Chapter 12

Biological oxidation

Li WeiFang

1. Introduction

2. Mitochondrial Structure and Function

3. Energy Generation

4. Electron Transport

5. Oxidative Phosphorylation

6. Shuttling Electron Carriers into the Mitochondrion

7. Respiratory control

1. Introduction

Metabolic oxidations

↓

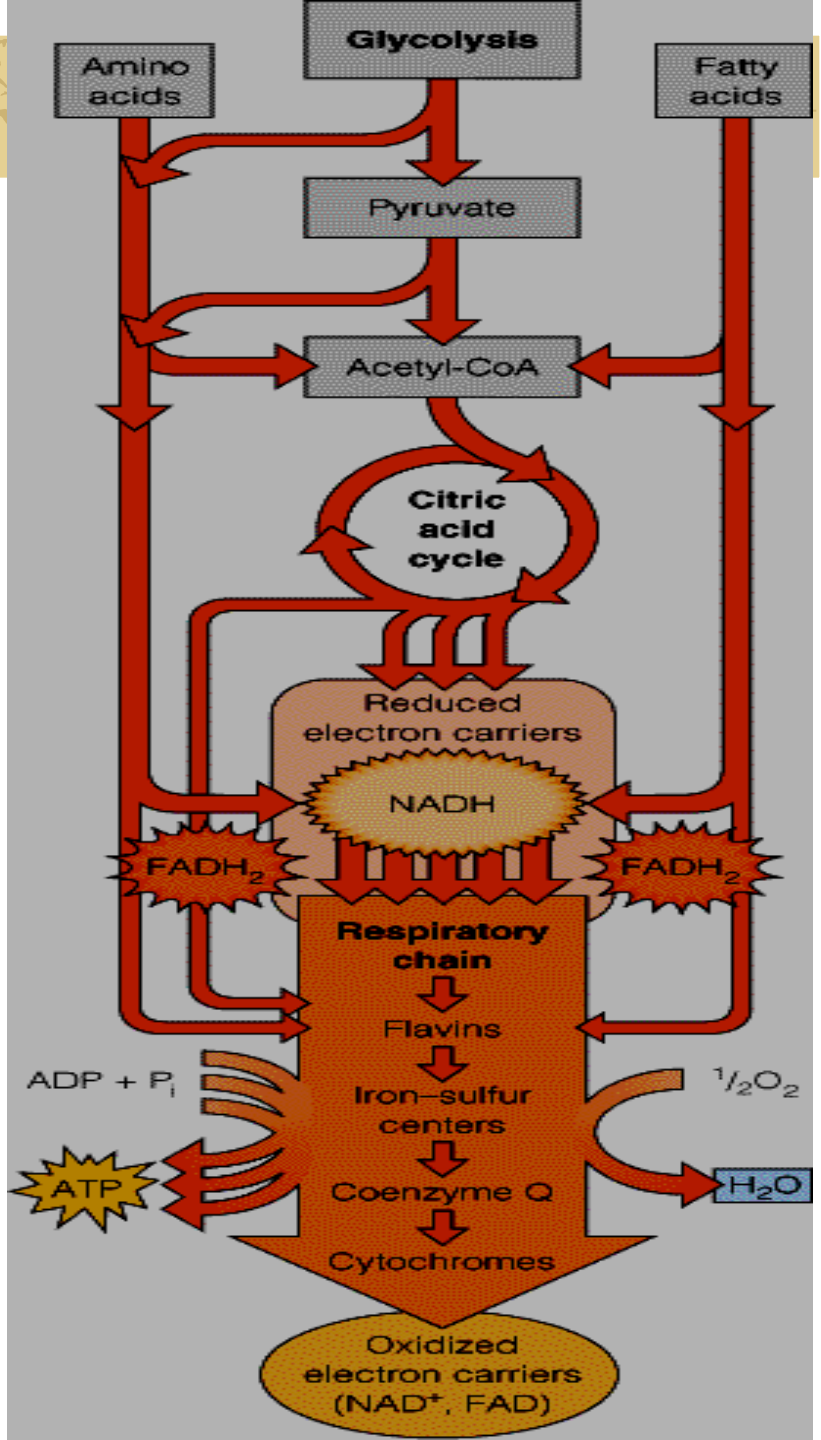
Reduced electron carriers

NADH and **FADH2**

Oxidation | Mitochondrion

↓

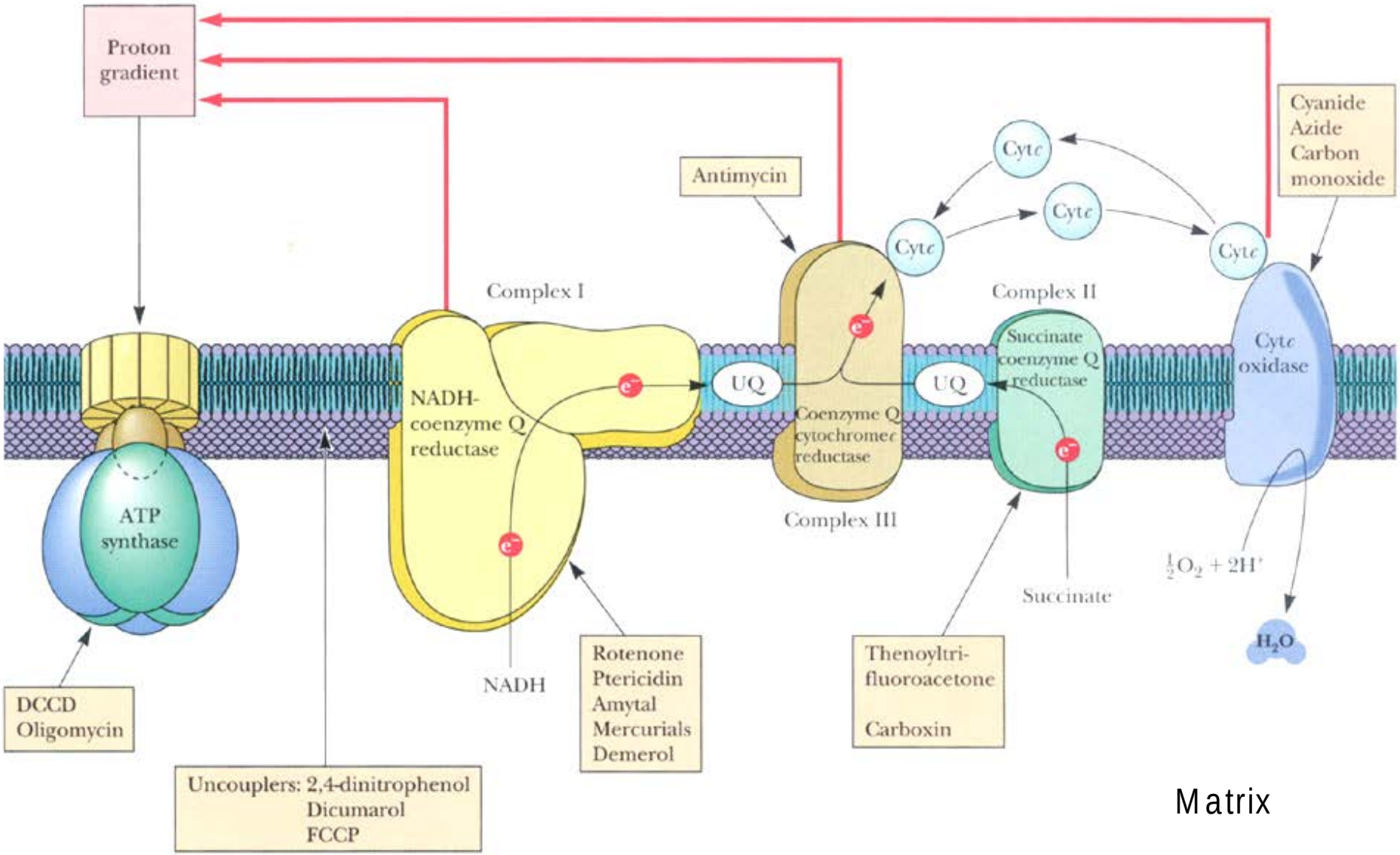
ATP



Biological oxidation: 有机分子在细胞内氧化分解成CO_2和H_2O并释放能量形成ATP的过程。

特点：

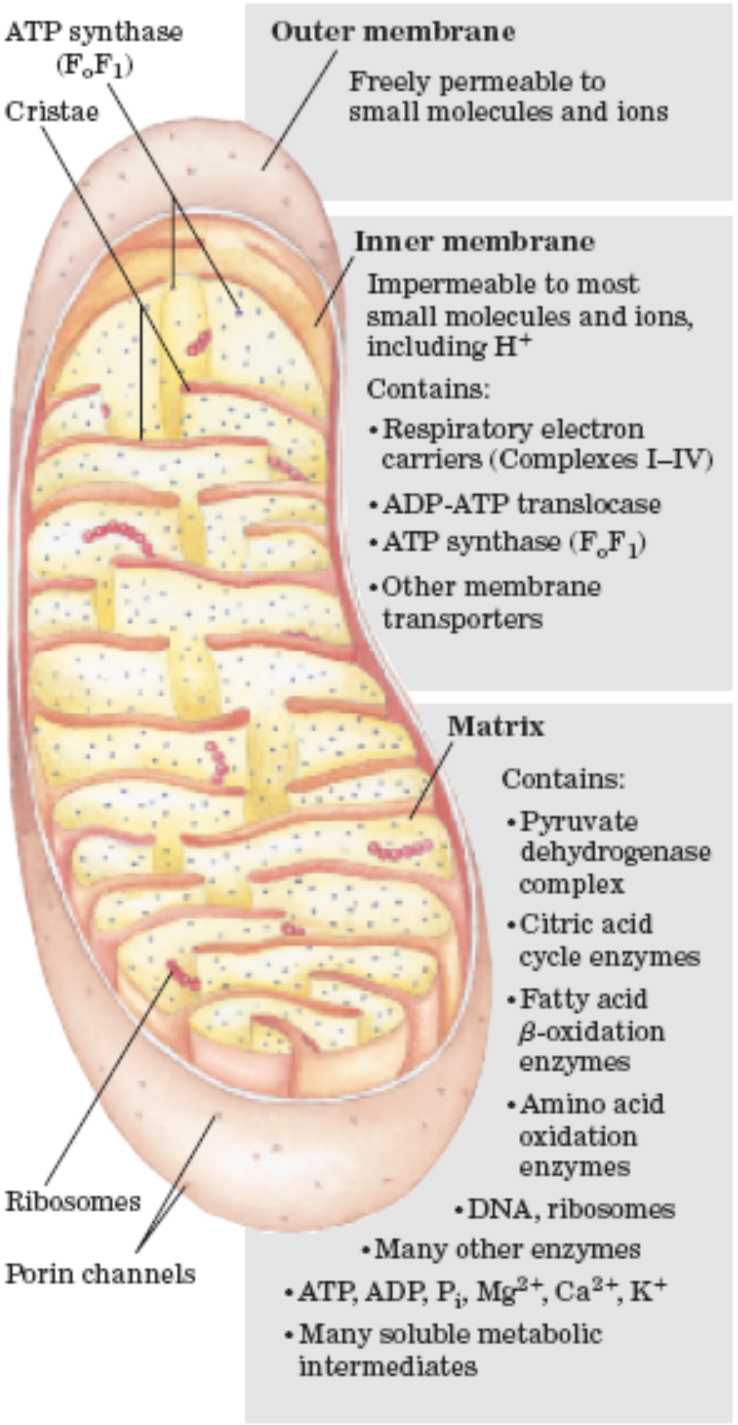
- 体温条件下进行；
- 产生的能量一般都贮存在ATP中。


Proton gradient
Cyanide
Azide
Carbon monoxide
Antimycin
Cyt c
Cyt c
Cyt c
Cyt c
Complex I
Complex II
Succinate coenzyme Q reductase
Cyt c oxidase
UQ
UQ
NADH-coenzyme Q reductase
Coenzyme Q cytochrome c reductase
Complex III
ATP synthase
$\frac{1}{2}O_2 + 2H^+$
Succinate
H_2O
DCCD
Oligomycin
NADH
Rotenone
Ptericidin
Amytal
Mercurials
Demerol
Thenoyltri-fluoroacetone
Carboxin
Uncouplers: 2,4-dinitrophenol
Dicumarol
FCCP
Matrix

2. Mitochondrial Structure and Function

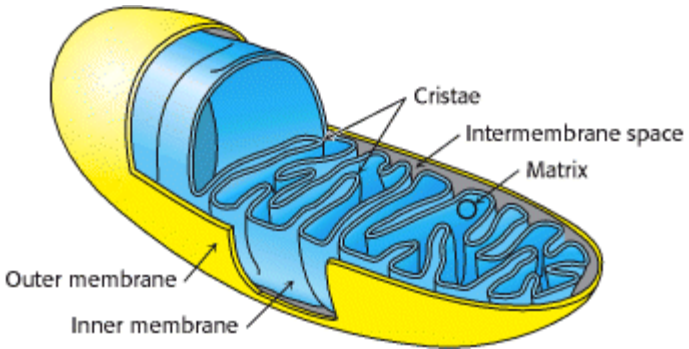
- **Outer membrane**： porous
- **Inner membrane**： much tighter， as a barrier to many biological metabolites, highly folded into cristae
- **Intermembrane space**
- **Matrix**

Most vertebrate 脊椎动物cells contain several hundred mitochondria



Outer mitochondrial membrane permeable to small molecules (*M*r 5,000) and ions--------- move freely through transmembrane channels formed by a family of integral membrane proteins called porins孔蛋白.

Inner membrane impermeable to most small molecules and ions, including protons (H)------ only through specific transporters components of the respiratory chain and the ATP synthase.



Respiratory proteins are bound to the inner membrane

Density of cristae corresponds to the respiratory activity of a cell

For example

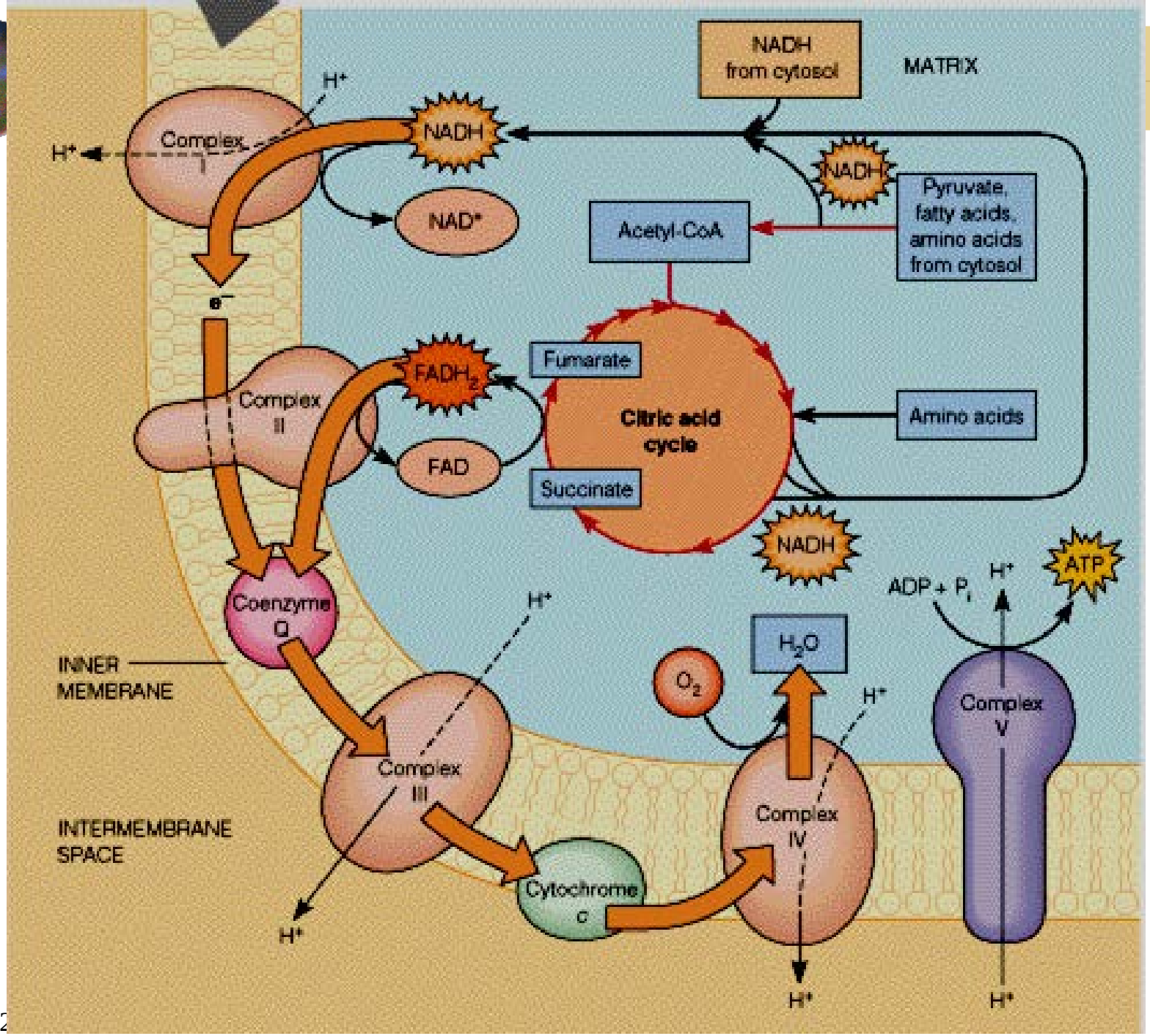
•Mitochondria in heart muscle cells (high rates of respiration) are densely packed with cristae

•Mitochondria in liver cells (low rates of respiration) have more sparsely distributed cristae

The total inner membrane of mitochondria is equal to 17 folds of plasma membrane

Processes occurring inside the mitochondrial matrix :

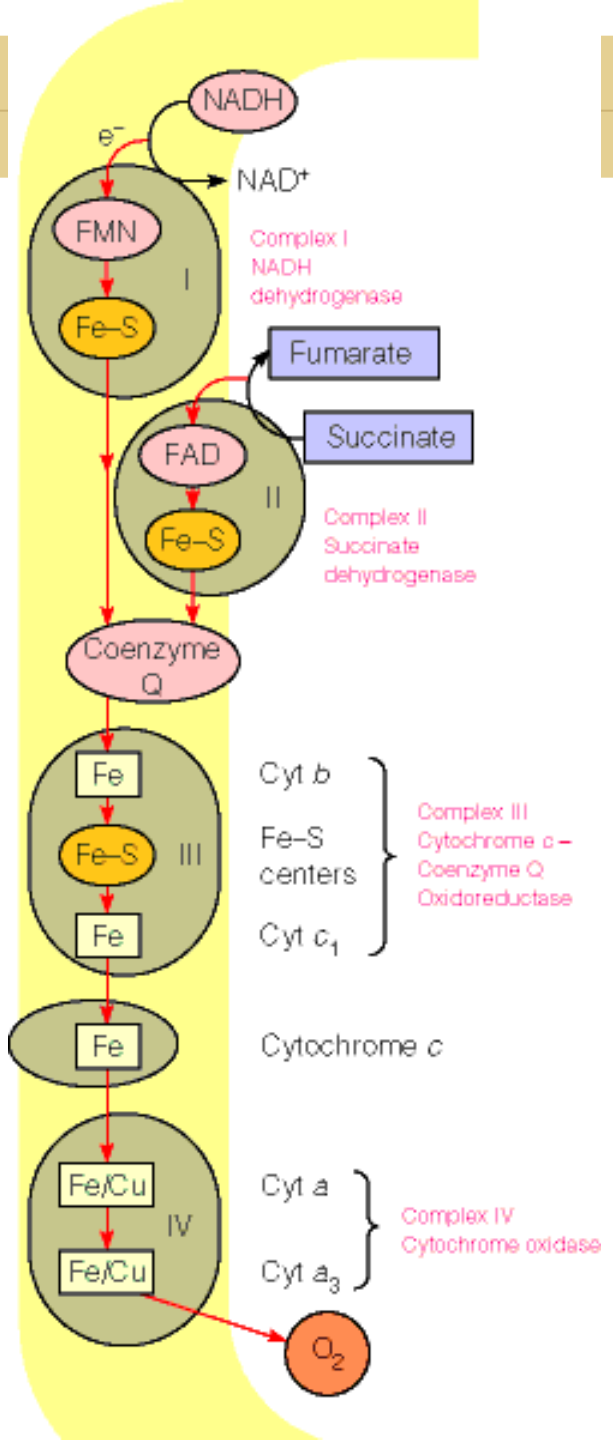
- **pyruvate oxidation**
- **fatty acid oxidation**
- **amino acid metabolism**
- **citric acid cycle**


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

Protein electron carriers-- embedded within the inner membrane

- Five multiprotein complexes named I, II, III, IV, and V
- Smaller carriers

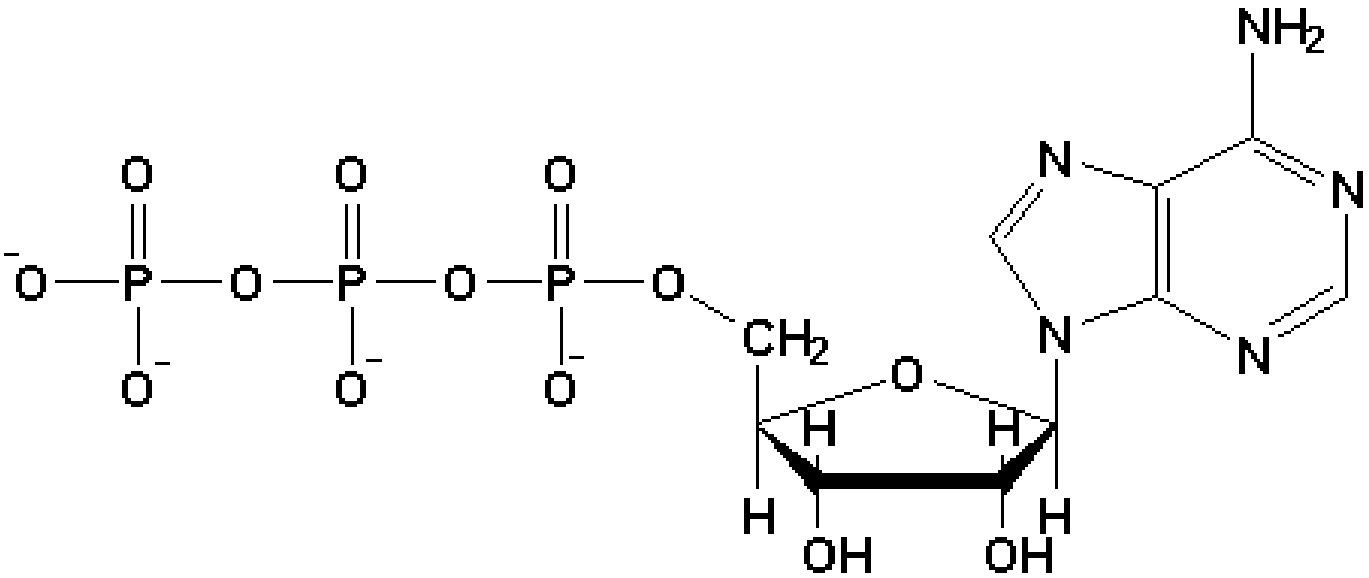
  coenzyme Q and cytochrome



3. Energy Generation

3. 1 ATP ------general "free energy currency"

drive countless biochemical reactions: cell motility, muscle contraction, and the specific transport of substances across membranes.

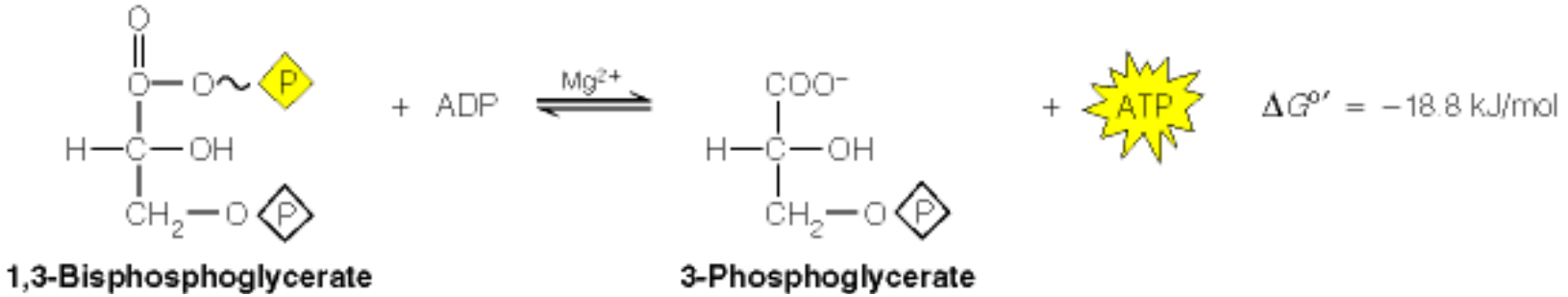


Adenosine triphosphate (ATP)

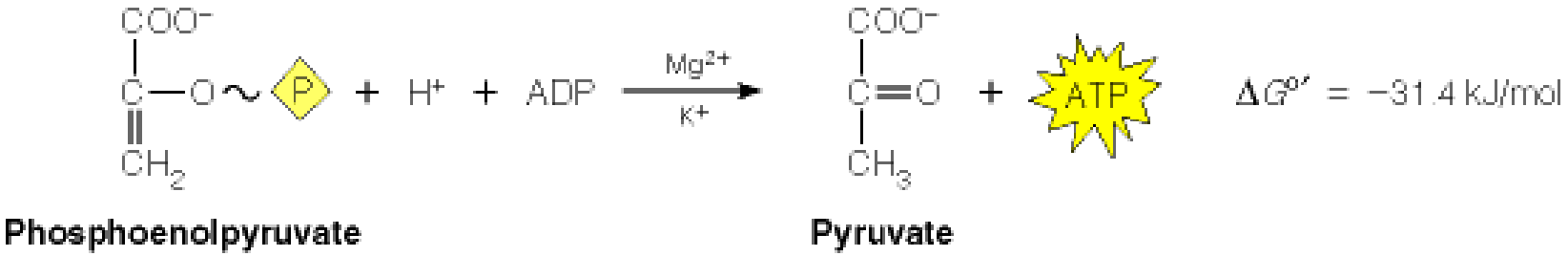
3.2 ATP is produced in the cell from ADP as a result of three types of phosphorylations –

- Substrate-level phosphorylations: occur when a "high-energy" phosphate containing molecule transfers phosphate to ADP in a chemical reaction to form ATP
- Oxidative phosphorylation
- Photosynthetic phosphorylation (in plants)

Substrate-level phosphorylations

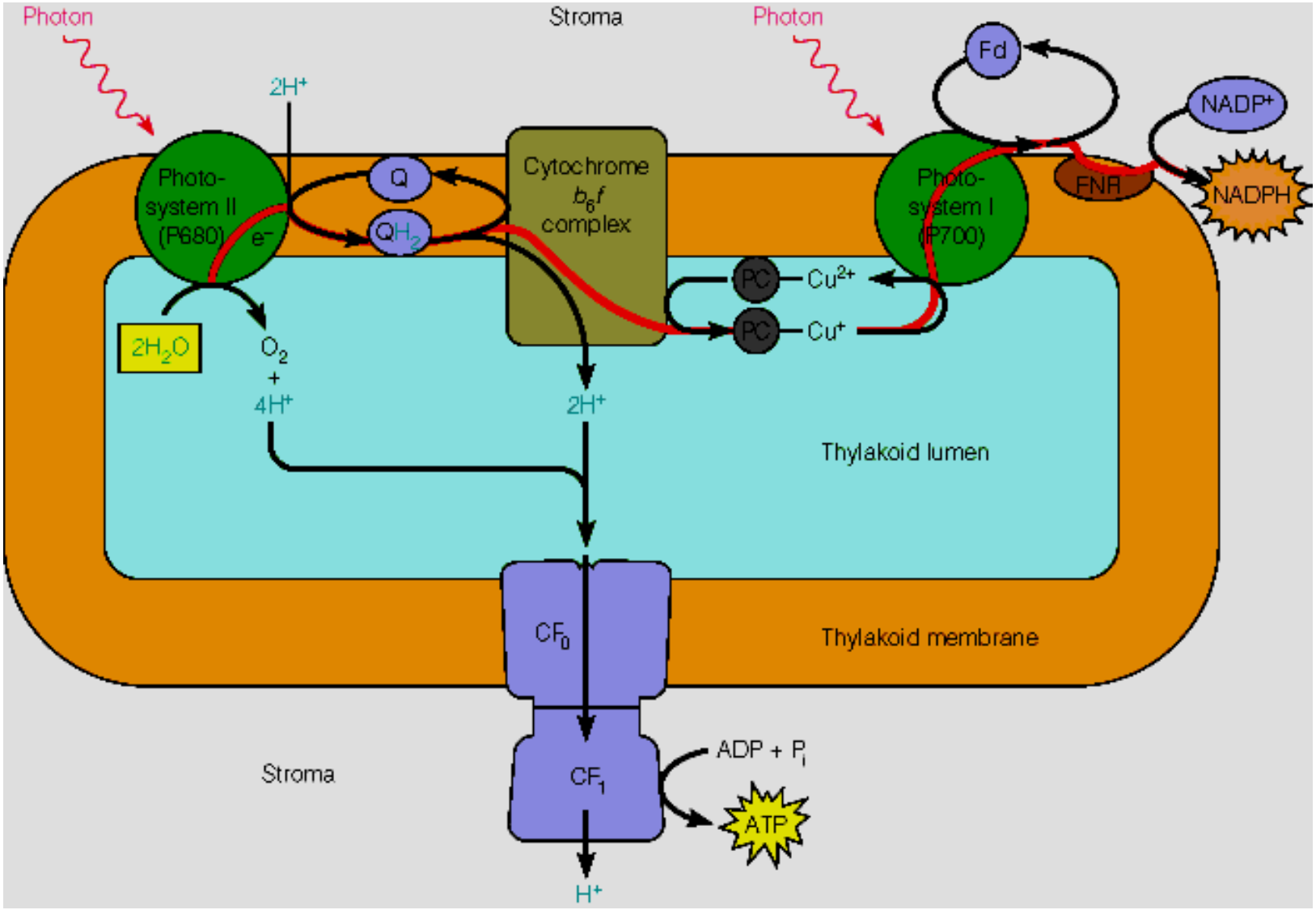


1,3BPG + ADP <=> 3PG + ATP



PEP + ADP <=> Pyruvate+ ATP

Photosynthetic phosphorylation: 叶绿体类囊体膜中



4. Electron Transport

- Biological oxidations generate reduced electron carriers NADH, $FADH_2$
- Reduced electron carriers donate their electrons to acceptor molecules and become reoxidized in the process
- The acceptor molecules are reduced

Reductant + oxidant <=>
Oxididized reductant (has lost electrons) + Reduced oxidant (has gained electrons)

Definition

Electron transport system (电子传递体系) is 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.

