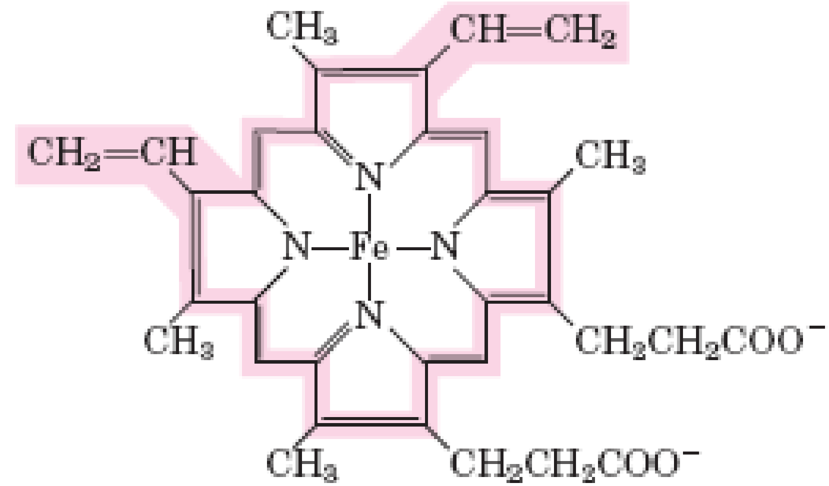
(b) Cytochrome *c*



(c) Cytochromes *a* and a_3

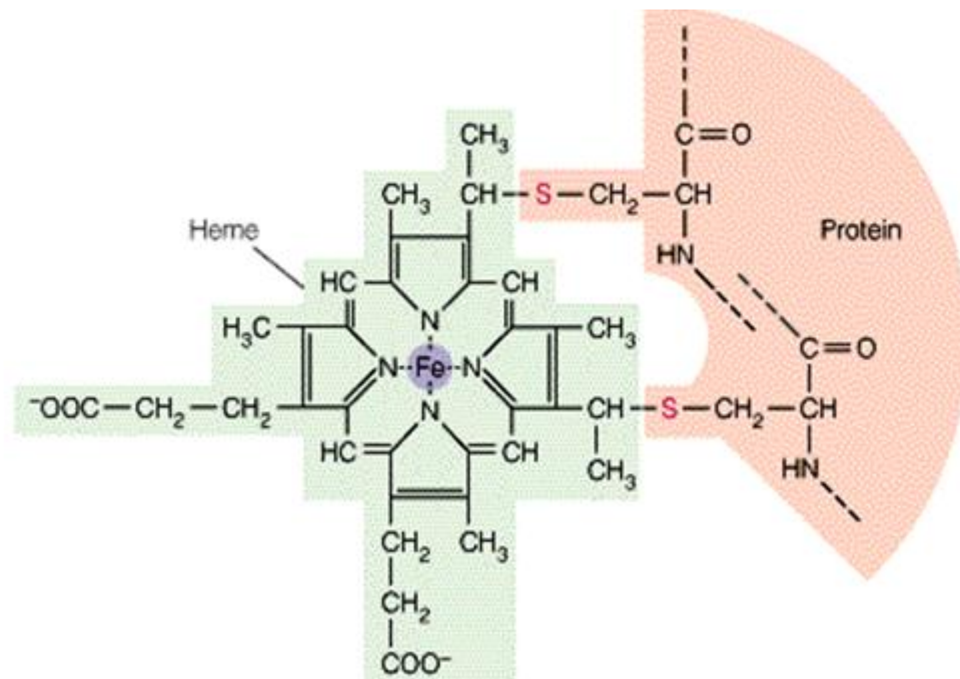


(b) Heme A in cytochromes *a* and a_3



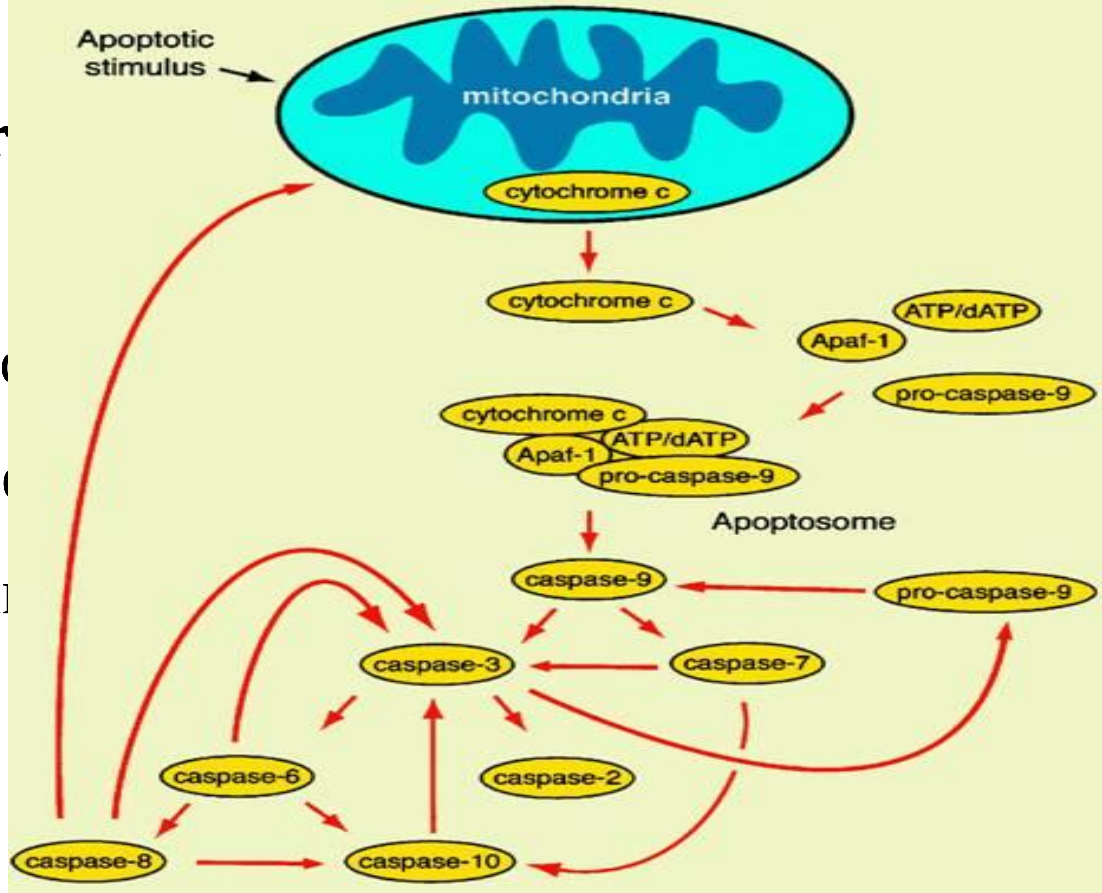
Iron protoporphyrin IX
(in *b*-type cytochromes)

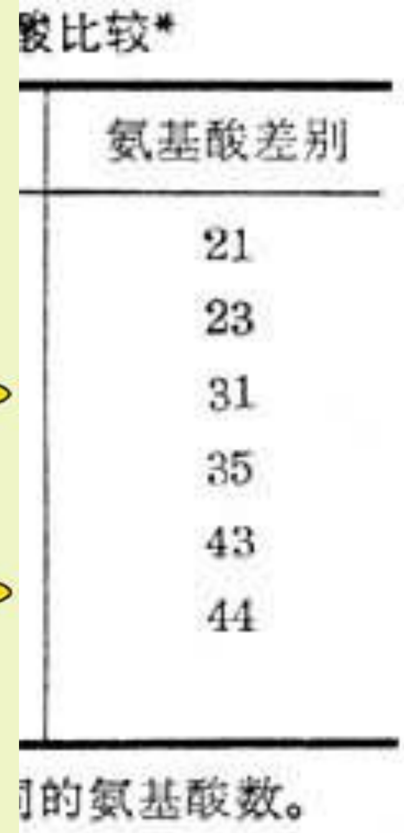
- **cyclic structure, consists of four five-membered, nitrogen-containing rings.**



(a) General structure of cytochromes *c* and c_1

Cytochr




酸比较*

氨基酸差别
21
23
31
35
43
44

间的氨基酸数。

- small pr
- readily
- The ami

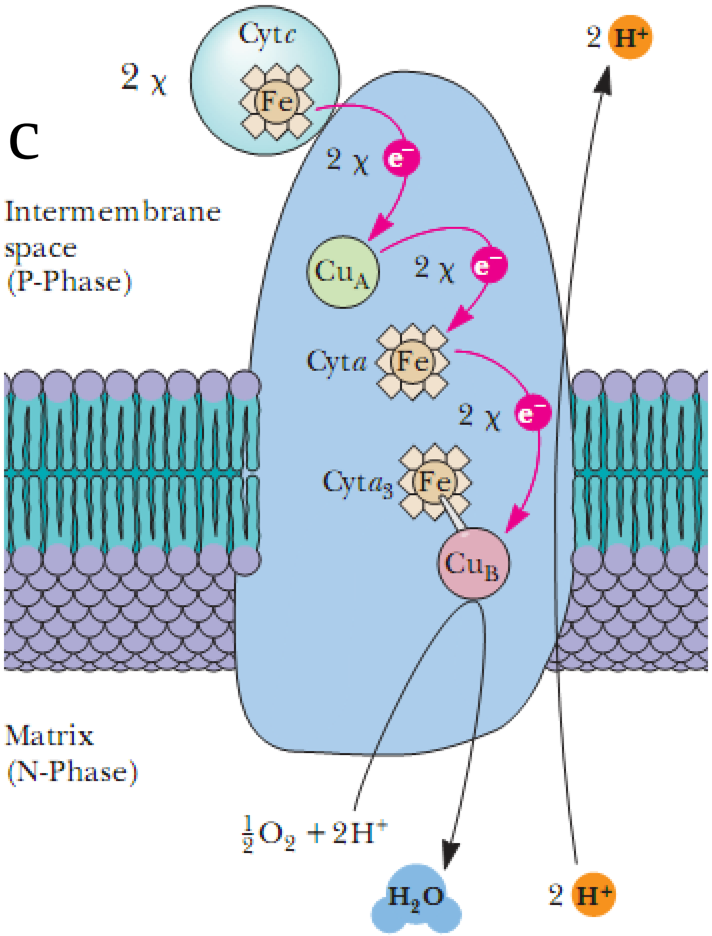
evolution,

- The other **cytochromes** are integral membrane proteins
- exceedingly difficult to dissociate from the membrane--------

less is known about their structure.

