

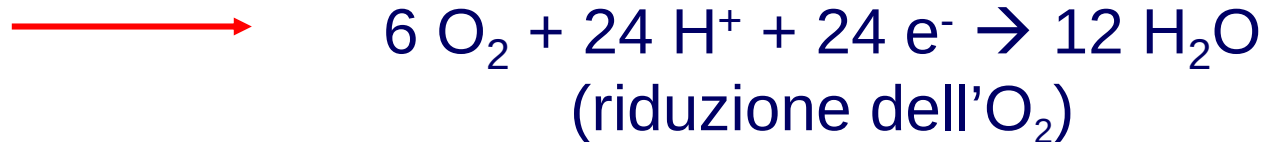
L'ossidazione completa del glucosio da parte dell'O<sub>2</sub>



può essere suddivisa in due semi-reazioni



e



NON diretta, ma mediata dai coenzimi NAD<sup>+</sup> e FAD

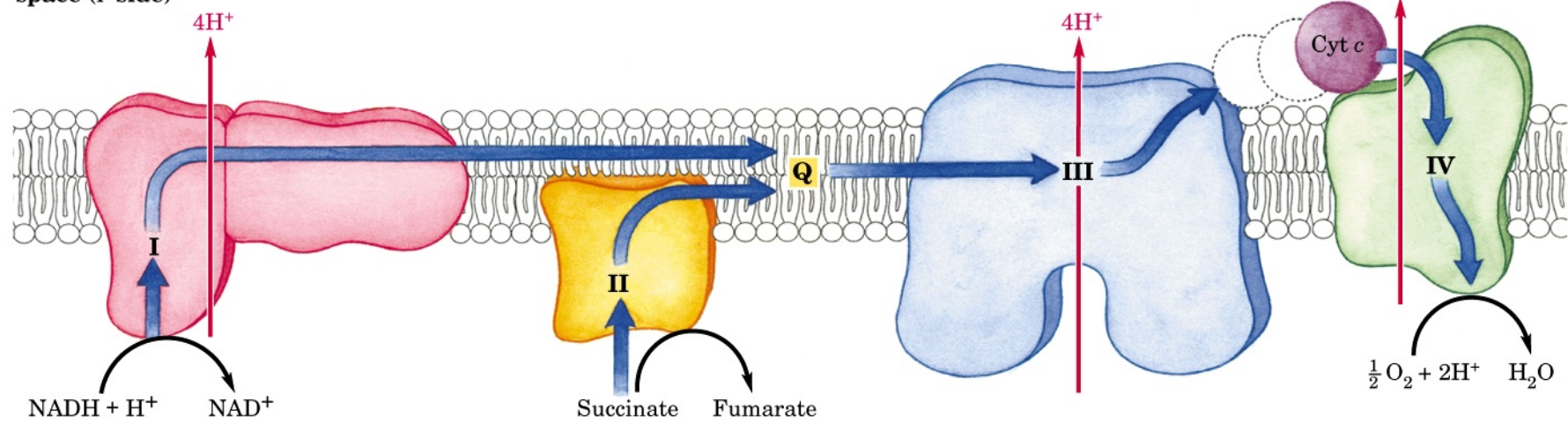
La **conservazione dell'energia** durante l'ossidazione di substrati può avvenire anche **mediante trasporto di elettroni** scambiati in reazioni di ossido-riduzione accoppiate.

Nella **fosforilazione ossidativa** il trasporto di elettroni e la formazione di un gradiente di protoni transmembrana promuove la **formazione di ATP**.