4.1 Some electron carriers in multienzyme complexs

The Respiratory Chain Consists of Four Complexes: Three Proton Pumps and a Physical Link to the Citric Acid Cycle

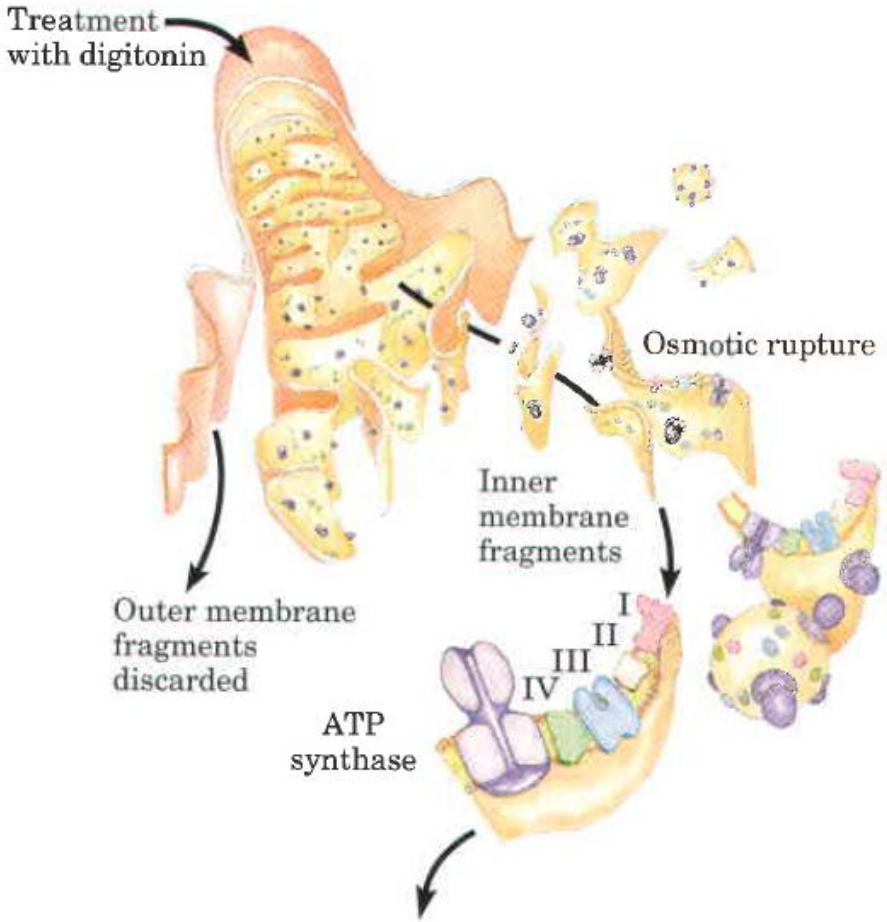
TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

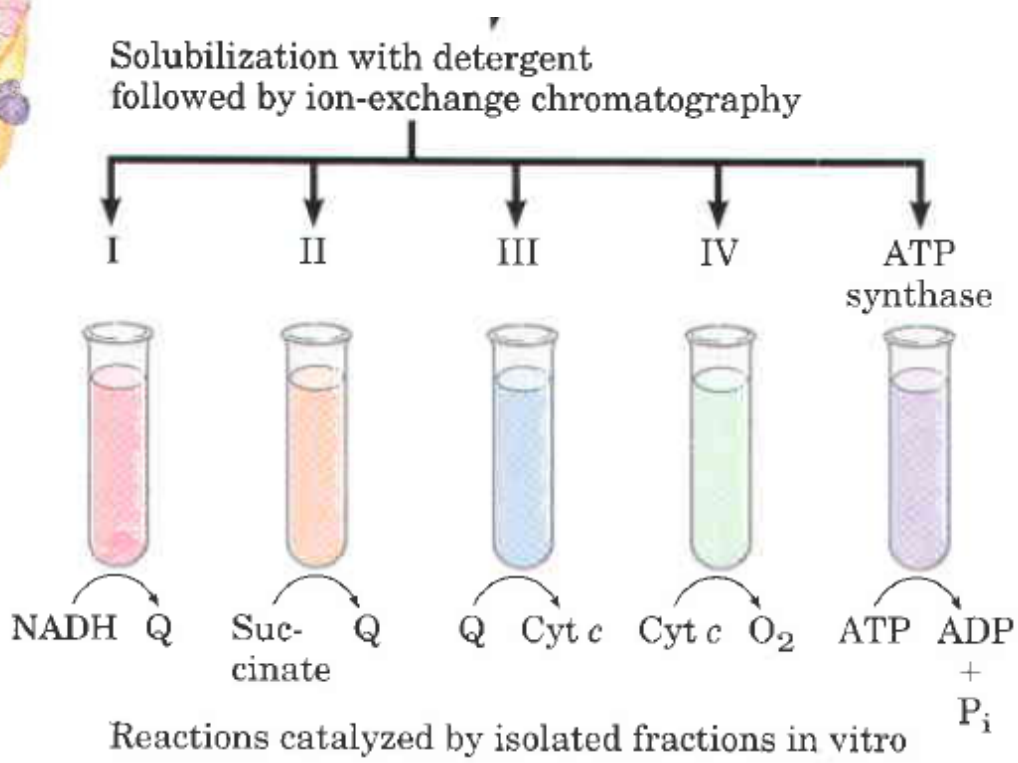
Enzyme complex/protein	Mass (kDa)	Number of subunits*	Prosthetic group(s)
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone:cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome *c*†	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

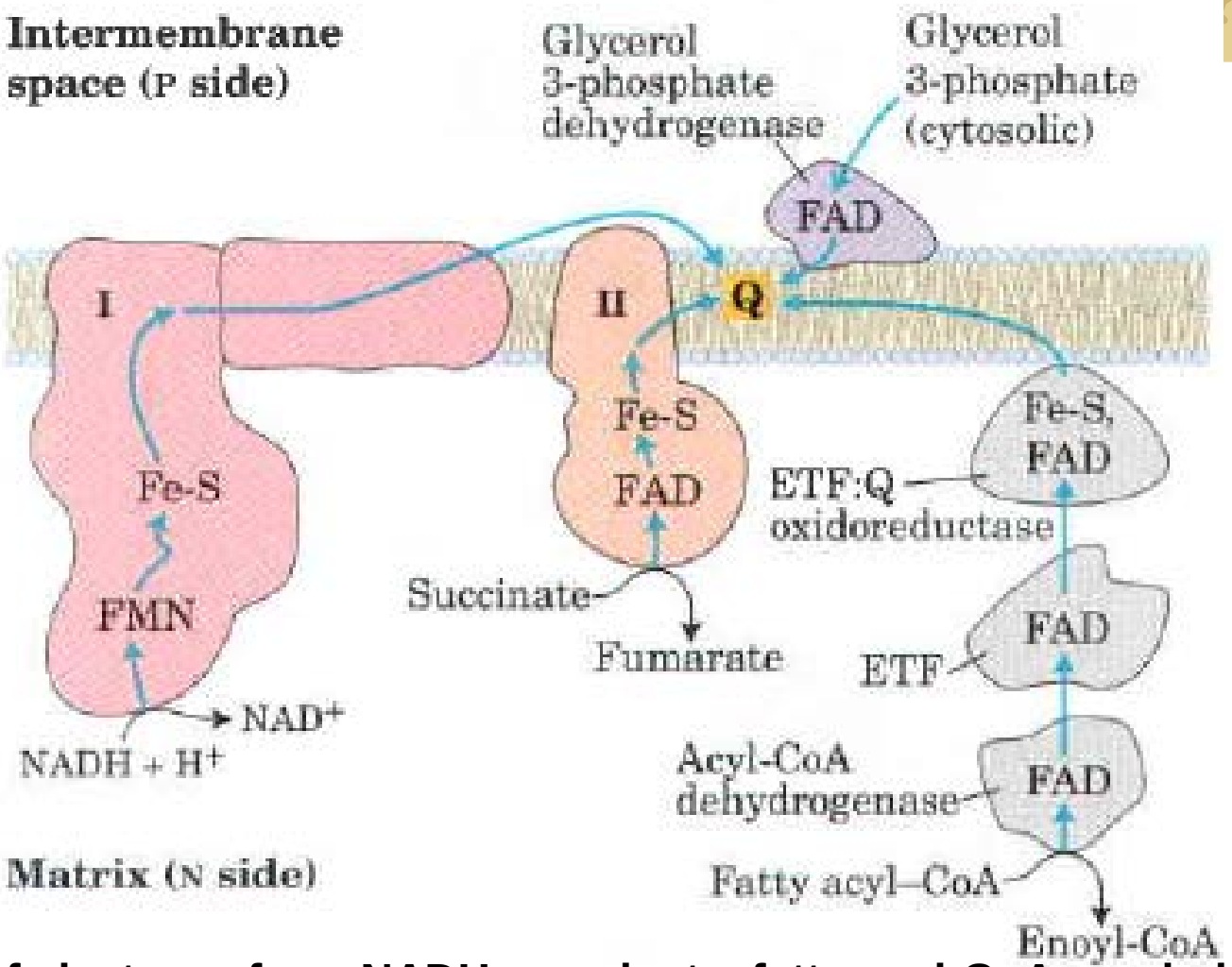
*Numbers of subunits in the bacterial equivalents in parentheses.

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

Separation of functional complexes of the respiratory chain.

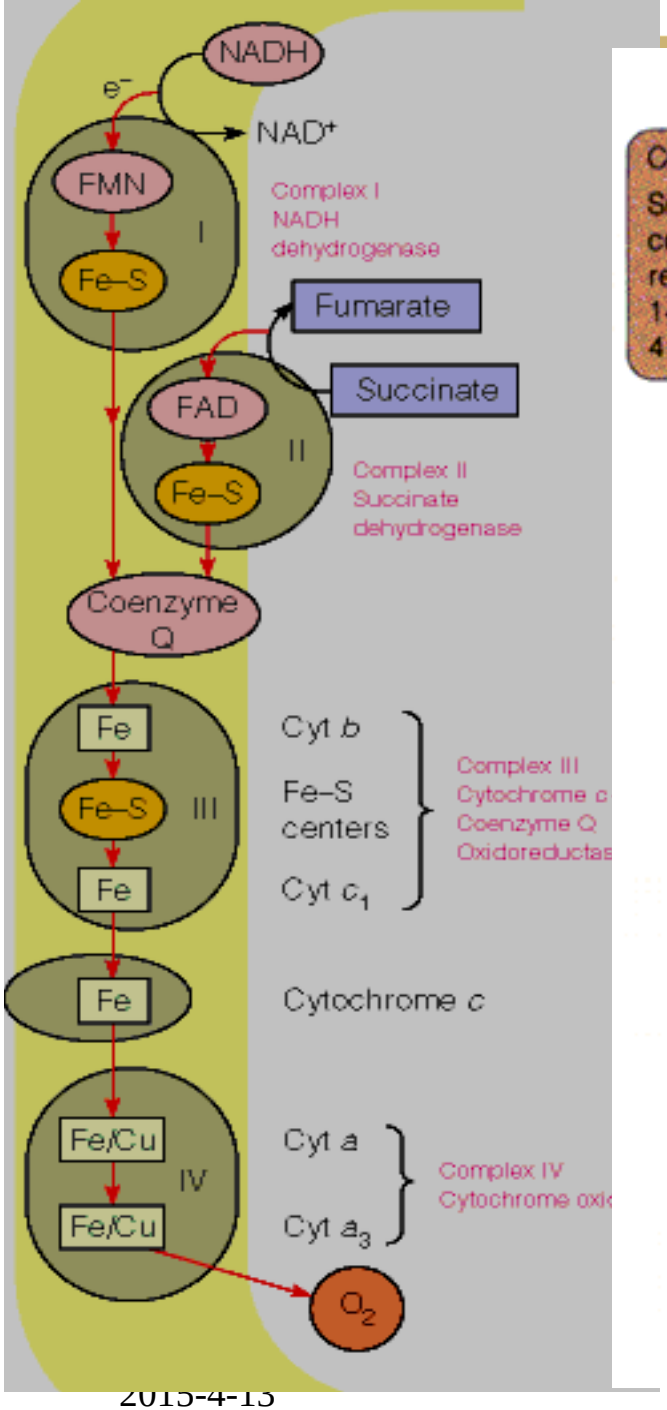


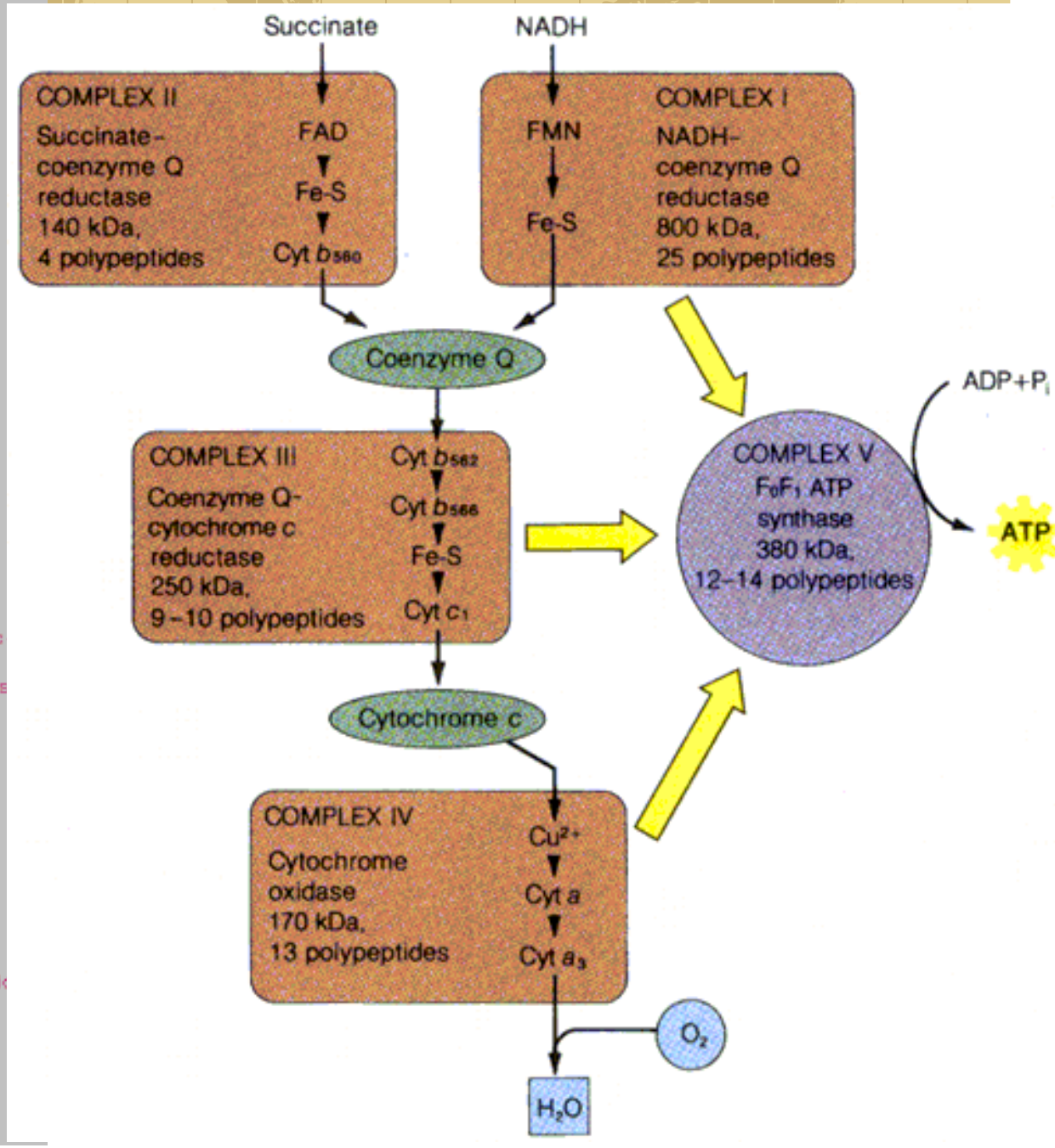




Path of electrons from NADH, succinate, fatty acyl-CoA, and glycerol 3 – phosphate to ubiquinone.

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 I I) on the way to Q


NADH
e⁻
NAD⁺
FMN
Fe–S
I
Complex I
NADH
dehydrogenase
Fumarate
FAD
Fe–S
II
Succinate
Complex II
Succinate
dehydrogenase
Coenzyme
Q
Fe
Fe–S
III
Fe
Cyt b
Fe–S
centers
Cyt c1
Complex III
Cytochrome c
Coenzyme Q
Oxidoreductas
Fe
Cytochrome c
Fe/Cu
IV
Fe/Cu
Cyt a
Cyt a3
Complex IV
Cytochrome oxi
O2


Succinate
NADH
COMPLEX II
Succinate–
coenzyme Q
reductase
140 kDa,
4 polypeptides
FAD
Fe-S
Cyt b560
FMN
Fe-S
COMPLEX I
NADH–
coenzyme Q
reductase
800 kDa,
25 polypeptides
Coenzyme Q
COMPLEX III
Coenzyme Q–
cytochrome c
reductase
250 kDa,
9–10 polypeptides
Cyt b562
Cyt b566
Fe-S
Cyt c1
COMPLEX V
F0F1 ATP
synthase
380 kDa,
12–14 polypeptides
ADP+Pi
ATP
Cytochrome c
COMPLEX IV
Cytochrome
oxidase
170 kDa,
13 polypeptides
Cu2+
Cyt a
Cyt a3
O2
H2O

4.1.2 The High-Potential Electrons of NADH Enter the Respiratory Chain at NADHQ Oxidoreductase.

$$NADH + Q + 5\,H^+_{matrix} \longrightarrow NAD^+ + QH_2 + 4\,H^+_{cytosol}$$

NADH is reoxidized to NAD^+ by complex I of the mitochondria (also called NADH dehydrogenase).

NADH dehydrogenase contains flavin mononucleotide (FMN)

NADH + H^+ + FMN <=> NAD^+ + FMNH2

Complex I contains about 25 separate polypeptide chains

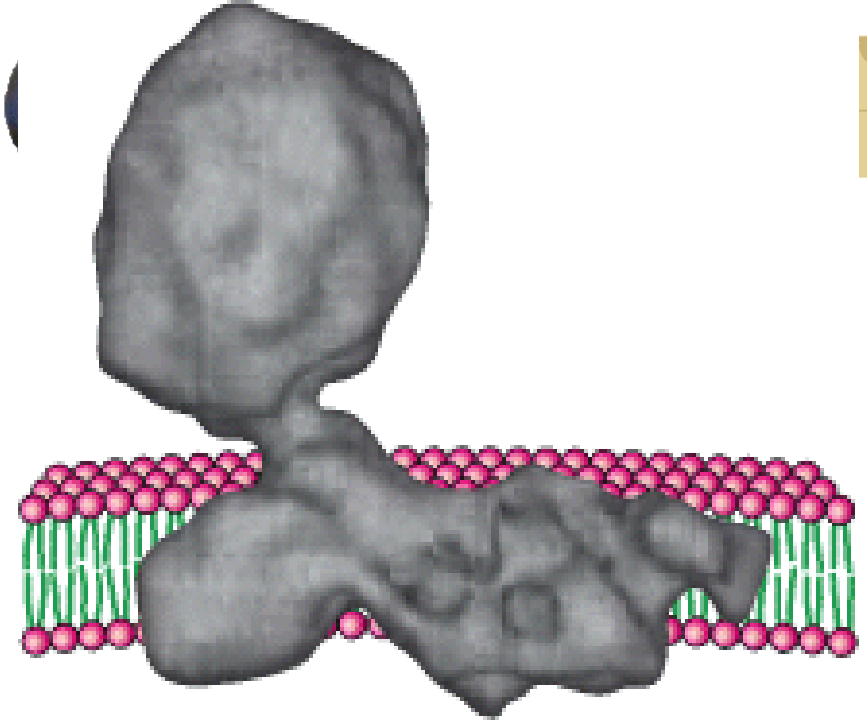
NADH is generated by numerous dehydrogenases in the cell

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

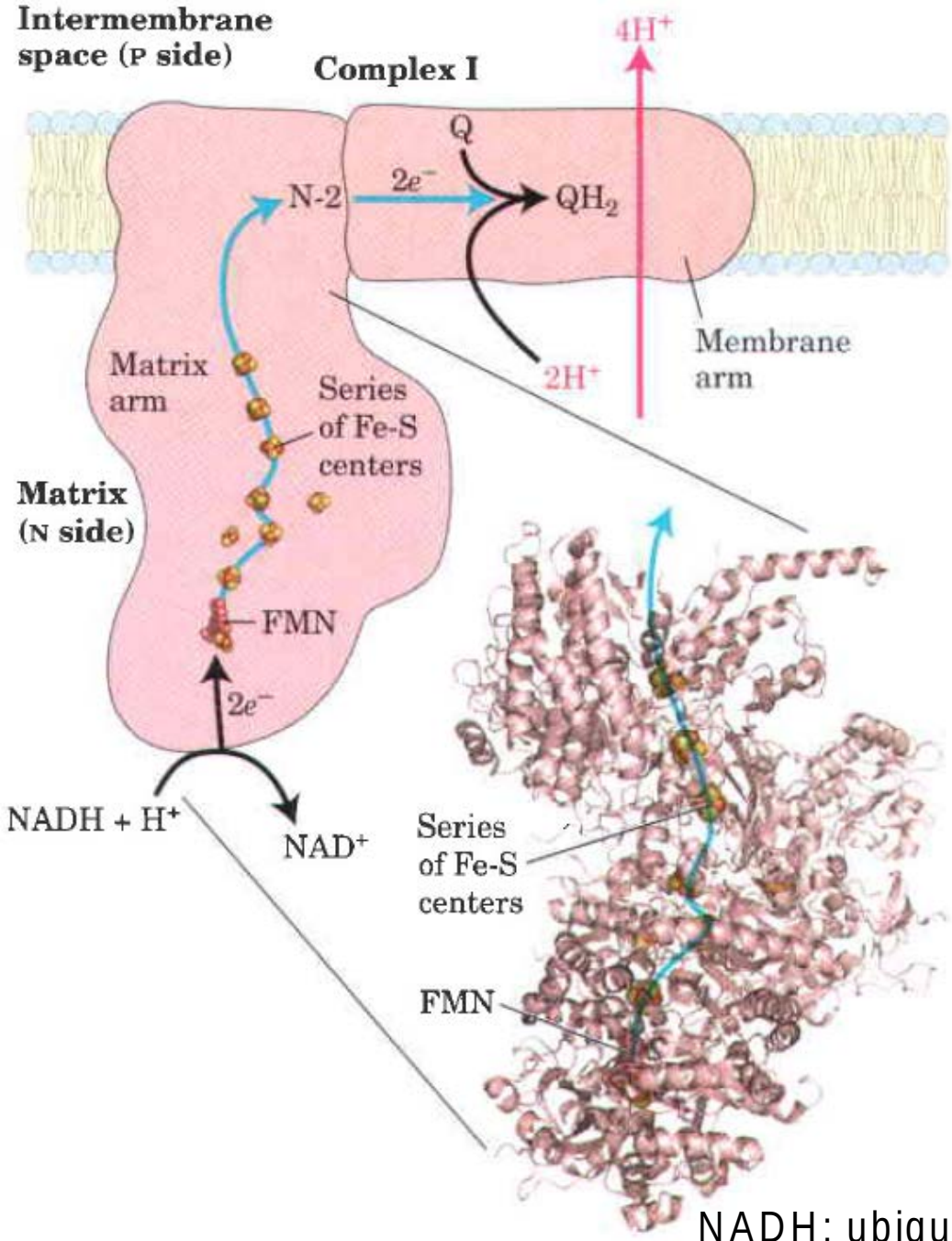
Reaction[*]	*Location*[†]
NAD-linked	
α-Ketoglutarate + CoA + $NAD^+ \rightleftharpoons$ succinyl-CoA + CO_2 + NADH + H^+	M
L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	M and C
Pyruvate + CoA + $NAD^+ \rightleftharpoons$ acetyl-CoA + CO_2 + NADH + H^+	M
Glyceraldehyde 3-phosphate + 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.



Structure of NADH-Q Oxidoreductase (Complex I).
The structure, determined by electron microscopy at 22-Å resolution， consists of a membrane-spanning part and a long arm that extends into the matrix. NADH is oxidized in the arm, and the electrons are transferred to reduce Q in the membrane.



NADH: ubiquinone oxidoreductase (Complex I).