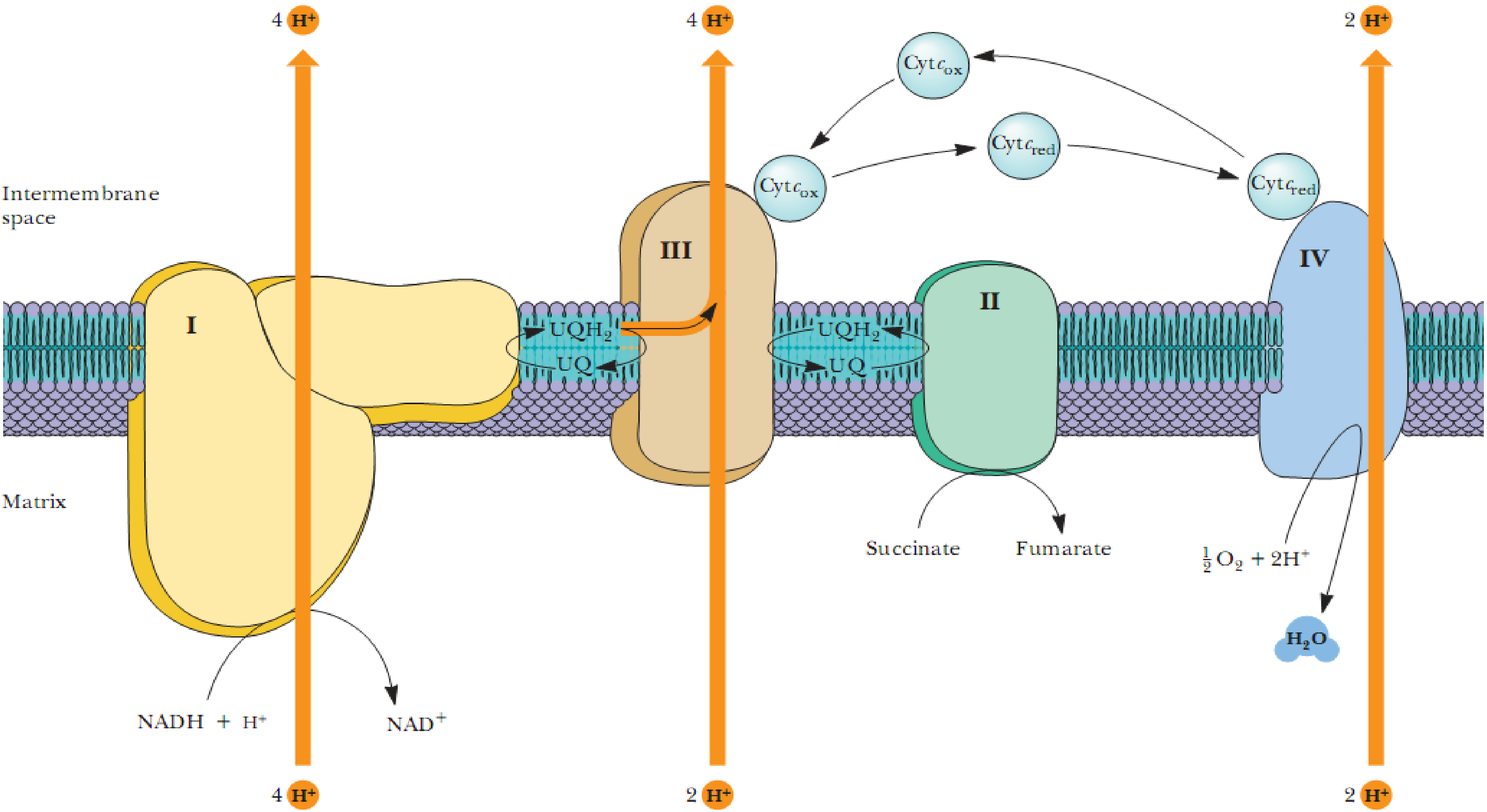
Complex IV (or cytochrome c oxidase 细胞色素氧化酶)

- contains Cyt a and a3
- it takes electrons from cytochrome c



The third proton pump

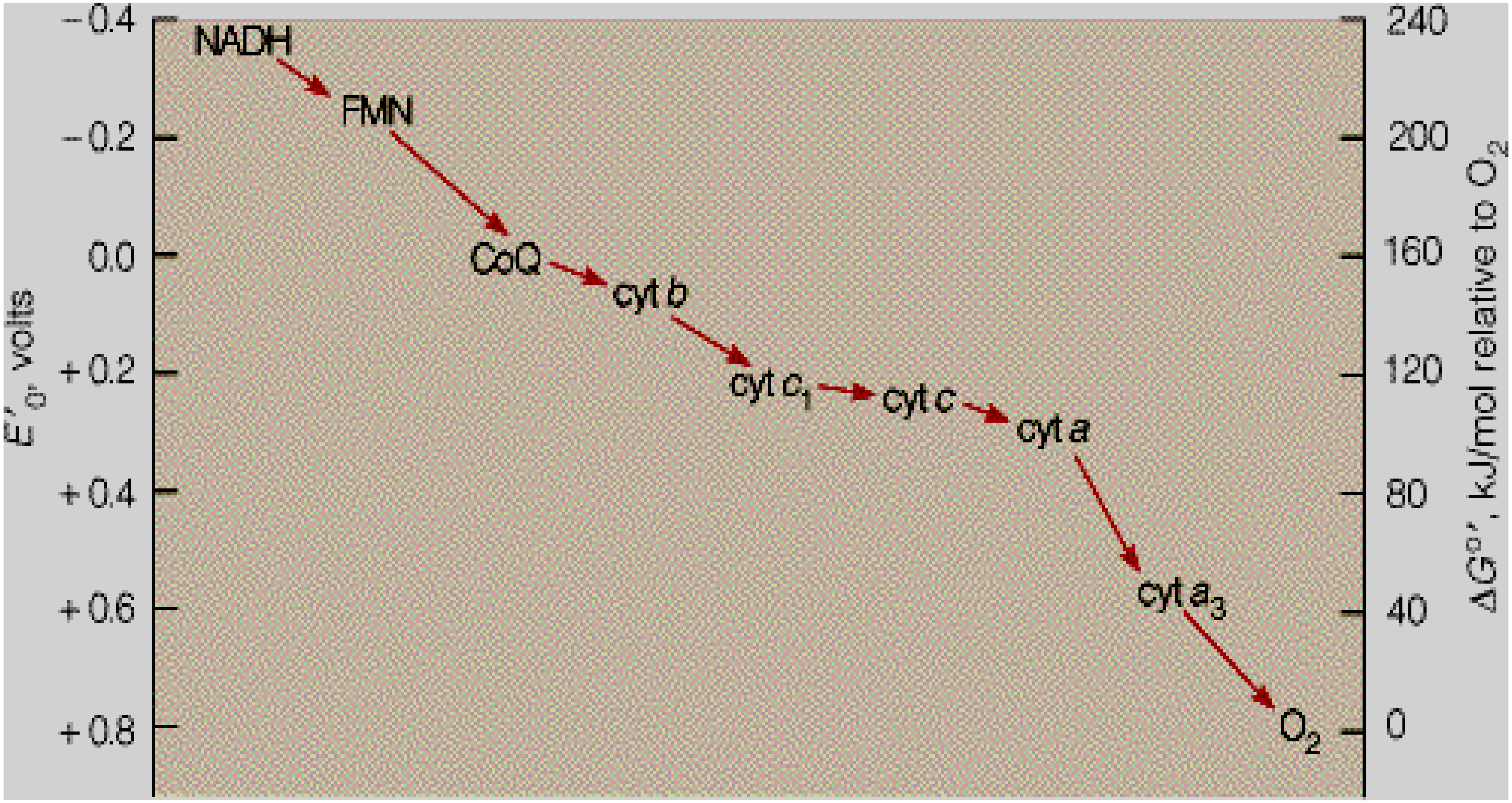
$$4\ \text{cyt}\ c\ (\text{Fe}^{2+}) + 4\ \text{H}^{+} + \text{O}_2 \longrightarrow 4\ \text{cyt}\ c\ (\text{Fe}^{3+}) + 2\ \text{H}_2\text{O}$$



• A model for the electron transport pathway in the mitochondrial inner membrane. UQ/UQH$_2$ and cytochrome *c* are mobile electron carriers and function by transferring electrons between the complexes. The proton transport driven by Complexes I, III, and IV is indicated.

4.2 Determining the Sequence of Respiratory Electron Carriers

- **standard reduction potential, E0' 标准还原势**



Inhibitors of electron transport:

$$\text{NADH} \xrightarrow{\text{rotenone}} \text{Q} \longrightarrow \text{Cyt } b \longrightarrow \text{Cyt } c_1 \longrightarrow \text{Cyt } c \longrightarrow \text{Cyt } (a + a_3) \longrightarrow \text{O}_2$$

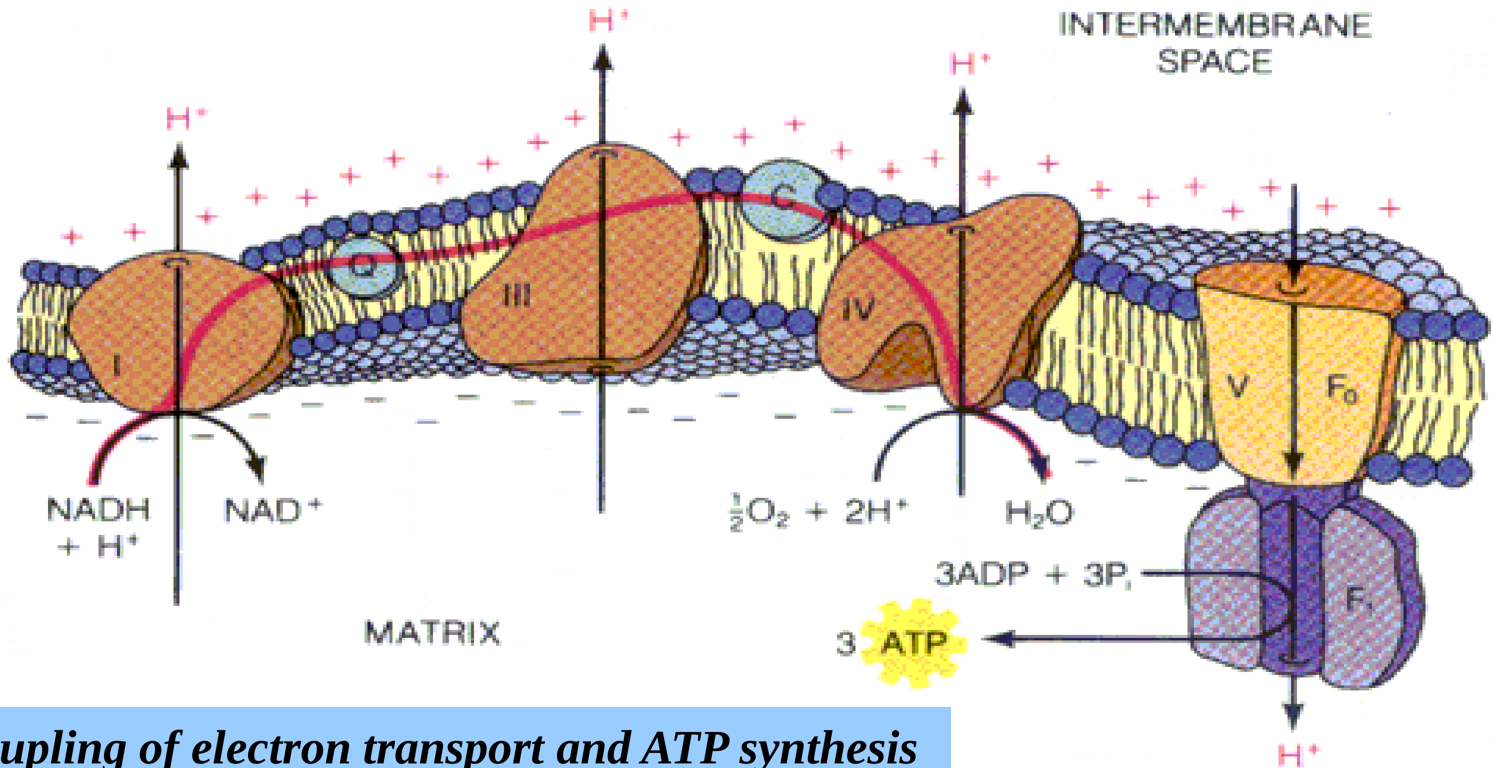
$$\text{NADH} \longrightarrow \text{Q} \longrightarrow \text{Cyt } b \xrightarrow{\text{antimycin A}} \text{Cyt } c_1 \longrightarrow \text{Cyt } c \longrightarrow \text{Cyt } (a + a_3) \longrightarrow \text{O}_2$$

$$\text{NADH} \longrightarrow \text{Q} \longrightarrow \text{Cyt } b \longrightarrow \text{Cyt } c_1 \longrightarrow \text{Cyt } c \longrightarrow \text{Cyt } (a + a_3) \xrightarrow{\text{CN}^- \text{ or CO}} \text{O}_2$$

5. Oxidative Phosphorylation

5.1 Definition

is a process where the energy of biological oxidation is ultimately converted to the chemical energy of ATP.

