Instructor: Zhang Rong

Department of Biochemistry & Molecular Biology

Sichuan University

CHAPTER 6

BIOLOGICAL OXIDATION

❖ Definition:

The process that substance is oxidazed into carbon dioxide (CO_2) and water(H_2O), and releases energy in organism.

❖ Characteristics of biological oxidation：

1、 The reaction is generally occurred in the condition that PH approaches neutral and the temperature is 37° C.

2、The produced energy is progressively released and can be storied .

3、The reaction requires water.

4、Use the decarboxylation way to produce CO_2.

5、Use the dehydrogenation way produce H_2O.

SECTION ONE

OXIDATION SYSTERM OF ATP PRODUCED

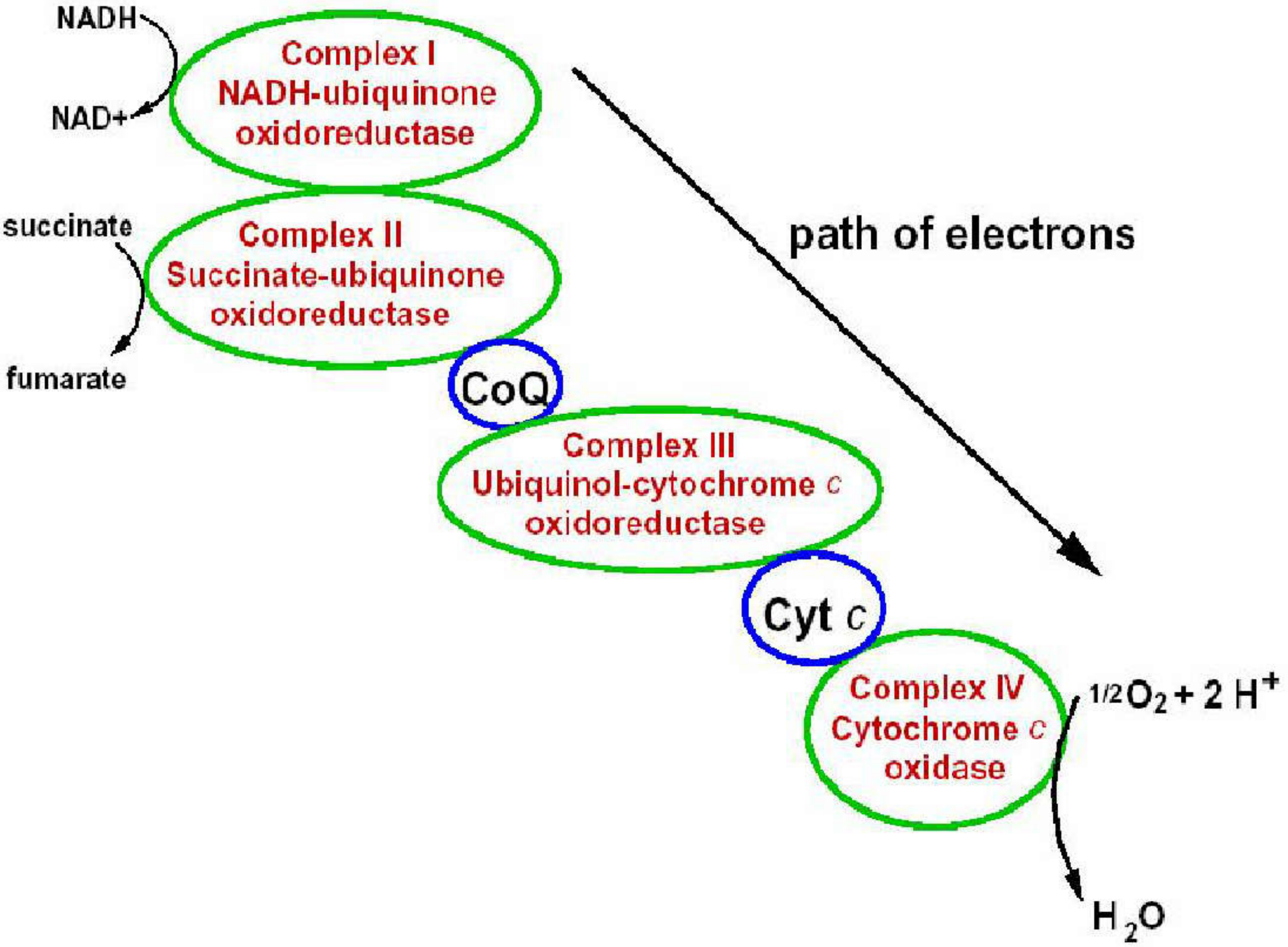
1、Respiratory chain

Definition： The chain reaction which is formed by the arrangement of a regular order of the enzymes and coenzymes on the inner membrane of mitochondria and is closely associated with the utilization of oxygen by cells is called respiratory chain.

（1）、Components of the respiratory chain

Four complex that still have the ability to transform electrons can be separated while using generally chemical methods

Flow of Electrons During Oxidative Phosphorylation



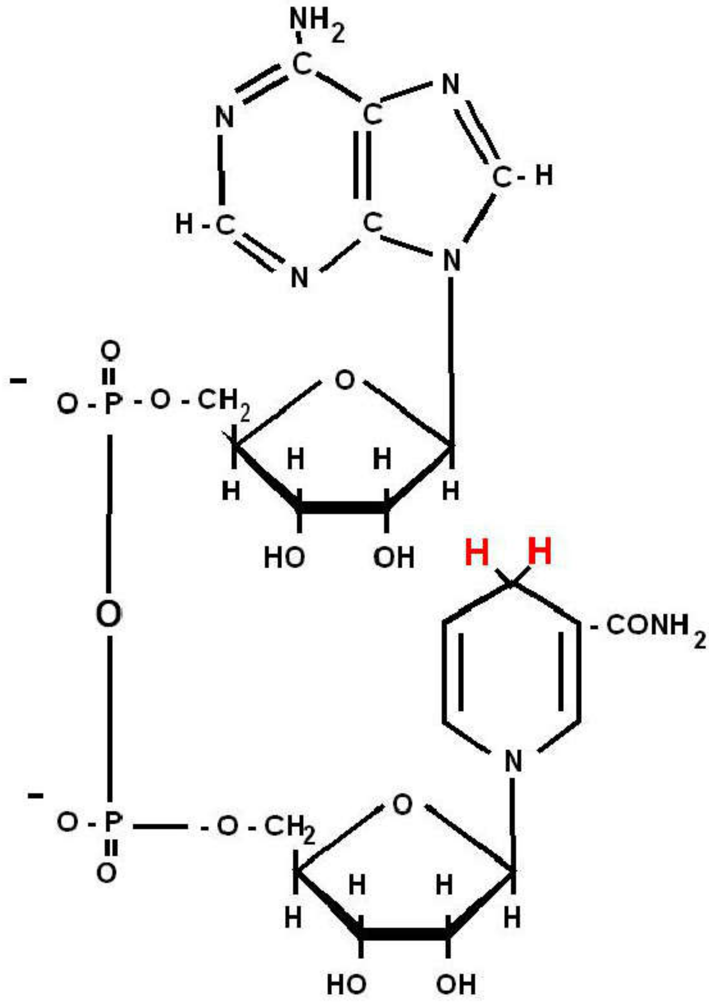
Structure and function of electron transmitter:

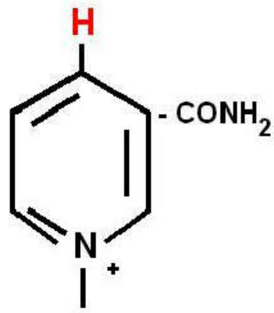
- Complex Ⅰ : NADH-ubiquinone oxidoreductase
  Pass the reducing equivalents of NADH to Q

NADH FMN Q
(FeS)

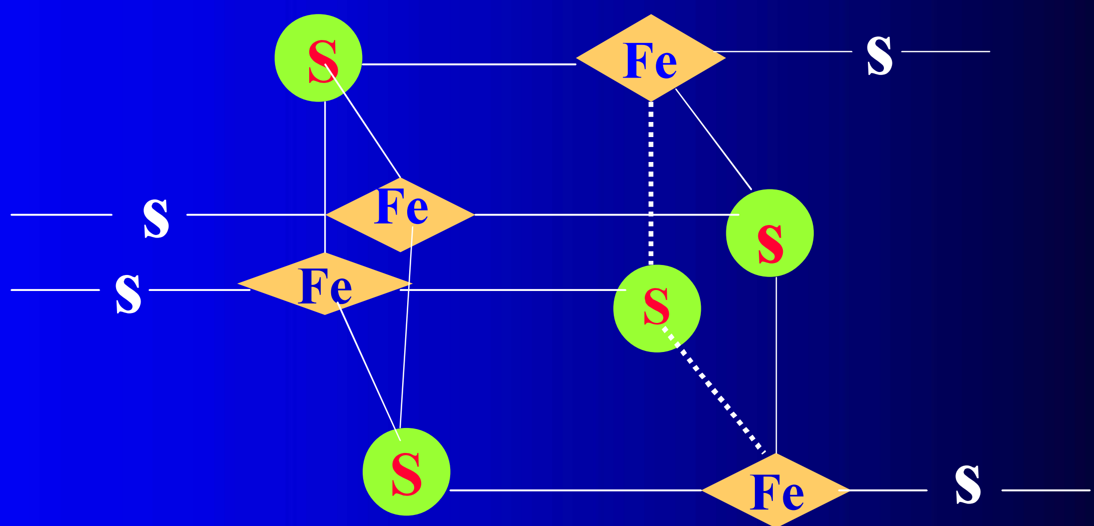
（1）Flavoprotein: The prosthetic group is FMN
（2）Iron-sulfur protein（FeS）