## MITOCONDRIO = CENTRALE ENERGETICA

- Trasformazione del piruvato ad acetil Coa;
  - Ciclo degli acidi tricarbossilici;
    - **Fosforilazione ossidativa**;
    - Catabolismo acidi grassi;
    - Ciclo dell'urea (in parte);
      - Serbatoio di  $\text{Ca}^{2+}$

Intermembrane  
space (P side)



Matrix (N side)

**table 19–3**

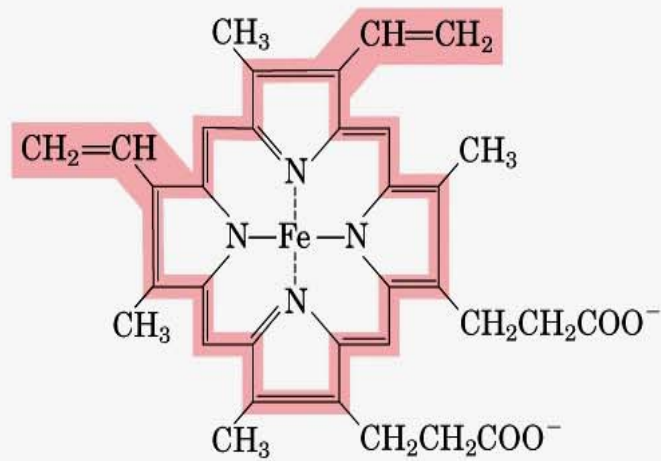
**Protein Components of the Mitochondrial Electron-Transfer Chain**

| Enzyme complex                                     | Mass (kDa) | Number of subunits* | Prosthetic group(s)                      |
|----------------------------------------------------|------------|---------------------|------------------------------------------|
| I NADH dehydrogenase                               | 850        | 42 (14)             | FMN, Fe-S                                |
| II Succinate dehydrogenase                         | 140        | 5                   | FAD, Fe-S                                |
| III Ubiquinone: cytochrome <i>c</i> oxidoreductase | 250        | 11                  | Hemes, Fe-S                              |
| Cytochrome <i>c</i> <sup>†</sup>                   | 13         | 1                   | Heme                                     |
| IV Cytochrome oxidase                              | 160        | 13 (3–4)            | Hemes; Cu <sub>A</sub> , Cu <sub>B</sub> |

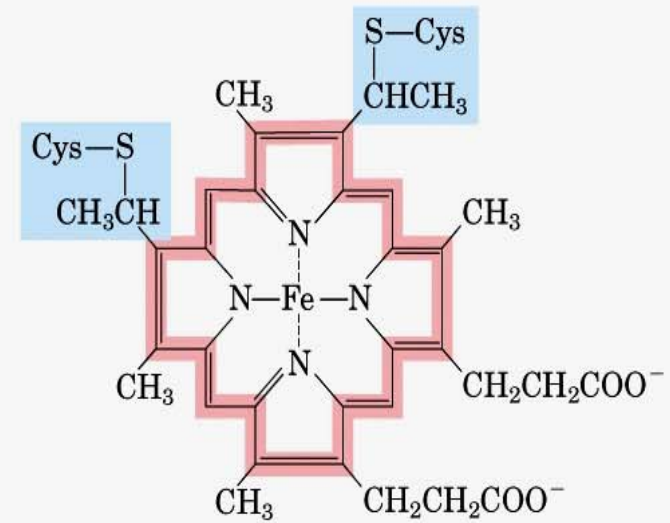
\*Numbers of subunits in the bacterial equivalents in parentheses.