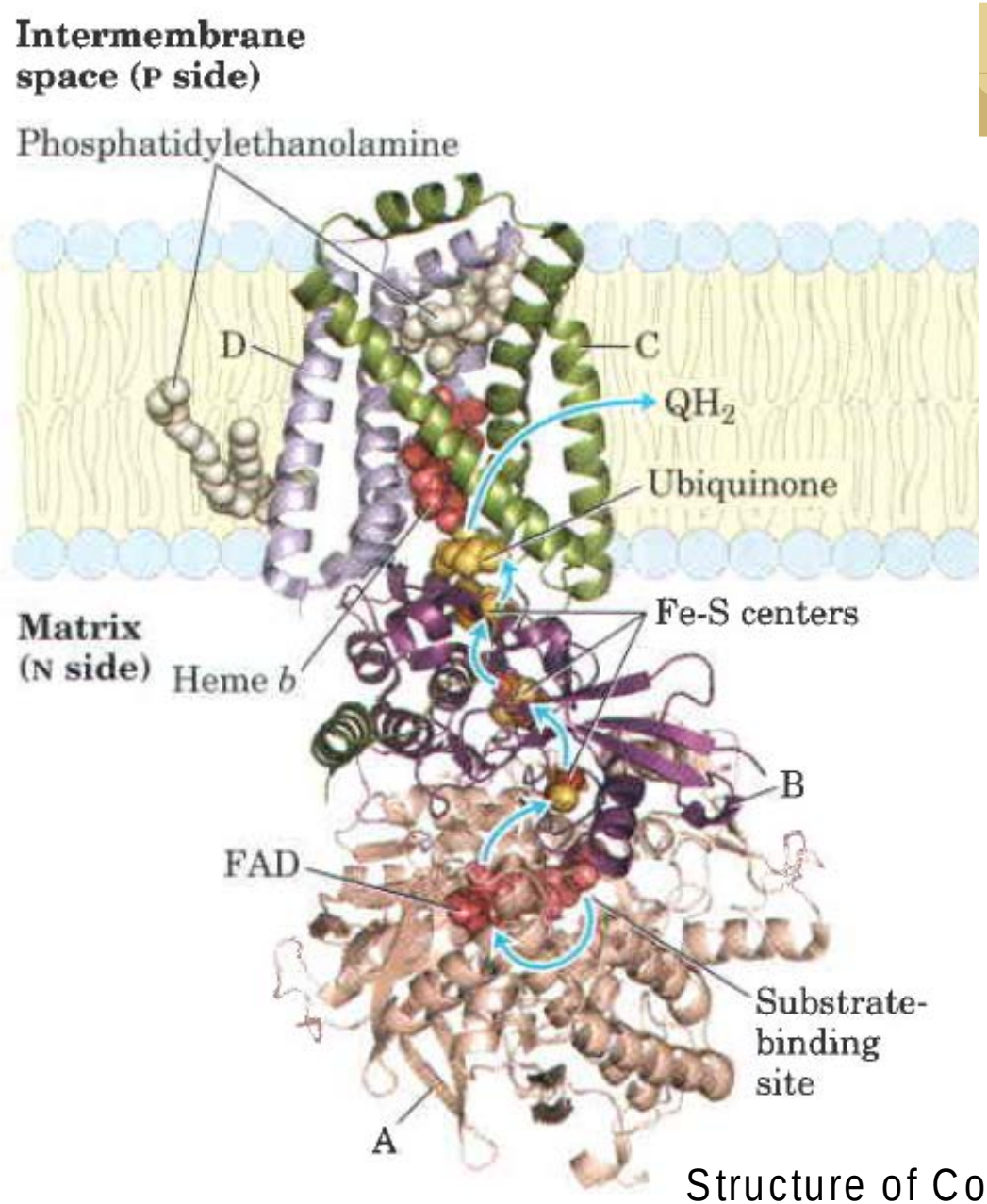
Complex II (succinate dehydrogenase)

Not in the path traveled by electrons from Complex I

Electrons from $FADH_2$ produced by the enzyme succinate dehydrogenase in the citric acid cycle

Both complexes I and II donate their electrons to the same acceptor, coenzyme Q

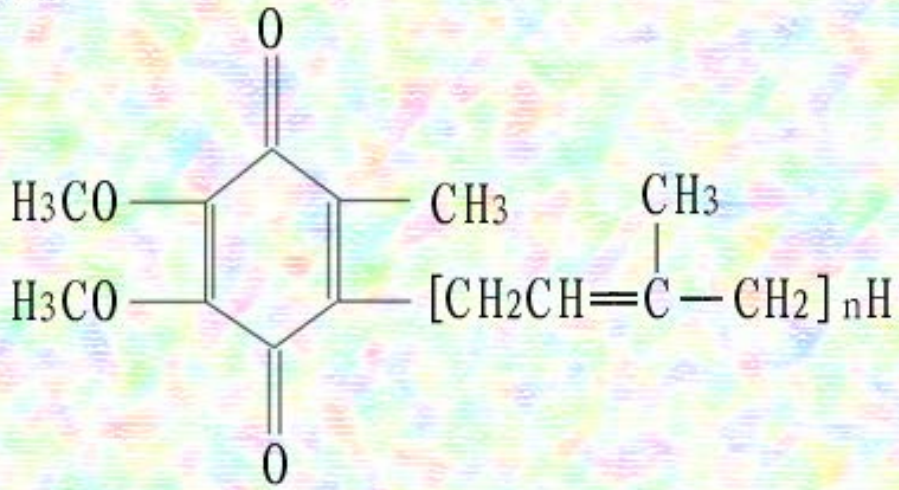
Contains iron-sulfur proteins, participate in electron transfer

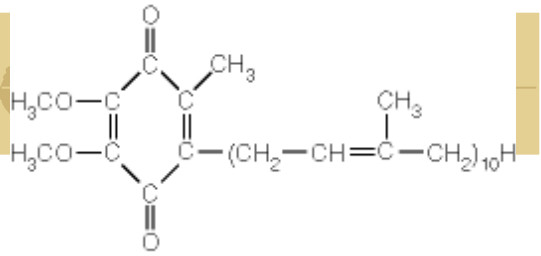


Structure of Complex II (succinated ehydrogenase). (PDB ID IZOY)

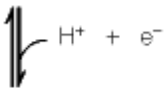
辅　酶　Q

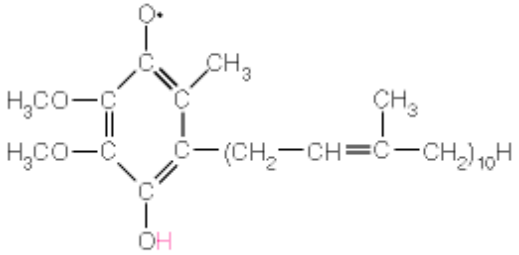
辅酶Q也是呼吸链中参与电子传递的一种辅酶。不同的辅酶Q只是侧链长度不同，其中醌基可被还原为羟基，称为氢醌。



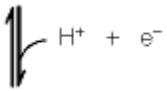


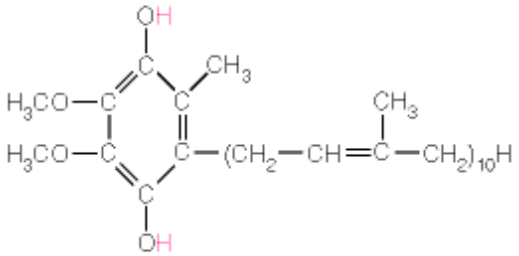
Oxidized coenzyme Q_{10} (CoQ)



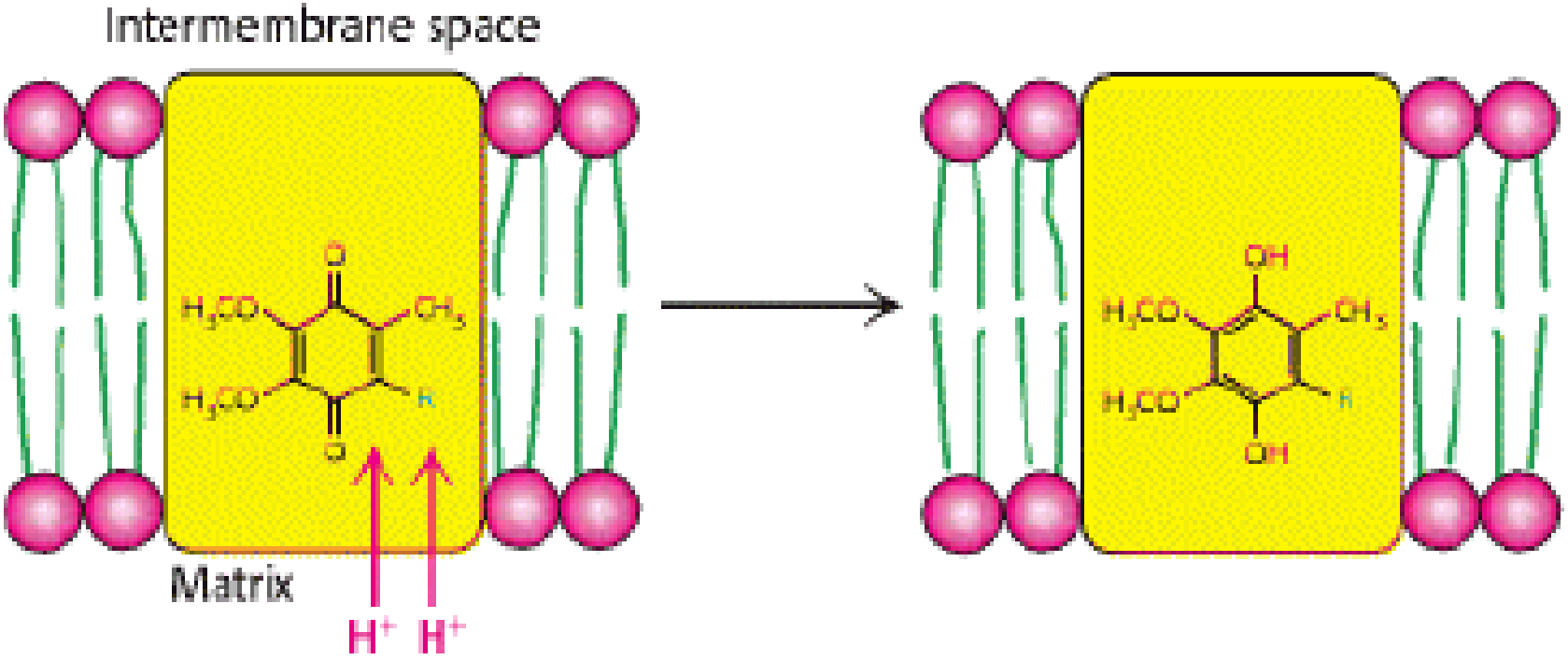


Semiquinone form of coenzyme Q





Reduced coenzyme Q_{10} ($CoQH_2$)



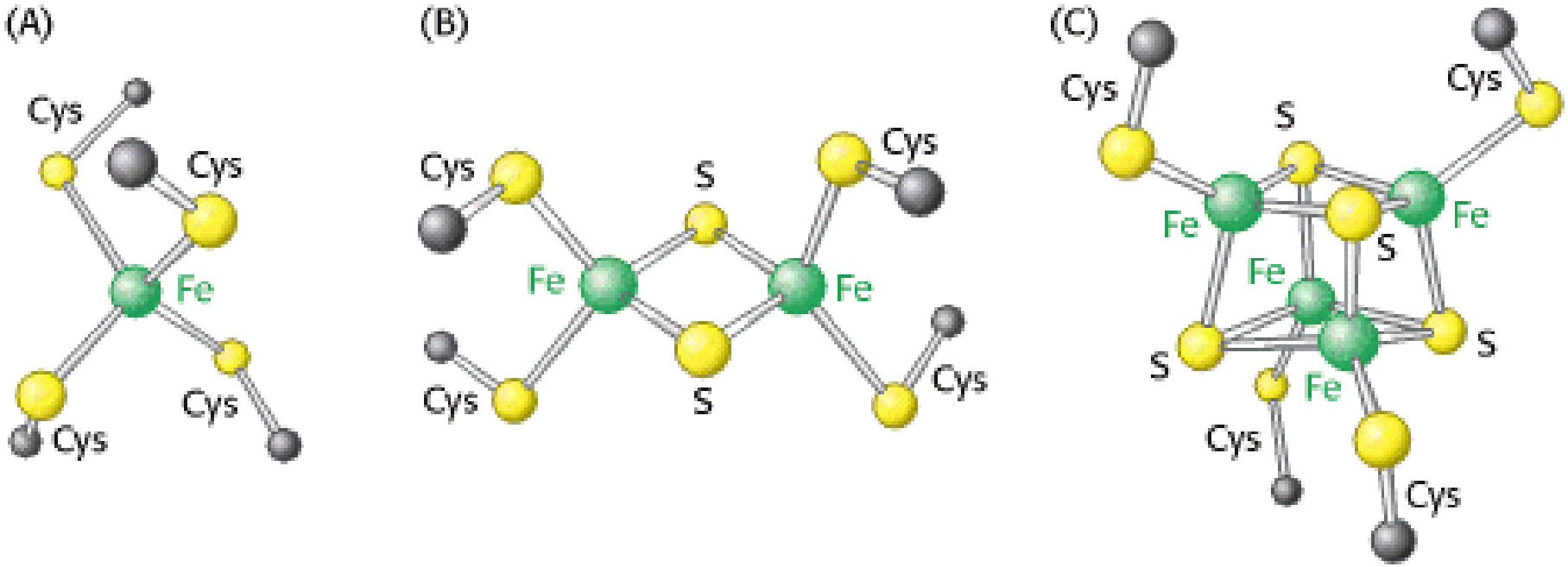
Coupled Electron-Proton Transfer Reactions

Complex III –

Contains a diversity of electron carrying proteins

Include cytochrome b, iron sulfur centers, and cytochrome C1.

$$QH_2 + 2\,\text{Cyt}\,c_{ox} + 2\,H^+_{matrix} \longrightarrow Q + 2\,\text{Cyt}\,c_{red} + 4\,H^+_{cytosol}$$



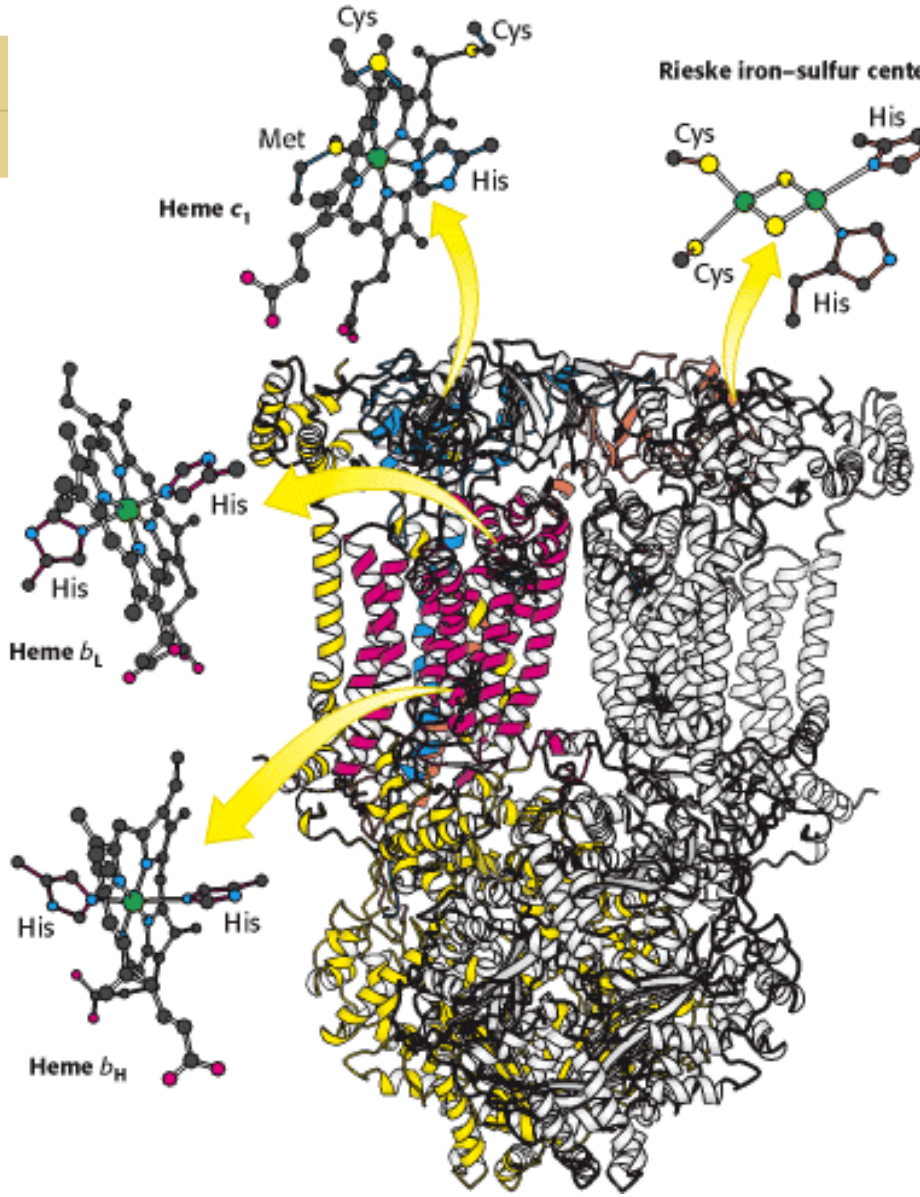
Iron-Sulfur Clusters.

(A)A single iron ion bound by four cysteine residues.

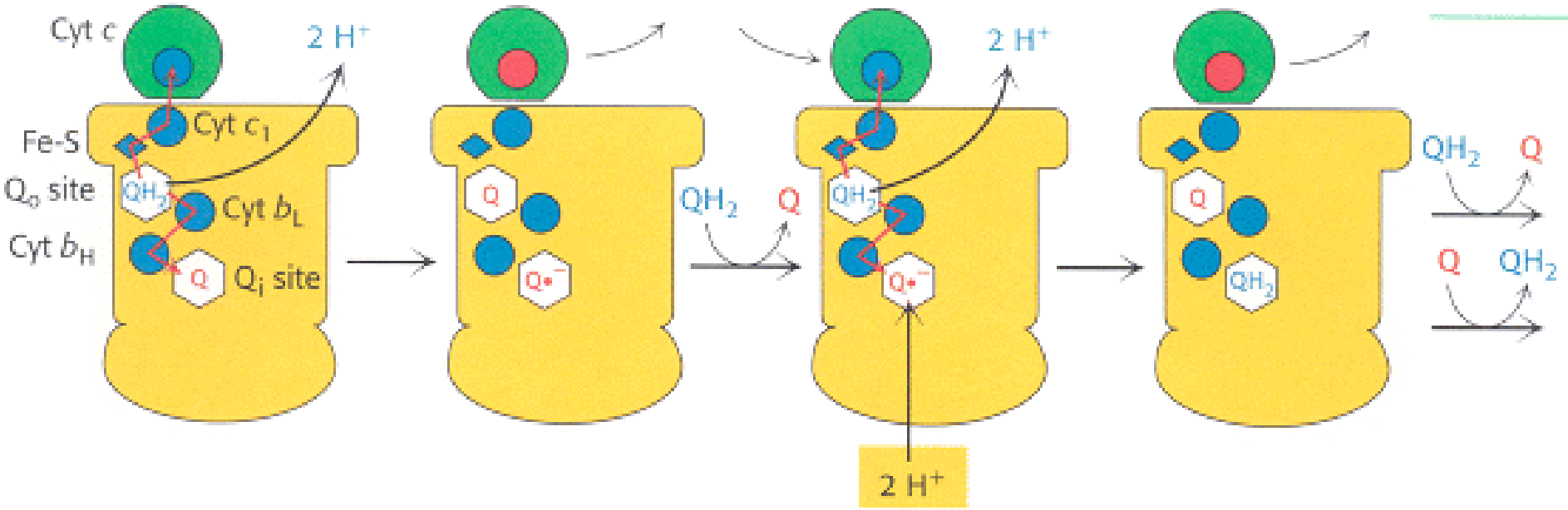
(B) 2Fe-2S cluster with iron ions bridged by sulfide ions.

(C) 4Fe-4S cluster.

Each of these clusters can undergo oxidation-reduction reactions.



Structure of Q-Cytochrome *C Oxidoreductase (Cytochrome BC1).*


Cyt c
2 H+
Cyt c1
Fe-S
Qo site
QH2
Cyt bL
Cyt bH
Q
Qi site
Q
Q•−
QH2
Q
2 H+
QH2
Q•−
Q
QH2
QH2
Q
Q
QH2
2 H+

Q Cycle

Cytochrome c

Small protein (Mr = 13,000)

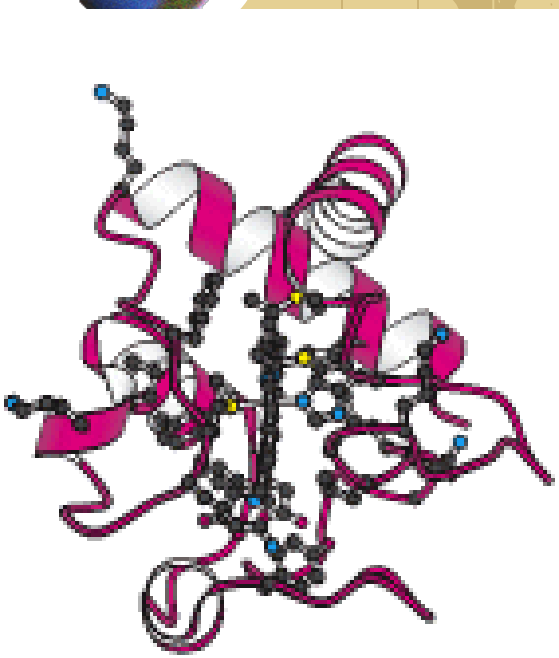
Associated with the inner membrane of the mitochondria

Readily extracted in soluble form.

The other cytochromes are integral membrane proteins.

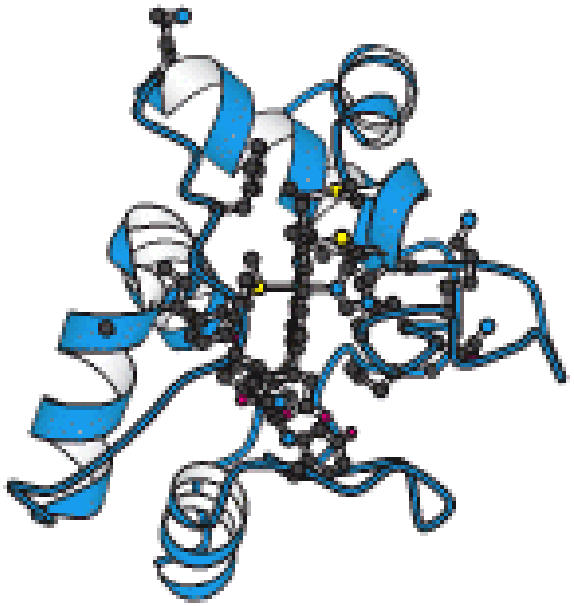
The amino acid sequence of the protein---highly conserved in evolution, nearly 50% identity (from yeast to human)

通过辅基中铁离子价的可逆变化，在复合体III与Ⅳ之间传递电子



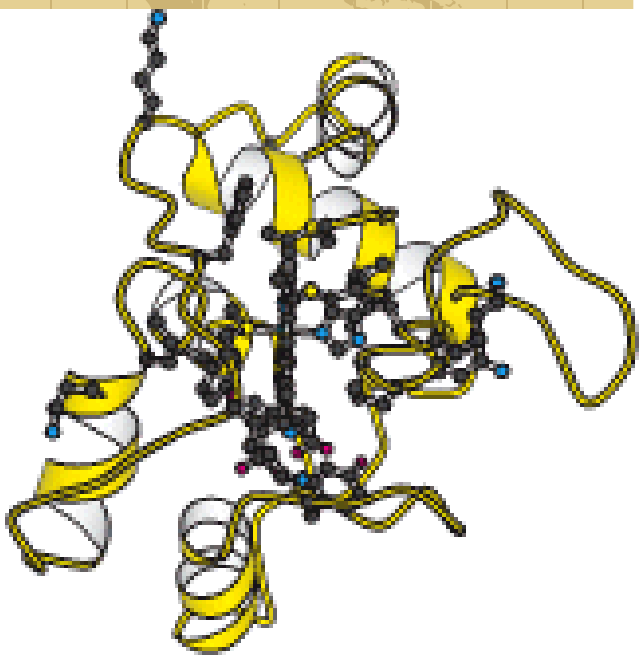
Tuna

金枪鱼



Rhodospirillum rubrum

红螺菌



Paracoccus denitrificans

. 副球菌

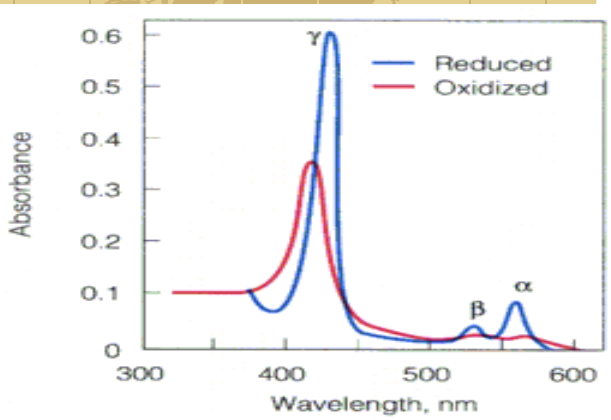
Conservation of the Three-Dimensional Structure of Cytochrome *C.*
The side chains are shown for the 21 conserved amino acids and the heme

在电子传递链的组分中UQ和Cytc是可移动的

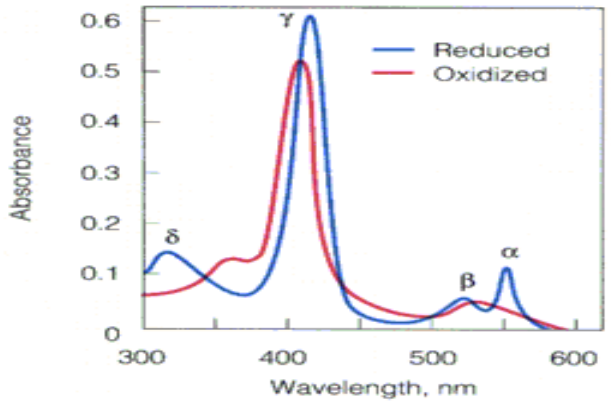
UQ是一类脂溶性的苯醌衍生物，能在膜脂质内自由移动，通过醌/酚结构互变，在传递质子、电子中起“摆渡”作用。是复合体Ⅰ/Ⅱ与Ⅲ之间的电子载体。

Cytochromes — are proteins, strong absorption of visible light.

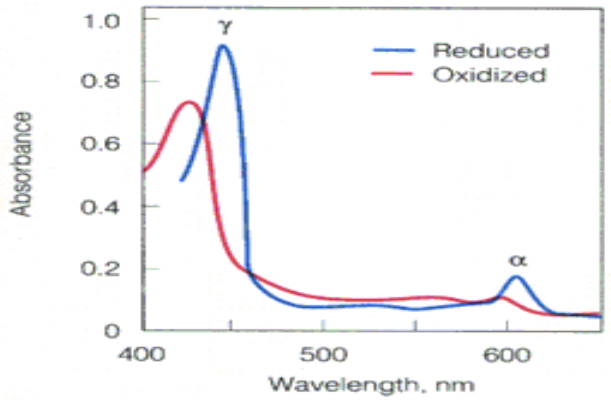
Each type of cytochrome in its reduced (Fe^{2+}) state has three absorption bands in the visible range.



(a) Cytochrome *b*



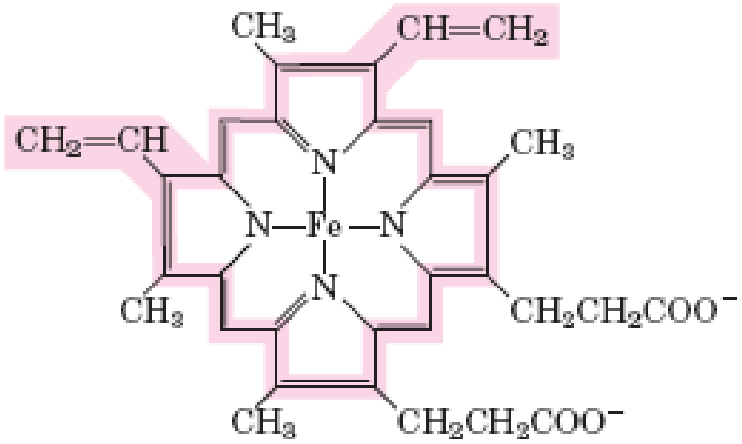
(b) Cytochrome *c*



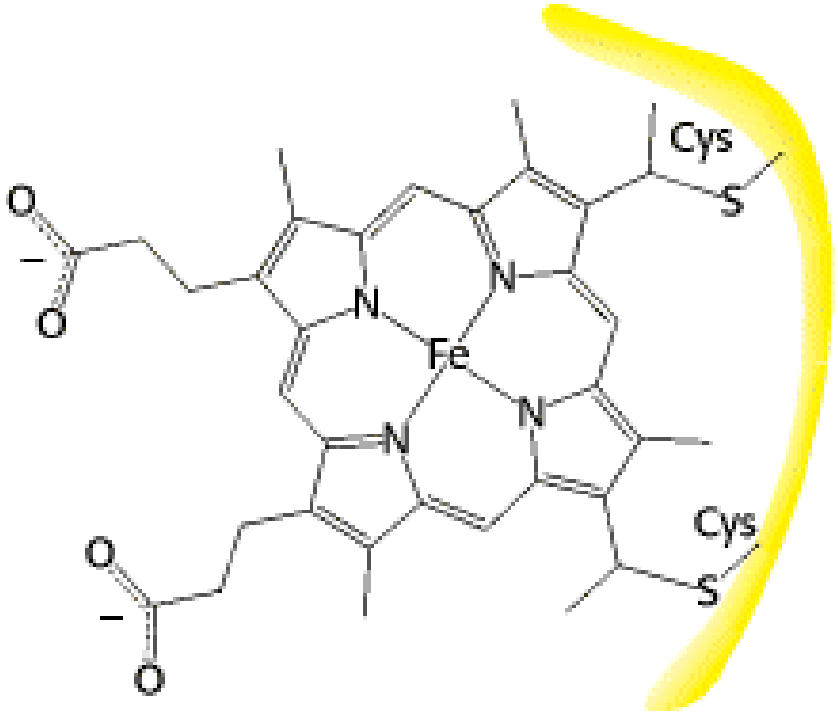
(c) Cytochromes *a* and a_3

Cytochromes are a group of red or brown **heme**亚铁血红素

porphyrin卟啉-- cyclic structure, consists of four five-membered, nitrogen-containing rings, The four nitrogen atoms are coordinated with a central Fe ion, either Fe^{2+} or Fe^{3+}.