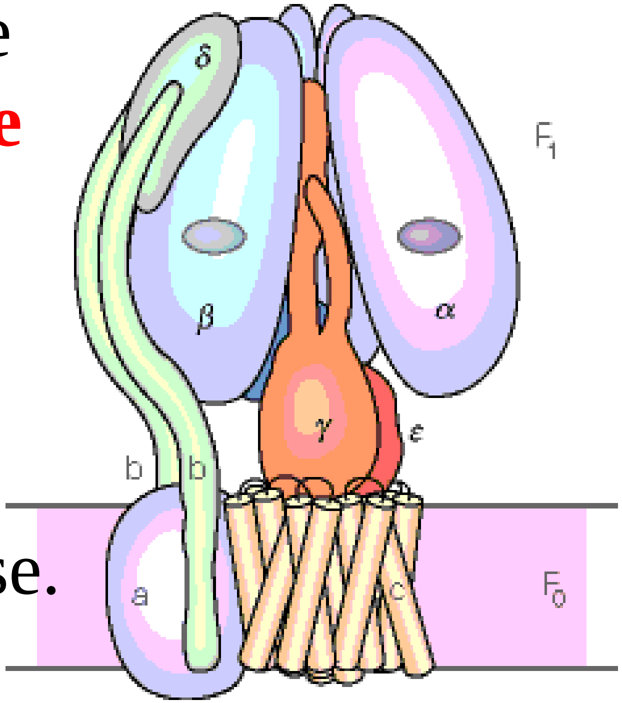


The coupling of electron transport and ATP synthesis

5.2 ATP合成部位

Complex V (**ATP synthase** , **FoF1 complex**). located on the inner mitochondrial cristae

- a top knob **---F1 (**projects into the mitochondrial matrix, contains **three dimers**)
- the base **---Fo** (in the inner mitochondrial membrane)
- a stalk--- joins the knob to the base.



5.3 Chemiosmotic hypothesis

Chemical coupling hypothesis (化学偶联假说）

1953, Edward Slater

Conformational coupling hypothesis （结构偶联假说）

1964, Paul Boyer

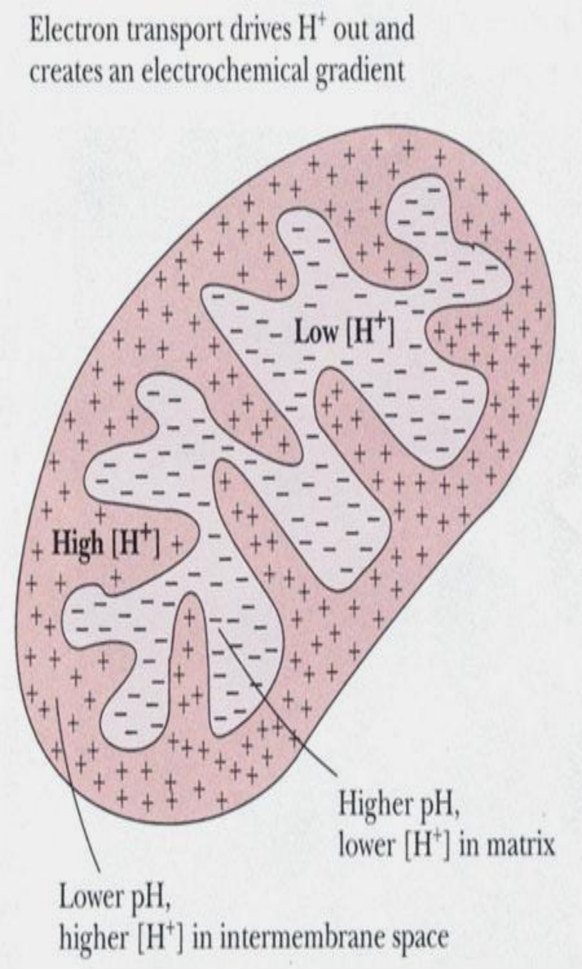
Chemiosmotic hypothesis（化学渗透假说）1978, Nobel Prize

1961, Peter Mitchell

1920-1992

Chemiosmotic hypothesis principles

1. movement of electrons through ETS (the **electron transport** system)
2. protons to be pumped from the mitochondrial matrix to the intermembrane space
3. the difference in potential
4. provides the energy source for making ATP in the mitochondrion



Evidence supporting the **chemiosmotic coupling hypothesis**:

1. Mitochondria pump protons and establish **a pH gradient** across their inner membrane.

2. Oxidative phosphorylation requires **an intact inner** membrane.

3. Agents that **uncouple ETS** from oxidative phosphorylation dissipate the proton gradient

◦ ◦ ◦ ◦ ◦ ◦

5.4 Binding-change mechanism

Paul Boyer

1997 Nobel

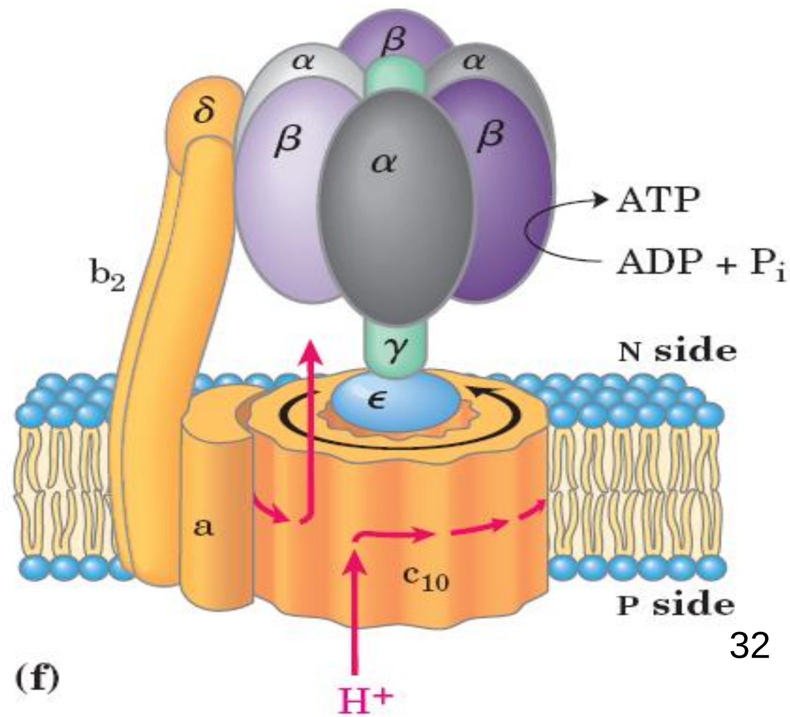
Paul Boyer

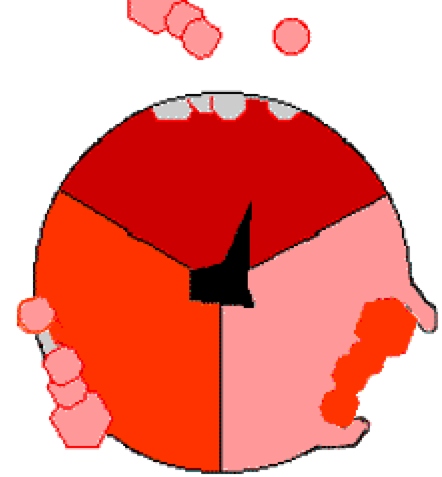
出生	1918年7月31日 （96岁） 美国犹他州普若佛
国籍	美国
研究领域	化学
任职于	洛杉矶加利福尼亚大学
母校	杨百翰大学、威斯康辛大学麦迪逊分校
著名成就	三磷酸腺苷生物合成机理
获奖	1997年诺贝尔化学奖