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

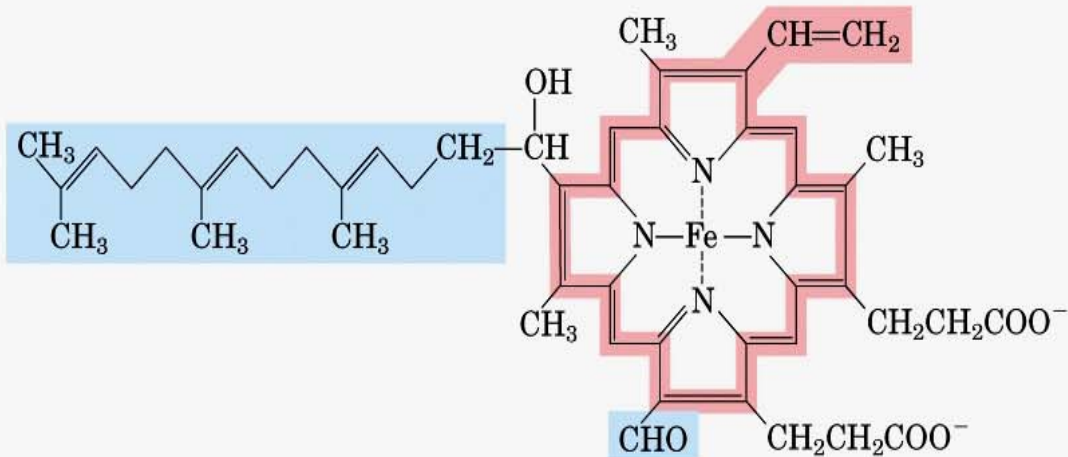
# CITOCROMI: David Keilin, 1925



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



Heme C  
(in *c*-type cytochromes)



Heme A  
(in *a*-type cytochromes)

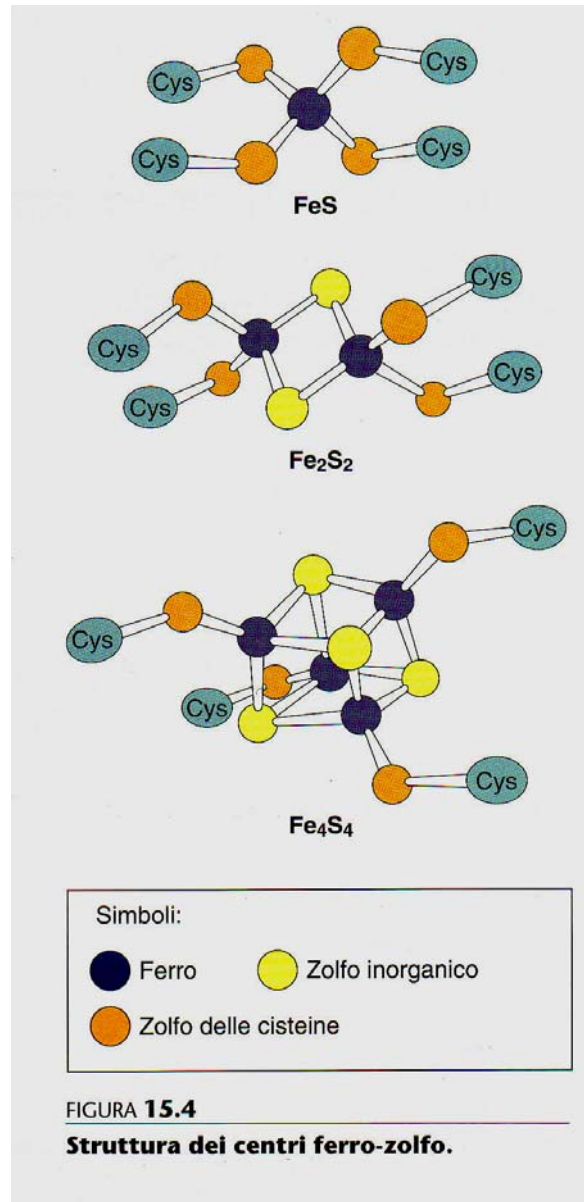


## CENTRI FERRO-ZOLFO: ferro non eme

[2Fe-2S]