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



Attachment of *C -Type Cytochromes*

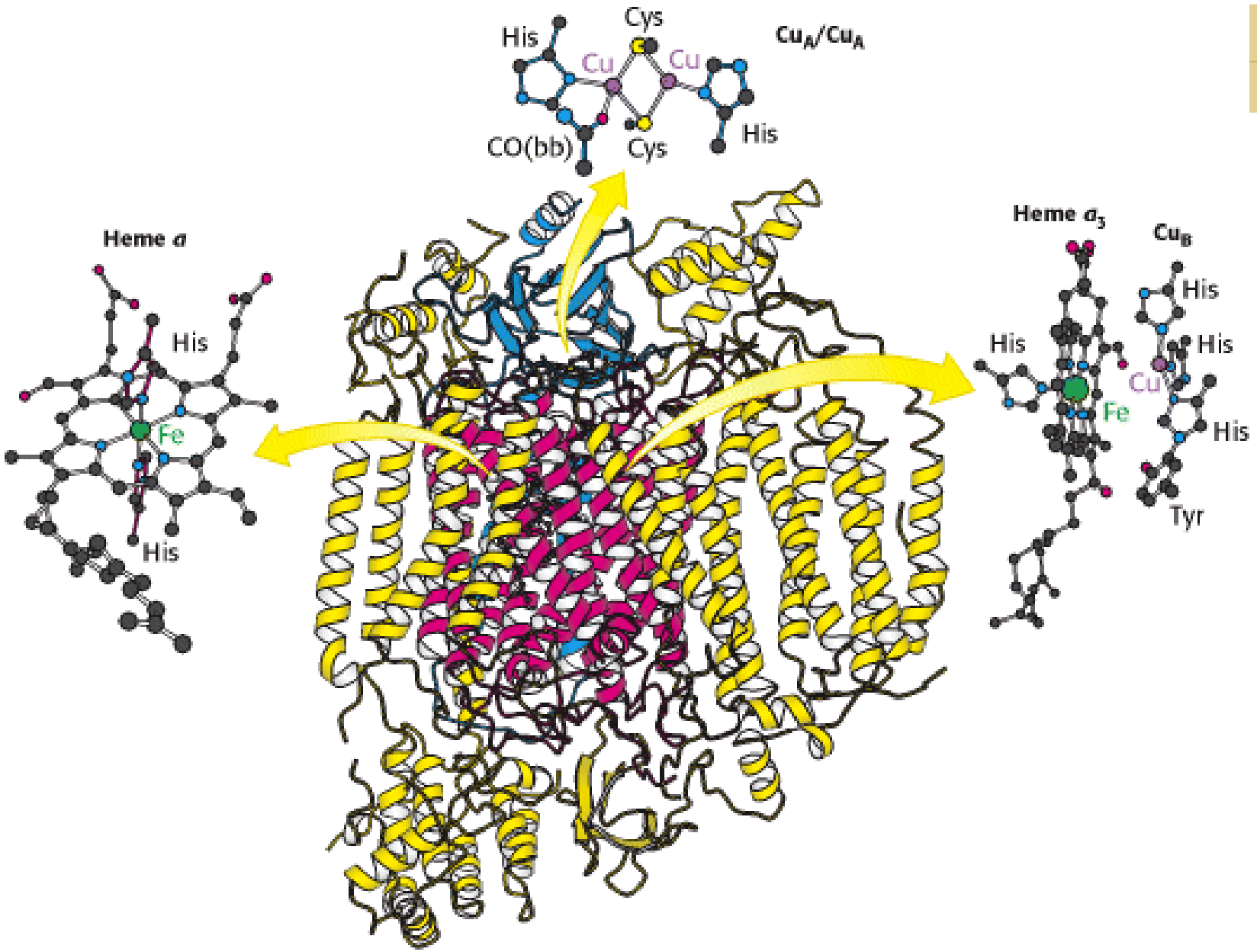
A heme group is covalently attached to a protein through thioether硫醚 linkages formed by the addition of sulfhydryl groups of cysteine residues to vinyl groups on protoporphyrin.

Complex IV

Cytochrome oxidase 细胞色素氧化酶

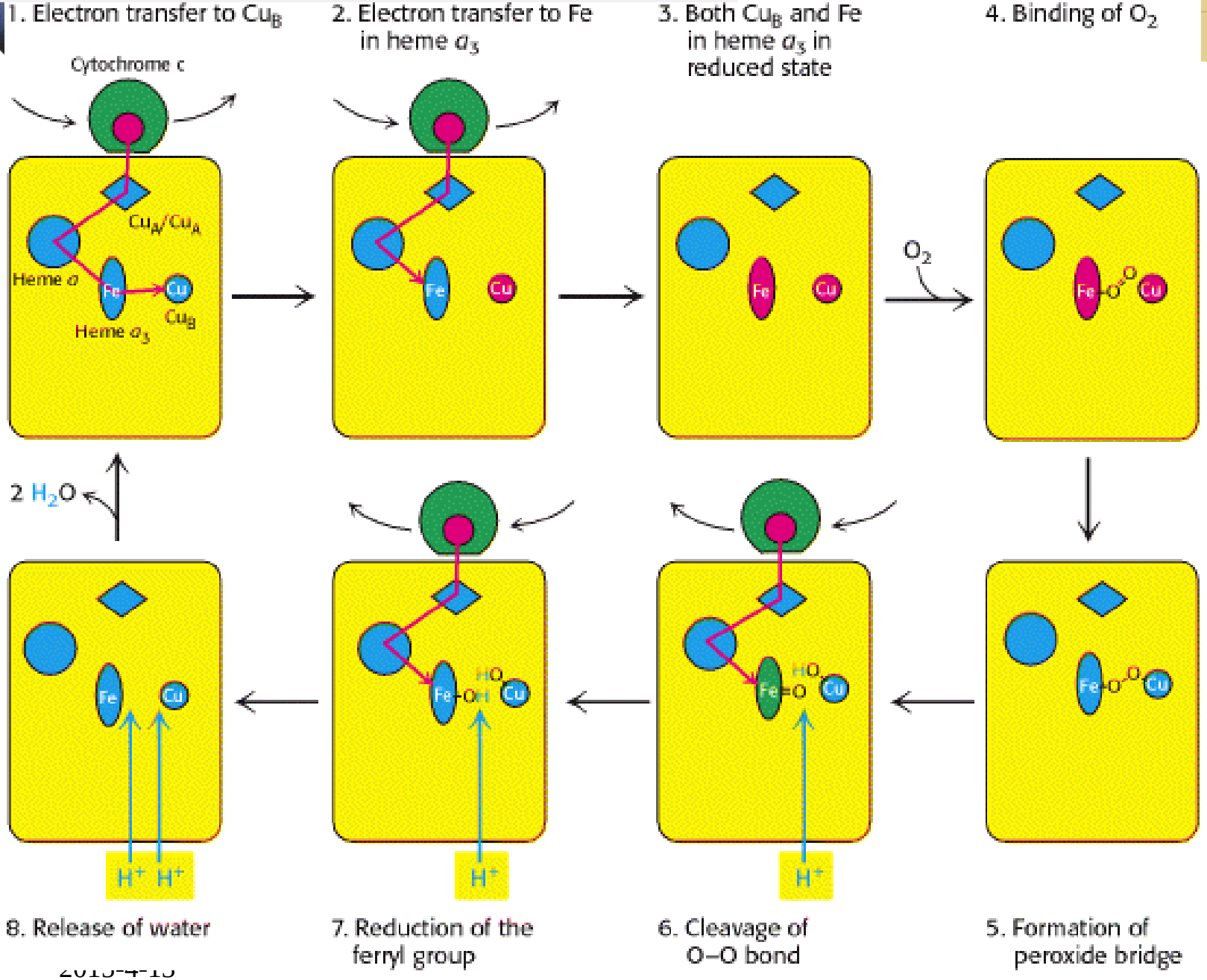
Contains cytochromes a and a3

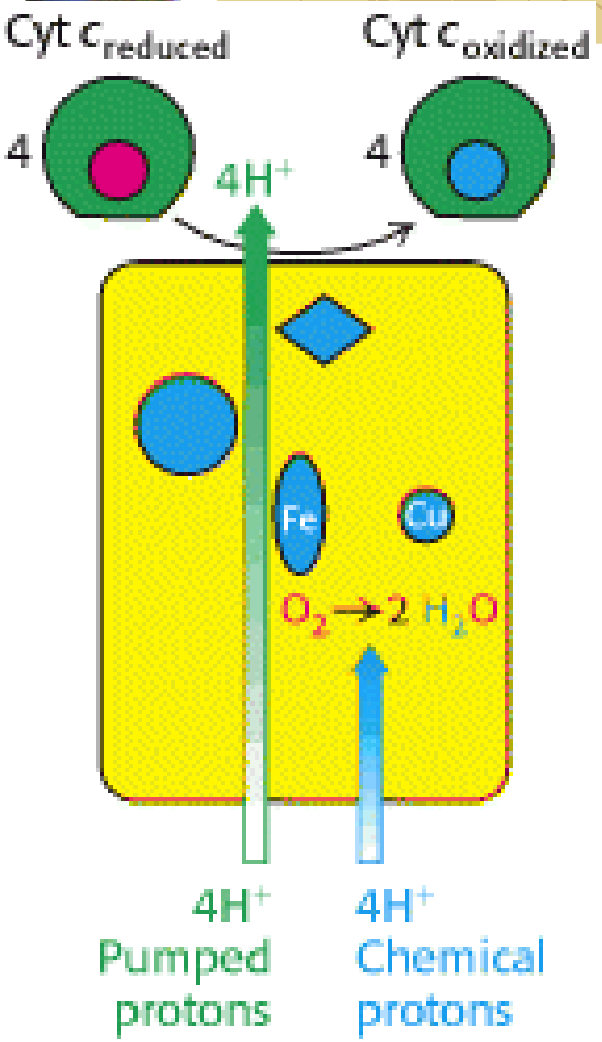
Takes electrons from cytochrome c



Structure of Cytochrome *C Oxidase.*

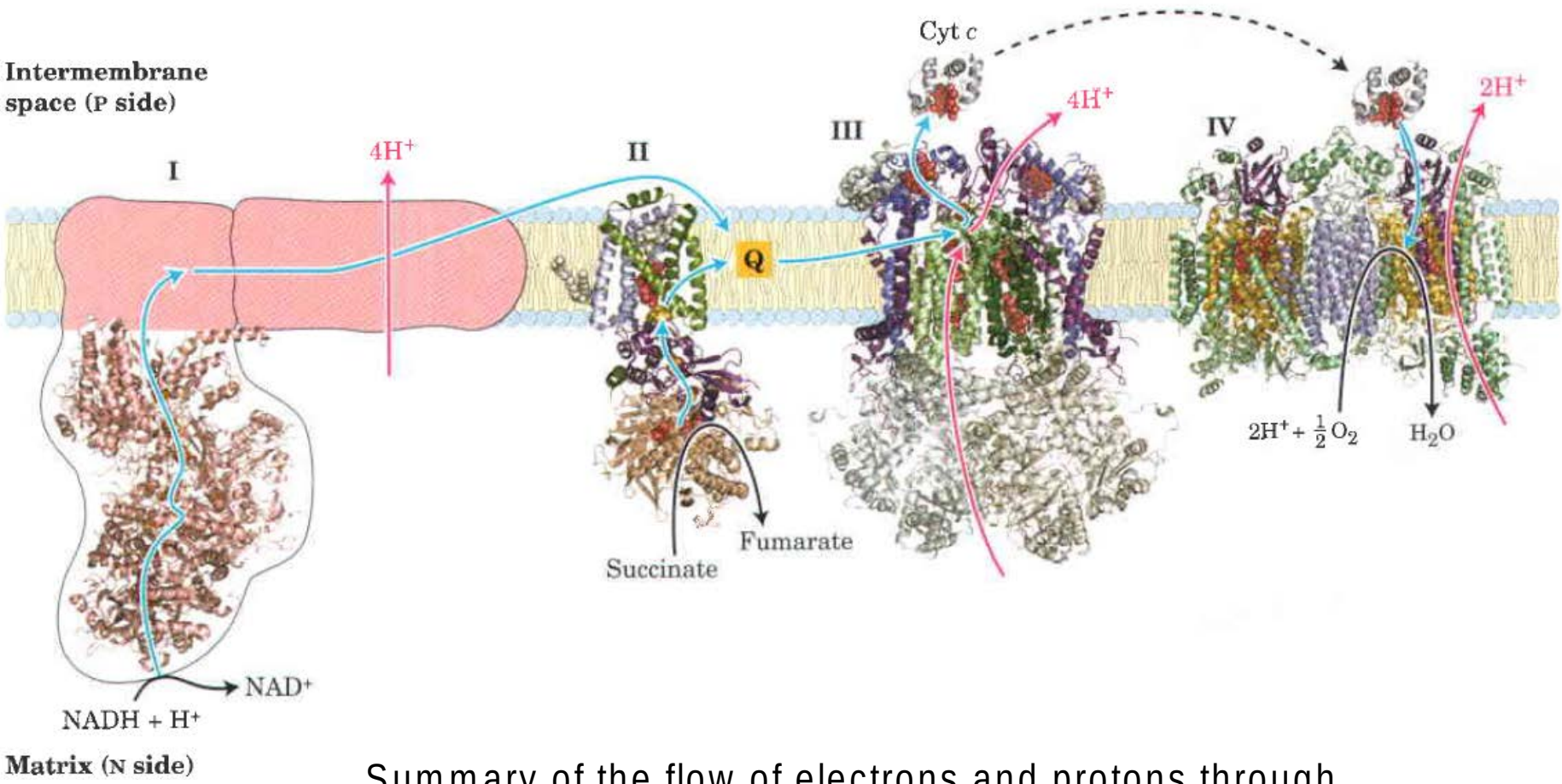
Cytochrome Oxidase Mechanism.





Proton Transport by Cytochrome *C Oxidase.*

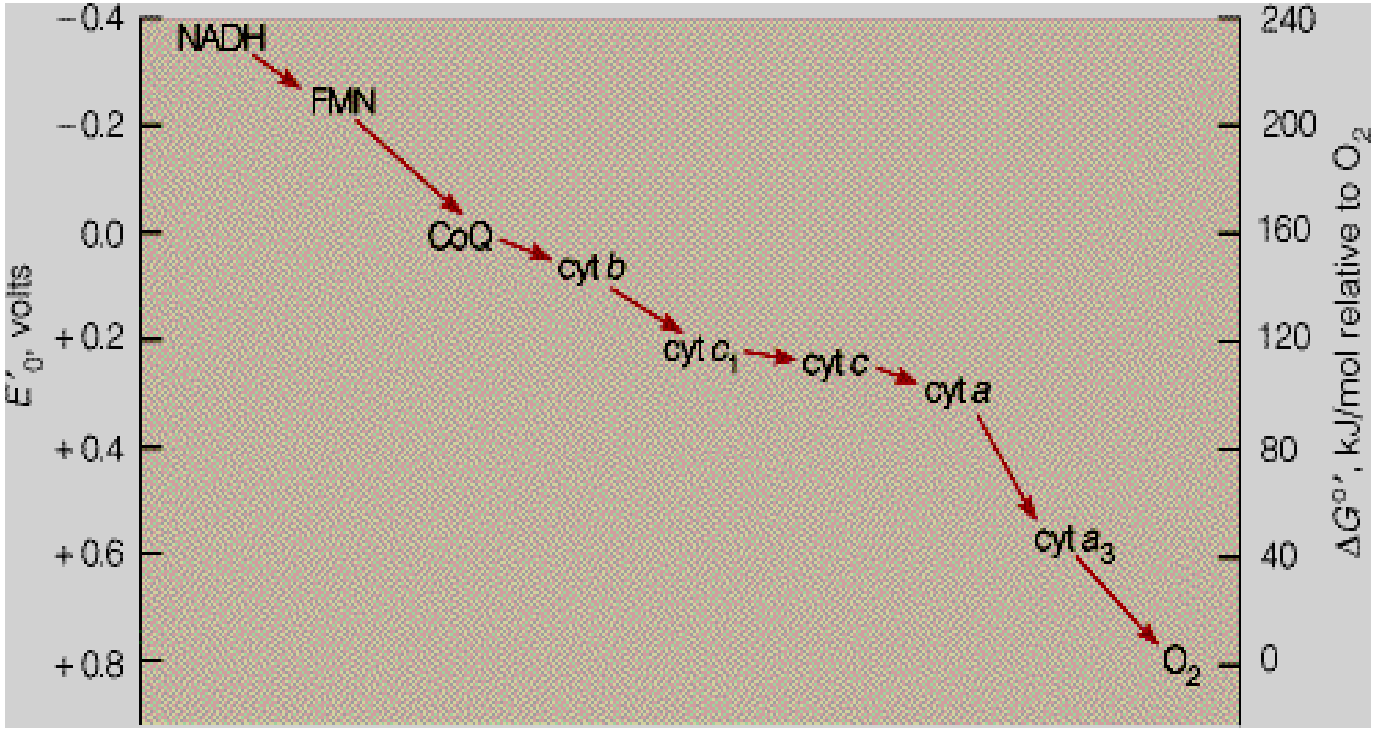
$$4\ \text{Cyt}\ c_{red} + 4\ H^+_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

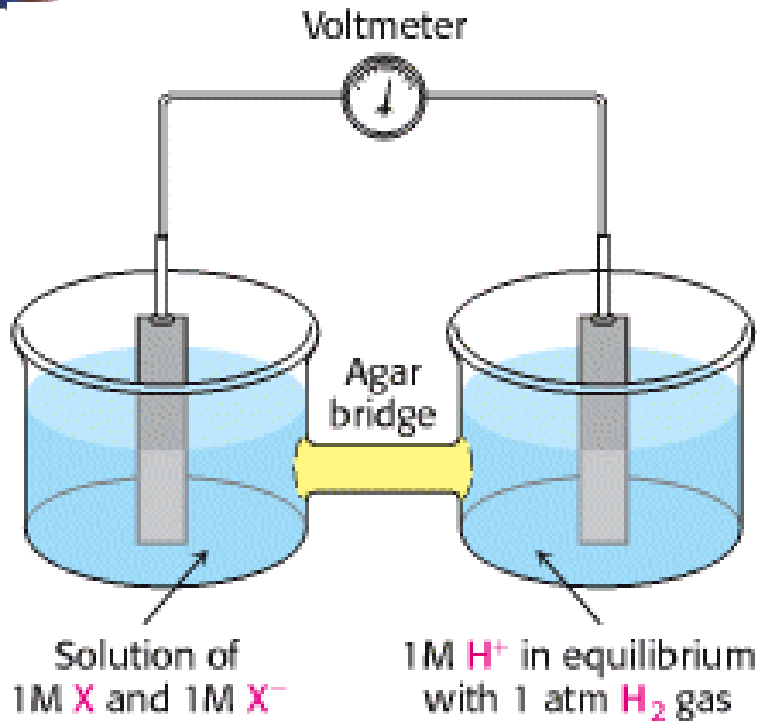


Summary of the flow of electrons and protons through the four complexes of the respiratory chain

4.2 Determining the Sequence of Respiratory Electron Carriers

Standard reduction potentials of the major respiratory electron carriers.



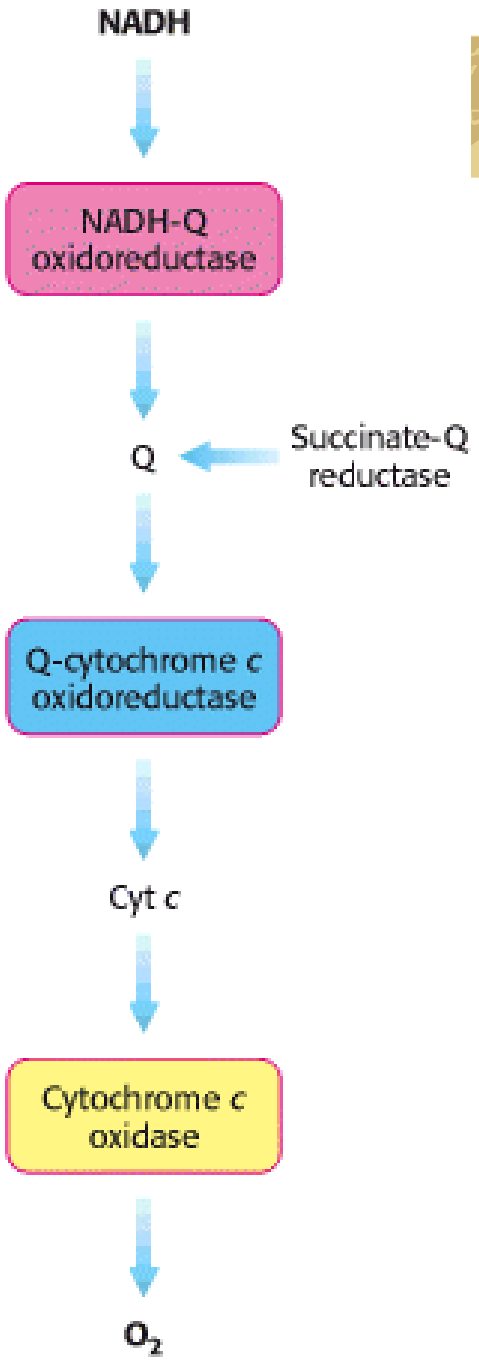


Standard reduction potential, E0'标准还原势

Species with a higher **standard reduction potential** tend to accept electrons from molecules with a lower **standard reduction potential**

$$\text{Oxidant} + e^- \longrightarrow \text{reductant}$$

Measurement of Redox Potential



Oxidant	Reductant	n	E'_0, V
Acetate + CO_2 + $2H^+$	Pyruvate + H_2O	2	−0.70
Succinate + CO_2 + $2H^+$	α-Ketoglutarate + H_2O	2	−0.67
Acetate + $3H^+$	Acetaldehyde + H_2O	2	−0.60
O_2	O_2^-	1	−0.45
Ferredoxin (oxidized)	Ferredoxin (reduced)	1	−0.43
$2H^+$	H_2	2	−0.42
Acetoacetate + $2H^+$	β-Hydroxybutyrate	2	−0.35
Pyruvate + CO_2 + H^+	Malate	2	−0.33
NAD^+ + H^+	NADH	2	−0.32
$NADP^+$ + H^+	NADPH	2	−0.32
FMN (enzyme-bound) + $2H^+$	$FMNH_2$ (enzyme-bound)	2	−0.30
Lipoate (oxidized) + $2H^+$	Lipoate (reduced)	2	−0.29
1,3-Bisphosphoglycerate + $2H^+$	Glyceraldehyde-3-phosphate + P_i	2	−0.29
Glutathione (oxidized) + $2H^+$	2 Glutathione (reduced)	2	−0.23
FAD + $2H^+$	$FADH_2$	2	−0.22
Acetaldehyde + $2H^+$	Ethanol	2	−0.20
Pyruvate + $2H^+$	Lactate	2	−0.19
Oxaloacetate + $2H^+$	Malate	2	−0.17
α-Ketoglutarate + NH_4^+ + $2H^+$	Glutamate + H_2O	2	−0.14
Methylene blue (oxidized) + $2H^+$	Methylene blue (reduced)	2	0.01
Fumarate + $2H^+$	Succinate	2	0.03
CoQ + $2H^+$	$CoQH_2$	2	0.04
Cytochrome b (+3)	Cytochrome b (+2)	1	0.07
Dehydroascorbate + $2H^+$	Ascorbate	2	0.08
Cytochrome c_1 (+3)	Cytochrome c_1 (+2)	1	0.23
Cytochrome c (+3)	Cytochrome c (+2)	1	0.25
Cytochrome a (+3)	Cytochrome a (+2)	1	0.29
$\frac{1}{2}O_2$ + H_2O	H_2O_2	2	0.30
Ferricyanide	Ferrocyanide	2	0.36
Nitrate + $2H^+$	Nitrite + H_2O	1	0.42
Cytochrome a_3 (+3)	Cytochrome a_3 (+2)	1	0.55
Fe (+3)	Fe (+2)	1	0.77
$\frac{1}{2}O_2$ + $2H^+$	H_2O	2	0.82

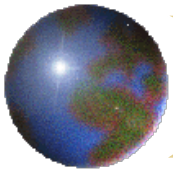
$$\tfrac{1}{2}O_2 + 2\,H^+ + 2\,e^- \longrightarrow H_2O \qquad E_0' = +0.82\ V \qquad \text{(a)}$$

$$NAD^+ + H^+ + 2\,e^- \longrightarrow NADH \qquad E_0' = -0.32\ V \qquad \text{(b)}$$

$$\tfrac{1}{2}O_2 + NADH + H^+ \longrightarrow H_2O + NAD^+ \qquad \text{(c)}$$

$$\Delta G^{\circ\prime} = -nF\Delta E_0'$$

$$\begin{aligned}\Delta G^{\circ\prime} &= -2 \times 23.06\ \text{kcal mol}^{-1}\,\text{V}^{-1} \times +0.82\ \text{V} - \\ &\quad (-2 \times 23.06\ \text{kcal mol}^{-1}\,\text{V}^{-1} \times -0.32\ \text{V}) \\ &= -37.8\ \text{kcal mol}^{-1} - (14.8\ \text{kcal mol}^{-1}) \\ &= -52.6\ \text{kcal mol}^{-1}\ (-220.1\ \text{kJ mol}^{-1})\end{aligned}$$



琥珀酸
↓
FAD
↓

$$NADH \rightarrow FMN \rightarrow CoQ \rightarrow Cytb \rightarrow Cytc1 \rightarrow Cytc \rightarrow Cytaa_3 \rightarrow O_2$$

A 1.14-Volt potential difference between NADH and O2 drives Electron transport through the chain and favors the formation of a proton gradient

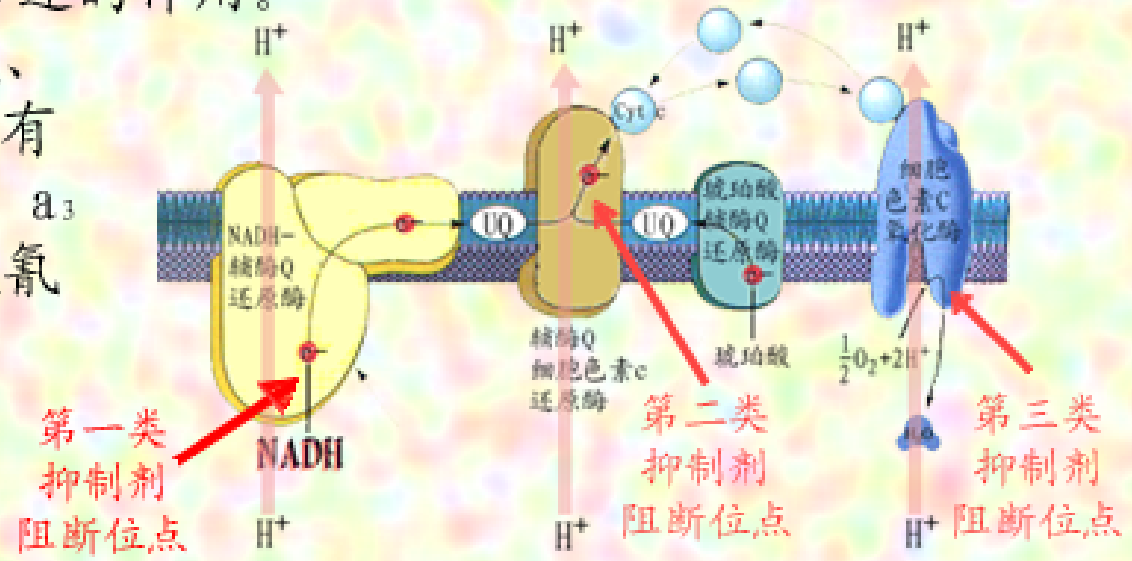
电子传递的抑制剂

能够切断呼吸链中某一部位电子流的物质称为电子传递抑制剂。利用某种特异的抑制剂切断某部位的电子流，再测定电子传递链中各组分的氧化—还原状态，是研究电子传递顺序的一种重要方法。已知的抑制剂有以下几类：

1. 鱼藤酮、安密妥以及杀粉蝶菌素，它们的作用是阻断电子由NADH向辅酶Q的传递。

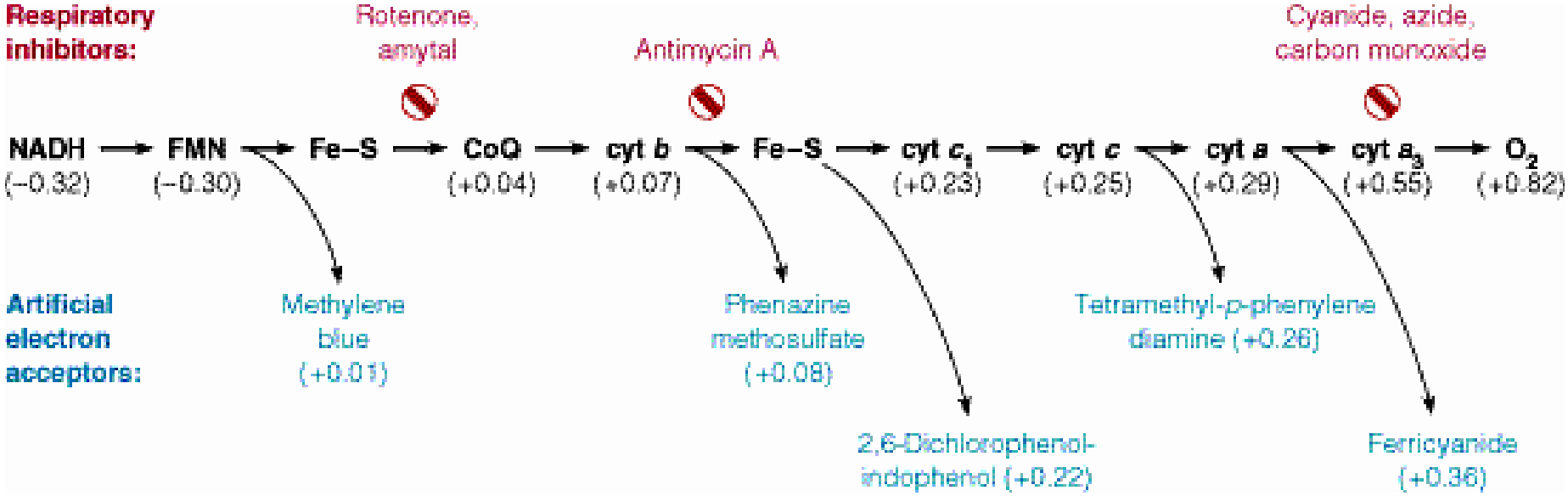
2. 抗霉素A，是由链霉菌分离出的抗菌素，有抑制电子从细胞色素b到细胞色素c_1传递的作用。