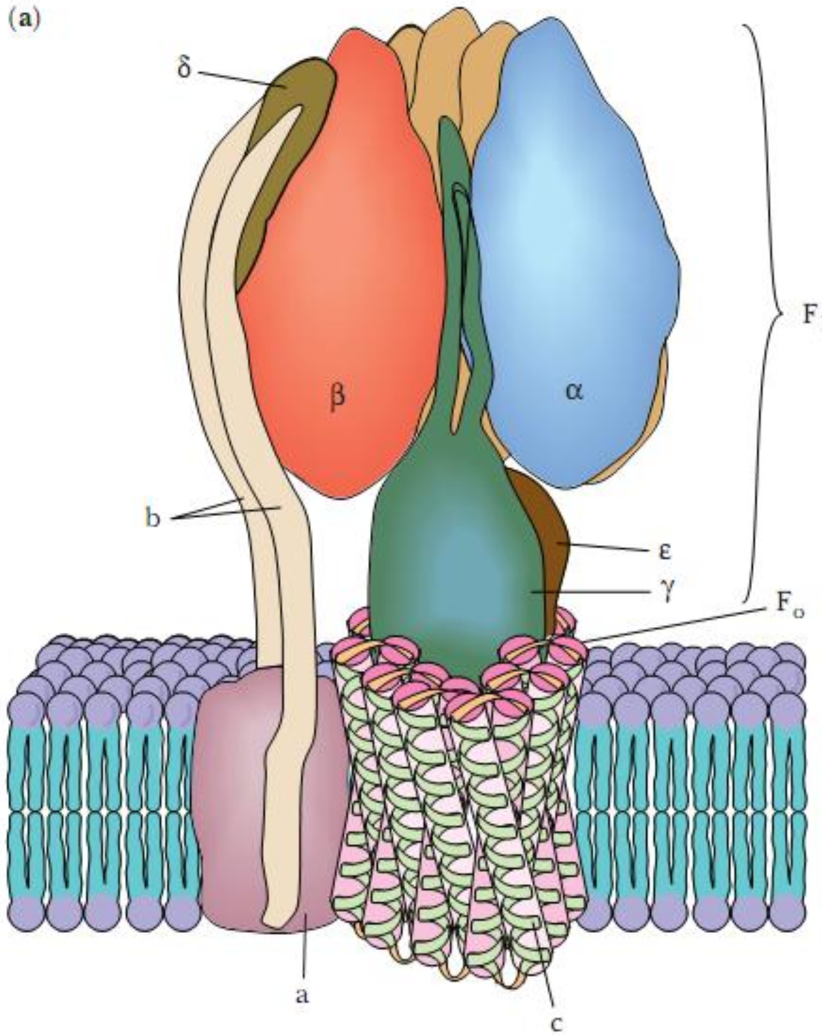
Rotational catalysis mechanism

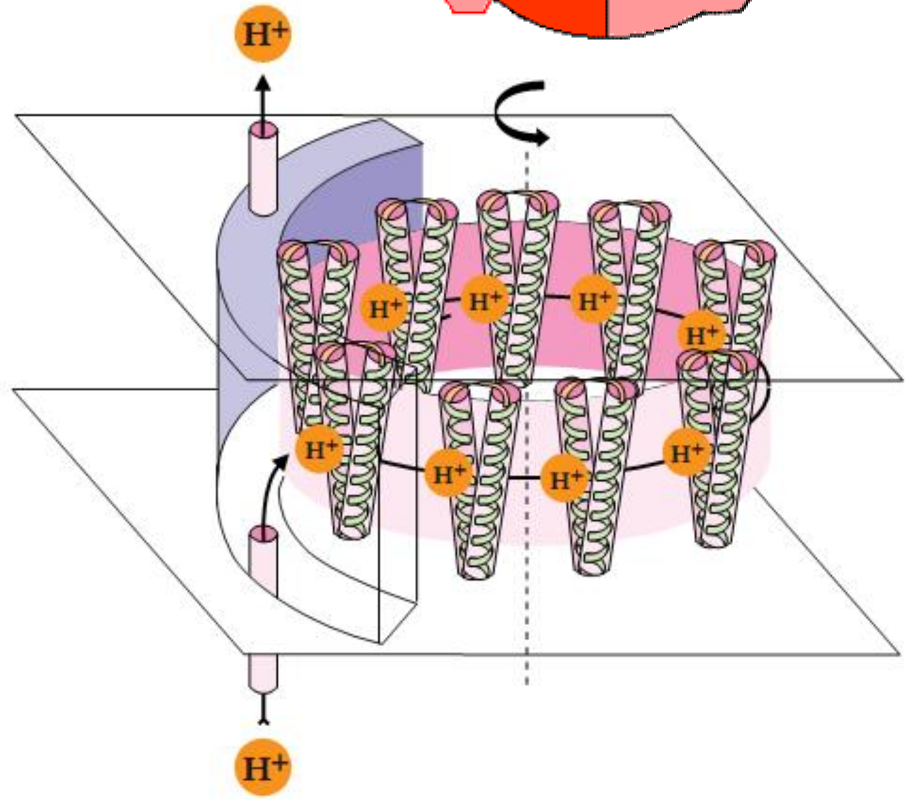
（旋转催化机制）

Movement of the protons through the **Fo complex** causes it to rotate

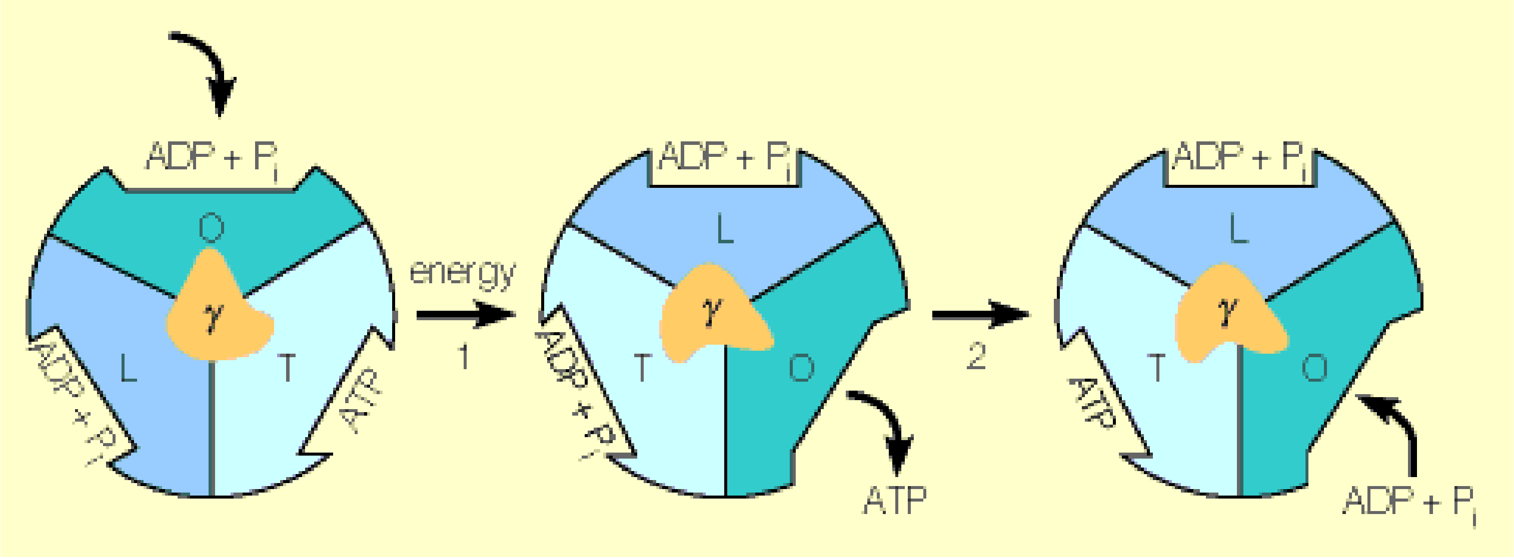





(a)
δ
β
α
b
ε
γ
F1
Fo
a
c


(b)
H+

F1 ATP synthase as a rotary engine driving the synthesis of ATP



F1 β subunit contains three binding sites for ATP or ADP + Pi:

T site (Tight) : bind to S tightly, have catalytic activity

L site (Loose): bind to S loosely， have no catalytic activity

O site (Open): bind to S with low affinity

The proton motivation------energy released by F_O------ γ subunit rotation

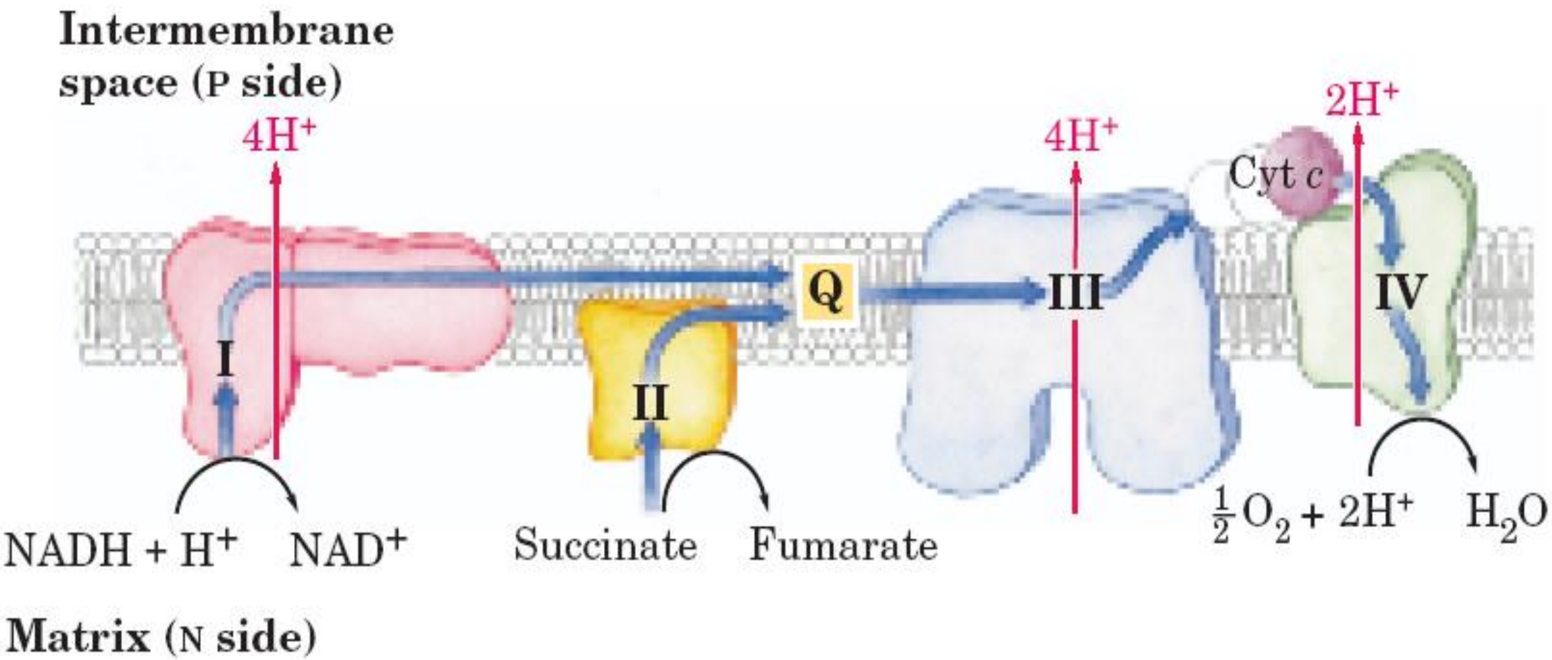
5.5 P/O Ratio

The efficiency of **oxidative phosphorylation**

$$xADP + xP_i + \frac{1}{2}O_2 + H^+ + NADH \longrightarrow xATP + H_2O + NAD^+$$

the **amount of ATP** made versus the amount of oxygen consumed— x (P/O ratio)

the molecules of ATP made per pair of electrons carried through the electron transport(P/2e- ratio) integer.



complex I from NADH : 10/4=2.5

complex II (FAD's electrons) : 6/4=1.5

opinion： H^+ 10 (NADH) / 6 (succinate) vs 2 e^-

ATP 1 vs 3 H^+ ($+H^+$)

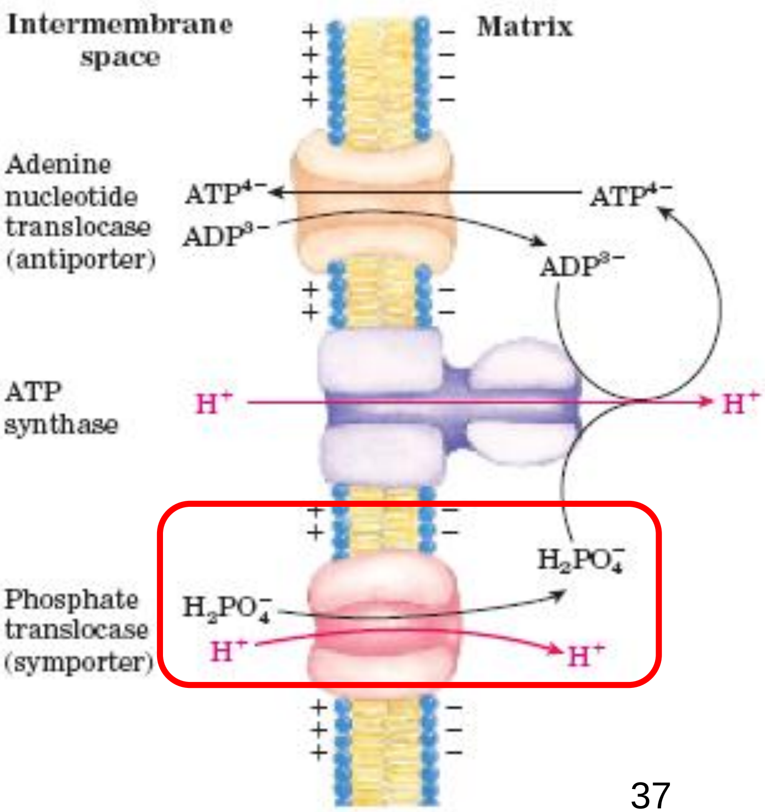
the surplus one may be used for transporting ATP, ADP, Pi