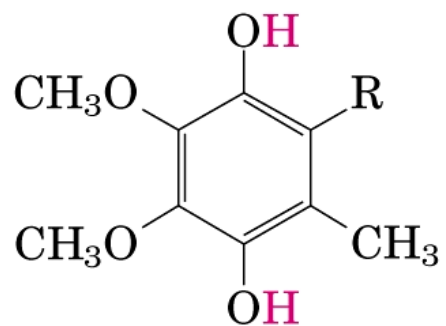
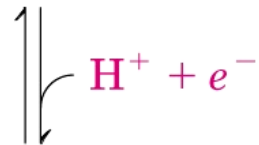
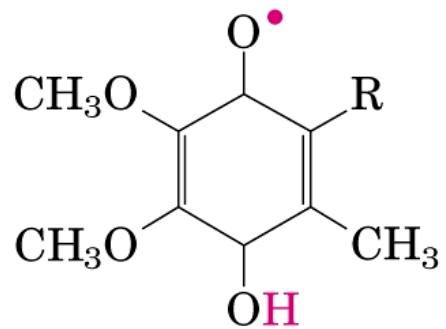
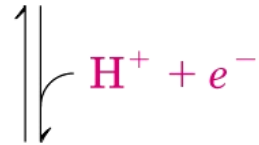
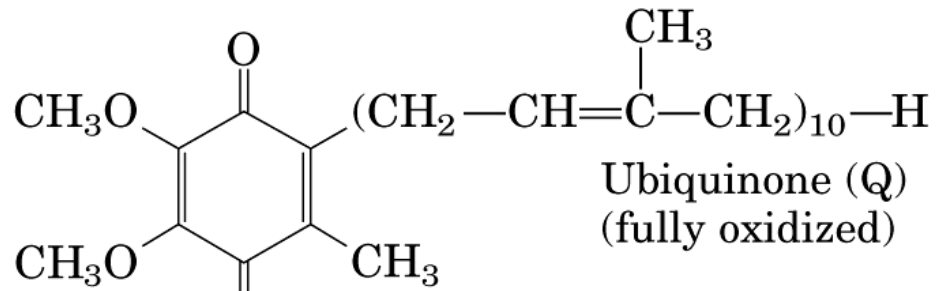
[4Fe-4S]