3. 氰化物、硫化氢、叠氮化物和一氧化碳等有阻断电子由细胞色素a、a_3传至氧的作用，这就是氰化物等中毒的原理。



Inhibitors

Specific **inhibitors**： rotenone 鱼藤酮, antimycin A 抗霉素A, cyanide 氰化物, azide 叠氮化物, and carbon monoxide



Rotenone 鱼藤酮, amytal 安米妥,能和NADH脱氢酶牢固结合

Piericidin－A 杀粉蝶菌素A为CoQ的结构类似物

除Cyt aa_3外, 其它cyt 的亚铁血红素形成的6个键都与Pr和卟啉环形成配位键

仅 Cyt aa_3 分子中所含的血红素A中的铁原子形成了5个配位键，还有一配位键能与O_2、CO、CN－结合。所以氰化物等能引起细胞的窒息死亡。

An **inhibitor** ----a specific target of inhibition--- crossover point

overall pathway is blocked

Before the crossover point, electron carriers----reduced state

After the crossover point, electron carriers ----oxidized state

5. Oxidative Phosphorylation

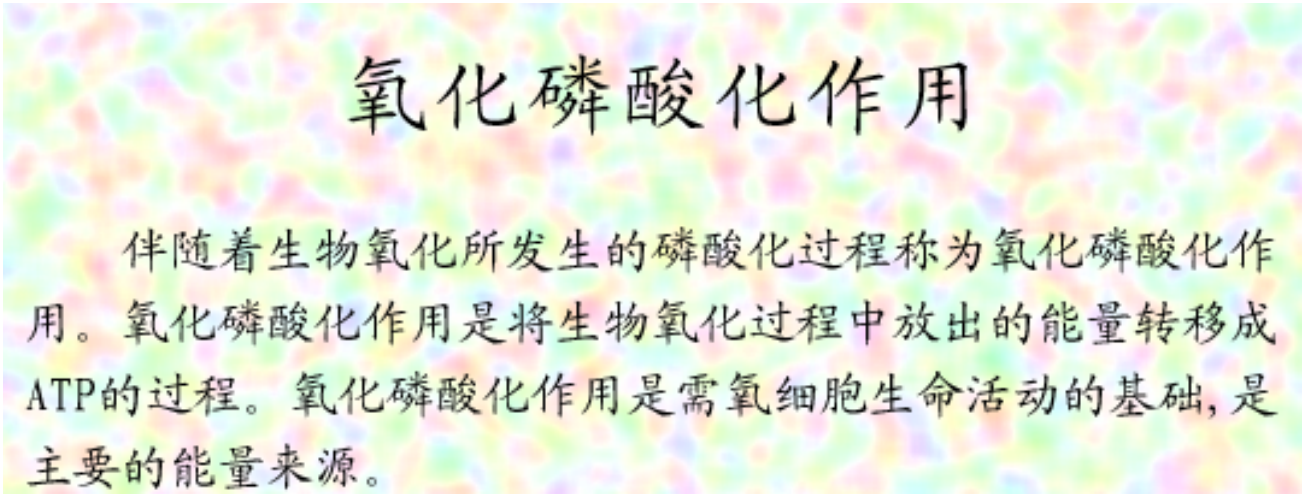
5.1 Definition

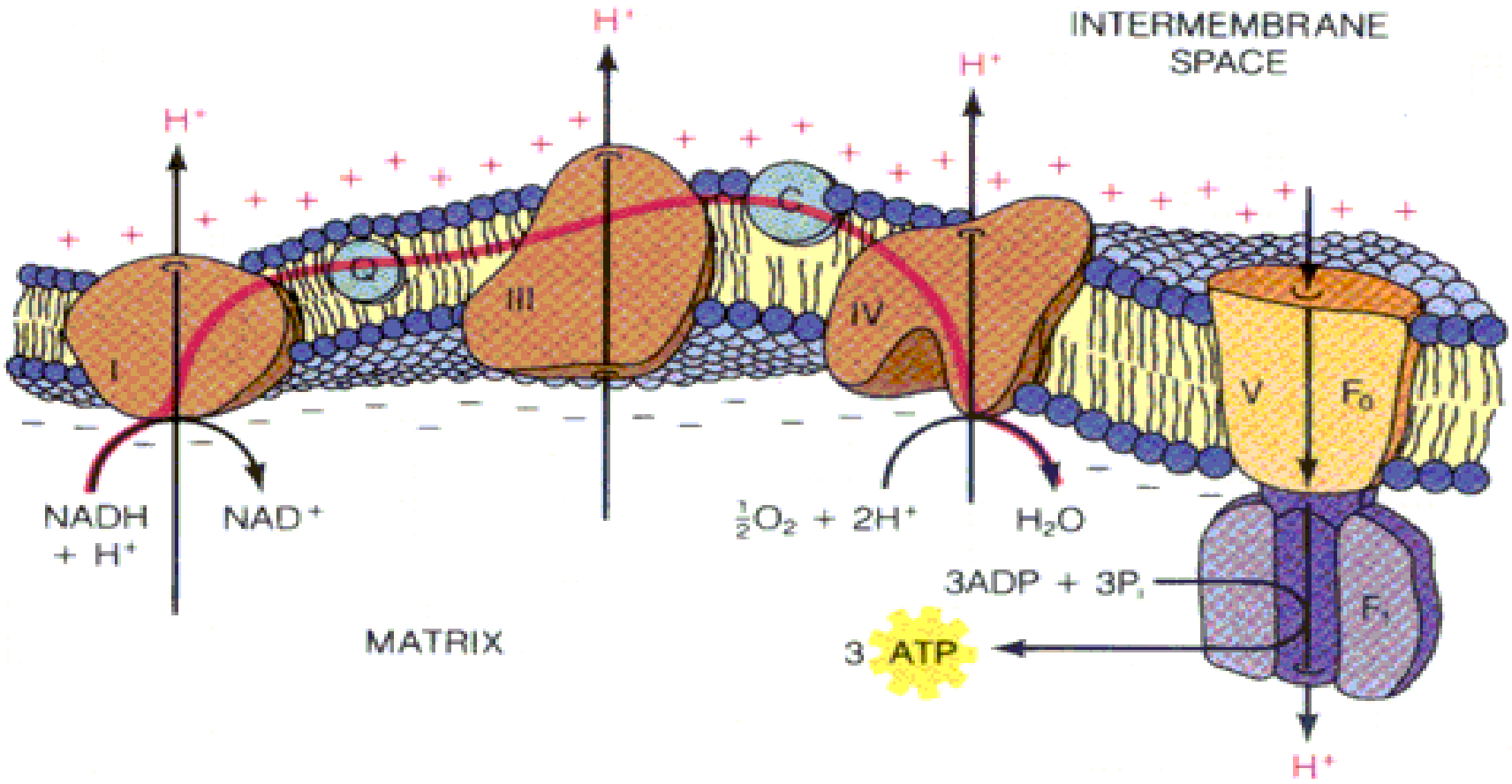
Oxidative phosphorylation ------

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

真核：线粒体内膜

原核：浆膜




H+
H+
H+
INTERMEMBRANE
SPACE
I
II
III
IV
V
Fo
F1
NADH
+ H+
NAD+
½O2 + 2H+
H2O
3ADP + 3Pi
3 ATP
MATRIX
H+

1. Movement of electrons through ETS (the **electron transport** system)
2. Protons to be pumped from the mitochondrial matrix of a cell to the intermembrane space
3. The difference in potential
4. Provides the energy source for making ATP in the mitochondrion

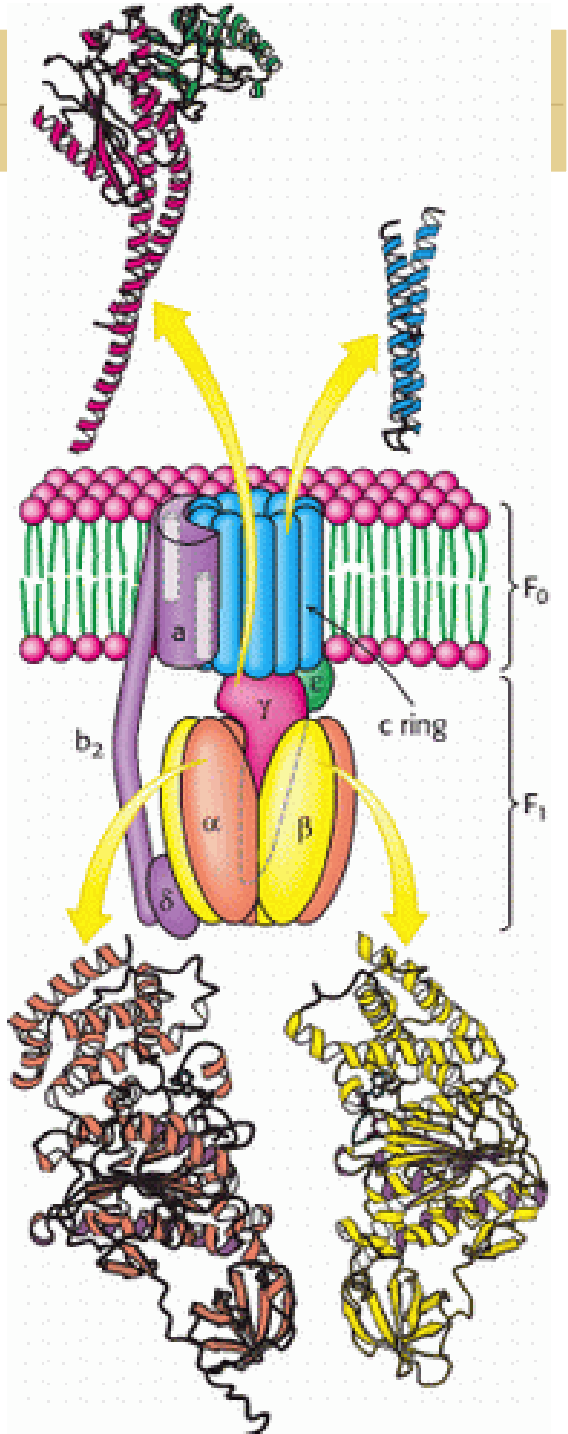
$$ADP + P_i + H^+ \rightleftharpoons ATP + H_2O$$

$$\Delta G^{\circ\prime} = +7.3\ \text{kcal mol}^{-1}\ (+30.5\ \text{kJ mol}^{-1})$$

$$NADH + \tfrac{1}{2}O_2 + H^+ \rightleftharpoons H_2O + NAD^+$$

$$\Delta G^{\circ\prime} = -52.6\ \text{kcal mol}^{-1}\ (-220.1\ \text{kJ mol}^{-1})$$

The World's Smallest Molecular Motor: Rotational Catalysis



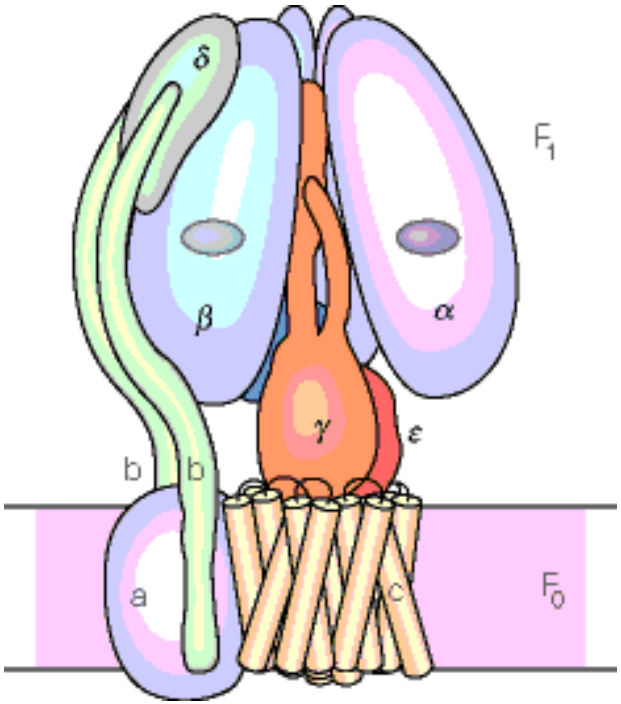
5.2 ATP合成部位

Complex V (**ATP synthase**, **F_OF1 complex**): located on the inner mitochondrial cristae

A top knob ---**F1** (projects into the mitochondrial matrix, contains three dimers arranged like segments of an orange around the stalk)

The base ---**Fo** (in the inner mitochondrial membrane)

A stalk--- joins the knob to the base.



F1 complex: synthesizes ATP, protons pass from the intermembrane space through the stalk and out the top of the **F1 complex** into the mitochondrial matrix

The stalk contains γ and ε proteins

Δ is attached to protein b ,a and c of the **Fo** base

F1 $(\alpha\beta)_3\gamma\varepsilon\delta$ ATP synthase

F_O subunits ab_2c_{10-15} Ion translocator

Fo complex

The antibiotic oligomycin 寡霉素binding site

---- blocks the flow of protons through the **Fo** channel

---- inhibits oxidative phosphorylation directly

5.3 Chemiosmotic hypothesis

Energy coupling hypothesis: **ATP synthesis** VS **ETS**

Chemical coupling hypothesis

1953, Edward Slater, 活泼的高能共价中间物

Conformational coupling hypothesis

1964, Paul Boyer, Mt 内膜蛋白质发生构象变化

Chemiosmotic hypothesis, 1978, Nobel Prize

1961, Peter Mitchell, explains how ATP is synthesized by mitochondria as a result of protons pumped during ETS.

1920-1992

The Nobel Prize in Chemistry 1978

"for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory”

Peter D. Mitchell

United Kingdom

Glynn Research Laboratories

Chemiosmotic coupling hypothesis principles: Fig 24-18

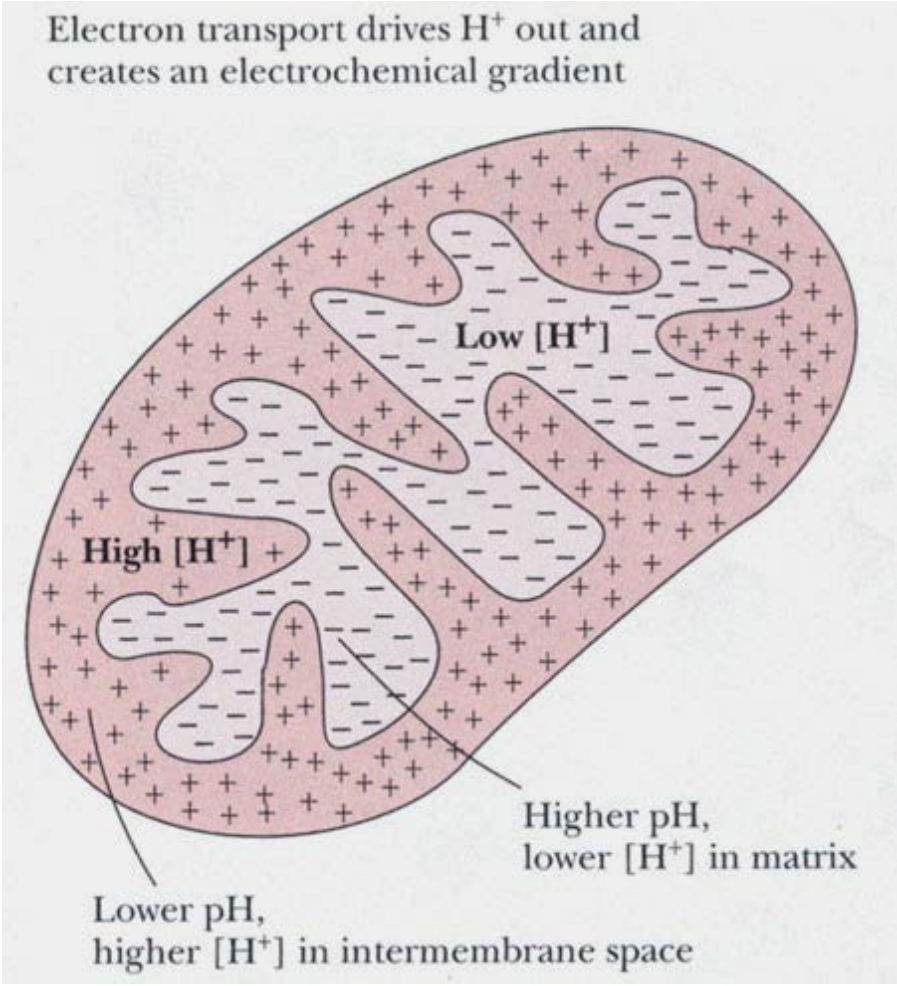
1. Energy from electron transport drives an active transport system.

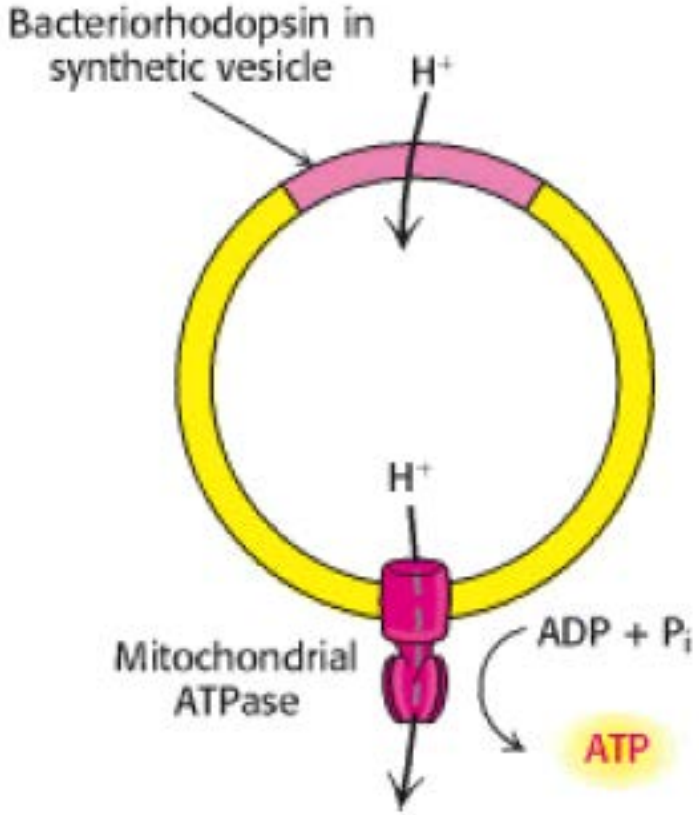
2. The active transport system pumps protons out of the mitochondrial matrix into the intermembrane space.

3. An electrochemical gradient of protons is created

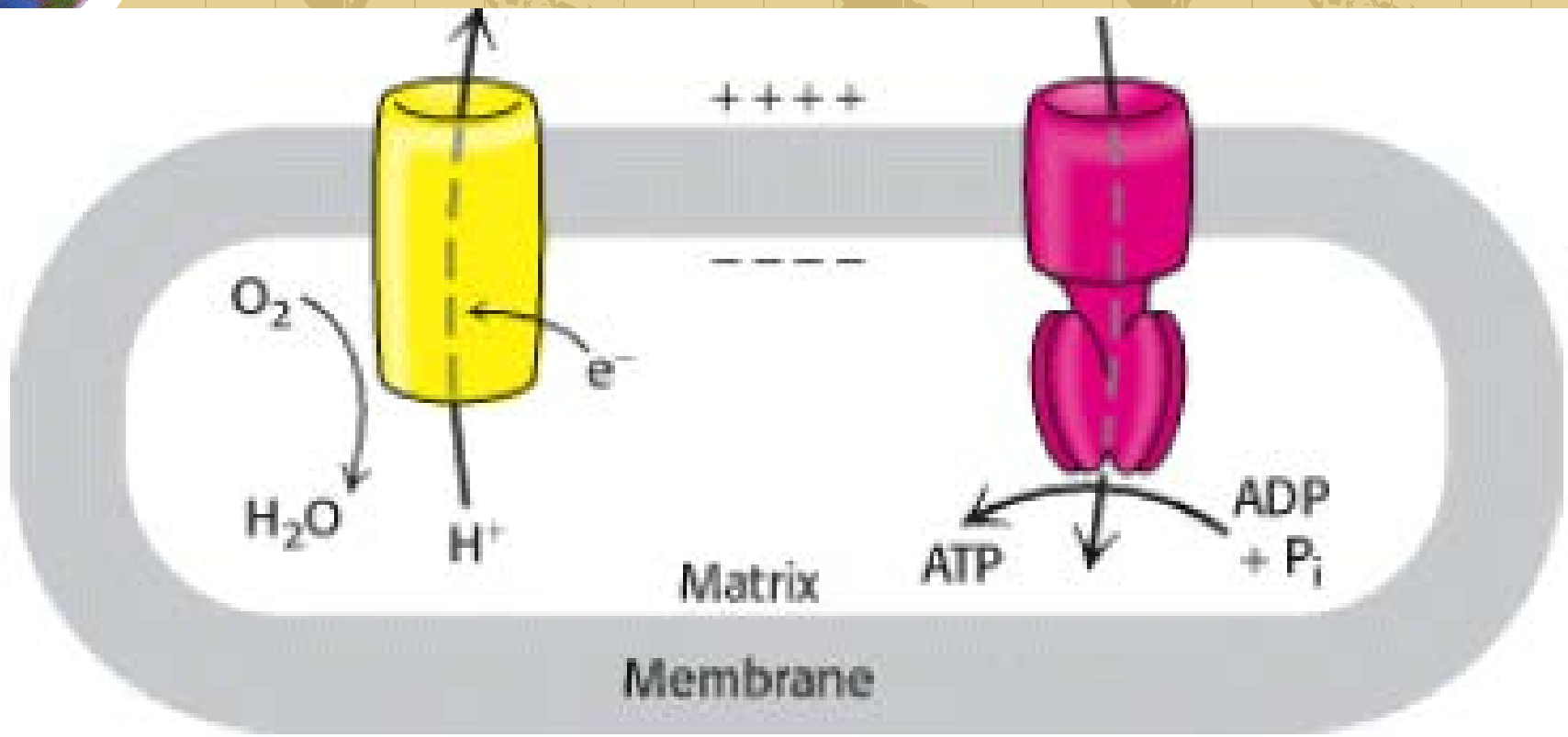
a lower pH value outside the mitochondrial membrane than inside.

The protons on the outside have a thermodynamic tendency to flow back in, so as to equalize pH on both sides of the membrane

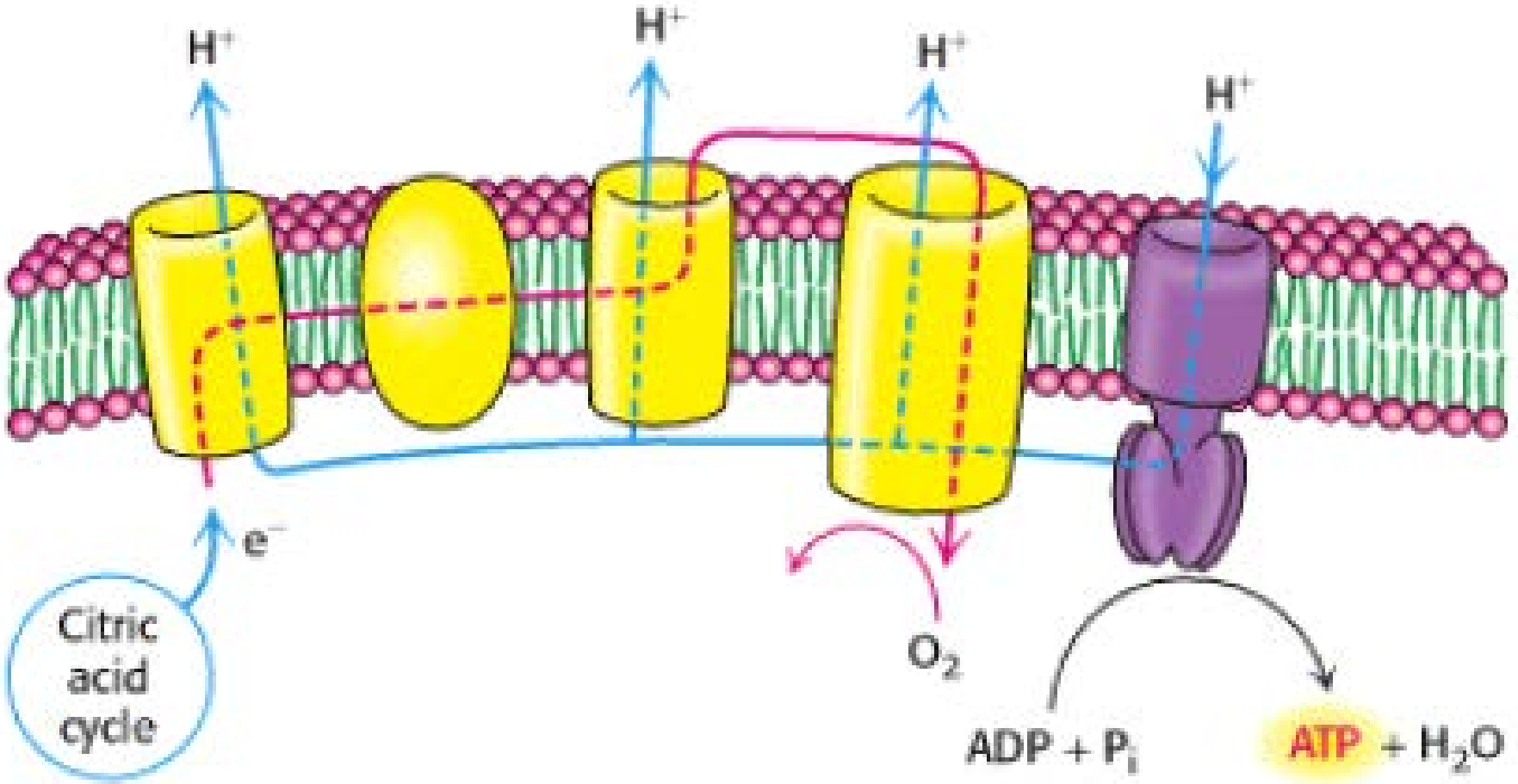




4. When protons do flow back into the matrix, the free energy arising from the gradient (21 kJ/mol of protons) is dissipated, with some of it being used to drive the synthesis of ATP.



Essence of Oxidative Phosphorylation.


H+
H+
H+
H+
e−
Citric acid cycle
O2
ADP + Pi
ATP + H2O

Evidence supporting the chemiosmotic coupling hypothesis:

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

2. Oxidative phosphorylation requires an intact inner membrane.

If the inner membrane is damaged, protons can leak back into the mitochondrial matrix and destroy the proton gradient. Thus, there would be no energy to make ATP

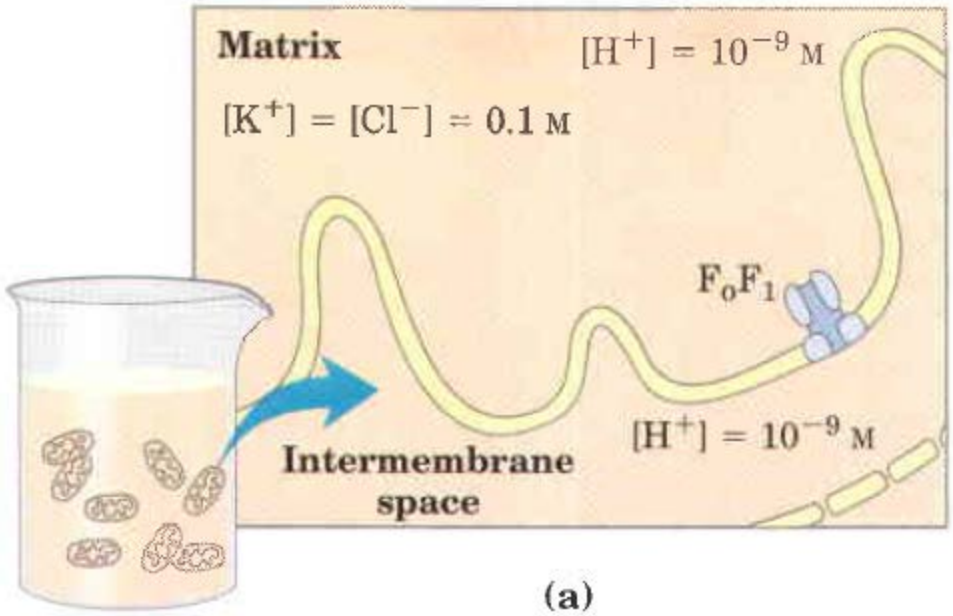
3. Key electron transport proteins span the inner membrane

perfectly positioned to serve as pumps.