NADH 2.5 ATP

FADH 1.5ATP

Adenine nucleotide and phosphate translocases

嘌呤核苷酸 - 磷酸转运酶



6. Shuttling Electron Carriers into the Mitochondrion

the inner membrane is not permeable to NADH

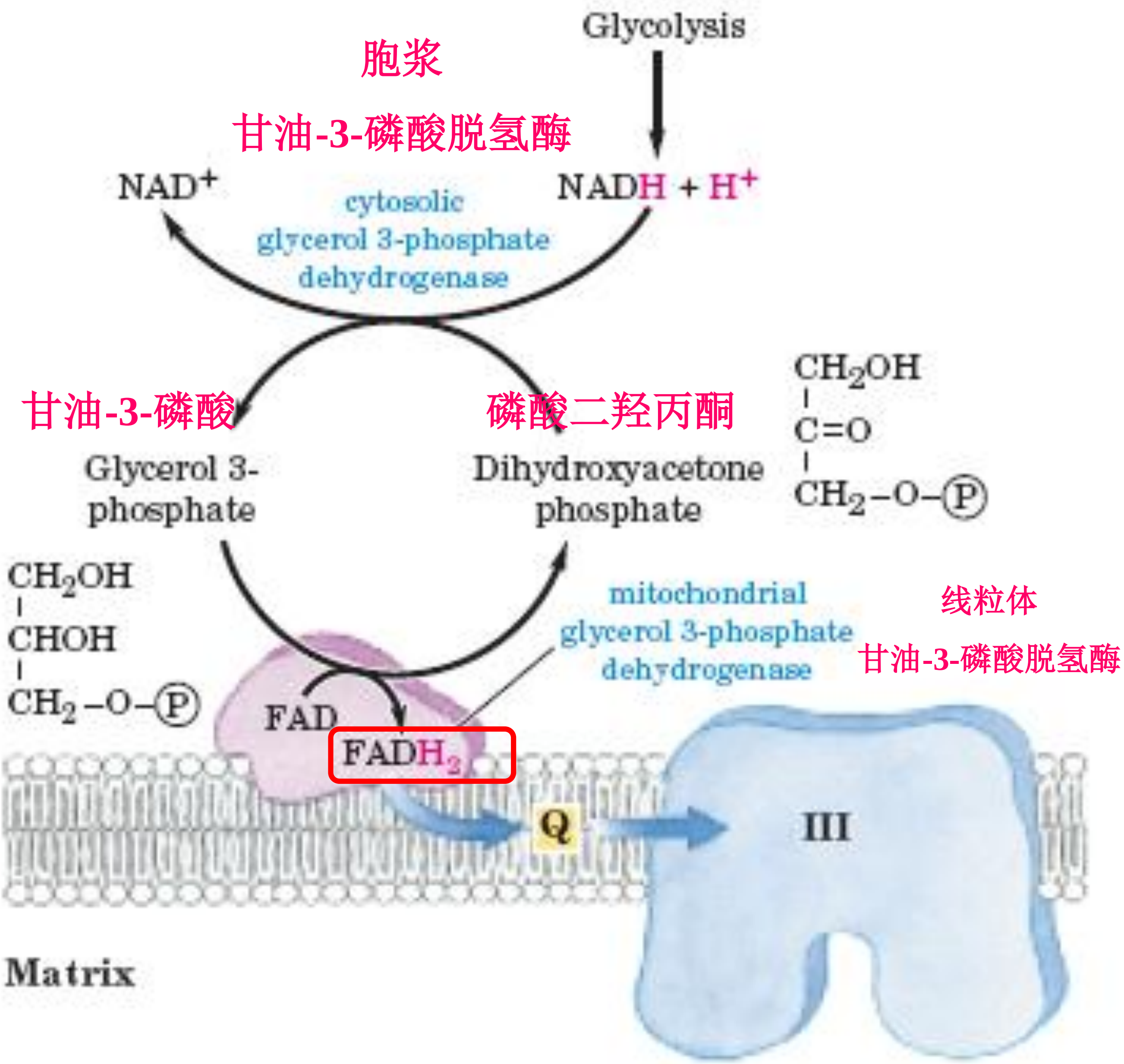
How can the NADH generated by glycolysis in the cytosol be reoxidized to NAD^+ by O_2 via the respiratory chain?

- **Glycerol-3-P shuttle system (甘油-3-磷酸穿梭途径）**
- **malate/aspartate shuttle system （苹果酸-天冬氨酸穿梭途径）**

骨骼肌和大脑

Glycerol-3-P shuttle system（甘油-3-磷酸穿梭途径）：

- In the **skeletal muscle** and **brain** mitochondria
- Note: the shuttle transfers electrons from NADH ultimately to make **$FADH_2$**
- Transfers electrons to CoQ bypassing complex I.
- **1.5ATPs.**

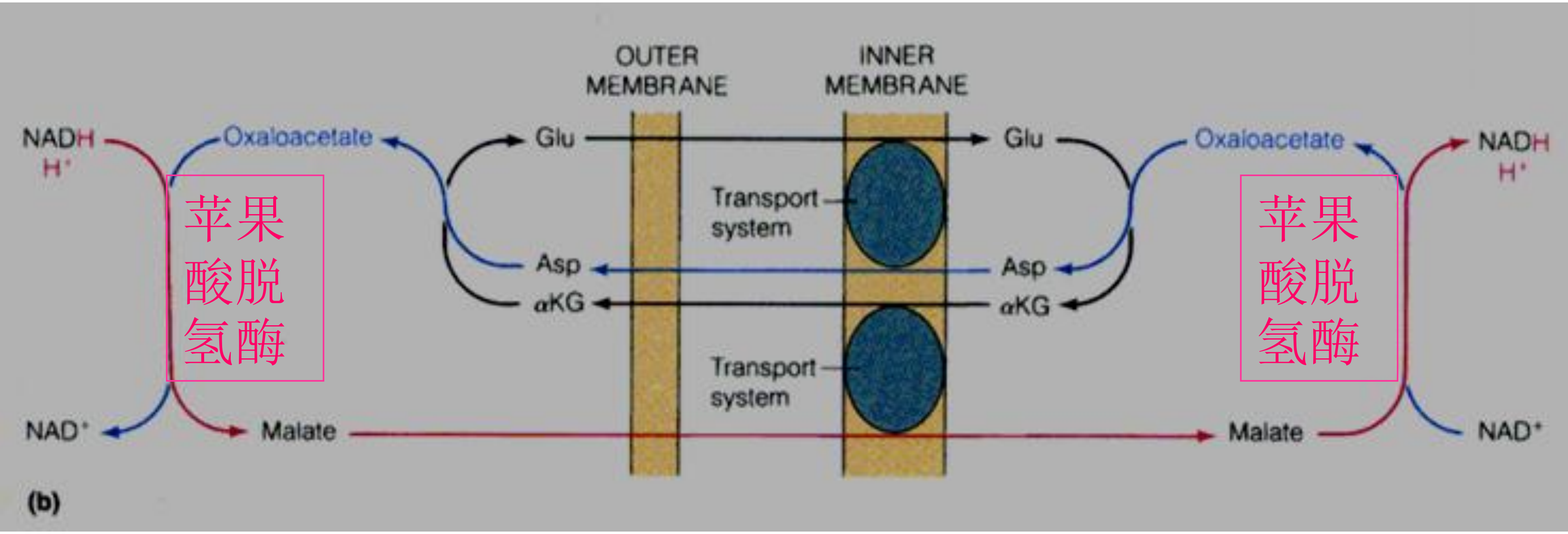


Glycolysis
胞浆
甘油-3-磷酸脱氢酶
NAD+
NADH + H+
cytosolic
glycerol 3-phosphate
dehydrogenase
甘油-3-磷酸
磷酸二羟丙酮
Glycerol 3-
phosphate
Dihydroxyacetone
phosphate
CH2OH
C=O
CH2–O–P
CH2OH
CHOH
CH2–O–P
mitochondrial
glycerol 3-phosphate
dehydrogenase
线粒体
甘油-3-磷酸脱氢酶
FAD
FADH2
Q
III
Matrix

心、肝、肾

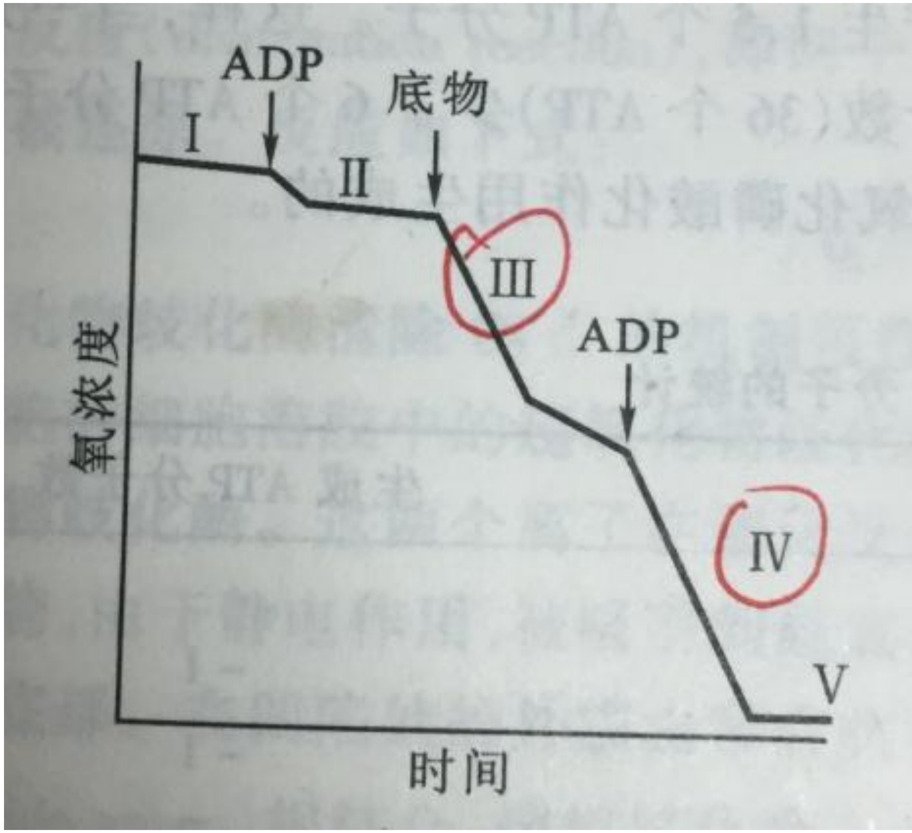
malate/aspartate shuttle system （苹果酸-天冬氨酸穿梭途径）：

- in **liver , kidney and heart** mitochondria.
- **2.5ATPs**



7. respiratory control

- Substrates： ADP, Pi, O_2, oxidizable metabolite
- **Respiration** is tightly coupled to the **synthesis of ATP**
- **ATP synthesis** absolutely dependent on continued **electron flow** from substrates to oxygen