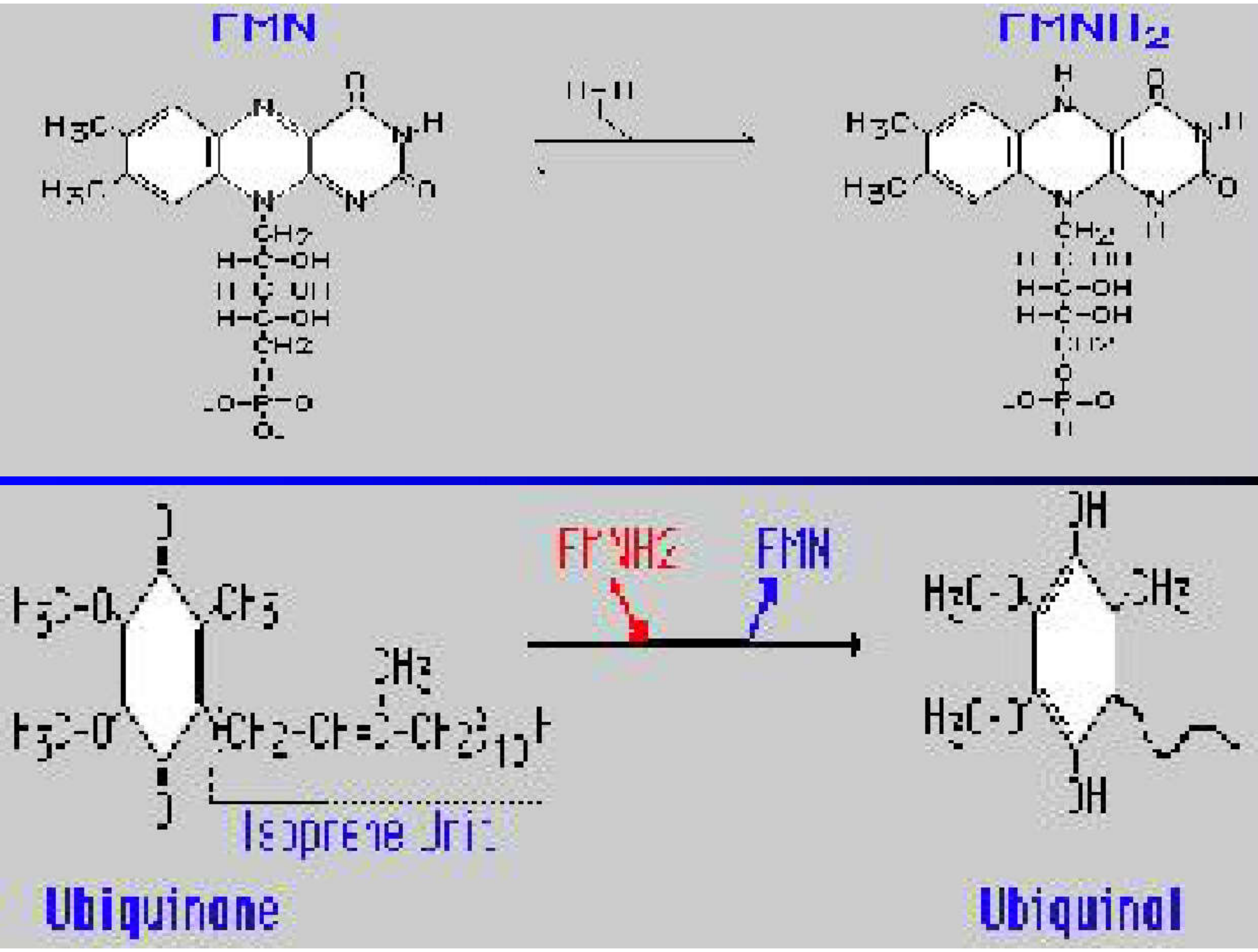
NADH





NAD^+


S
Fe
S
S
Fe
S
S
Fe
S
S
S
Fe
S


FMN
FMNH2
FMNH2
FMN
Ubiquinone
Ubiquinol

Complex Ⅱ： succinate-ubiquinone oxidoreductase

Pass the electrons from succinate to ubiquinone

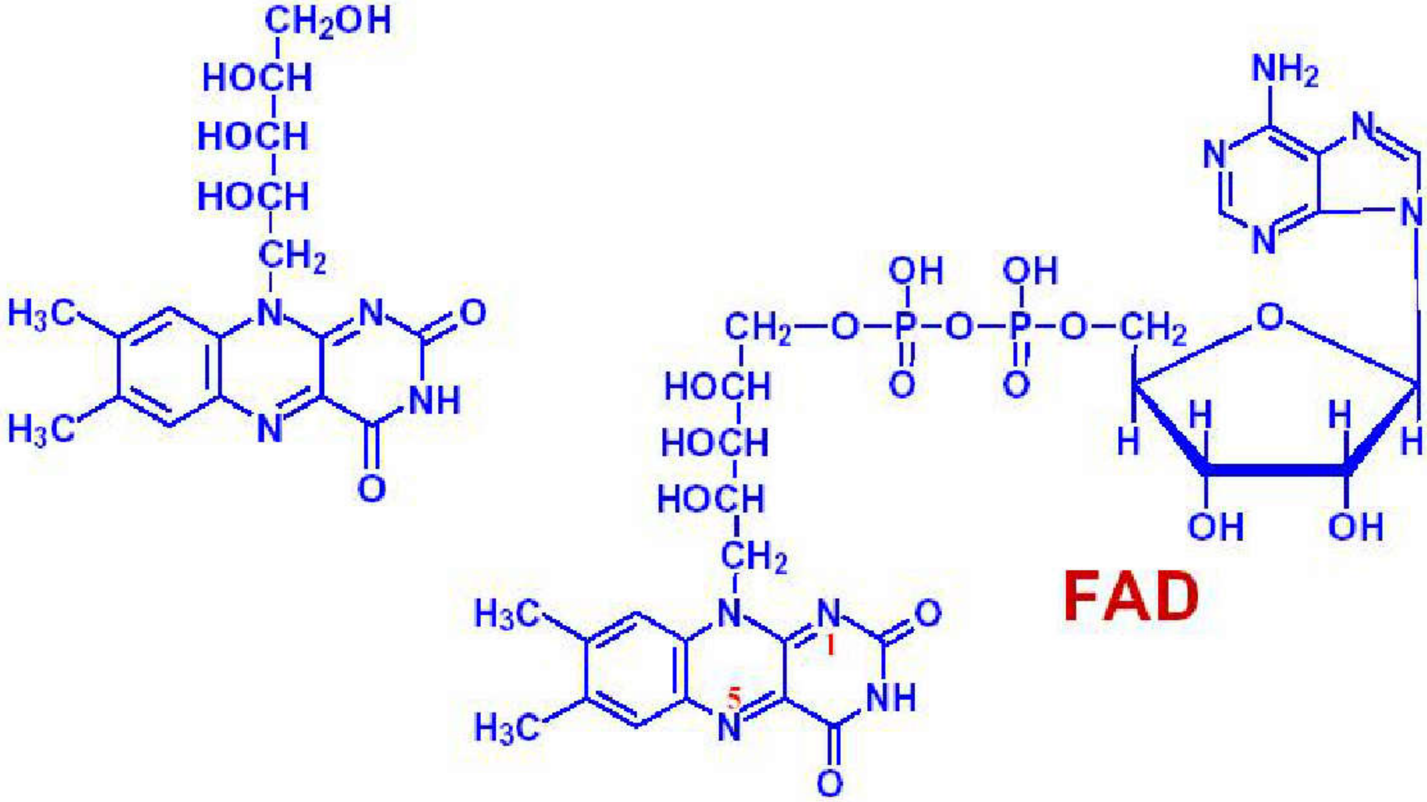
succinate FAD Q
(FeS)

（1） Flavoprotein: Prosthetic group is FAD

（2）Iron-sulfur protein (FeS)

(3) Cytochrome$_{560}$

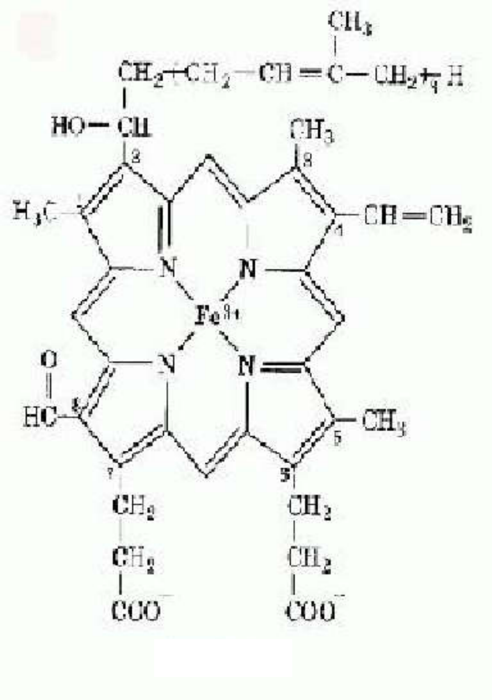
Riboflavin (B_2)