Fe e S sono uniti a Cys con legami di coordinazione con simmetria tetraedrica

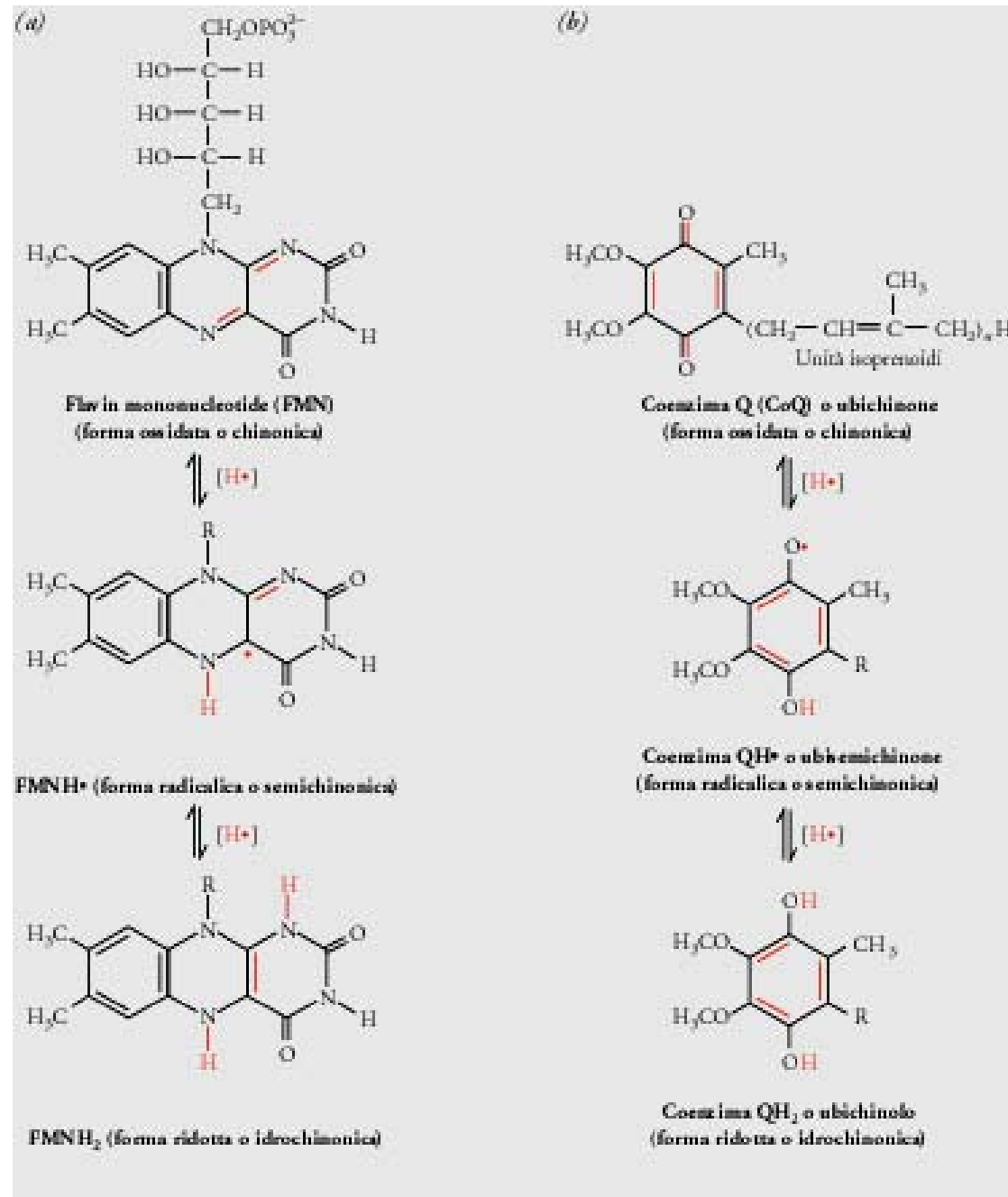
Possono subire reazioni redox ( $\text{Fe}^{2+} \longleftrightarrow \text{Fe}^{3+}$ )

# CoenzimaQ



## FLAVINE e CoQ

Hanno 3 stati di ossidazione possibili perché possono accettare o donare o uno o due elettroni