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

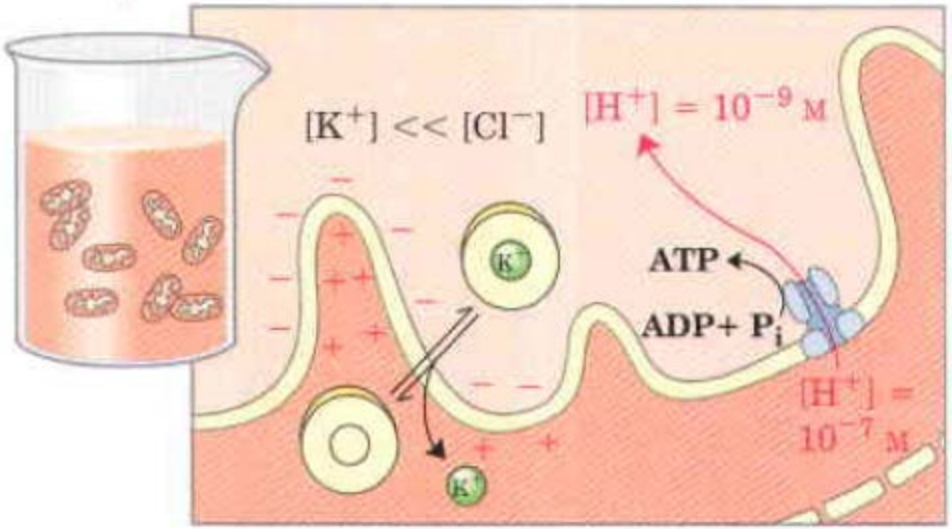
5. If one creates an artificial proton gradient across the mitochondrial inner membrane by incubating mitochondria in an acid solution, ATP is produced by the mitochondria in the absence of electron transport.

Evidence for the role of a proton gradient in ATP synthesis



(a)

pH lowered from 9 to 7; valinomycin present; no K^+



(b)

电子传递  跨膜的质子梯度

1. 氧化－还原回路机制

Mitchell 提出

FMN，CoQ起着（$H^+ + e^-$）载体作用

Fe-S聚簇和cyt是单纯的电子载体

2. 质子泵机制　　复合体的构象变化

电子传递 → 氨基酸侧链PK值改变 →

发挥质子泵作用的侧链交替暴露在膜的内外侧 → 质子发生移位

3. And now, the fact is that ????

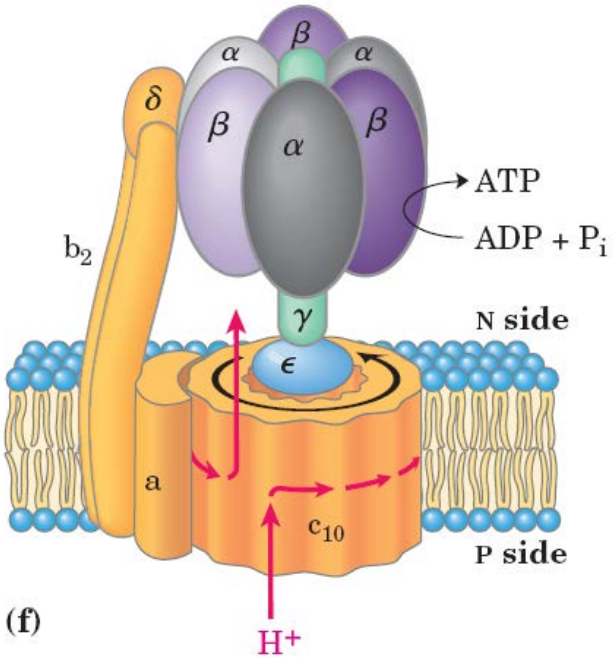
5.4 Binding-change mechanism

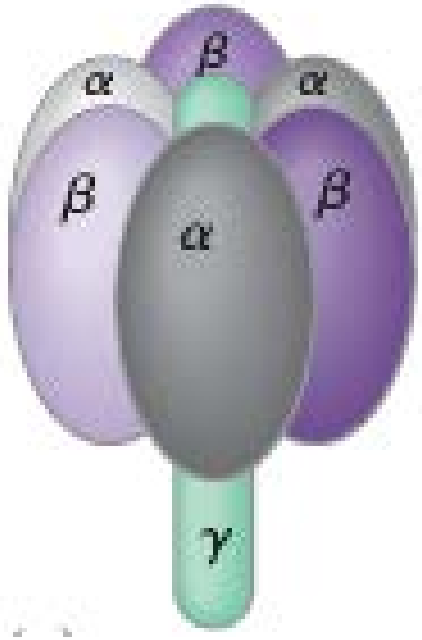
Paul Boyer

Paul Boyer

Rotational catalysis mechanism

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





Structure of the F1 complex

three *a* and three β subunits are

arranged like the segments of an orange

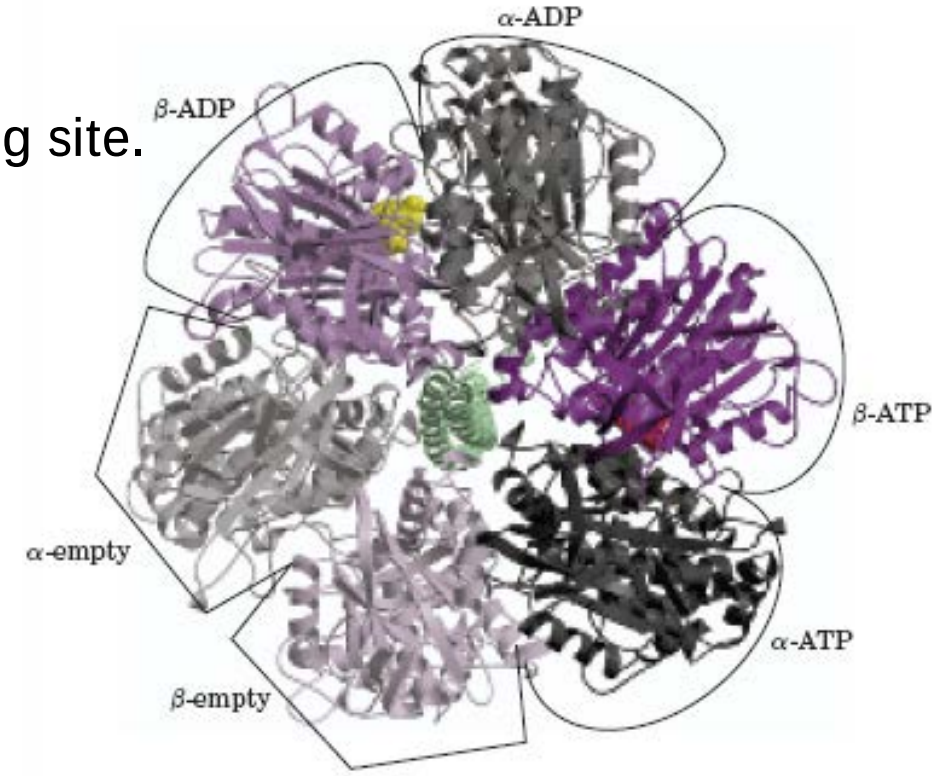
F1 viewed from above

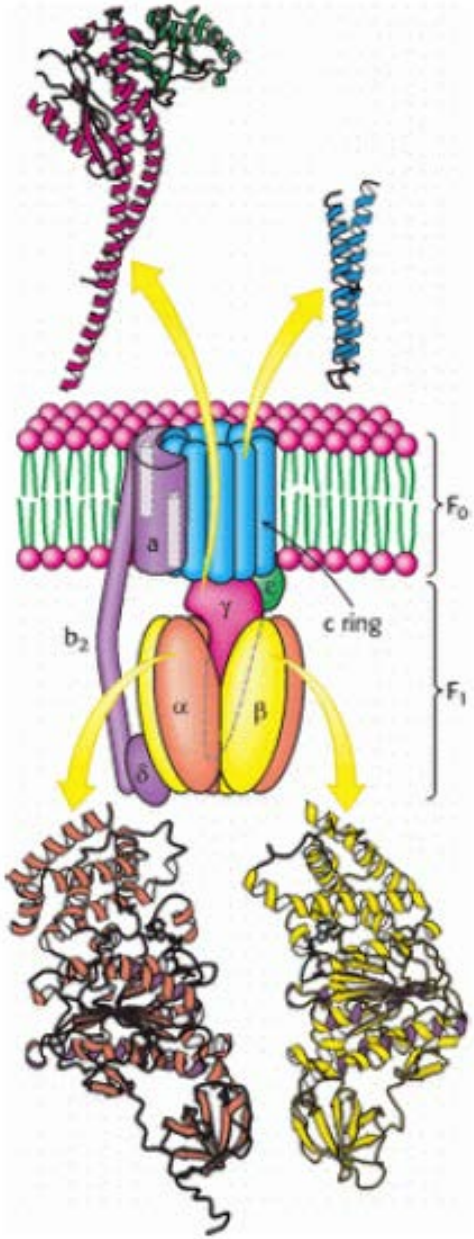
The single γ subunit associates primarily with one of the three αβ pairs, forcing each of the three β subunits into slightly different conformations, with different nucleotide-binding sites.

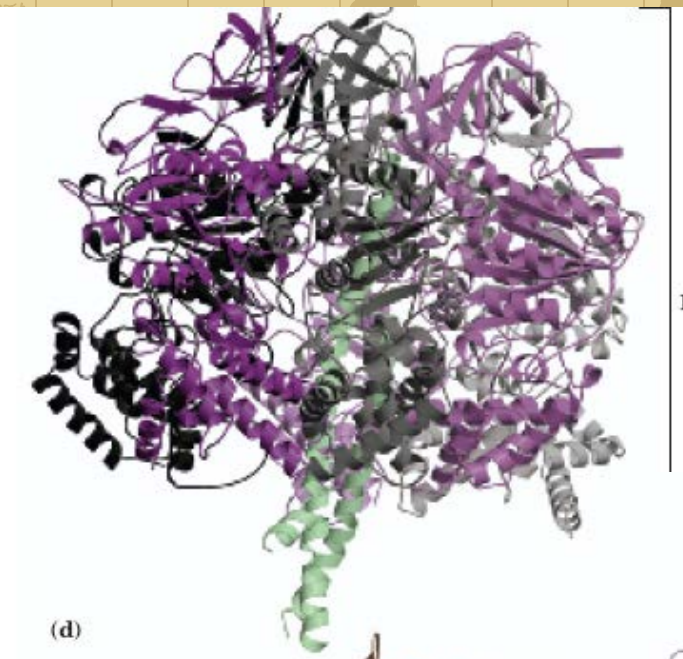
β-ADP has ADP (yellow) in its binding site.

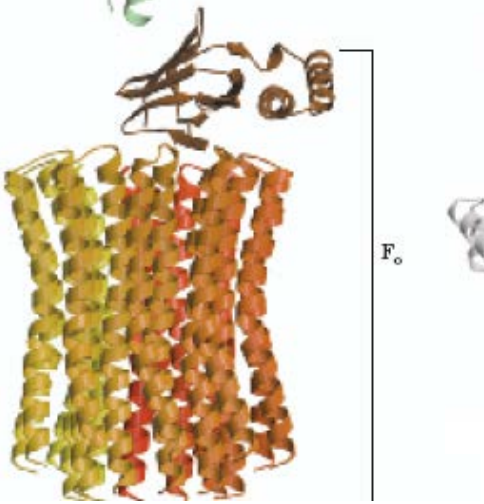
β-ATP has ATP (red).

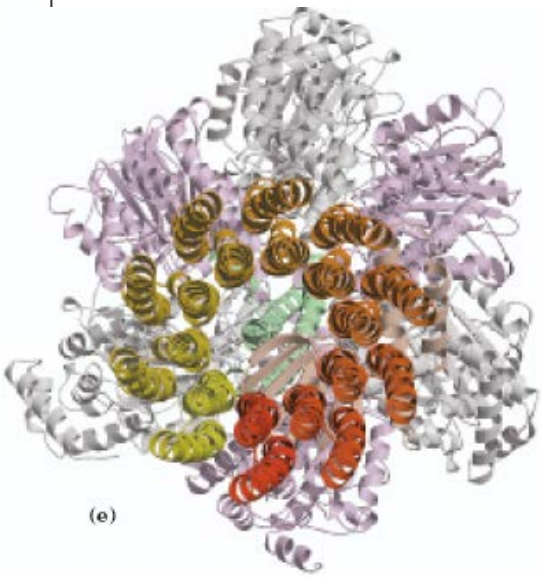
β-empty has no bound nucleotide.



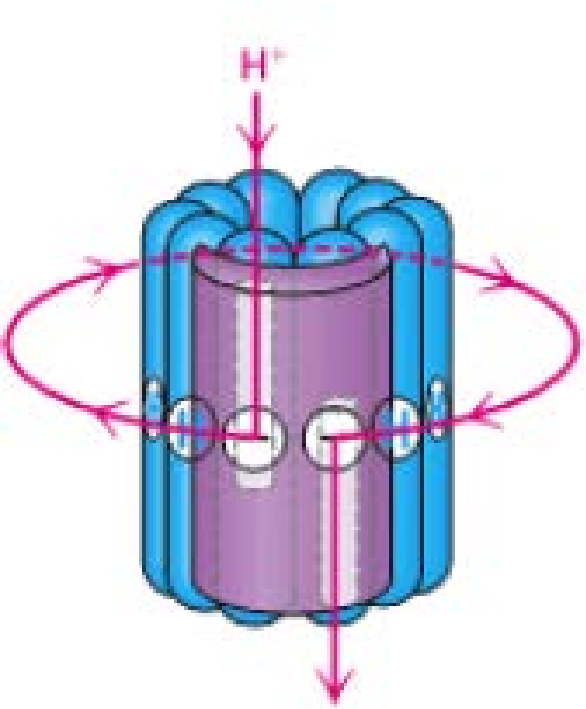

a
γ
ε
c ring
b_2
α
β
δ
F_0
F_1

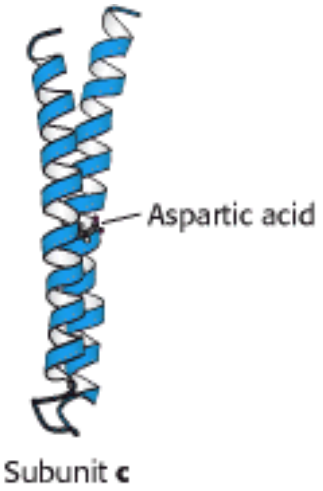

F_1
(d)

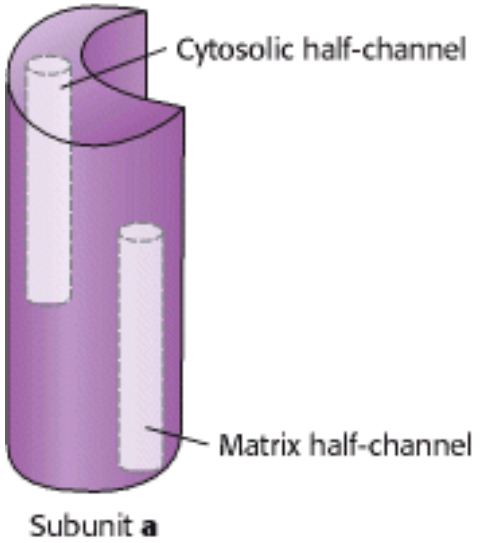

F_o


(e)

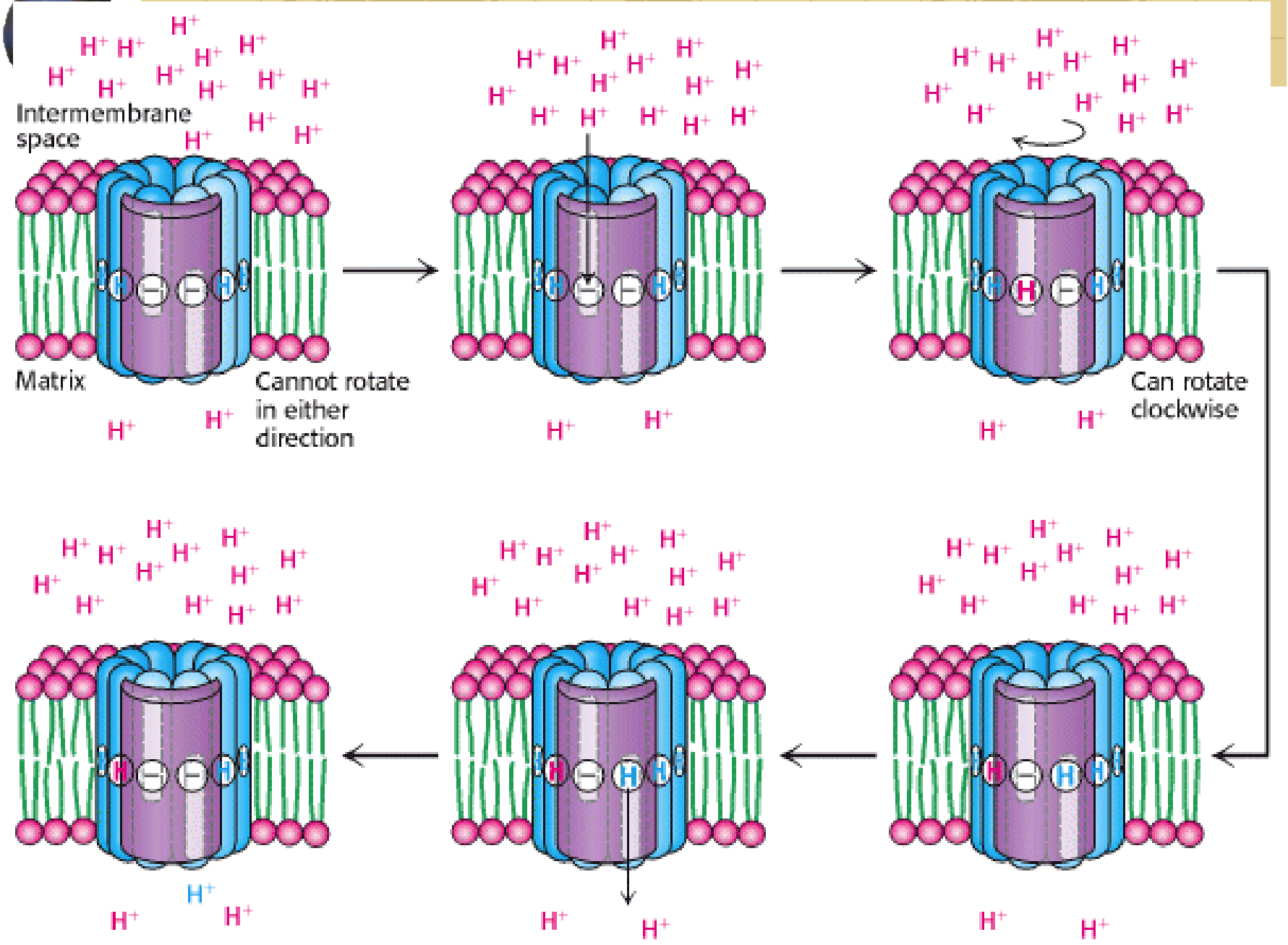
Proton Path Through the Membrane



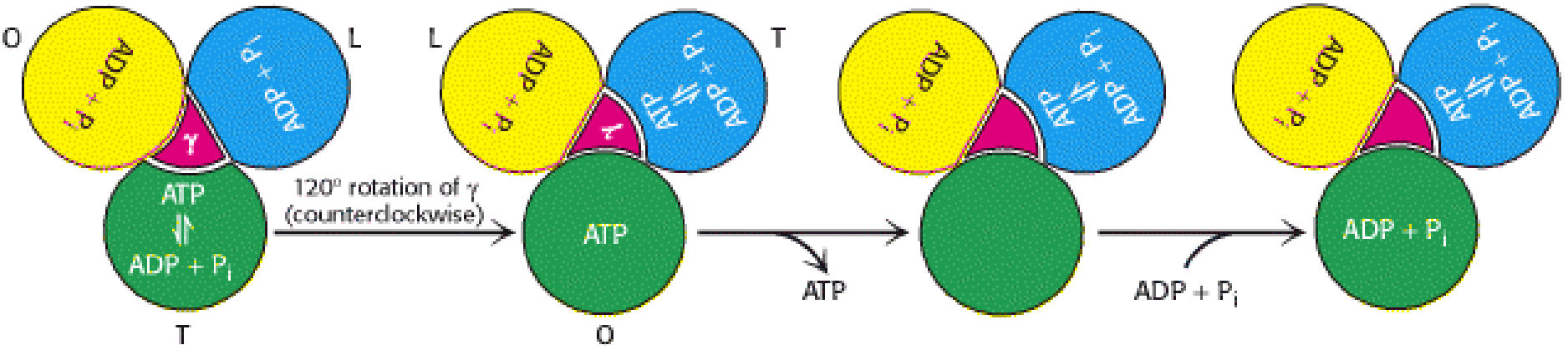




Each proton enters the cytosolic half-channel, follows a complete rotation of the **c** ring, and exits through the other half-channel into the matrix.


Intermembrane space
Matrix
Cannot rotate in either direction
Can rotate clockwise

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



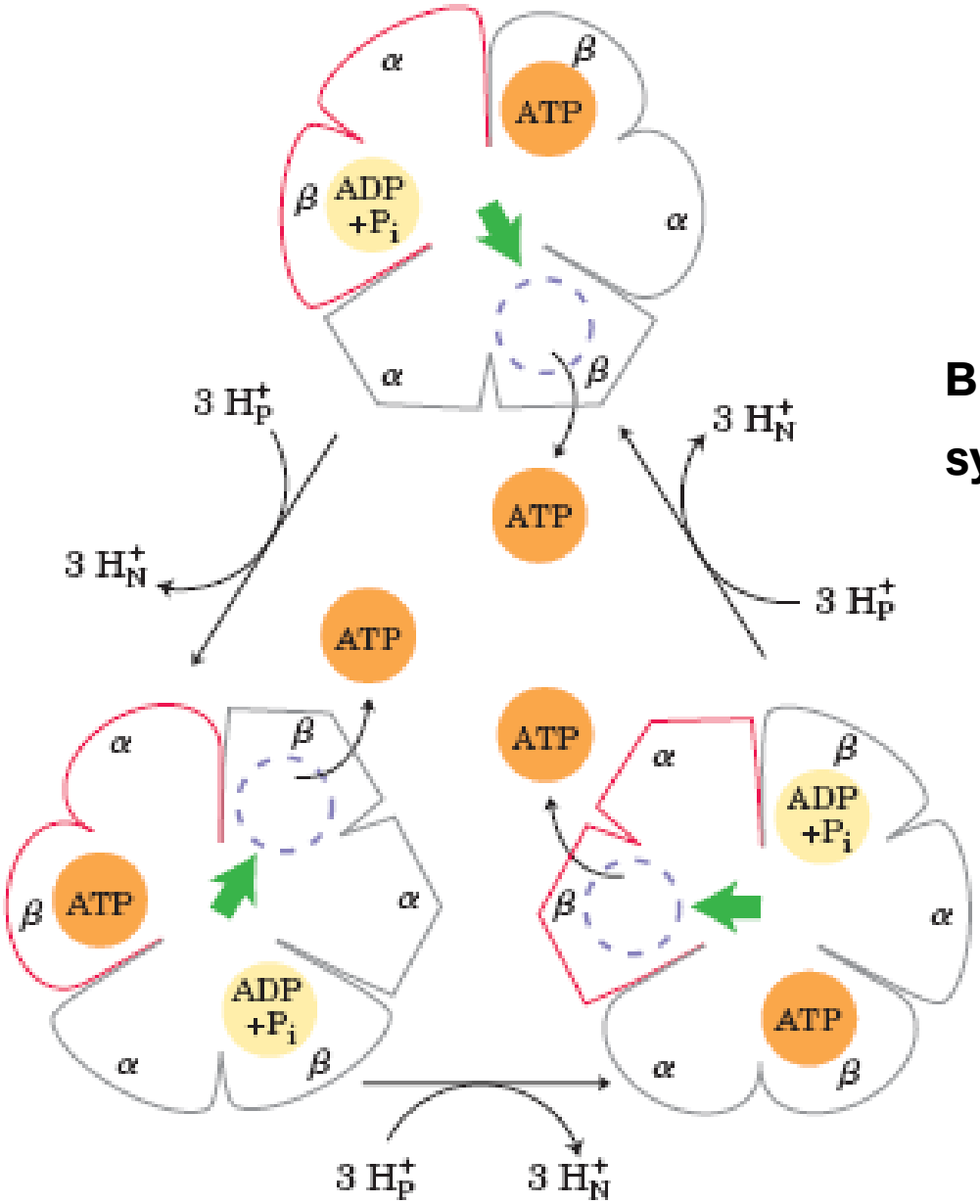
F1 β subunit contains three similar, but not identical, binding sites for ATP or ADP + Pi.

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

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

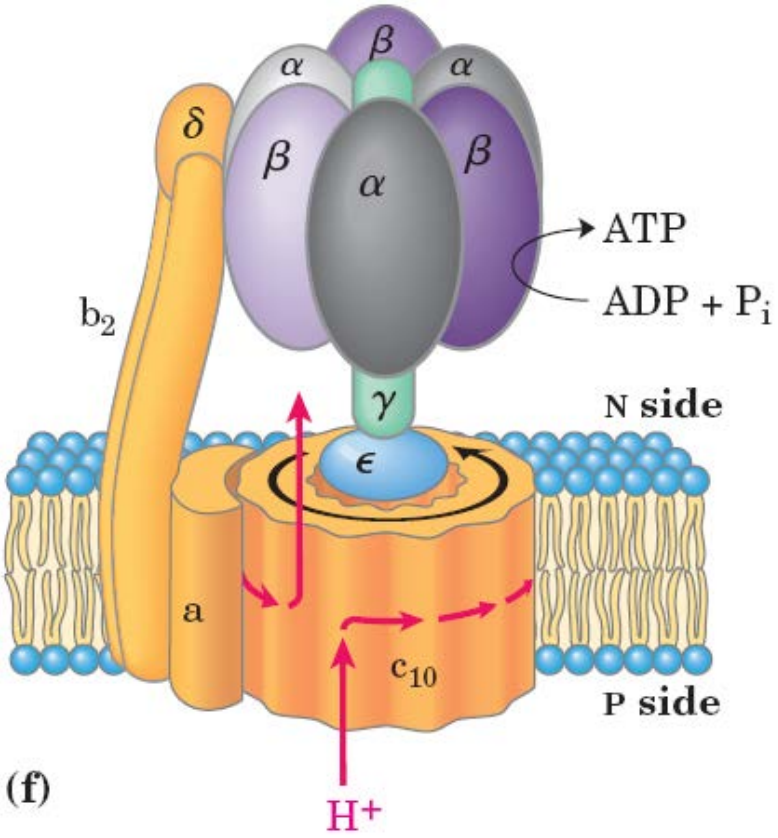
O site (Open)： bind to S with low affinity

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

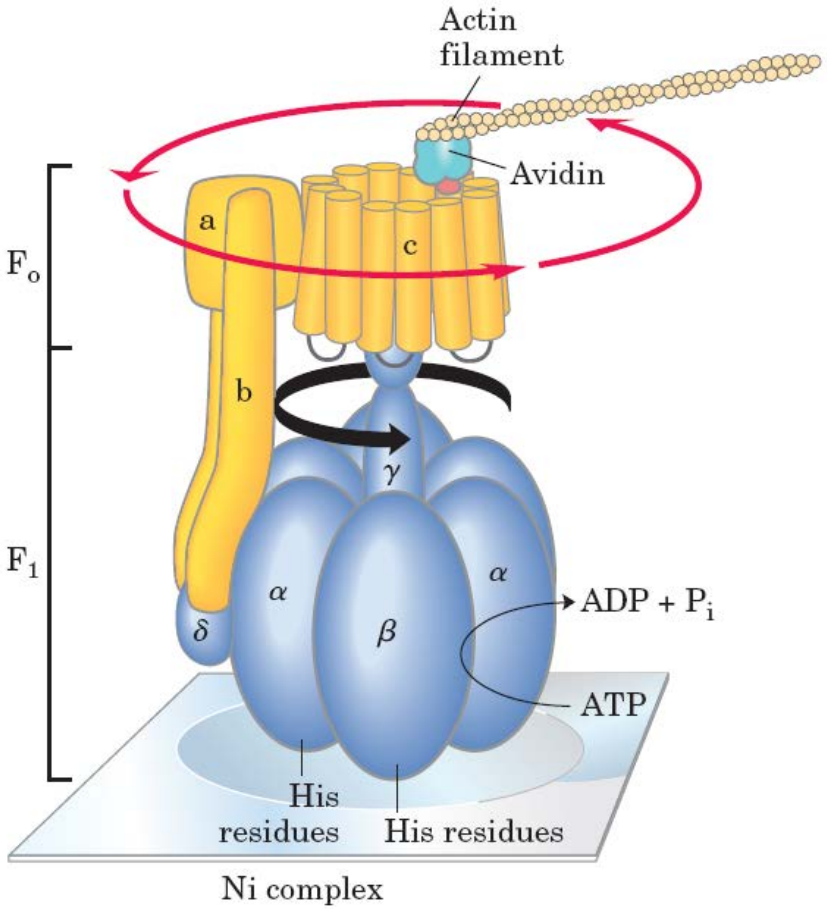


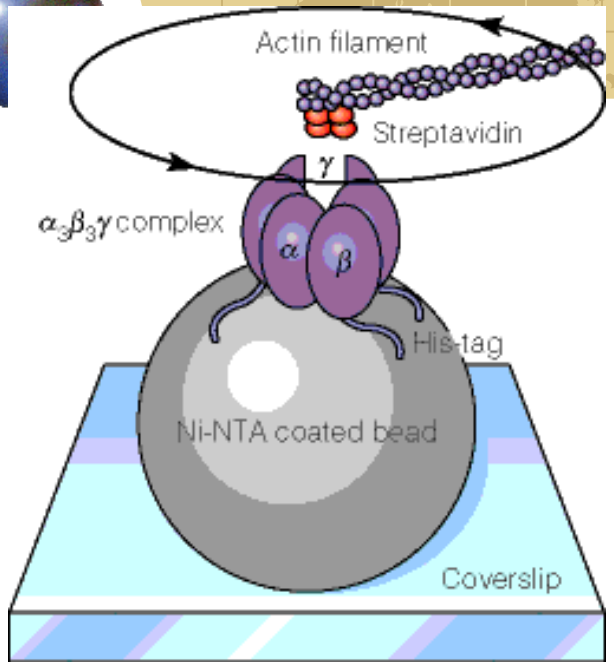
Binding-change model for ATP synthase.