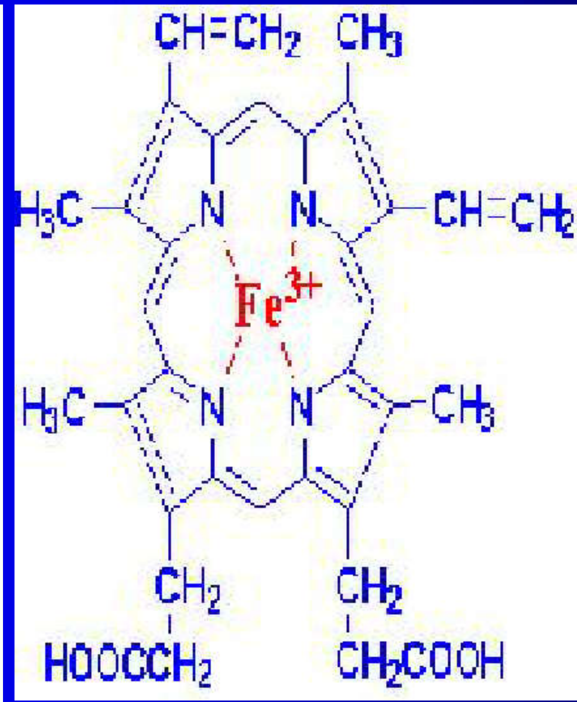


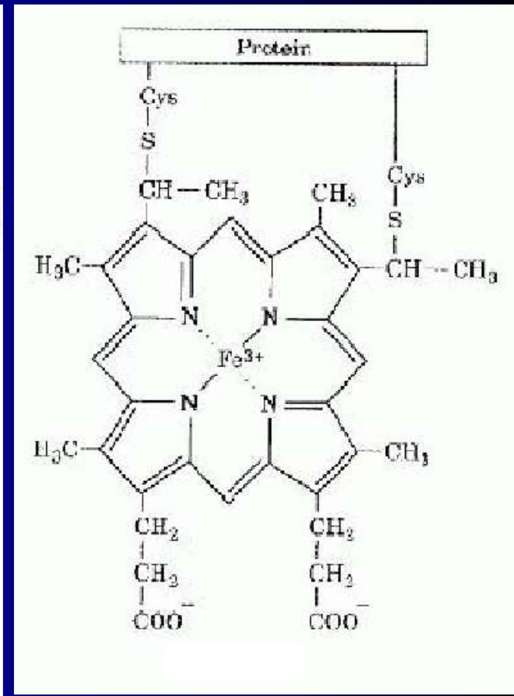
- **Complex Ⅲ：Pass the electrons from Q to CytC_1.**
  - **（1）Iron-sulfur protein (FeS)**
  - **（2）Cytochrome: Cytb_{562} , Cytb_{566}, Cytc_1**

Q   Cyt b_{562} Cyt b_{566} Cyt c_1

Cyt a | **Cyt b** | **Cyt c**







Complex Ⅳ： Cytochrome c oxidase

Pass the electrons from Cytc to oxygen.

Cyt c　　Cyt a　　Cyt a_3　　$1/2O_2$

This complex contain Cyta and Cyta3

Cytochrome C is the only soluble cytochrome and , togethe with uquinone (Q), it seems to be a more mobile component of the respiratory chain.

（2）The sequence of respiratorychain:

A、Determine the redox potential of all the electrons transmitters of the respiratory chain （$\Delta E^{0'}$）.

Some redox potentials of special interest in mammalian oxidation systems.

Oxidation-reduction couple	$\triangle E^{0/}$(V)
$2H^+ + 2e \longrightarrow H_2$	-0.42
$NAD^+ + 2H^+ + 2e \longrightarrow NADH + H^+$	-0.32
$FMN + 2H^+ + 2e \longrightarrow FMNH_2$	-0.12
$FAD + 2H + 2e \longrightarrow FADH_2$	-0.06
$CoQ + 2H^+ + 2e \longrightarrow CoQ$	0.04
$Cytb(Fe^{3+}) + e \longrightarrow Cytb(Fe^{2+})$	0.07
$CytC_1(Fe^{3+}) + e \longrightarrow Cyt\ C_1(Fe^{2+})$	0.22
$CytC(Fe^{3+}) + e \longrightarrow CytC(Fe^{2+})$	0.25
$Cyta(Fe^{3+}) + e \longrightarrow Cyta(Fe^{2+})$	0.29
$Cyta_3(Fe^{3+}) + e \longrightarrow Cyta_3(Fe^{2+})$	0.39
$1/2O_2 + 2H^+ + 2e \longrightarrow H_2O$	0.82

B、Add some respiratory chain inhibitors which the affect locus are afferent.

C、Analyse with the specially absorption spectrums properties of the electrons transmitter.

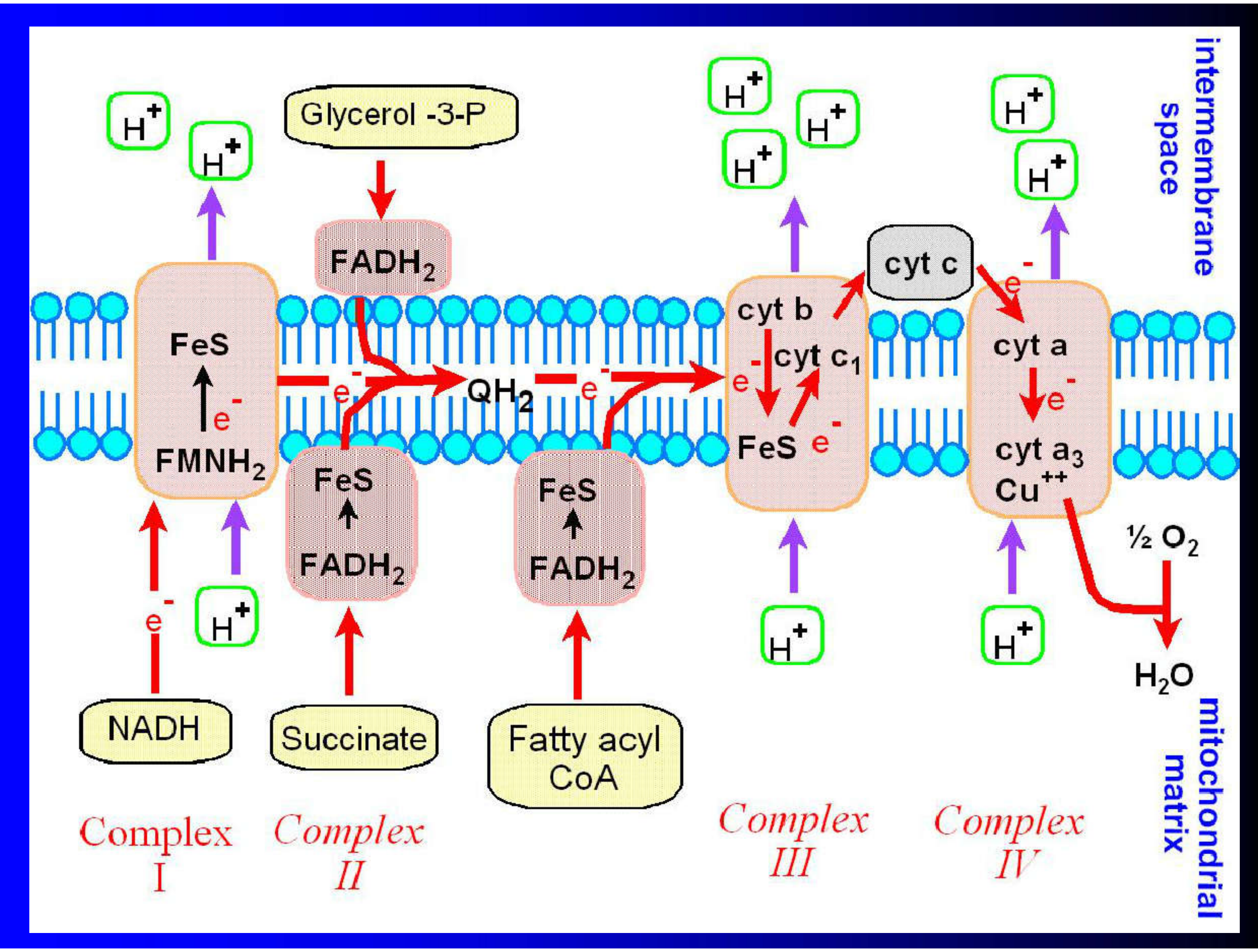
D、 Rebuild the electrons transmitter in external

- The two respiratory chain which their arrangements are known till now :
- a、NADH-oxidation-reduction respiratory chain ：

b、Succinate oxidation-reduction respiratory chain：

succinate FAD（FeS）

NADH FMN CoQ Cytb c_1 c aa_3 O_2



intermembrane space
Glycerol -3-P
FADH2
cyt c
cyt b
cyt c1
FeS
FMNH2
QH2
cyt a
cyt a3
Cu++
½ O2
H2O
NADH
Succinate
Fatty acyl CoA
Complex I
Complex II
Complex III
Complex IV
mitochondrial matrix

2、Oxidation phosphorylation

Definition: Redox reaction of substrates producing reducing equivalents(H or electrons) , There are funneled into the respiratory chain to their final reaction with oxygen to form water, and released free energy that used to synthesize ATP from ADP and Pi , in this way, oxidation is closely coupled to phosphorylation.

（1）The location of for oxidative phosphorylation

①、P/O ratio:

Definition : When substrates are oxidated producing equivalents(H or electrons), these are funneled into the respiratory chain ,their final reaction with oxygen to form water . The mol of the Pi that is consumed when one gram of mol oxygen (O_2) is consumed is called P/O ratio.

Substrates

oxygen

Equivalents FAD

NADH FMN CoQ Cytb c_1 c aa_3 O_2

H_2O

2ADP + Pi 2ATP P/O Ratio = 2

3ADP + Pi 3ATP P/O Ratio = 3

- **P/O ratio is measured in extra-somatic mitochondria:**

Substrate	Electron transfer channel	P/O ratio	ATP quality
ß-羟丁酸	NADH FMN CoQ Cyt O_2	2.4-2.8	3
琥珀酸	FAD CoQ Cyt O_2	1.7	2
抗坏血酸	Cytc $Cytaa_3$ O_2	0.88	1
细胞色素C	$Cytaa_3$ O_2	0.61-0.68	1