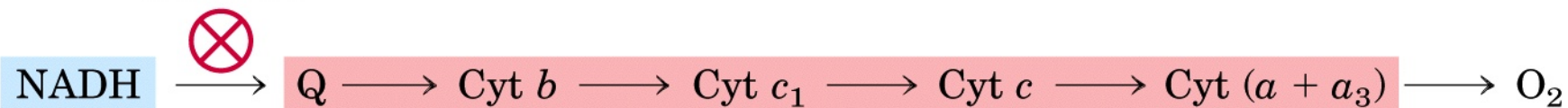


## Potenziali di riduzione standard di alcuni componenti della catena di trasporto degli elettroni

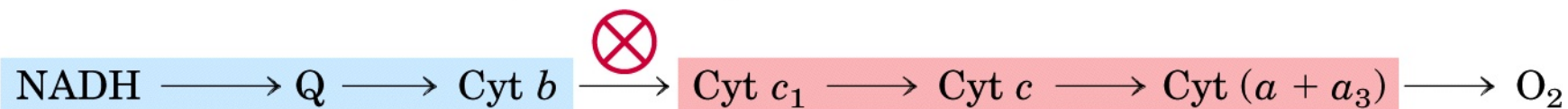
| Redox reaction (half-reaction)                                                                                              | $E'^{\circ}$ (V) |
|-----------------------------------------------------------------------------------------------------------------------------|------------------|
| $2\text{H}^{+} + 2\text{e}^{-} \longrightarrow \text{H}_2$                                                                  | -0.414           |
| $\text{NAD}^{+} + \text{H}^{+} + 2\text{e}^{-} \longrightarrow \text{NADH}$                                                 | -0.320           |
| $\text{NADP}^{+} + \text{H}^{+} + 2\text{e}^{-} \longrightarrow \text{NADPH}$                                               | -0.324           |
| $\text{NADH dehydrogenase (FMN)} + 2\text{H}^{+} + 2\text{e}^{-} \longrightarrow \text{NADH dehydrogenase (FMNH}_2\text{)}$ | -0.30            |
| $\text{Ubiquinone} + 2\text{H}^{+} + 2\text{e}^{-} \longrightarrow \text{ubiquinol}$                                        | 0.045            |
| $\text{Cytochrome } b (\text{Fe}^{3+}) + \text{e}^{-} \longrightarrow \text{cytochrome } b (\text{Fe}^{2+})$                | 0.077            |
| $\text{Cytochrome } c_1 (\text{Fe}^{3+}) + \text{e}^{-} \longrightarrow \text{cytochrome } c_1 (\text{Fe}^{2+})$            | 0.22             |
| $\text{Cytochrome } c (\text{Fe}^{3+}) + \text{e}^{-} \longrightarrow \text{cytochrome } c (\text{Fe}^{2+})$                | 0.254            |
| $\text{Cytochrome } a (\text{Fe}^{3+}) + \text{e}^{-} \longrightarrow \text{cytochrome } a (\text{Fe}^{2+})$                | 0.29             |
| $\text{Cytochrome } a_3 (\text{Fe}^{3+}) + \text{e}^{-} \longrightarrow \text{cytochrome } a_3 (\text{Fe}^{2+})$            | 0.55             |
| $\frac{1}{2}\text{O}_2 + 2\text{H}^{+} + 2\text{e}^{-} \longrightarrow \text{H}_2\text{O}$                                  | 0.816            |