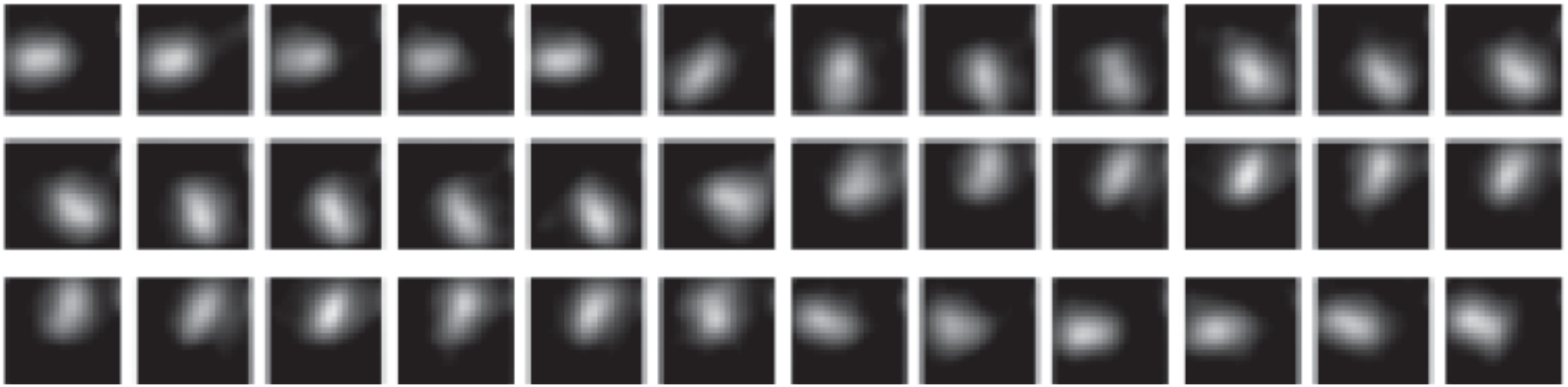
FoF1 complex



Rotation of Fo andy experimentally demonstrated




Actin filament
Streptavidin
γ
$\alpha_3\beta_3\gamma$ complex
α
β
His-tag
Ni-NTA coated bead
Coverslip



Proton movement through the complex (F_O)

↓

Each rotation (120^0) of the rotor converts all three

binding sites into new conformations

↓

At each turn of the wheel, 3 ATP is released.

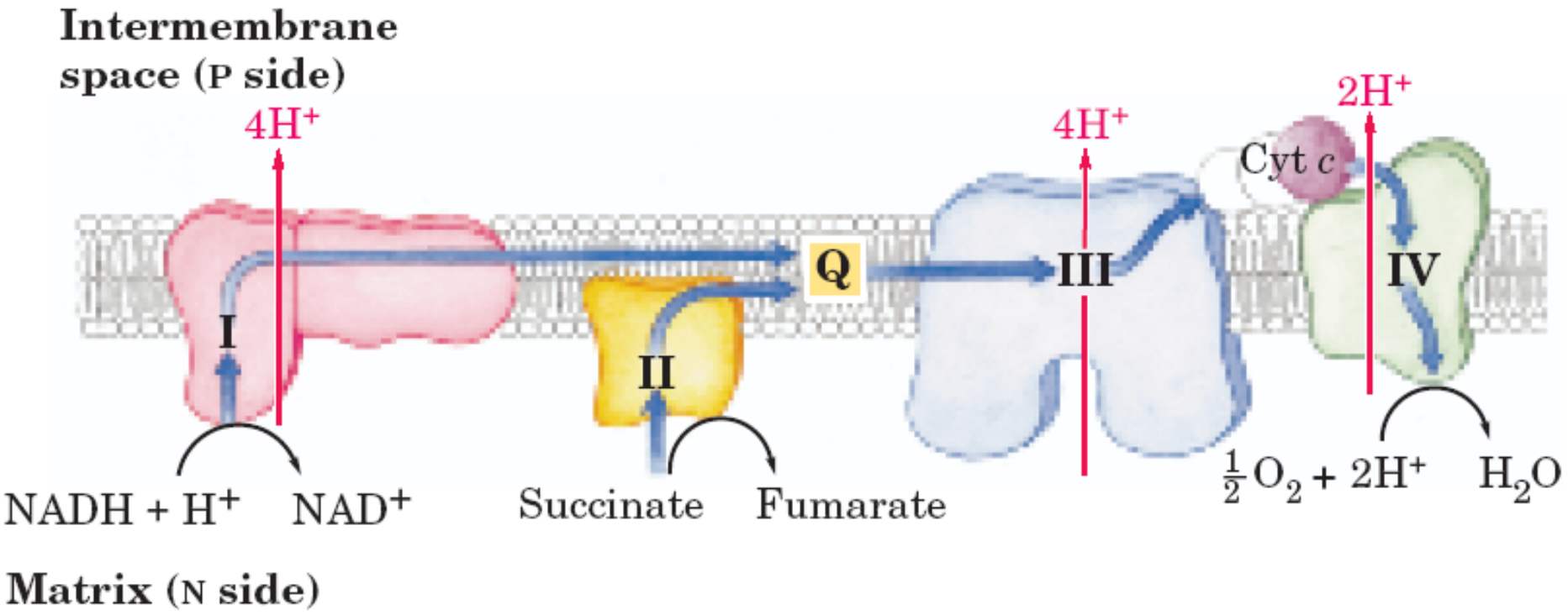
5.5 P/O Ratio

The efficiency of **oxidative phosphorylation**

$$x\mathrm{ADP} + x\mathrm{P_i} + \tfrac{1}{2}\mathrm{O_2} + \mathrm{H^+} + \mathrm{NADH} \longrightarrow x\mathrm{ATP} + \mathrm{H_2O} + \mathrm{NAD^+}$$

The amount of ATP made versus the amount of oxygen consumed--x

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


Intermembrane
space (P side)
4H+
4H+
2H+
Cyt c
Q
I
II
III
IV
NADH + H+
NAD+
Succinate
Fumarate
½ O2 + 2H+
H2O
Matrix (N side)

Balanced equation for the mitochondrial oxidation of NADH :

Complex I from NADH : about (2.5/1) 3:1

Complex II (FAD's electrons) :about (1.5/1) 2:1

ADP + Pi <=> **ATP** (= -31 kJ/mol)

3 **ATP** -93 kJ

about 42%, of the energy released

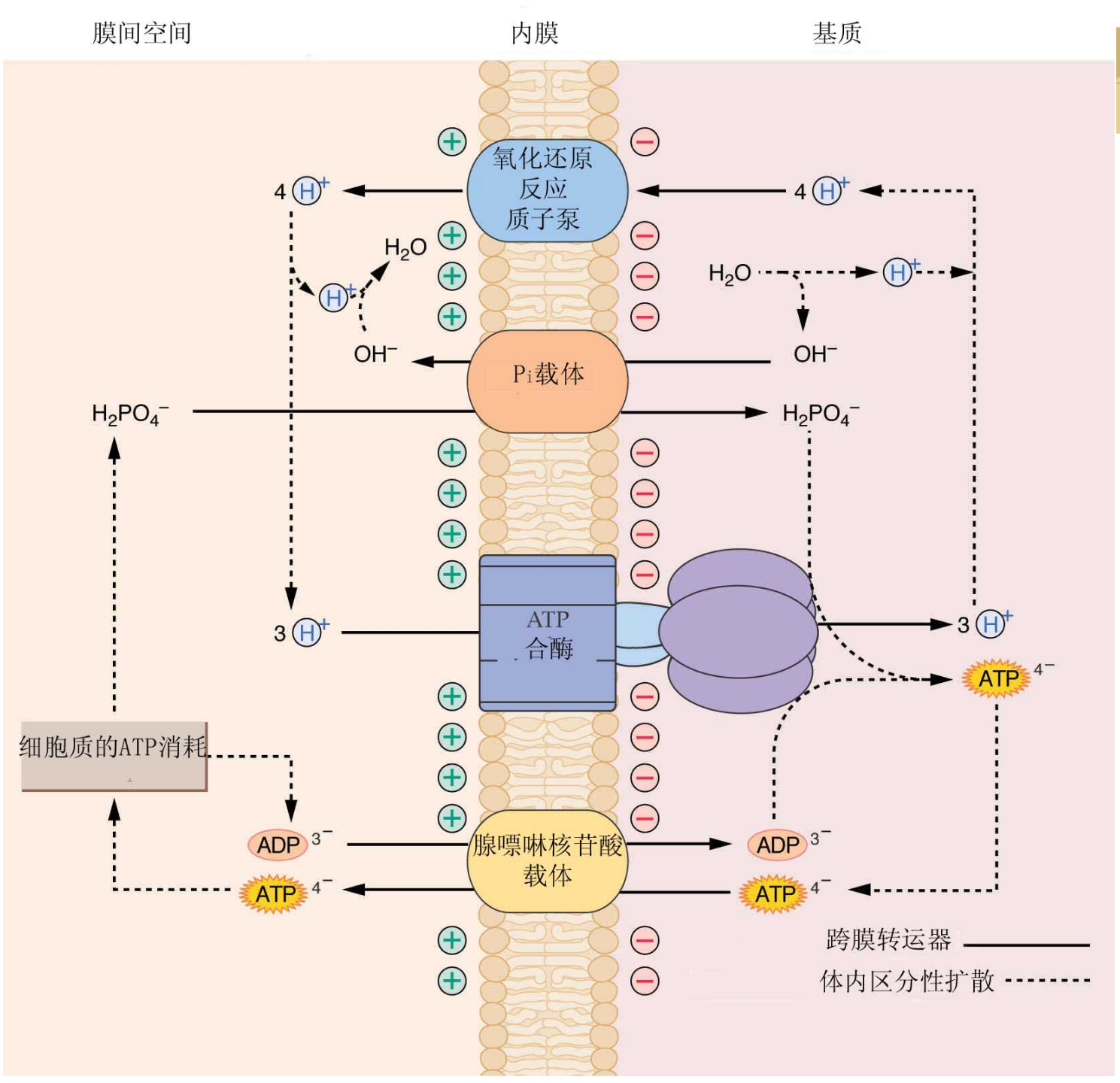
opinion：H^+ 10 (NADH) /6 (succinate) vs 2 e^-

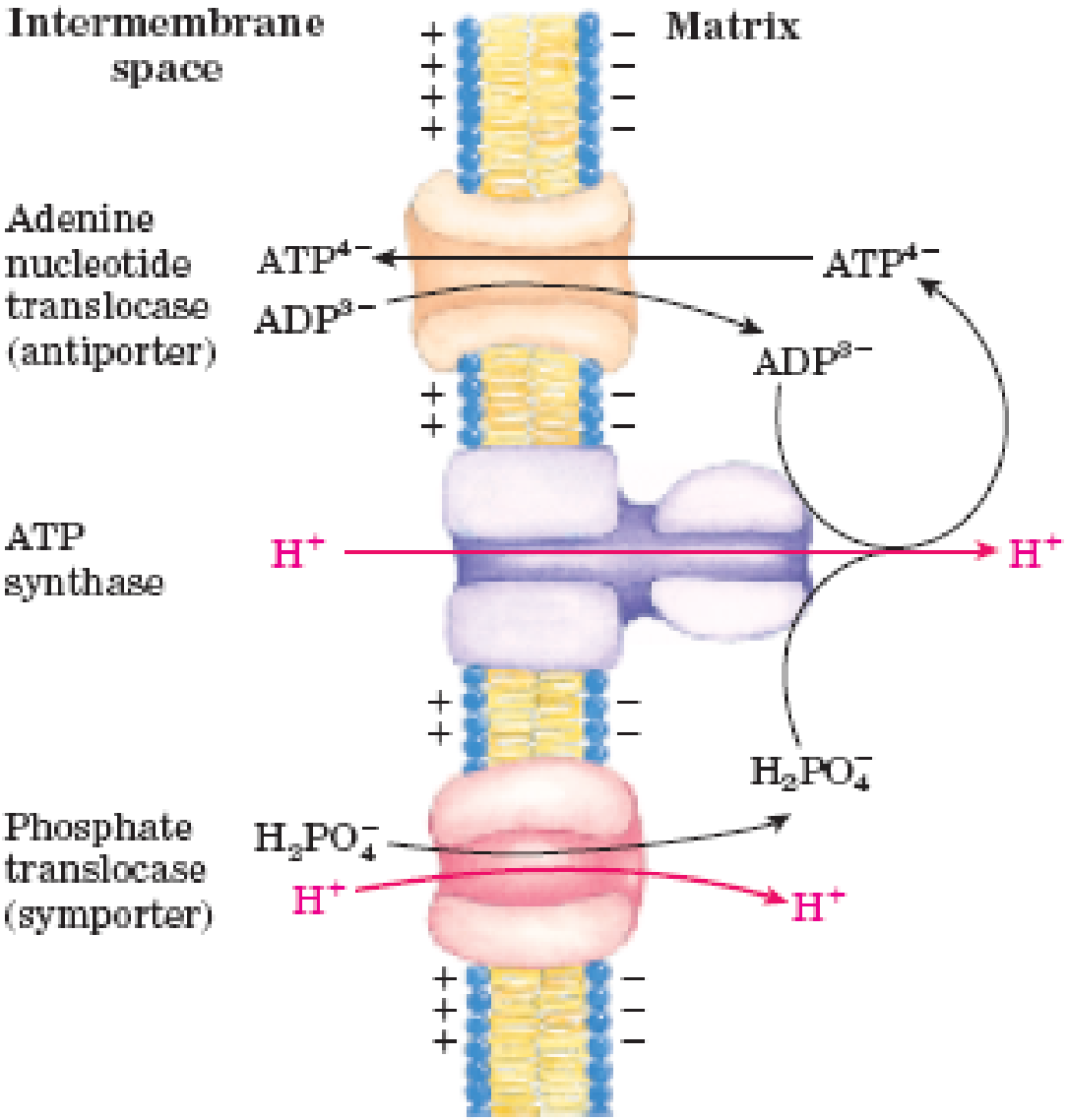
ATP 1 vs 3 H^+ ($+H^+$)

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

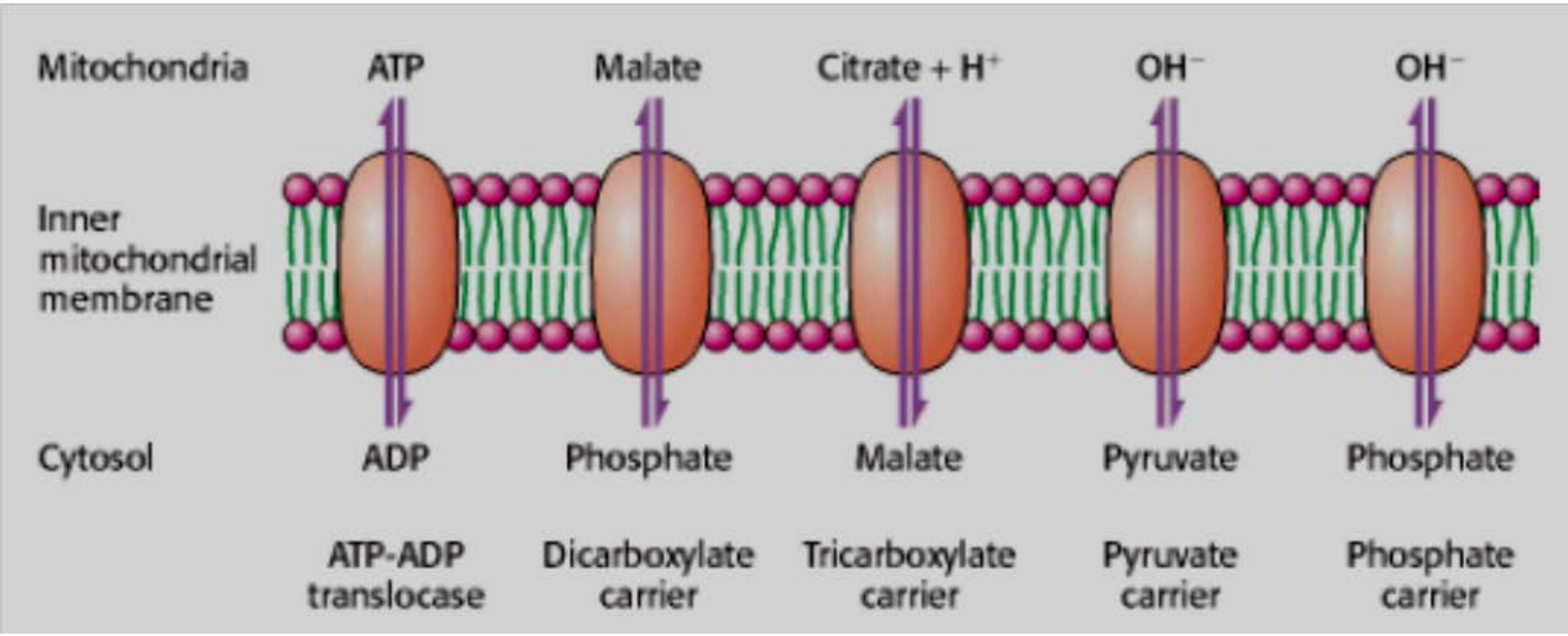
NADH 2.5 ATP

FADH 1.5 ATP


膜间空间
内膜
基质
氧化还原
反应
质子泵
4 H+
H2O
H+
OH−
Pi载体
H2PO4−
3 H+
ATP
合酶
ATP 4−
细胞质的ATP消耗
ADP 3−
腺嘌呤核苷酸
载体
跨膜转运器
体内区分性扩散



Adenine nucleotide and phosphate translocases



Mitochondrial Transporters.

6. Shuttling Electron Carriers into the Mitochondrion

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?

DHAP/ Glycerol-3-P shuttle system

skeletal muscle and brain mitochondria

Malate/aspartate shuttle system

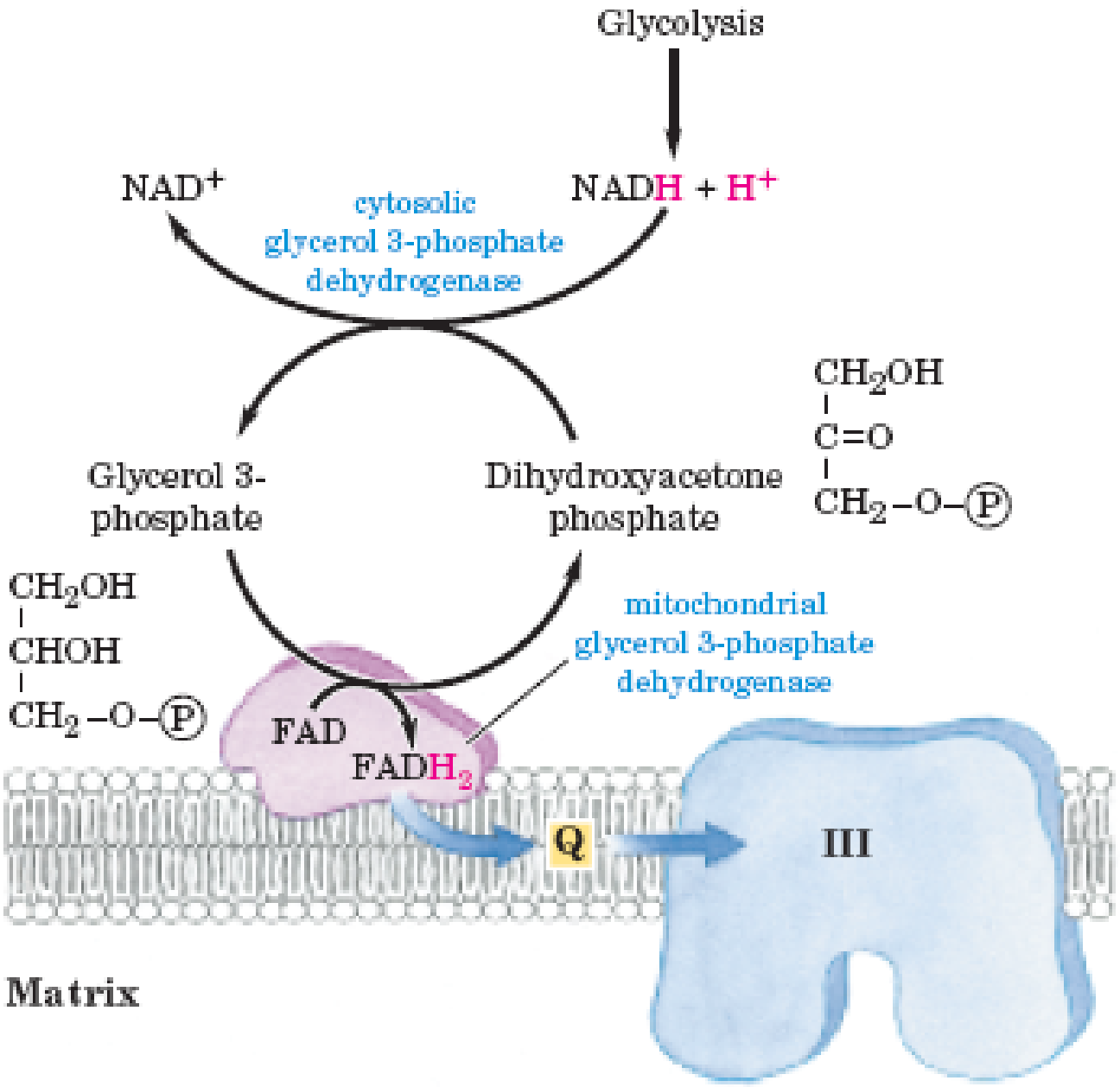
liver , kidney and heart mitochondria

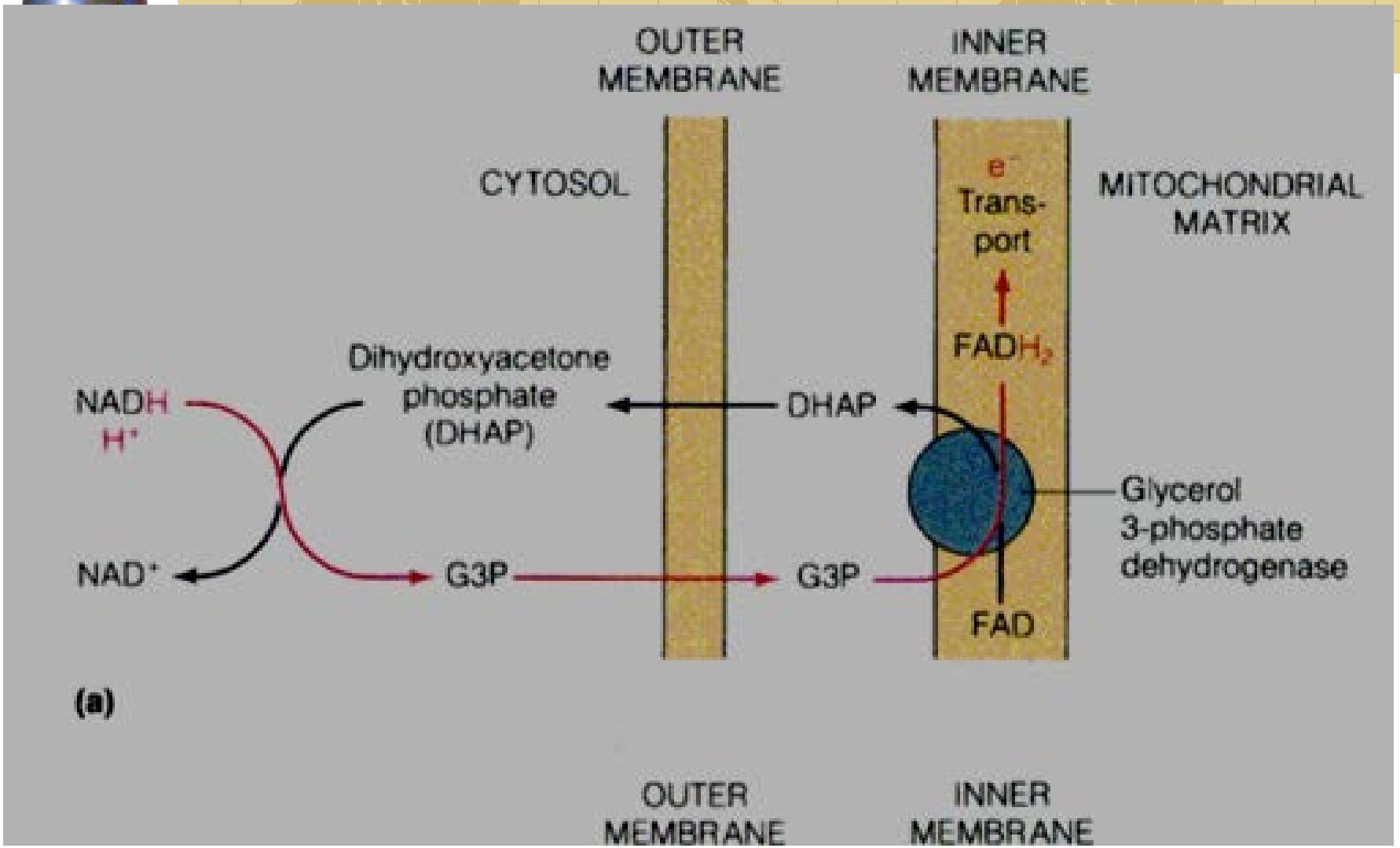
DHAP/ Glycerol-3-P shuttle system :

Note: the shuttle transfers electrons from NADH ultimately to make $FADH_2$

Transfers electrons to CoQ bypassing complex I.

Inefficient, **1.5 ATPs**.