$FADH_2$

NADH FMN CoQ Cytb $Cytc_1$ Cytc Cytaa3 O2

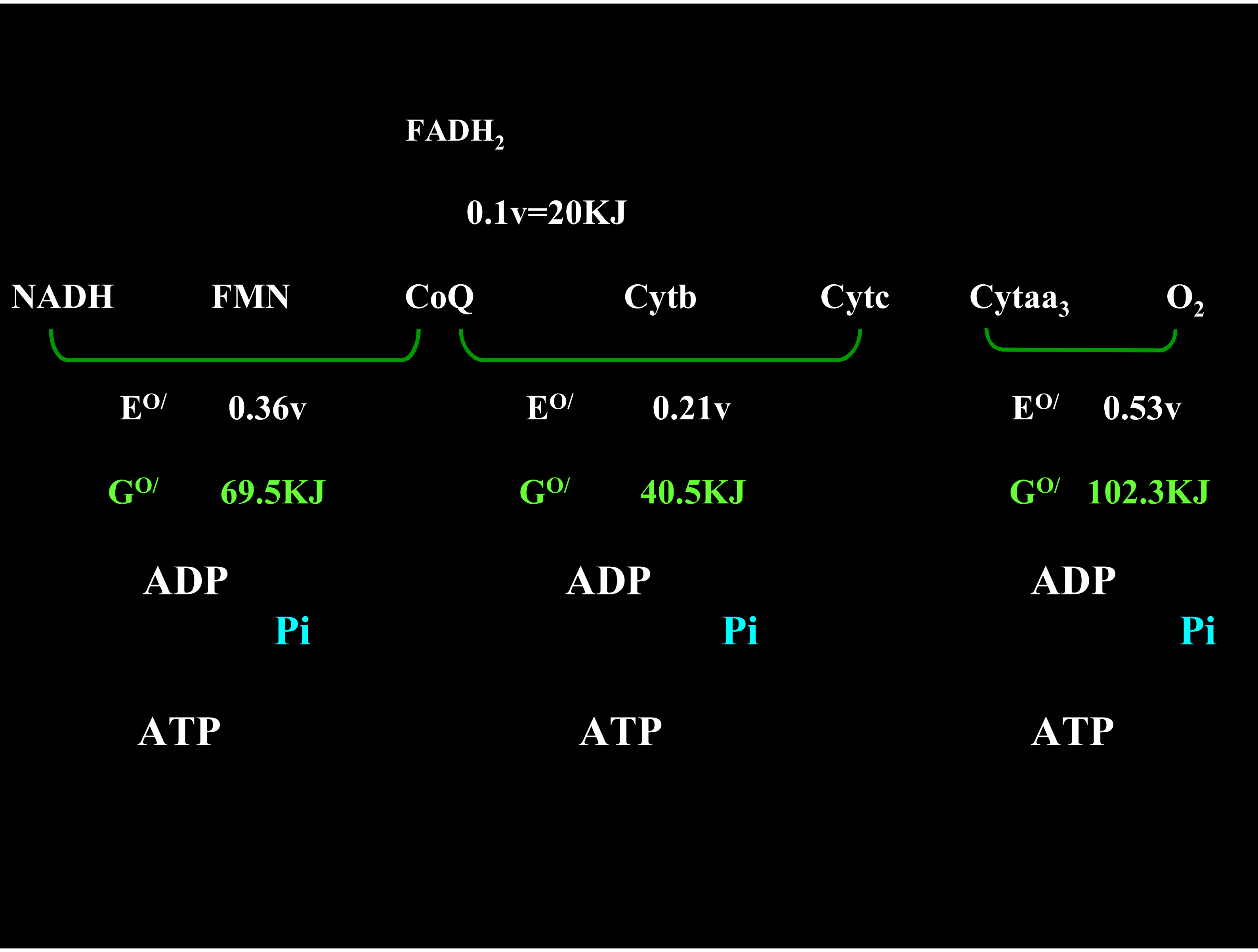
②、Inferring from the free energy released in different phases:

It is clear that, an approximate 30.5KJ/mol free energy is needed to from one gram molecular of ATP, which is equal to 0.2v of $E^{O/}$ respiratory chain. Therefore we can infer the location the ATP produced from the numerical value of the potential difference between two neighbor carriers on the respiratory chain.

Then **$\Delta E^{O/}$ is used to calculate $\Delta G^{O/}$:**

$$\triangle G^{O/} = - nF\triangle E^{O/}$$

$$F = 96.5KJ/mol . V$$

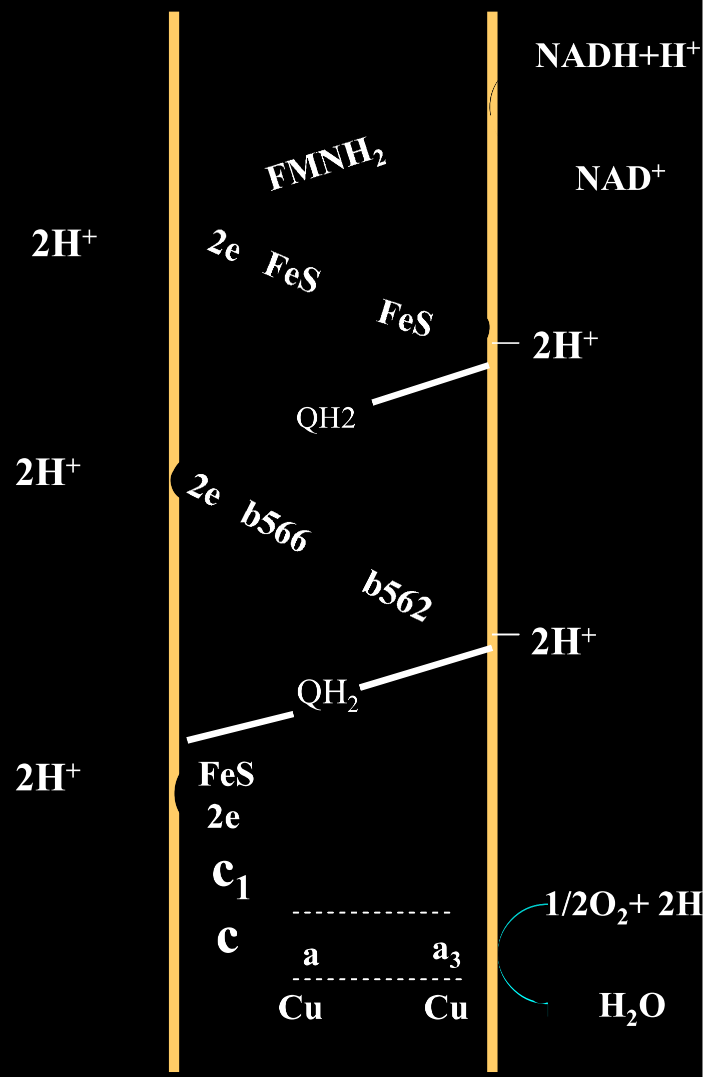


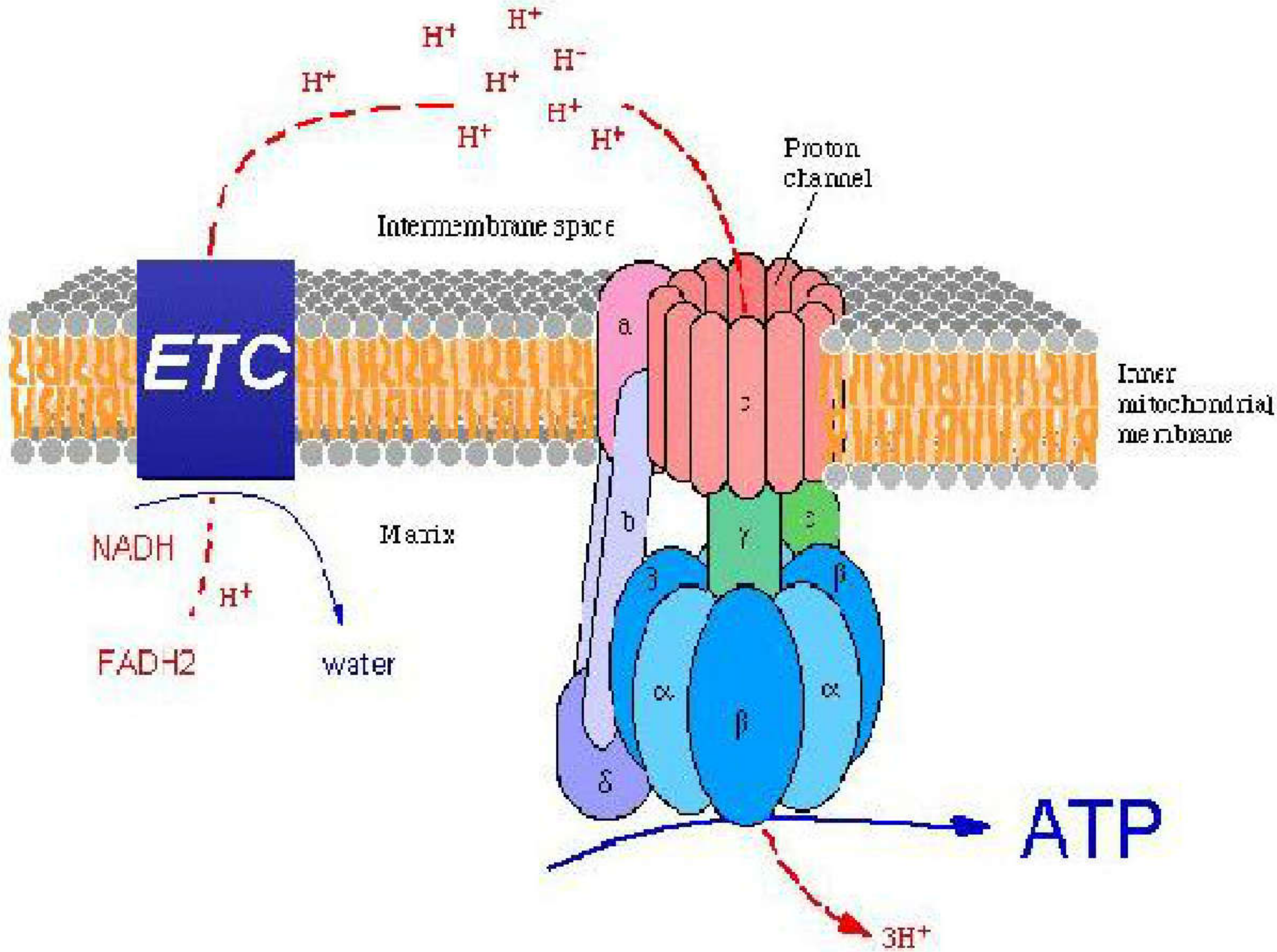
FADH2
0.1v=20KJ
NADH
FMN
CoQ
Cytb
Cytc
Cytaa3
O2
EO/ 0.36v
EO/ 0.21v
EO/ 0.53v
GO/ 69.5KJ
GO/ 40.5KJ
GO/ 102.3KJ
ADP
Pi
ATP
ADP
Pi
ATP
ADP
Pi
ATP

（2）The couple mechanism of oxidative phosphorylation

1、The chemo-osmosis theory:

In the early 1960_s Peter Mitchell suggested the chemo-osmosis hypothesis . He was given Nobel Chemistry Prize in 1978.