ATP is consumed at high rates

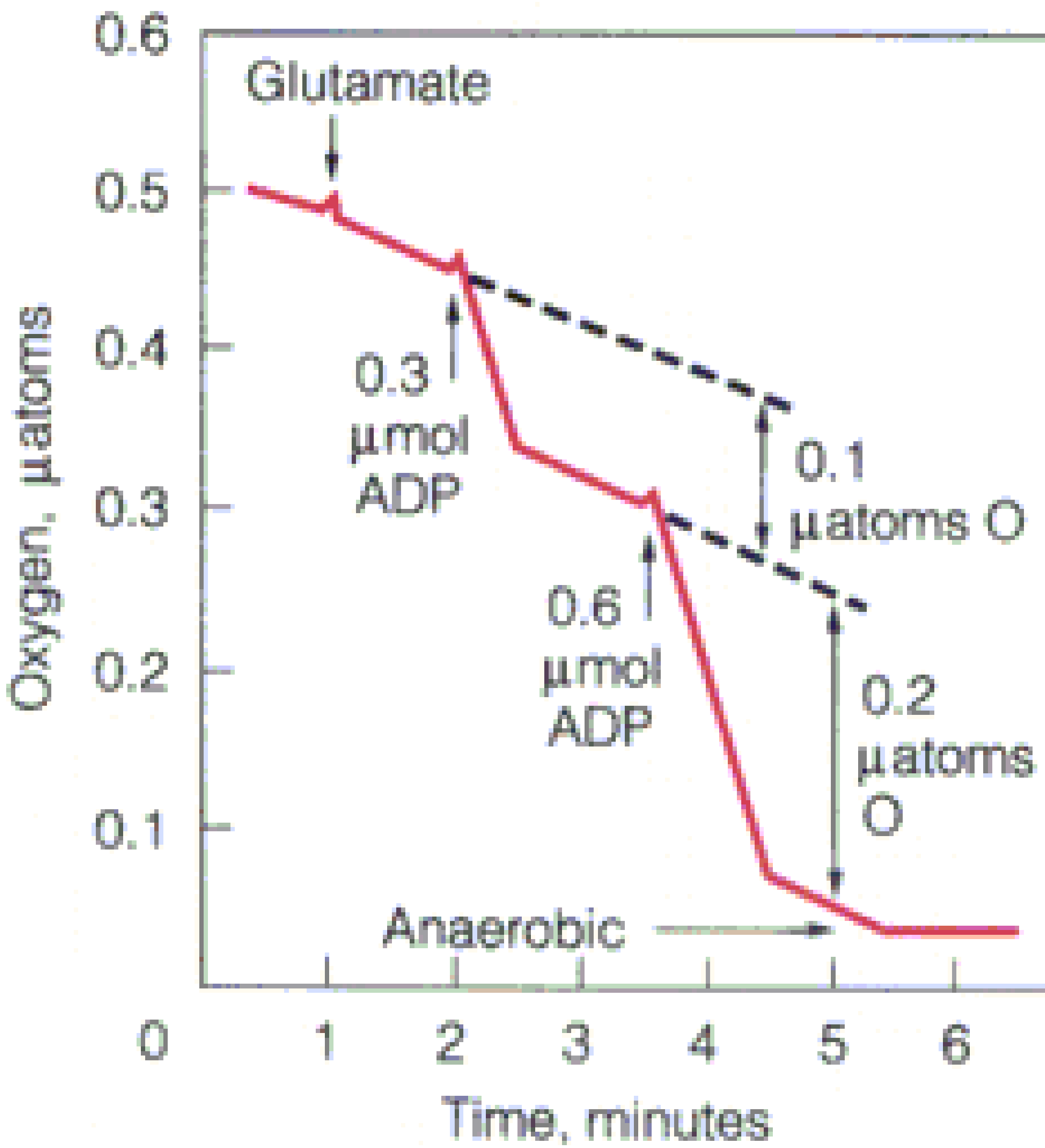
- accumulation of **ADP**
- stimulates respiration
- activation of ATP resynthesis

Biological sense

- **ensures that substrates will not be oxidized wastefully**
- **their utilization is controlled by the physiological need for ATP**
- **Respiration** depends on **ADP**

energy-generating capacity $\xleftrightarrow{\text{ADP}}$ **energy demands**

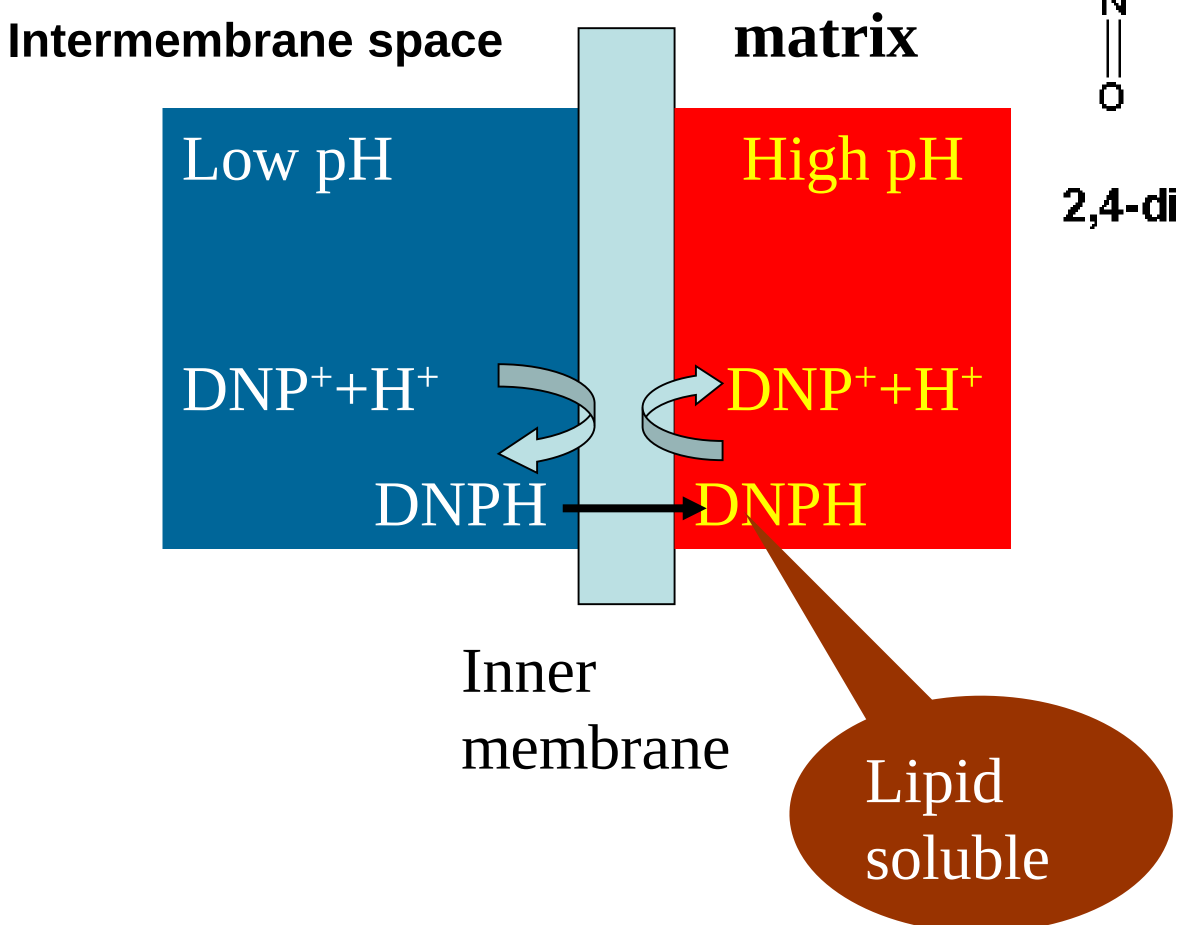
ADP is important !

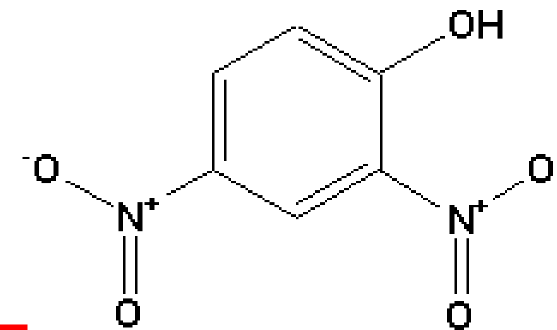


氧化磷酸化影响因素

1）**Chemical uncouplers**: dissipate the proton gradient

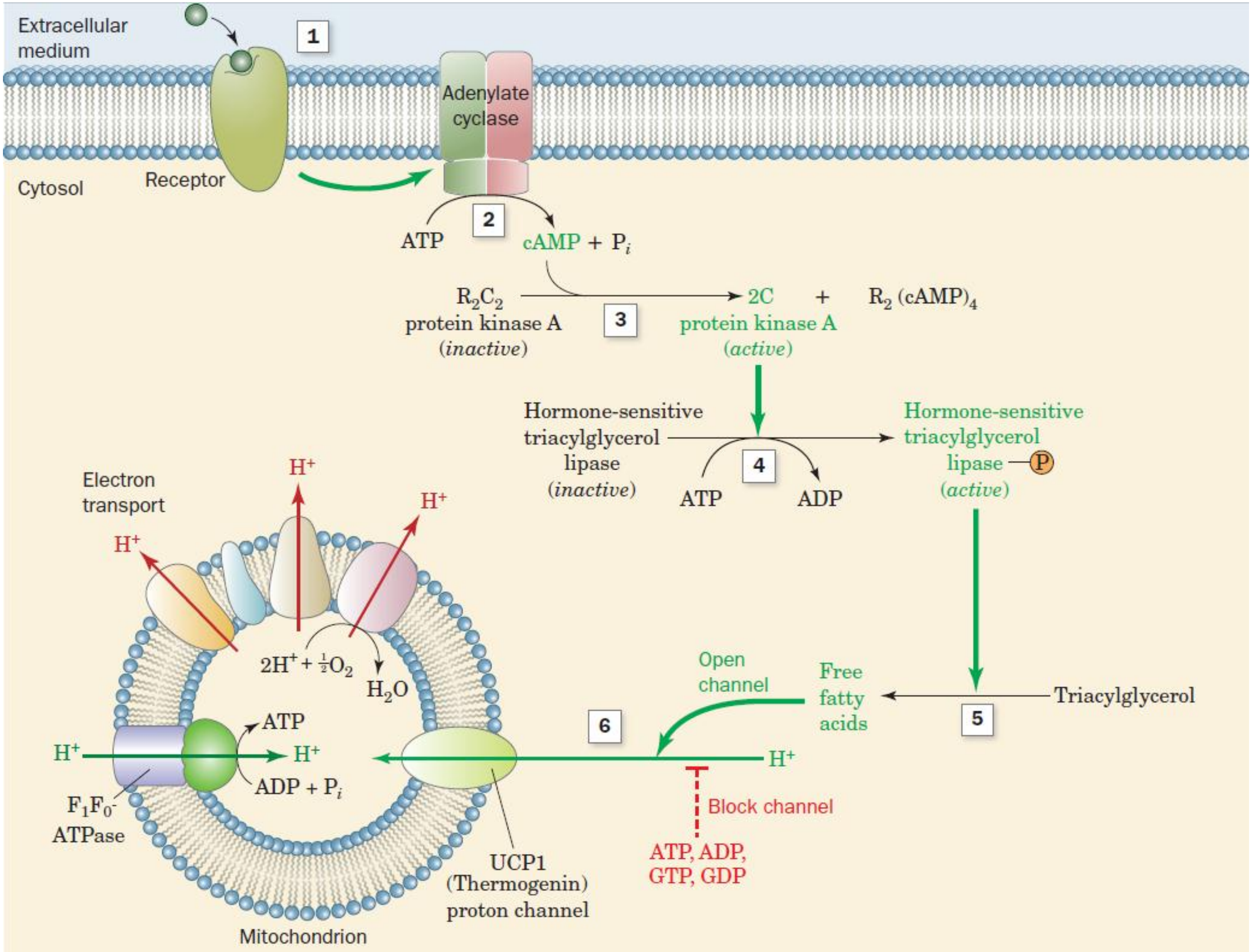
- **DNP**（2，4-二硝基苯酚）
- **FCCP**（三氯甲氧基苯腙羰基氰化物）