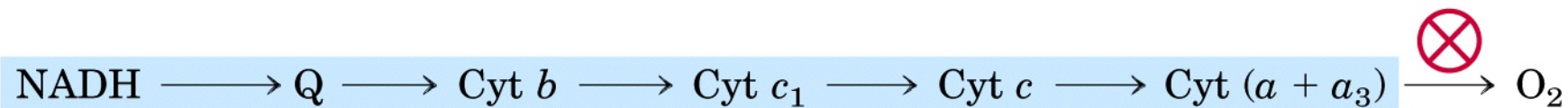
rotenone



antimycin A



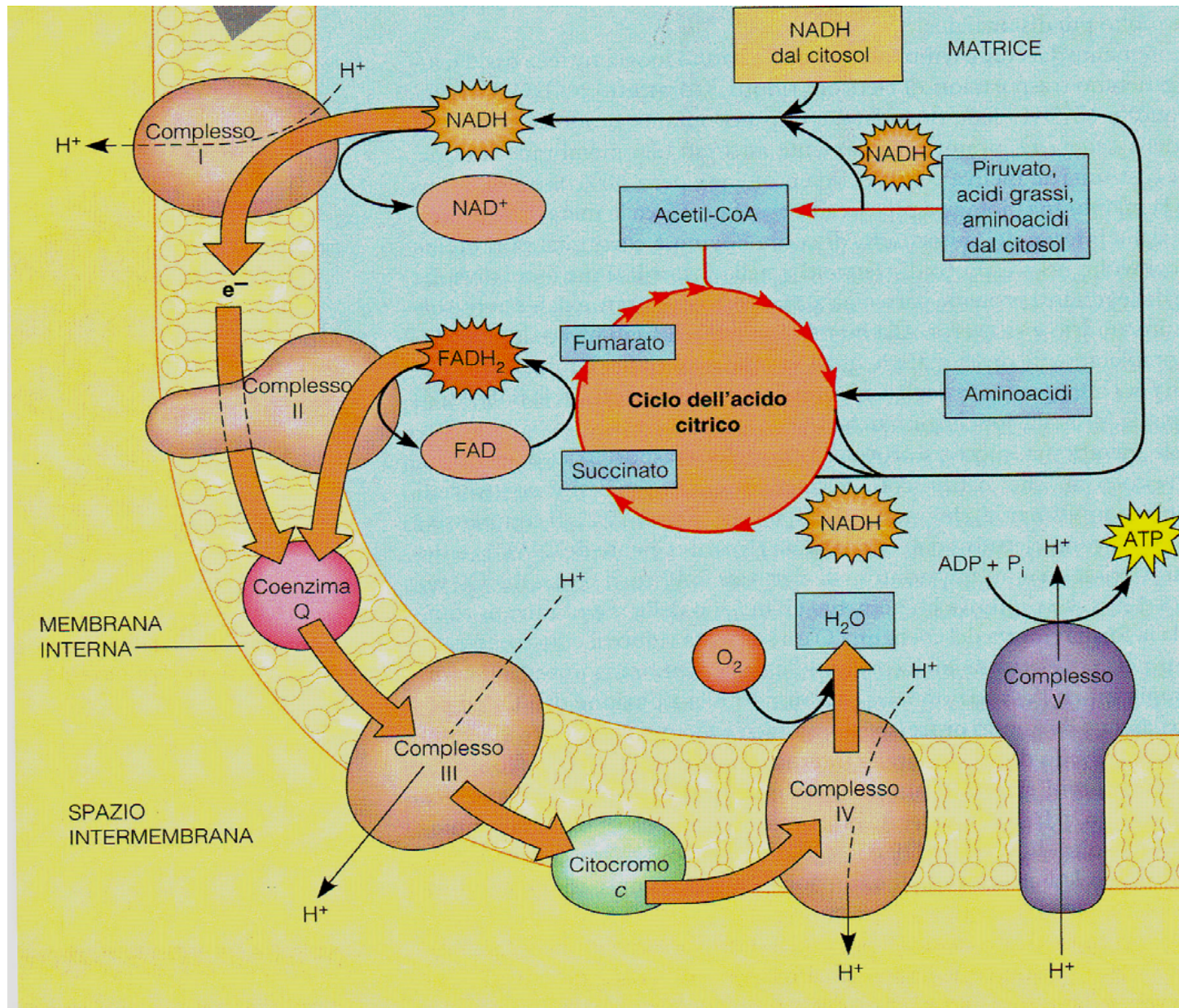
CN<sup>-</sup> or CO



# CATENA DI TRASPORTO DEGLI ELETTRONI

Gli elettroni trasportati dai 10 NADH e 2 FADH<sub>2</sub> prodotti da Glicolisi e ciclo di Krebs passano nella catena di trasporto di elettroni in cui:  
4 complessi enzimatici contenenti circa 10 centri redox  
assemblati sulla **membrana interna** del mitocondrio;

1. NADH e FADH<sub>2</sub> vengono **riossidati** a NAD<sup>+</sup> e FAD;
2. Gli **elettroni vengono trasferiti** secondo una sequenza dettata dal potenziale ossidoriduttivo dei trasportatori (verso potenziali redox via via crescenti), fino ad arrivare all'ossigeno;
3. Durante il trasferimento (che è un processo **ESOERGONICO**) vengono **espulsi protoni** nello spazio intermembrana;
4. L'energia conservata in questo gradiente protonico permette la sintesi di ATP (**FOSFORILAZIONE OSSIDATIVA**), nel momento in cui i protoni tornano nella matrice.