H+
H+
H-
H+
H+
H+
H+
H+
Proton channel
Intermembrane space
ETC
a
c
Inner mitochondrial membrane
b
γ
ε
β
β
α
β
α
δ
NADH
H+
FADH2
Matrix
water
ATP
3H+

2、ATP synthetase：

（1） structure of ATP synthetase：

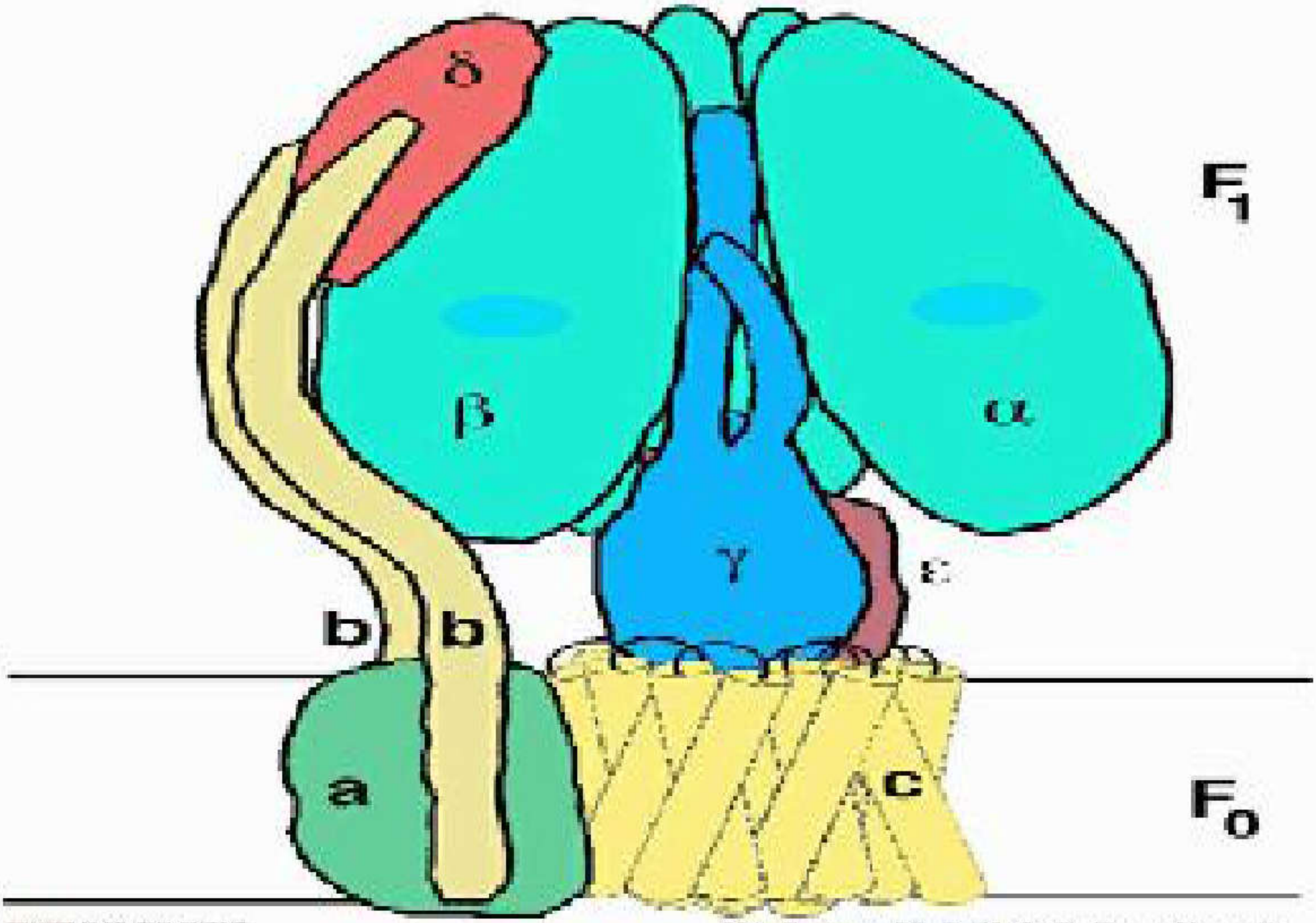
ATP synthetase is a large membrane-protein complex .

The ATP-synthesizing enzyme complex of the inner mitochondrial membrane has two major components, F_1 and F_0.

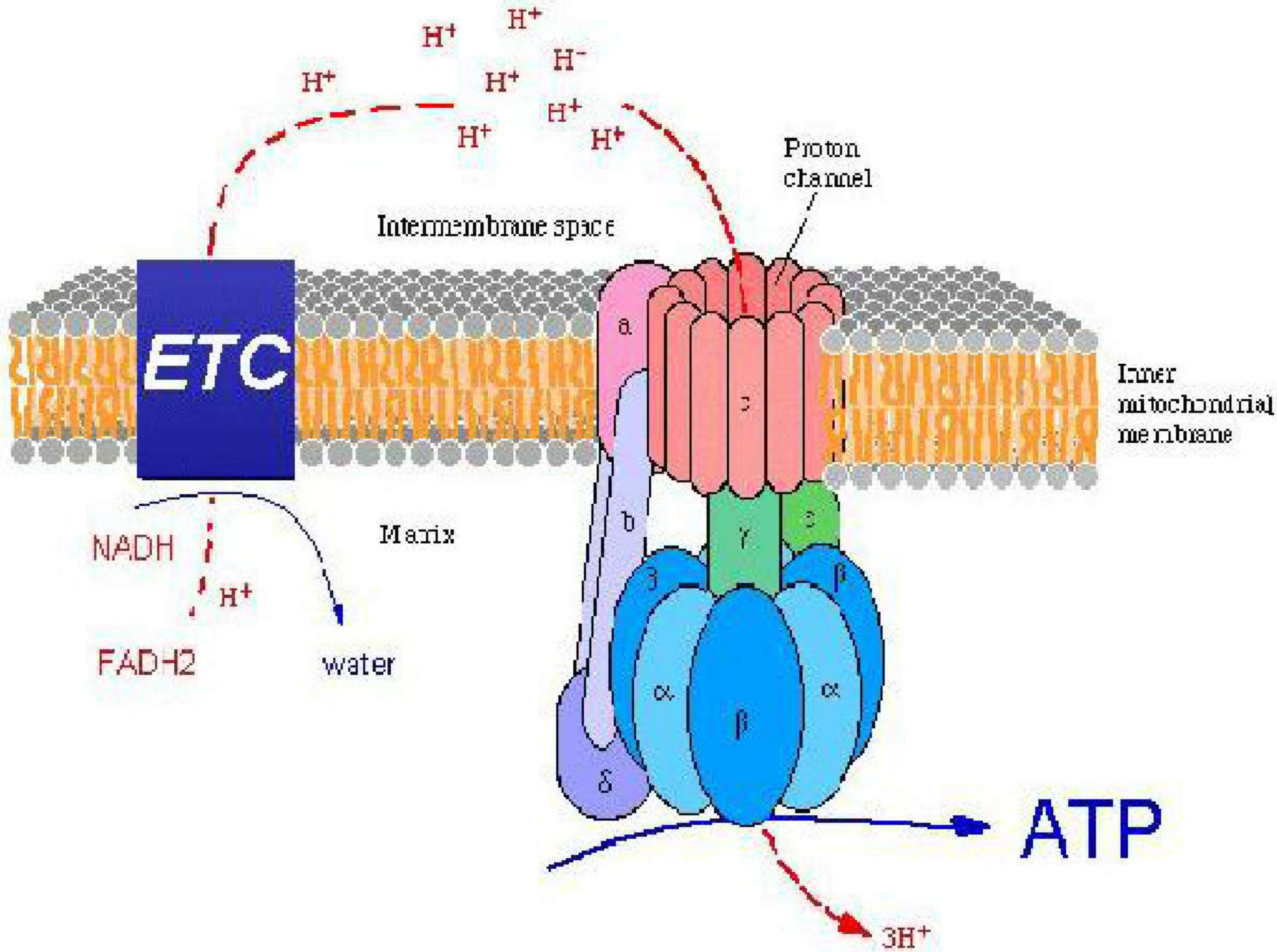
- F_1： F_1 dissolves in water.

The F_1 complex is consisted with the proportion of $\alpha_3\beta_3\gamma\delta\varepsilon$. MW: 371Kd

A tight binding site for ATP, apparently identical to the catalytic site, is located on each β subunit, or perhaps between each β and its associated α subunit.