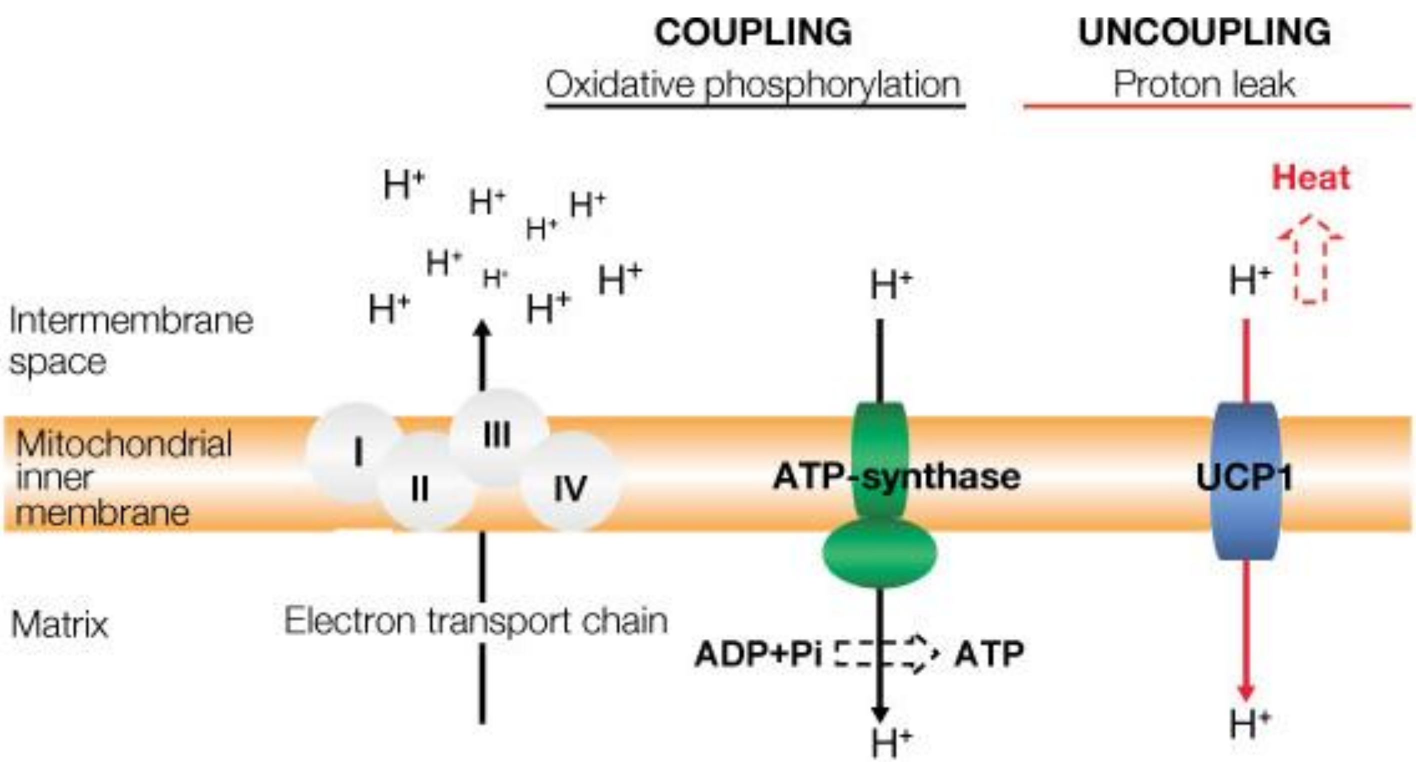
2,4-dinitrophenol (DNP)

Action of Thermogenin （产热素）

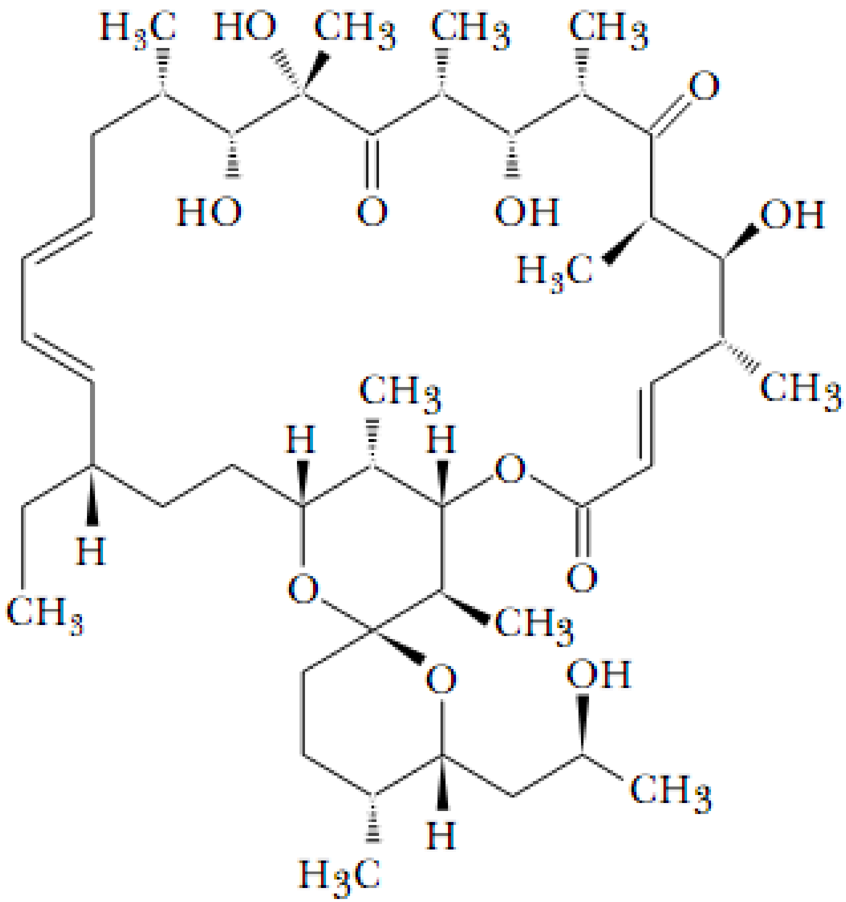


Brown fat (BF)

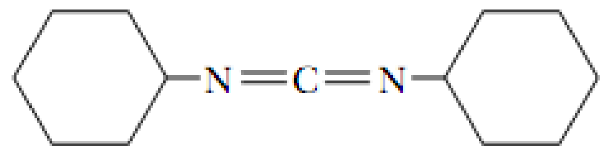
- Rich mitochondria
- Oxidize FAs for heat production



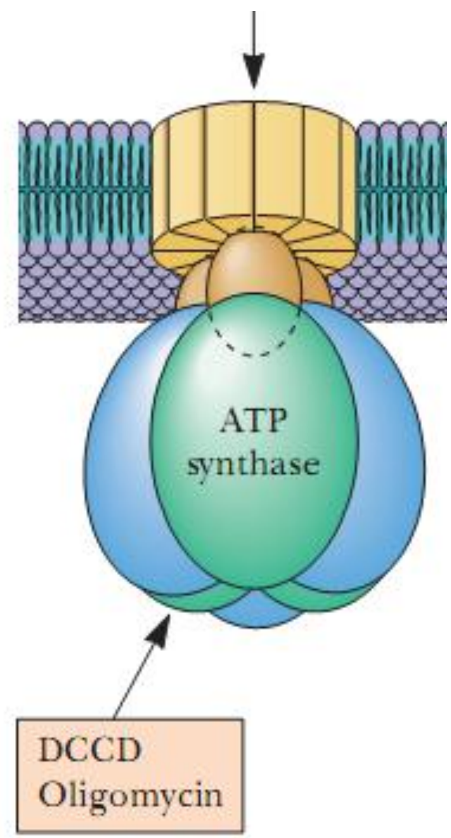
2）inhibitors of oxidative phosphorylation（氧化磷酸化抑制剂）：



Oligomycin A



Dicyclohexylcarbodiimide (DCCD)



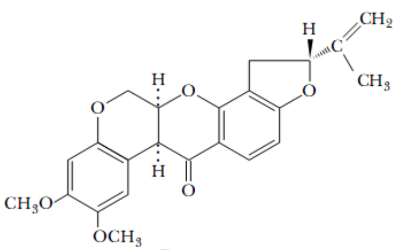
3）ionophores（离子载体抑制剂 ）：脂溶性物质

Valinomycin　缬氨霉素　K^+

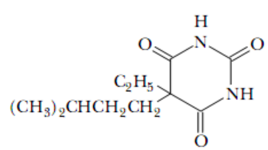
Gramicidin　短杆菌肽　K^+　Na^+

转运阳离子到基质中时消耗了自由能，降低了质子动力，抑制ATP的形成

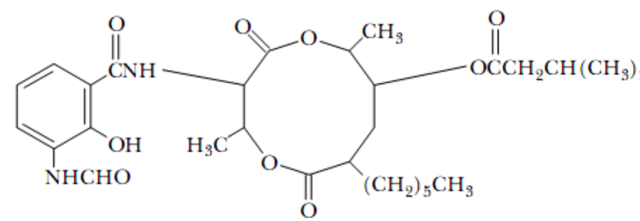
4）Inhibitors of electron transport：



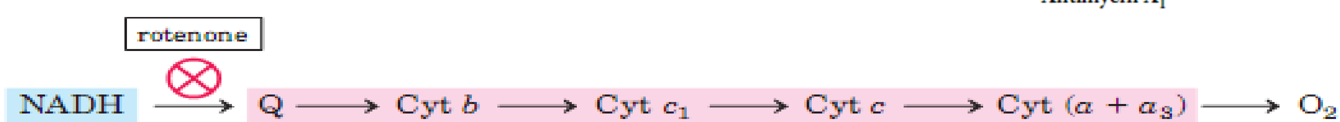
Rotenone

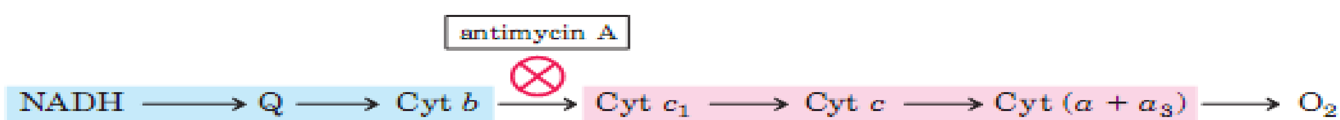


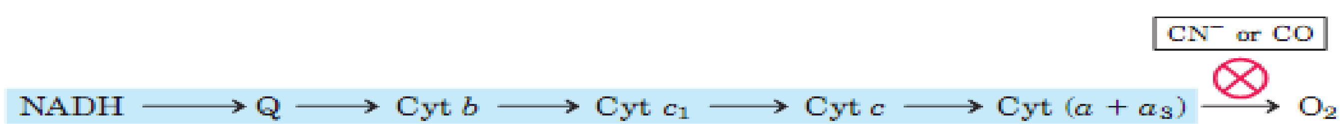
Amytal
(amobarbital)