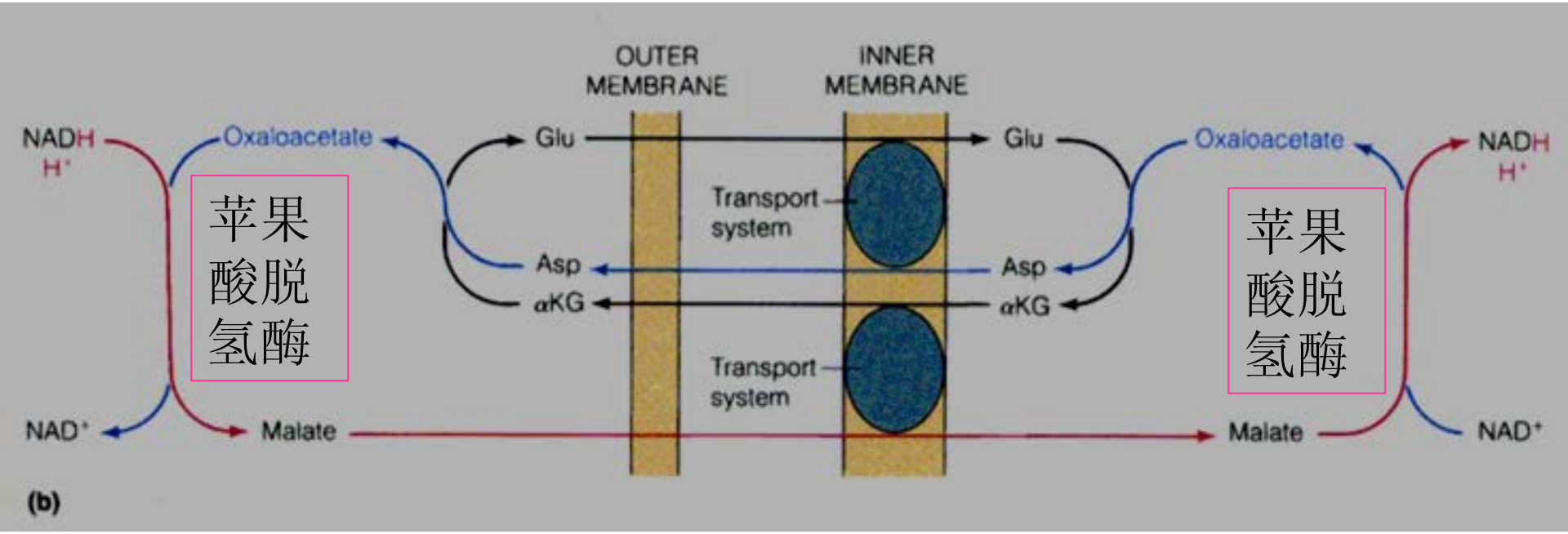

Glycolysis
NAD+
NADH + H+
cytosolic
glycerol 3-phosphate
dehydrogenase
Glycerol 3-
phosphate
Dihydroxyacetone
phosphate
CH2OH
C=O
CH2–O–P
CH2OH
CHOH
CH2–O–P
mitochondrial
glycerol 3-phosphate
dehydrogenase
FAD
FADH2
Q
III
Matrix

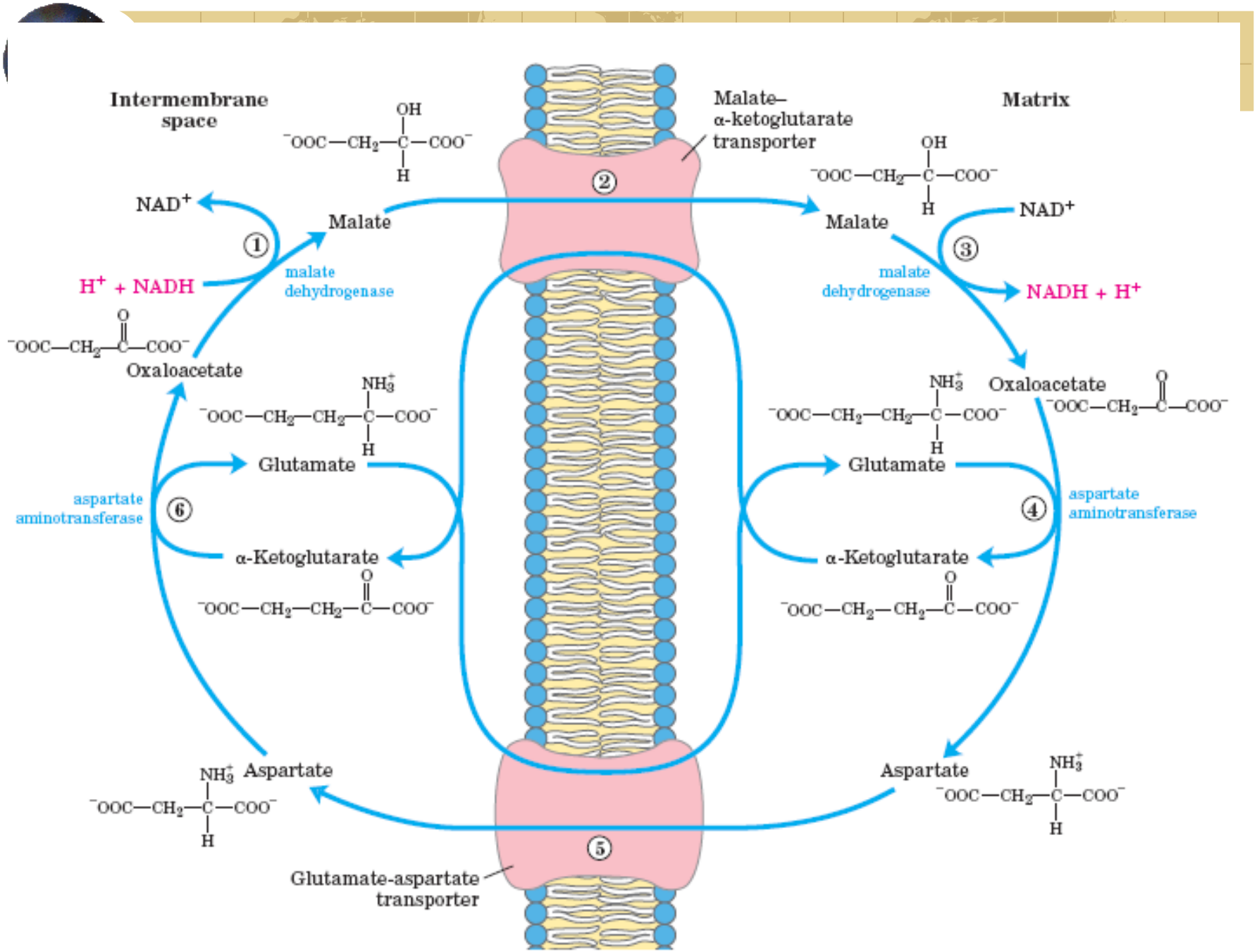

OUTER MEMBRANE
INNER MEMBRANE
CYTOSOL
e⁻ Trans-port
MITOCHONDRIAL MATRIX
FADH₂
Dihydroxyacetone phosphate (DHAP)
NADH H⁺
DHAP
Glycerol 3-phosphate dehydrogenase
NAD⁺
G3P
G3P
FAD
(a)
OUTER MEMBRANE
INNER MEMBRANE

甘油-3-磷酸脱氢酶

Malate/aspartate shuttle system:

2.5 ATPs per pair of electrons from NADH.




Intermembrane space
Matrix
Malate–α-ketoglutarate transporter
NAD+
Malate
malate dehydrogenase
H+ + NADH
Oxaloacetate
Glutamate
aspartate aminotransferase
α-Ketoglutarate
Aspartate
Glutamate-aspartate transporter
NADH + H+
1
2
3
4
5
6

7 Respiratory control

Substrates： ADP, Pi, O_2, oxidizable metabolite

Respiration is tightly coupled to the **synthesis of ATP**

respiratory control

•ATP synthesis absolutely dependent on continued electron flow from substrates to oxygen

•Electron flow in normal mitochondria occurs only when ATP is being synthesized

Biological sense

- Ensures that substrates will not be oxidized wastefully
- Their utilization is controlled by the physiological need for ATP

In most aerobic cells : [ATP] = (4- to 10-) *[ADP]

Respiration depends on ADP as a substrate for phosphorylation

The cell: ATP is consumed at high rates

So that———

accumulation of ADP

stimulates respiration

activation of ATP resynthesis

relaxed and well-nourished cell

ATP accumulates at the expense of ADP

the depletion of ADP limits the rate of both electron transport

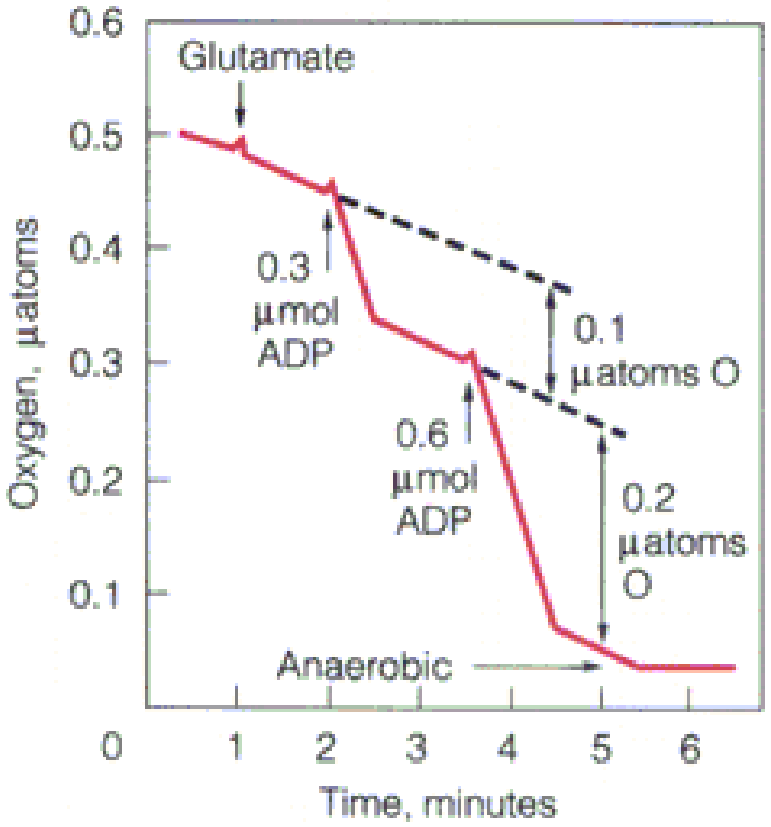
and its own phosphorylation to ATP

energy-generating capacity ⟺ **energy demands**

Addition of an oxidizable substrate

such as glutamate, has but a small effect on the respiration rate.

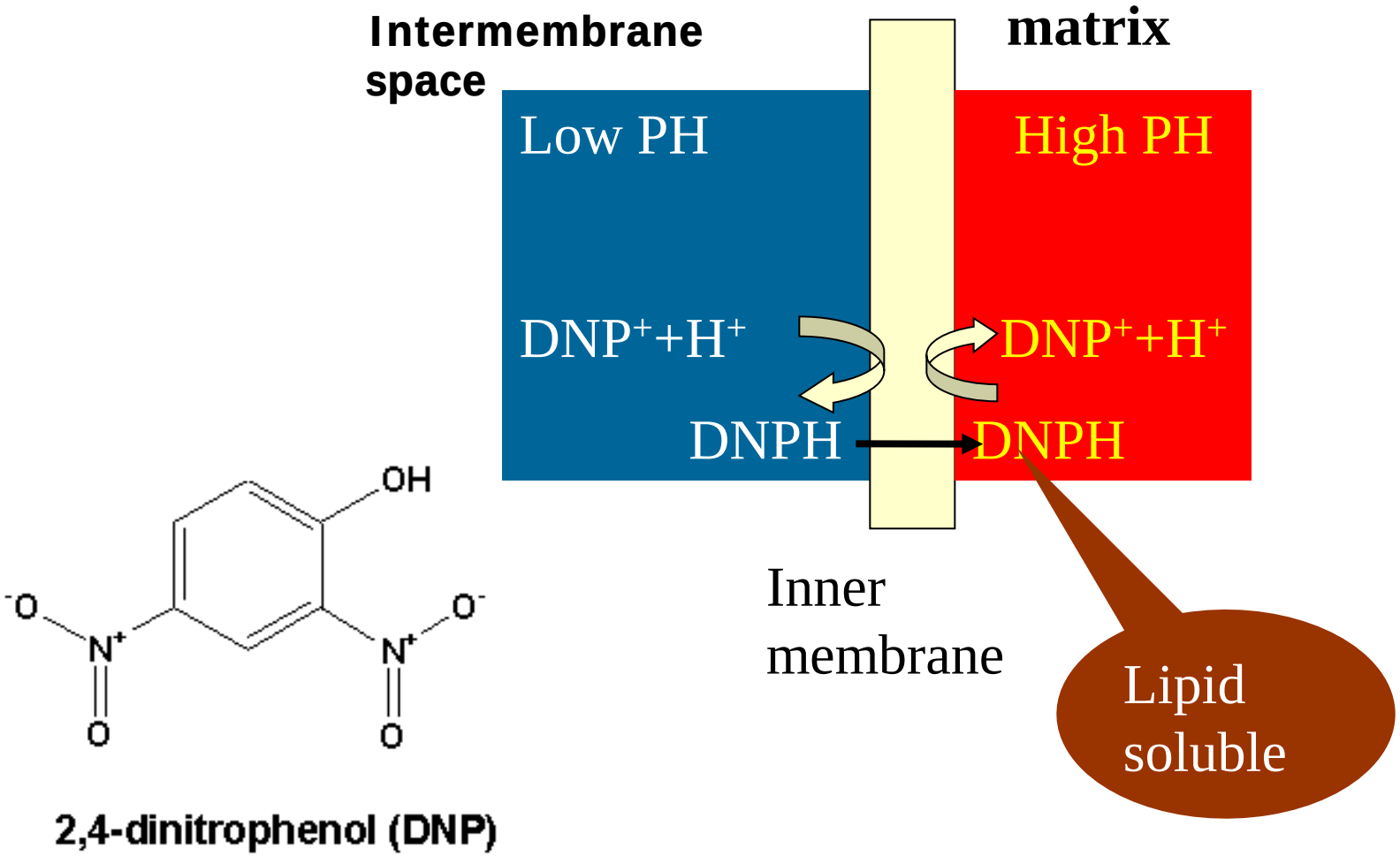
ADP is added, however, oxygen uptake proceeds at an enhanced rate until all of the added ADP has been converted to ATP, and then oxygen uptake returns to the basal rate.



氧化磷酸化的解偶连

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）


Intermembrane space
matrix
Low PH
High PH
DNP+ +H+
DNP+ +H+
DNPH
DNPH
Inner membrane
Lipid soluble
OH
-O
N+
O
O
N+
O-
O
2,4-dinitrophenol (DNP)

- permeabilize the inner mitochondrial membrane to protons
- destroy the proton gradient
- **uncouple** the ETS from the **oxidative phosphorylation** system.

electrons continue to pass through ETS

reduce oxygen to water, but **ATP** is not synthesized

•以前曾有人考虑使用DNP作为减肥药，但不久就停止使用，Why？

After Indian student's death in UK, university warns students against weight-loss pills

Edited by Amit Chaturvedi (With PTI inputs) | Updated: February 24, 2013 16:15 IST

Photo credit: from the Facebook page of Sarmad Alladin

London: The death of 18-year-old student from Hyderabad has shocked many in the UK. Reports say that Sarmad Alladin died after apparently taking 'lethal' bodybuilding pills to help him lose weight.

Known as 'Mr Muscles', Alladin was living in university accommodation in Epsom, Surrey, while attending the specialist art and design university in nearby Farnham University. He was taken to hospital hours after taking tablets which contained the drug Dinitrophenol (DNP), which has been linked to several deaths.

英国利兹大学女生萨拉·休斯顿死亡案经验尸后，死因确认为服用网上购买的含二硝基酚的违禁减肥药物而死，其家人呼吁政府在市场上彻底取缔含有这种物质的药物。这是最近的一则导报，比较讽刺的是，其家长和他本人都是学医的，但是由于贪食症，死者生前购买了二硝基酚，实际上这已经是2013年报道的第二起了，而服用二硝基酚减肥的死亡案例历史上可能过百。

让我们引用一下最近英国女学生死亡前的一些报道描述**“2012年9月休斯顿的室友在其卧室发现她死亡，死前她曾感到燥热、呼吸急促”**

1：**二硝基酚**在1930年左右很是热销，其售出可能上万份，直到1938年才受到美国药监局的重视，原因则是因为太多死亡病例，后来因为战争的原因，政府的关注度就消了。

2：二硝基酚现在依然在一些肥胖症的治疗中作为药物，但是请注意——只是少数病例，这不是私人可以使用的东西，因为你不是医生，看见英国那名死者了么？他是医学院的学生...“内行”尚且如此，何况只是欲望驱使，别用自己的小命搭上。

3：英国去世的这名死者出现病症加剧和他同时服用抗抑郁类药物有关，这里也多说一句，减脂的产品所有的都在内，都不能和抗抑郁和抗兴奋的药物一起服用。

氧化磷酸化抑制剂：

抑制ATP的形成，抑制O_2的消耗

寡霉素阻止膜间的H^+通过ATP合成酶的F_o进入线粒体基质，阻止ATP生成，还会维持和加强质子动力势，对电子传递产生反馈抑制，O_2的消耗就会相应减少。

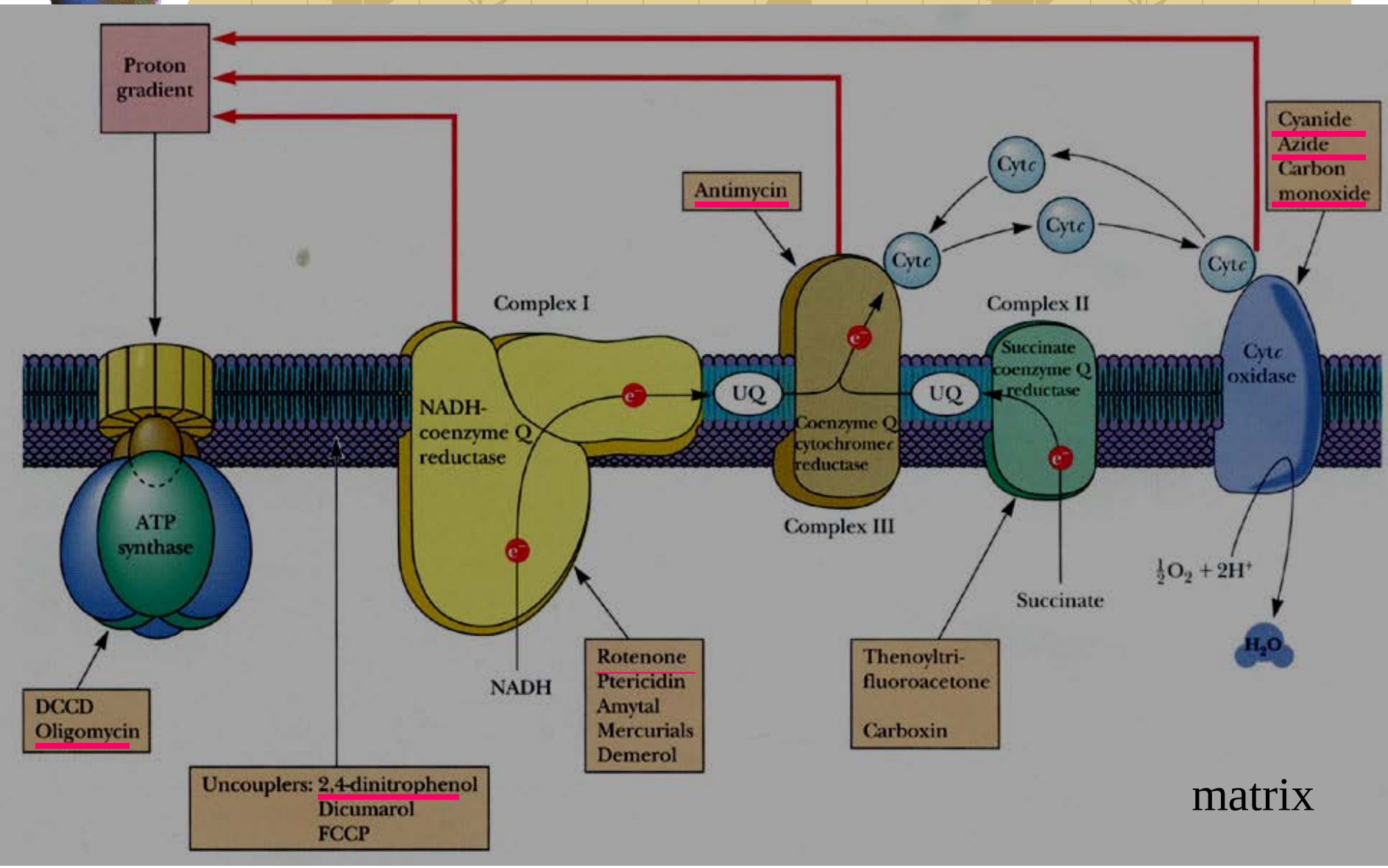
离子载体抑制剂：

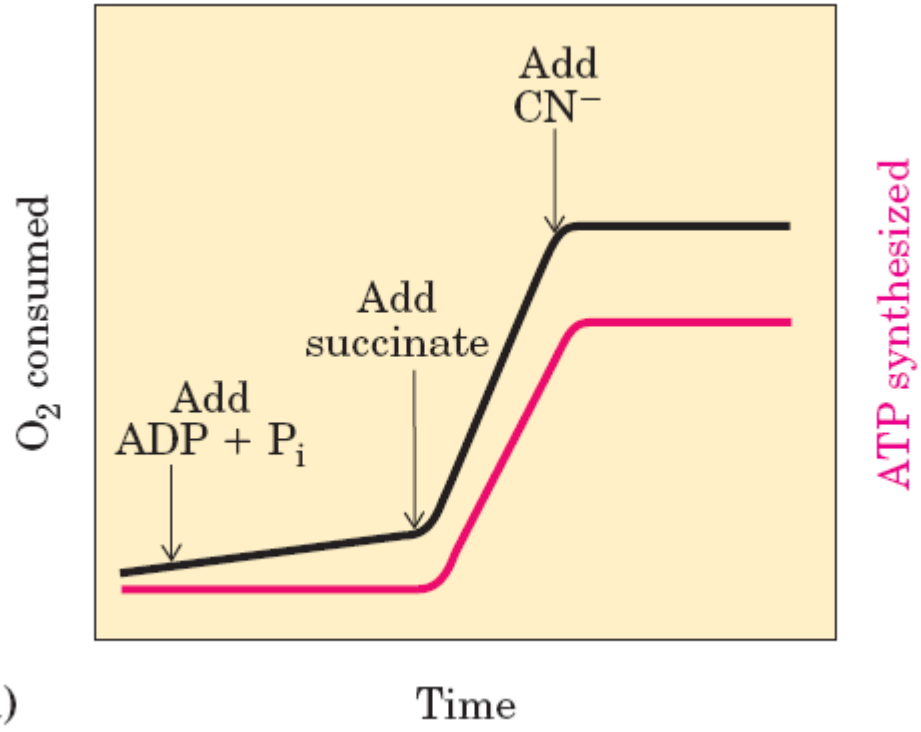
脂溶性物质

Valinomycin 缬氨霉素　K^+

Gramicidin 短杆菌肽　K^+　Na^+

在转运阳离子到基质中时消耗了自由能，降低了质子动力，从而抑制了ATP的形成

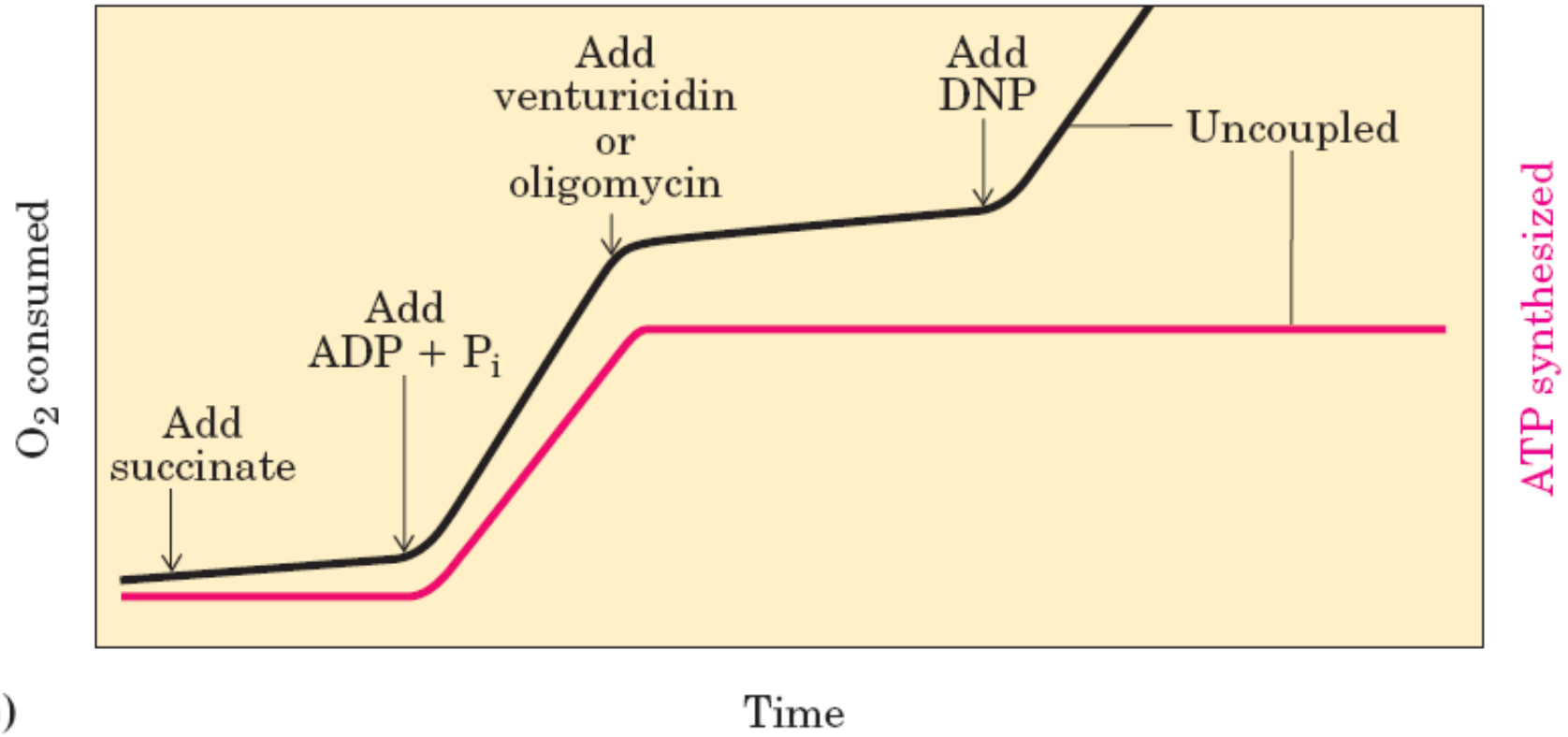

Proton gradient
Cyanide
Azide
Carbon monoxide
Antimycin
Cyt c
Cyt c
Cyt c
Cyt c
Complex I
Complex II
Succinate coenzyme Q reductase
Cyt c oxidase
NADH-coenzyme Q reductase
UQ
UQ
Coenzyme Q cytochrome c reductase
Complex III
ATP synthase
$\frac{1}{2}O_2 + 2H^+$
Succinate
H_2O
Rotenone
Ptericidin
Amytal
Mercurials
Demerol
Thenoyltri-fluoroacetone
Carboxin
DCCD
Oligomycin
NADH
Uncouplers: 2,4-dinitrophenol
Dicumarol
FCCP
matrix



Addition of ADP and Pi alone results in little or no increase in either respiration (O2 consumption) or ATP synthesis

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.



(b) Mitochondria provided with succinate respire and synthesize ATP only when ADP and Pi are added.
Subsequent addition of venturicidin 杀黑星菌素or oligomycin, inhibitors of ATP synthase, blocks both ATP synthesis and respiration.

Dinitrophenol (DNP) is an uncoupler, allowing respiration to continue without ATP synthesis.

Effect of Rotenone and Antimycin A on Electron Transfer

Rotenone, a toxic natural product from plants, strongly inhibits NADH dehydrogenase of insect and fish mitochondria. Antimycin A, a toxic antibiotic, strongly inhibits the oxidation of ubiquinol.

(a) Explain why rotenone ingestion is lethal to some insect and fish species.

(b) Explain why antimycin A is a poison.

(c) 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.

	ATP synthase activity (nmol of ATP formed min^{-1} mg^{-1}
Controls	3.0
Patient 1	0.25
Patient 2	0.11
Patient 3	0.17

	ATP hydrolysis (nmol of ATP hydrolyzed min^{-1} mg^{-1})
Controls	33
Patient 1	30
Patient 2	25
Patient 3	31

编码ATP synthase 基因的一突变型已经被鉴定，具有该突变的患者呈现肌无力，运动紊乱，色盲等症状。提取患者的亚线粒体颗粒，鉴定 ATP synthase 的功能。首先，加入琥珀酸，测定ATP synthase 的活性（结果如左表所示）。

问：

1）该实验中加入琥珀酸的目的是什么？

2）该突变对于琥珀酸-偶联ATP合成体系有什么影响？

同时将亚线粒体在加入了ATP，但缺乏琥珀酸的缓冲液中孵育测定 ATPase 活性（结果如右表所示）。

问：

3）为什么在ATPase活性测定体系中不能有琥珀酸？

4）该突变对于ATP的水解有什么影响？

5）结合上述两个实验，请简单阐述该突变的特性

课本上在讲果糖-2，6-二磷酸的代谢过程中提到了一种PFK2酶和FBPase2酶，这两种酶是 在一条肽链上，但是催化反应的活性部位不同。似乎催化的是两个可逆的反应： PFK2酶催化果糖-6-磷酸，在C2位磷酸化成为果糖-2，6-二磷酸。而FBPase2酶催化果糖-2 ，6-二磷酸水解成为果糖-6-磷酸。虽然书上说两种酶的催化机制不同，但是，由于酶在反应 中扮演的角色是催化剂，而对反应的平衡常数没有影响，可是书上的描述看来是PFK2明显有利 于果糖-2，6-二磷酸的积累而FBPase2有利于果糖-2，6-二磷酸的降解。难道这种酶对这个反 应的平衡常数有影响？还是当中需要通过别的不同的反应途径？

课本上P140上图24-29中的malate-aspartate shuttle模

型中关于α-ketoglutarate的箭头是不是画反了？

- 在ppt第109页的图中，寡毒素首先作用于F_O，使质子梯度得到维持和加强，电子传递受到抑制 （课件上给出的原因是反馈抑制作用）；但这时ADP的积累为什么没有促进电子传递的作用呢 ？为什么只有等到加入解偶联剂后才会出现呼吸速率的上升（电子传递加速）呢？