W. Junge, H. Lill & S. Engelbrecht
TIBS 22, 420 - 423 (1997)

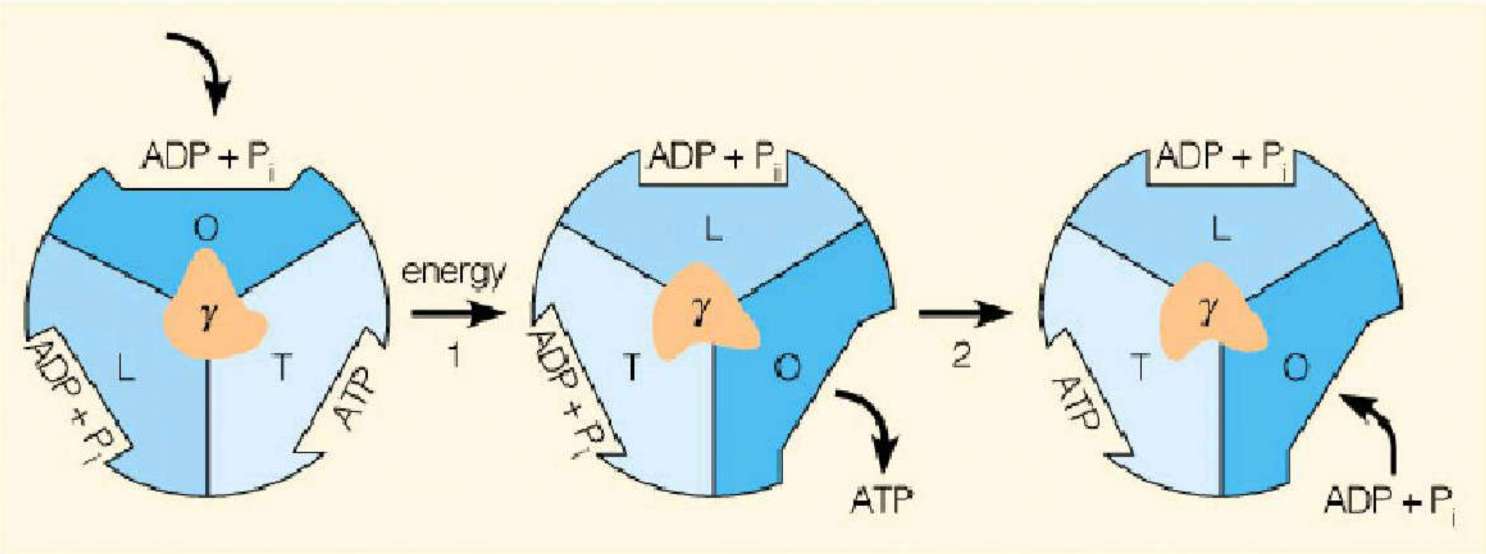

H+
H+
H+
H-
H+
H+
H+
H+
Proton channel
Intermembrane space
ETC
a
c
Inner mitochondrial membrane
b
γ
NADH
Matrix
H+
FADH2
water
α
β
α
δ
ATP
3H+

- F_0 : F_0 is liposoluble

  the F_0 complex consists of **a** subunit ,**b** subunit,**c** subunit and oligomycin-sensitivity-conferring protein(OSCP). Its structure is ab_2c_n

  The primary role of F_0 is transmitting the energy produced by the proton gradient to F_1 .

（2）The detailed mechanism of the synthesis of ATP



Adopted from "Biochemistry" by Mathews, Van Holde and Ahern
Addison Wesley Longman Press 2000

3、The factors influenced of oxidation and phosphorylation

(1)、ADP+Pi/ATP ratio is the primary factor to regulate the oxidation phosphorylation

(2)、Oxidative phosphorylation is also regulated by hormone：

The thyroxine is able to facilitate the reaction of hydrolysis to resolve ATP into ADP and Pi.

(3)、The inhibitors of the oxidation phosphorylation

Inhibitor may be divided into the inhibitors of the respiratory chain proper the inhibitors of the oxidative phosphorylation, and the uncouples of the oxidative phosphorylation

① **The inhibitors of the oxidative phosphorylation :**

Medicines: rotenone, piericidin A , amobarbital, antimycin A, dimercaptopropanol (BAL).

Poisons: CO, CN^- , N_3^+

FAD

NADH FMN | CoQ Cytb | $Cytc_1$ Cytc $Cytaa_3$ | O_2

Rotenone, Piericidin A, Amobarbitol (FMN | CoQ)

BAL, Antimycin A (Cytb | $Cytc_1$)

N_3, CO, CN^- ($Cytaa_3$ | O_2)

②、**Uncouples:**

Dinitrophenol, Pentachlorophenol, dinitrocresol

The action of the uncouples is to dissociate oxidation in the respiratory chain from phosphorylation. P/O ratio decreases the quality of ATP synthesized is decreased, and speed up the oxidative of substrates.

③ **Oligomycin:**

The oligomycin completely blocks the electrons from flowing back to F_1 from F_0. This inhibitors prevent ATP from being synthesized from ADP and Pi . It lead to the uncouples of the oxidative phosphorylation.

(4) The mutation of the mitochondrial DNA (mt DNA)

4、 High-energy phosphates —— ATP

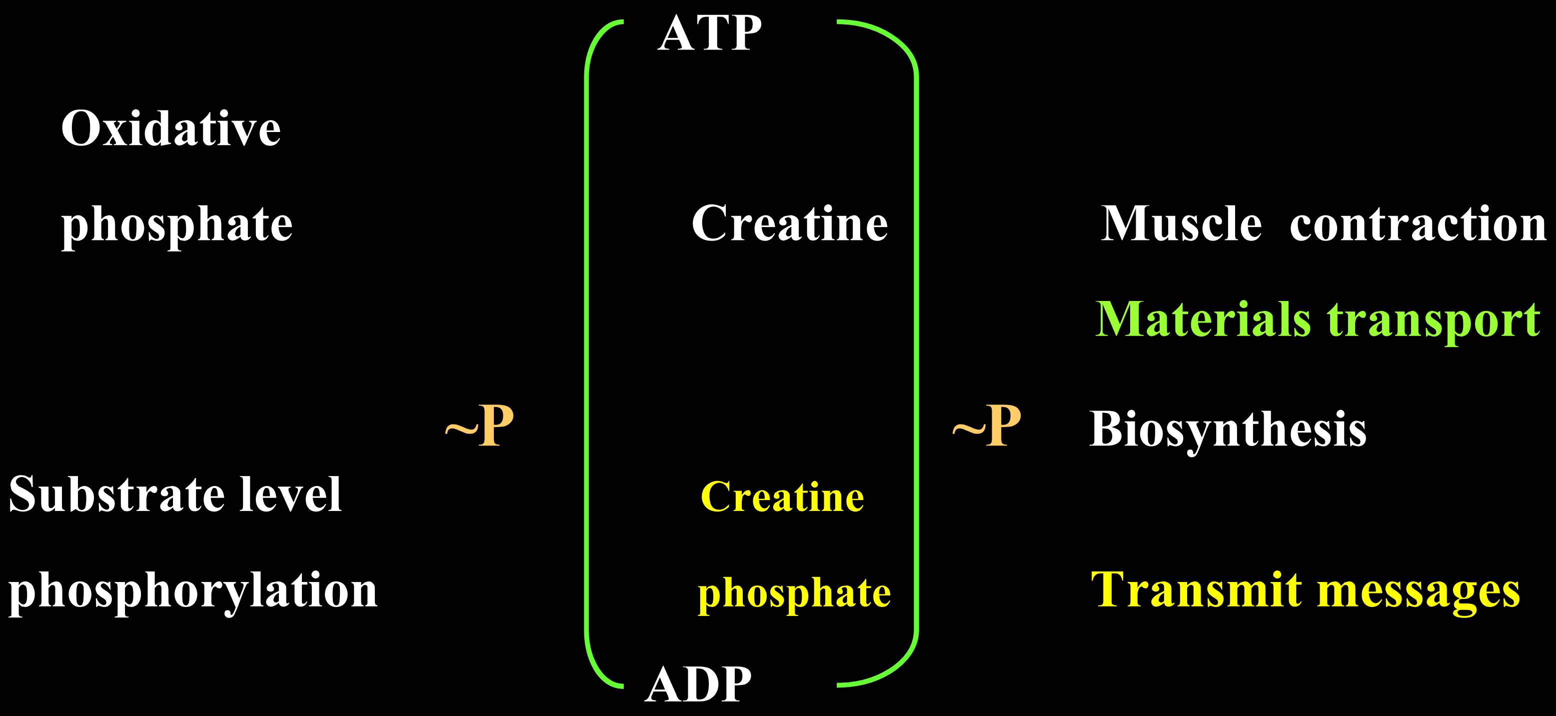
(1)、 High-energy phosphates bond

If the free energy that released when the phosphate ester-bond is hydrolyzing is more than 21KJ/mol ,we call it the high energy phosphate bond . Lipmann introduced the symbol "~P" to represent it.

The compounds contained the high energy phosphate bond are called high energy phosphate compounds.

(2)、 The common some high-energy compounds in organism:

(3)、ATP cycle：


ATP
Oxidative phosphate
Creatine
Muscle contraction
Materials transport
~P
~P
Biosynthesis
Substrate level phosphorylation
Creatine phosphate
Transmit messages
ADP

The role of ATP cycle :

- **Exchanging energy:** ATP acts as the "energy currency"of the cell, transferring free energy derived from substances of higher energy potential to those of lower energy potential.

- Connect the phosphorylation and dephosphorylation in the progress of metabolism.

(4) The transformation of the high-energy phosphate bond

① Adenylate kinase interconversts adenine nucleosides :

The enzyme adenylate kinase is prevent in most cells. It catalyzed the interconversion of ATP and AMP on the hand and ADP on the other:

Adenosine kinase

ATP + AMP ADP + ADP

② Other nucleoside tri-phosphates participate in the transfer of high-energy phosphate.