Antimycin A_1







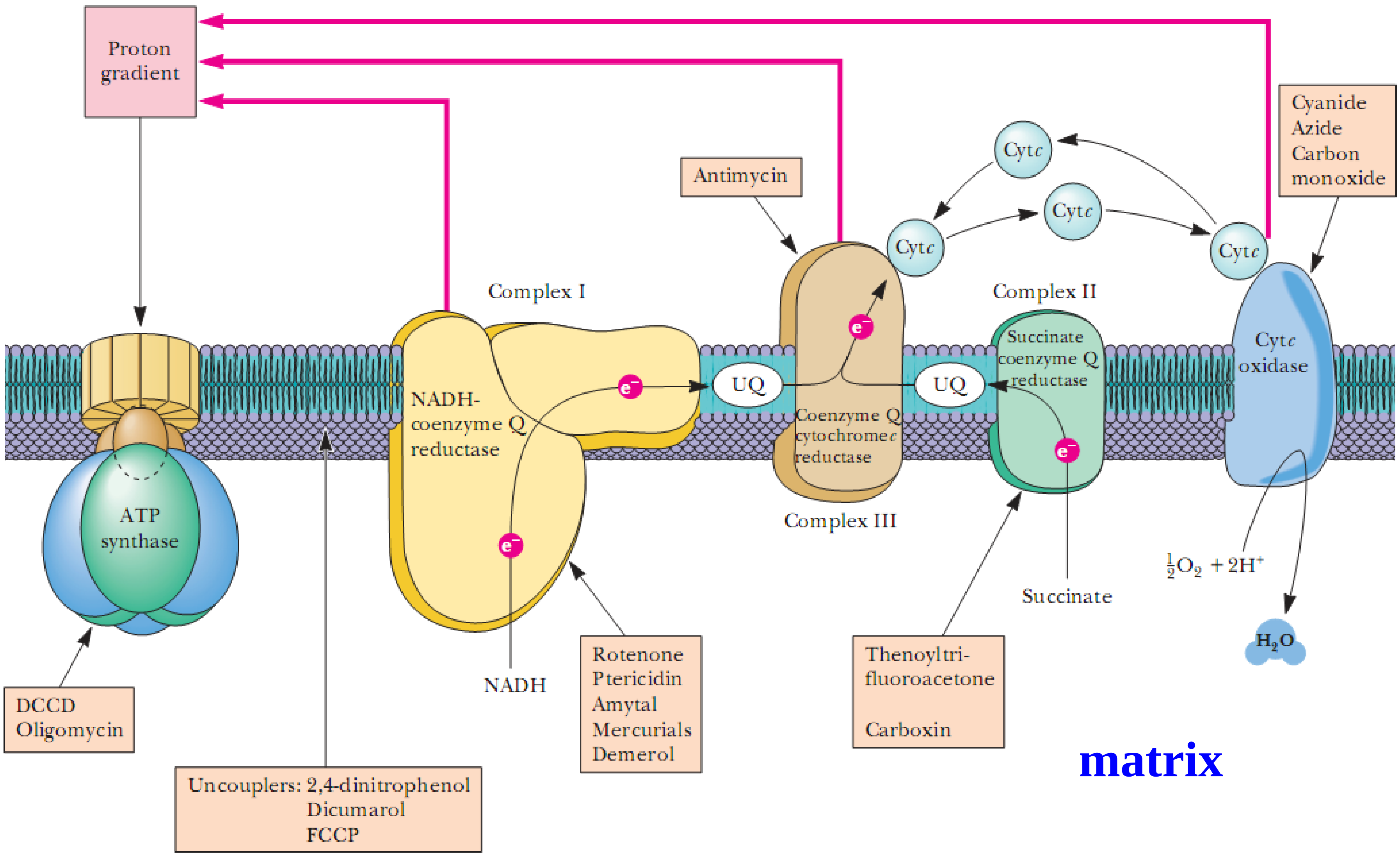
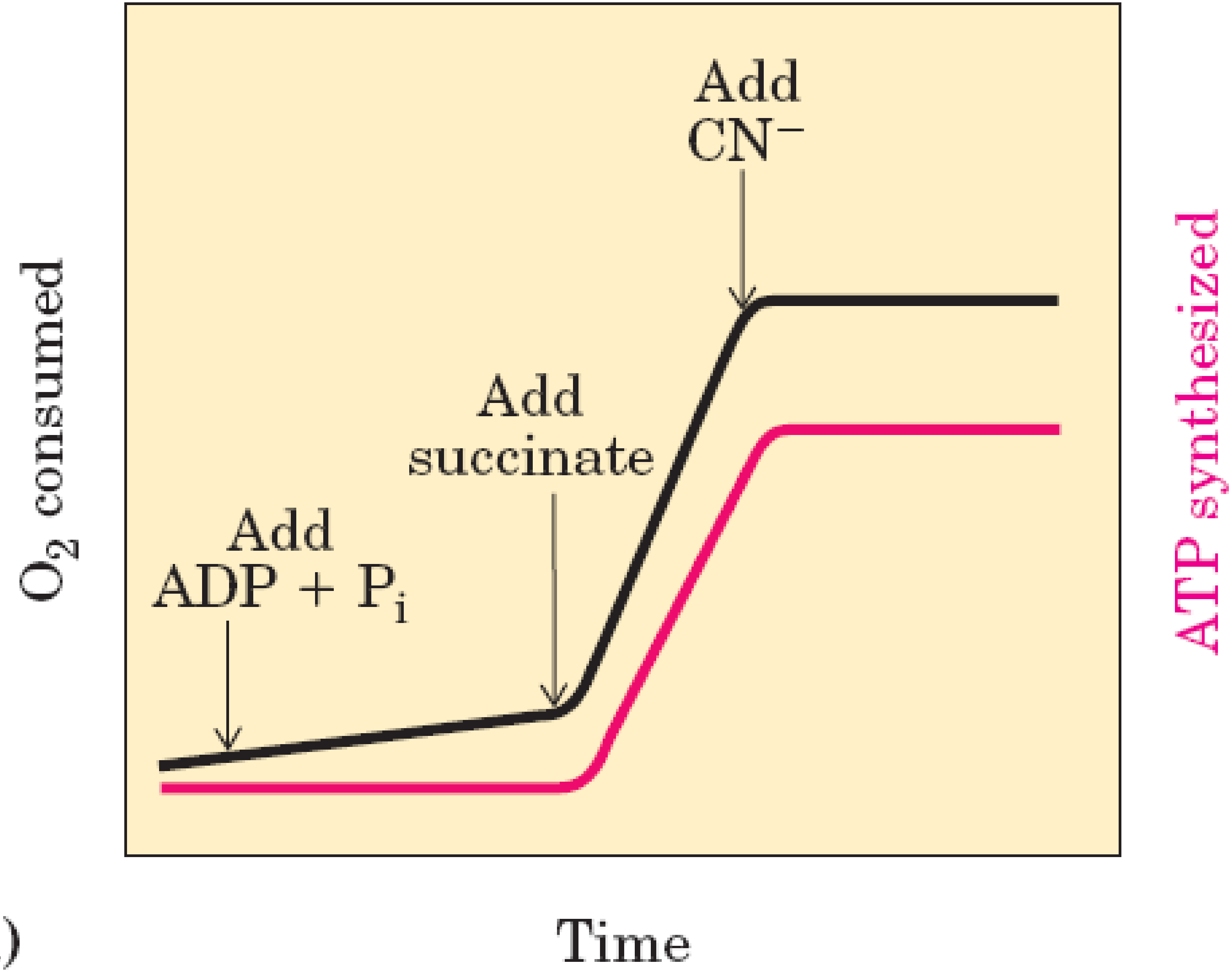


FIGURE 21.30 • The sites of action of several inhibitors of electron transport and/or oxidative phosphorylation.

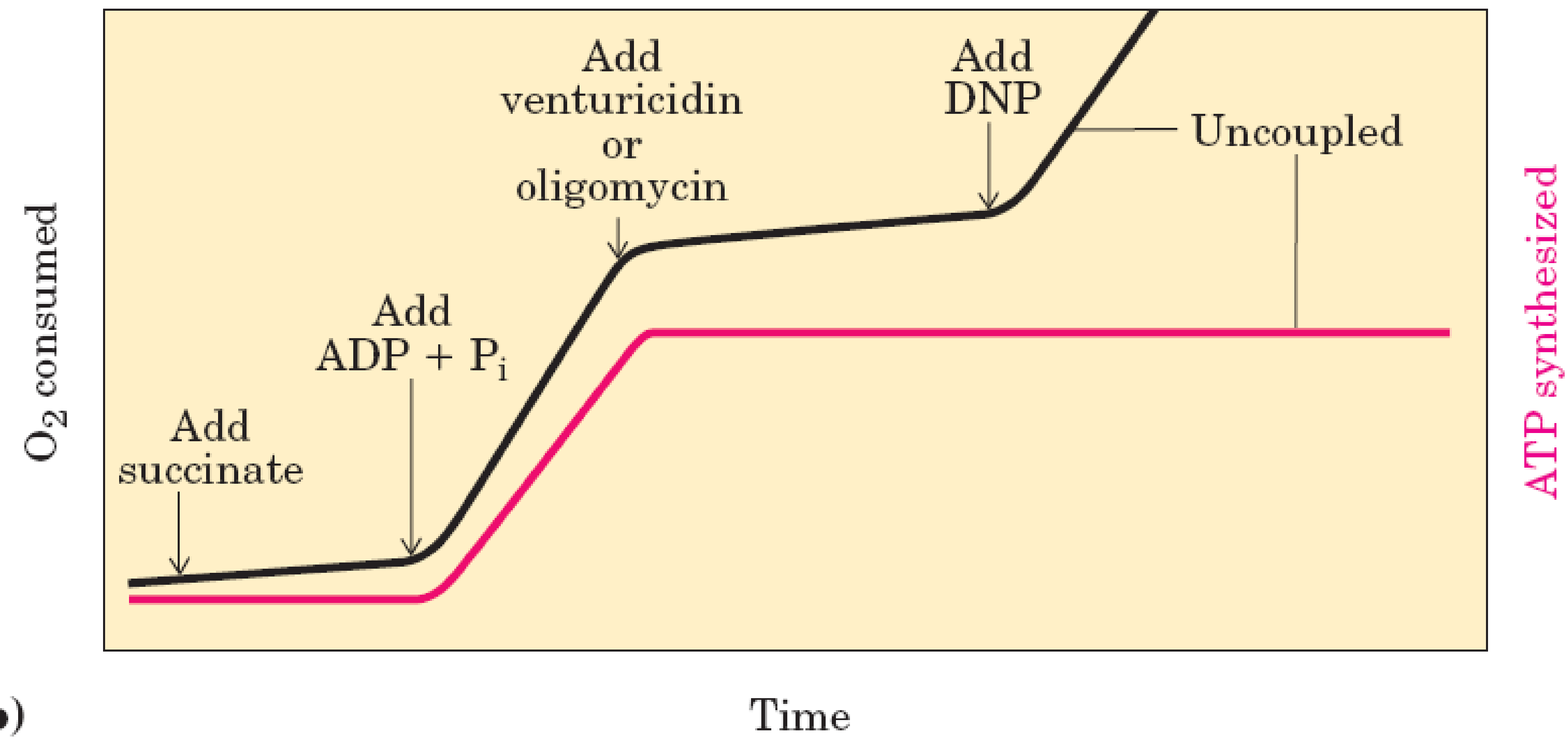
Summary

- ATP – glycolysis or TCA--**substrate-level** phosphorylation.
- Electrons stored in **NADH** and **$FADH_2$**, are passed through an highly organized chain of protein and coenzymes, the electron transport chain, finally reaching O_2 .
- Electron transport and oxidative phosphorylation locate at the **inner mitochondrial membrane**.
- In the course of electron transport, an **electrochemical proton energetic gradient** established across the inner mitochondrial membrane, which drives ATP synthesis with ATP synthase.
- Mitochondria also plays important role in regulating **apoptosis**, by secreting Cyt*c*

1. 什么是电子传递链？有哪两条传递链？是如何进行传递的？
2. 什么是氧化磷酸化？化学渗透假说主要内容包括哪几点？有哪些证据？
3. 什么是解偶联剂？氧化磷酸化抑制剂？离子载体抑制剂？常见的电子传递抑制剂有哪些？作用机制是什么？
4. **DNP**作为减肥药为何被禁用？是否影响底物水平磷酸化？
5. 胞浆中**NADH**是通过哪两条途径进行再氧化的？
6. 什么是**P/O**比？
7. **Given that rotenone and antimycin A are equally effective in blocking their respective sites in the electrontransfer chain, which would be a more potent poison? Explain.**
8. 如何解释下面两幅图？


Add
CN^-
Add
succinate
Add
ADP + P_i
O_2 consumed
ATP synthesized
Time

(a)


Add
venturicidin
or
oligomycin
Add
DNP
Uncoupled
Add
ADP + P_i
Add
succinate
O_2 consumed
ATP synthesized
Time
)