By means of the enzyme nucleoside di-phosphate kinase ,nucleoside tri-phosphates similar to ATP but containing an alternative base to adenine can be synthesized from their di-phosphates, eg:

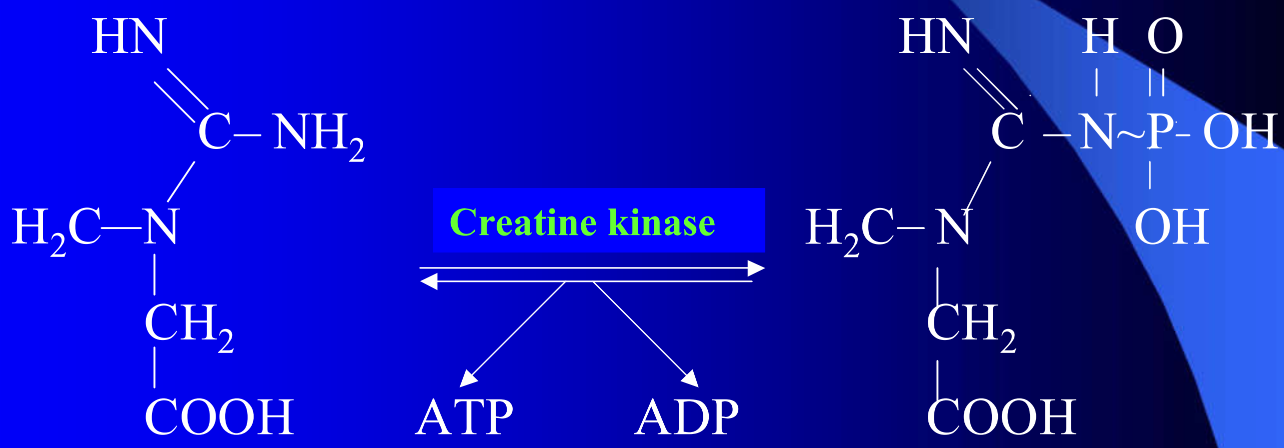
$$ATP + UDP \xrightarrow{\text{nucleoside diphosphate kinase}} ADP + UTP$$

$$ATP + GDP \xrightarrow{\text{nucleoside diphosphate kinase}} ADP + GTP$$

$$ATP + CDP \xrightarrow{\text{nucleoside diphosphate kinase}} ADP + CTP$$

③. Creatine phosphate shuttle:

The phosphagens, act as storage forms of high-energy phosphate, occurring in vertebrate skeletal muscle, heart and brain, under physiologic conditions phosphagens permit ATP concentrations to be maintained in muscle when ATP is rapidly being utilized as a source of energy for muscular contraction. On the other hand ,when ATP is plentiful and the ATP/ADP ratio is high, their concentration can up to act as a store of high-energy phosphate.



Creatine

Phosphate creatine

5. Substance transporter systems of the mitochondrial inner membrane

（1） Shuttle systems are required for mitochondrial oxidation of cytosolic NADH:

The dehydrogenase of the inner mitochondrial membrane of animal cells can accept electrons only from NADH in the matrix, given that the inner membrane is not permeable to cytosolic NADH, how can the NADH generated by glycolysis outside mitochondrial be re-oxidized to NAD^+ by O_2 via the respiratory chain?

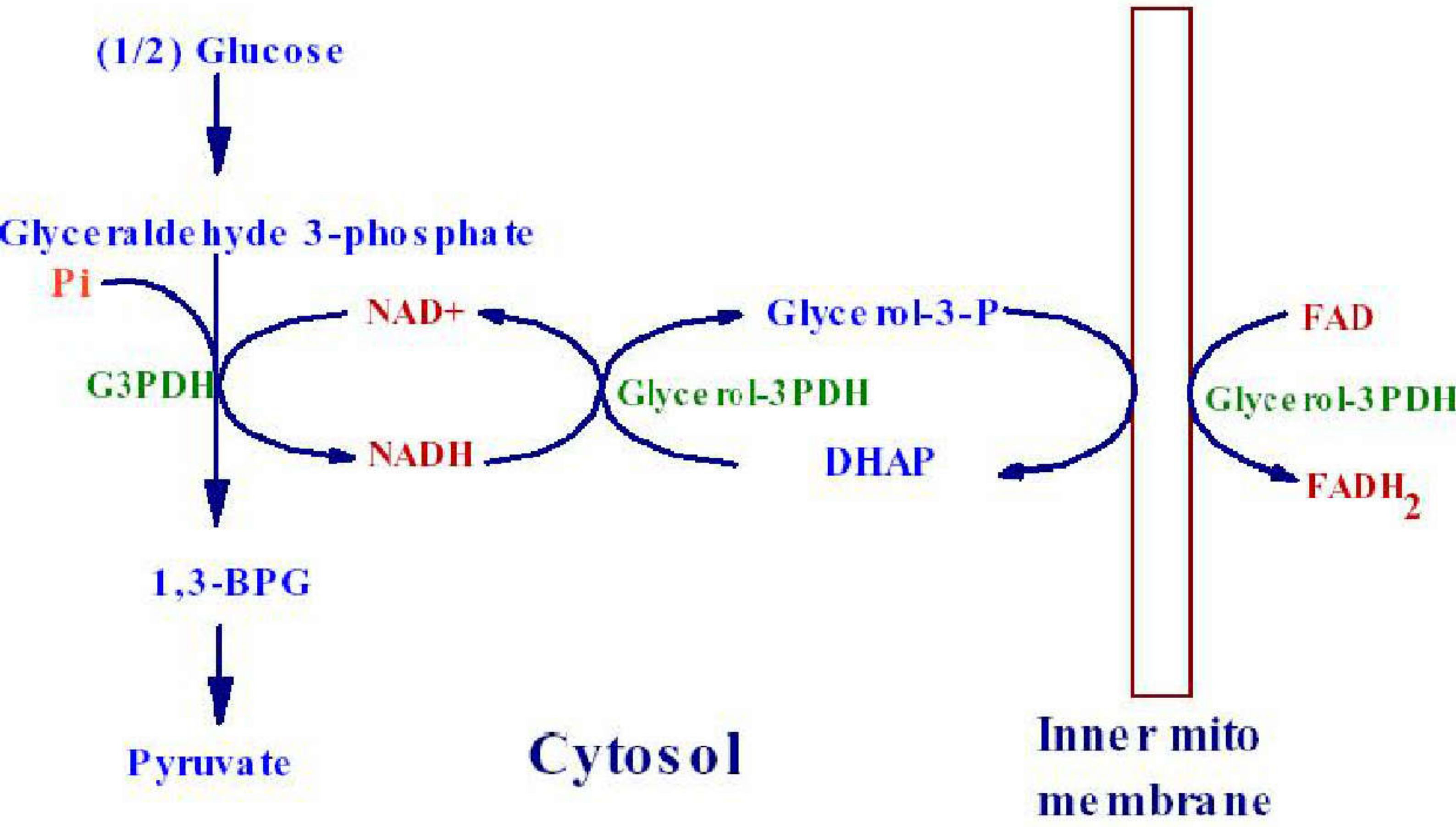
Special shuttle systems carry reducing equivalents from cytosolic NADH into mitochondrial by an indirect route.

The most active NADH shuttle , is the malate-aspatate shuttle and the glycerol-3-phosphate shuttle.

①. The glycerol-3-phosphate shuttle:

This shuttle in skeletal muscle and brain, in that it delivers the reducing equivalents from NADH into complex Ⅱ(into succinate oxidation respiratory chain) not complex Ⅰ, providing only enough energy to synthesize two ATP(2 ATP)molecules per pair of electrons.

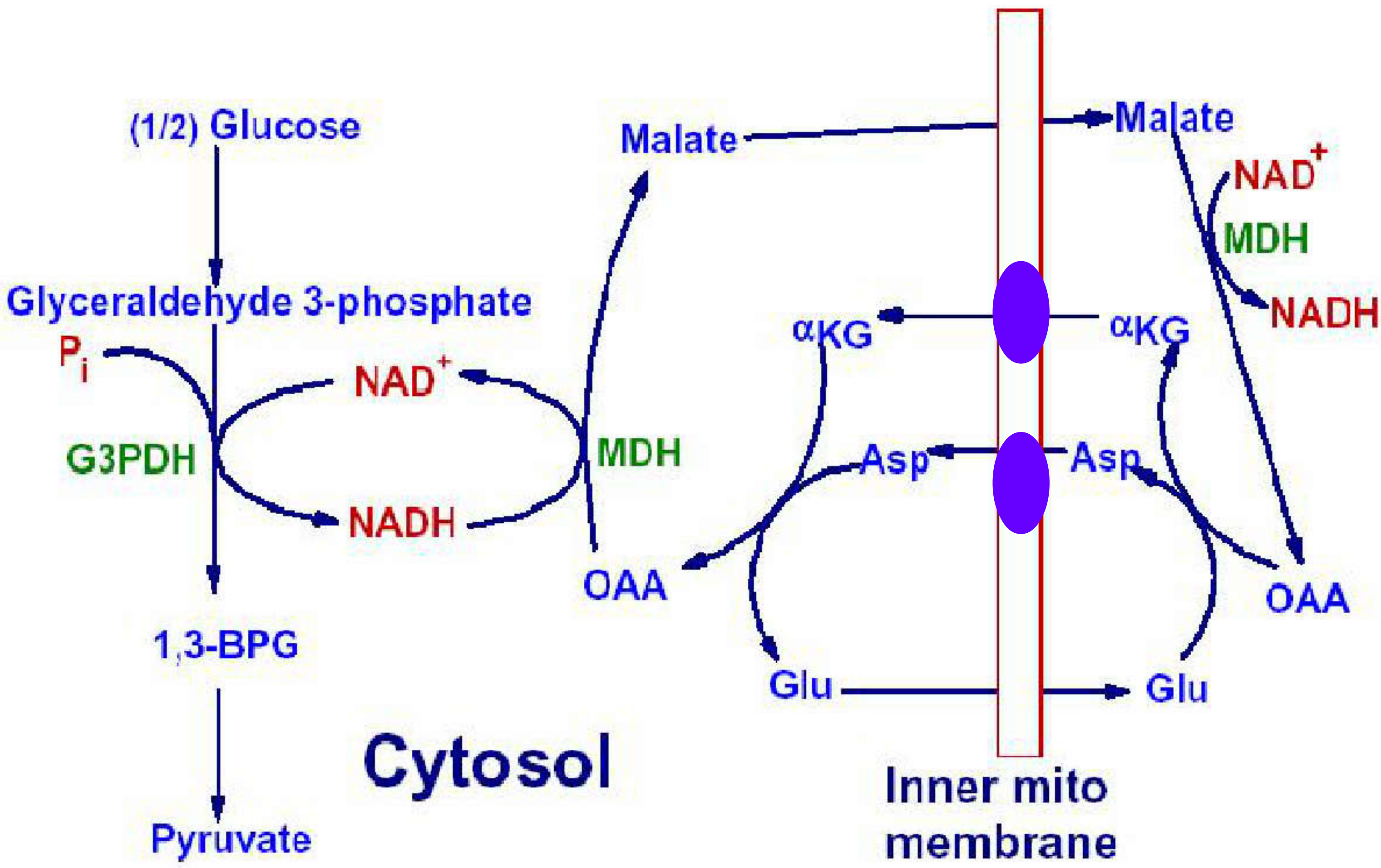
Glycerol Phosphate Shuttle



②. The malate – asparate shuttle:

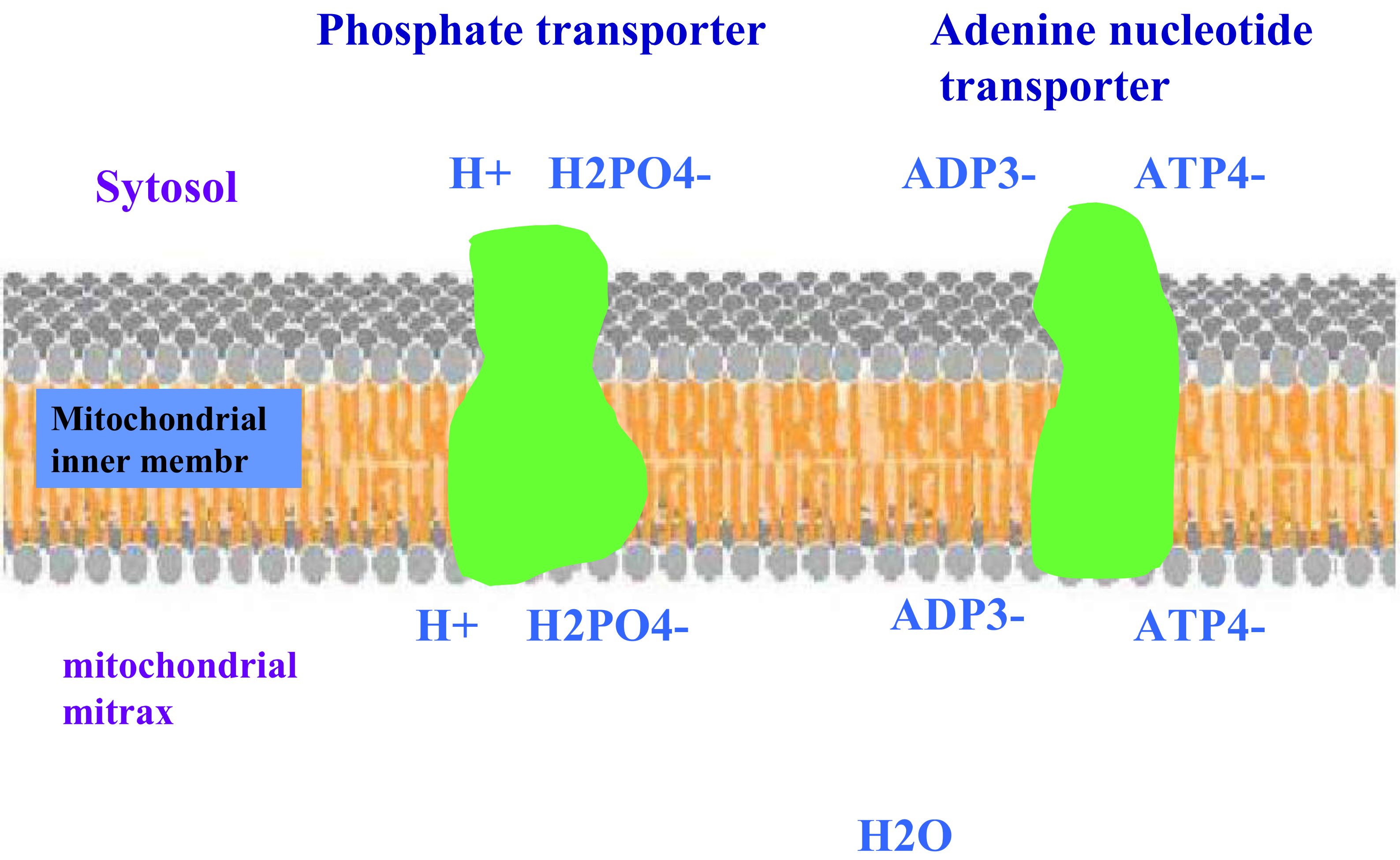
This shuttle in liver, kidney and heart mitochondria,in that it delivers the reducing equivalents from NADH into complex Ⅰ (into NADH oxidation respiratory chain), three molecules of ATP are generated as this pairs of electrons passes to O_2 .

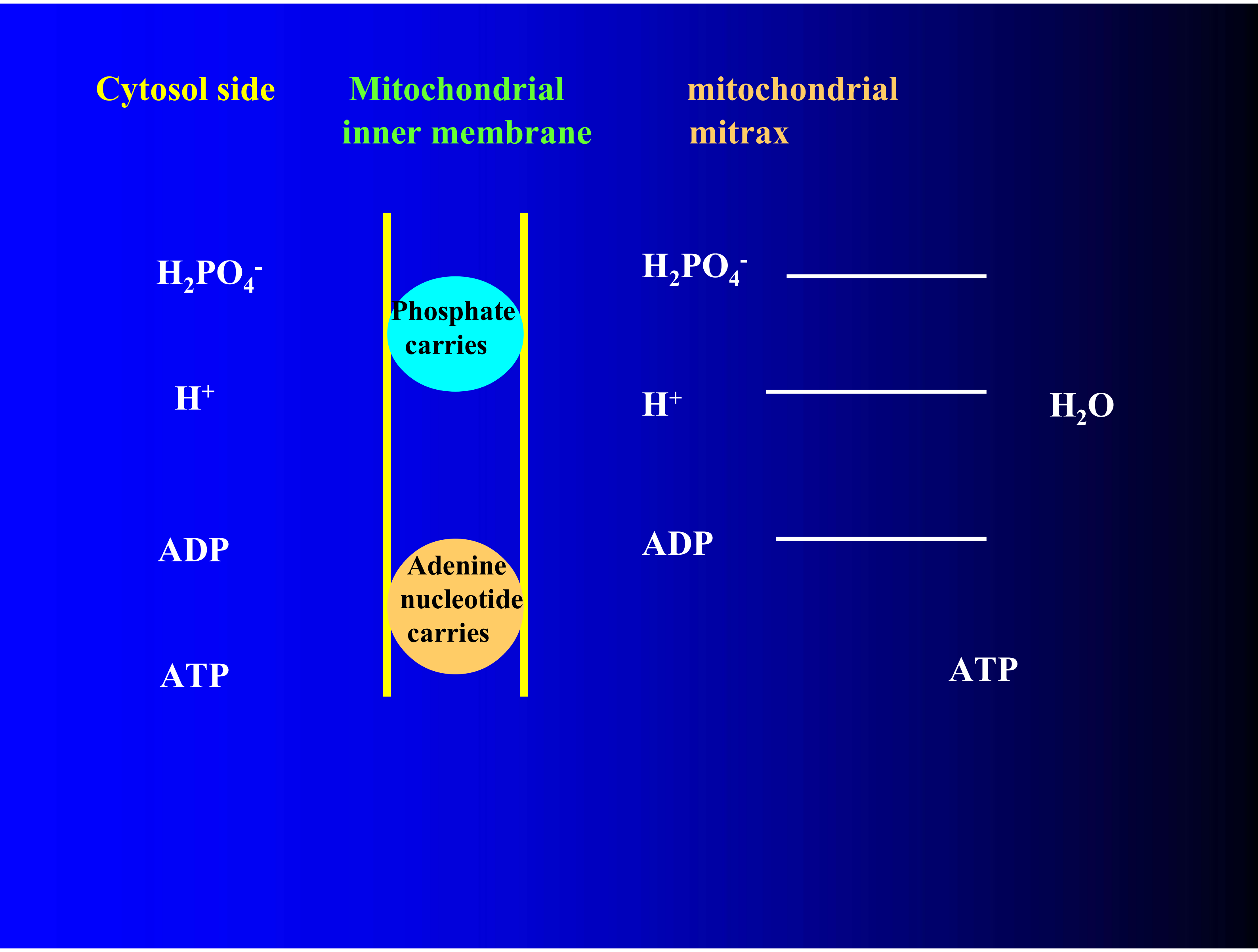
Malate-Aspartate Shuttle



（2）Adenine nucleotide transporter (ATP-ADP carrier)

The adenine nucleotide transporter allows the exchange of ATP and ADP but not AMP. It is vital in allowing ATP exit from mitochondria to the sites of extra-mitochondria utilization and allowing the return of ADP for ATP production within the mitochondrial.


Phosphate transporter
Adenine nucleotide transporter
Sytosol
H+
H2PO4-
ADP3-
ATP4-
Mitochondrial inner membr
H+
H2PO4-
ADP3-
ATP4-
mitochondrial mitrax
H2O


Cytosol side
Mitochondrial inner membrane
mitochondrial mitrax
$H_2PO_4^-$
Phosphate carries
H^+
ADP
Adenine nucleotide carries
ATP
$H_2PO_4^-$
H^+
H_2O
ADP
ATP

(3) The protein transports across

the mitochondrial membrane into the mitochondria by receptors that existed in membrane .

总　　　　结

生物氧化的概念、特点

生成ATP的氧化体系

一、呼吸链
定义、组成、排列顺序、两条主要的呼吸链

二、氧化磷酸化
定义、偶联部位、偶联机制

三、影响氧化磷酸化的因素
ADP、激素、抑制剂、解偶联剂、线粒体**DNA**突变

四、高能磷酸化合物—— **ATP**

高能磷酸键、常见的高能化合物、**ATP**循环、高能磷酸键的转移。

五、通过线粒体内膜的物质转运

胞液中**NADH**的氧化：**a-**磷酸甘油穿梭

苹果酸**-**天冬氨酸穿梭

腺苷酸载体

线粒体蛋白的跨膜转运

生物氧化复习题

一、名词解释

呼吸链　　磷氧比值　　生物氧化　**ATP**循环
高能化合物　　氧化磷酸化

二、问答题

1、简述呼吸链的组成成分及主要功能？
2、试述**ATP**在能量转换的核心作用？
3、简述线粒体外产生的**NADH**如何进行氧化磷酸化？