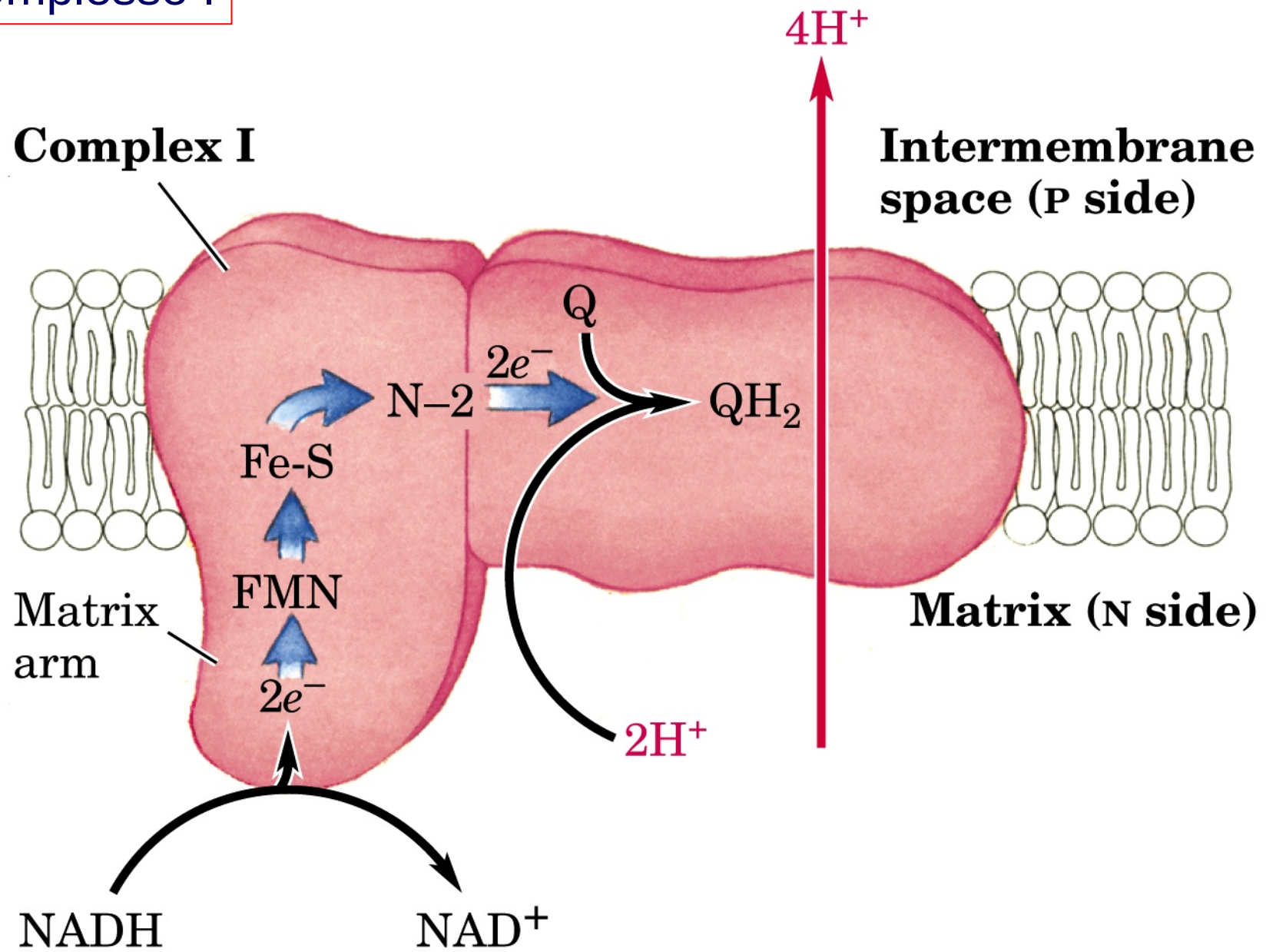


## Complesso I

- 43 catene polipeptidiche
- 850 kD
- 1 FMN (= FAD-AMP) che partecipa alle reazioni redox
- 6 o 7 centri ferro-zolfo che partecipano al trasporto di  $e^-$

Durante il trasporto di  $e^-$  , 4 protoni vengono trasferiti fuori dalla matrice nello spazio intra-membrana

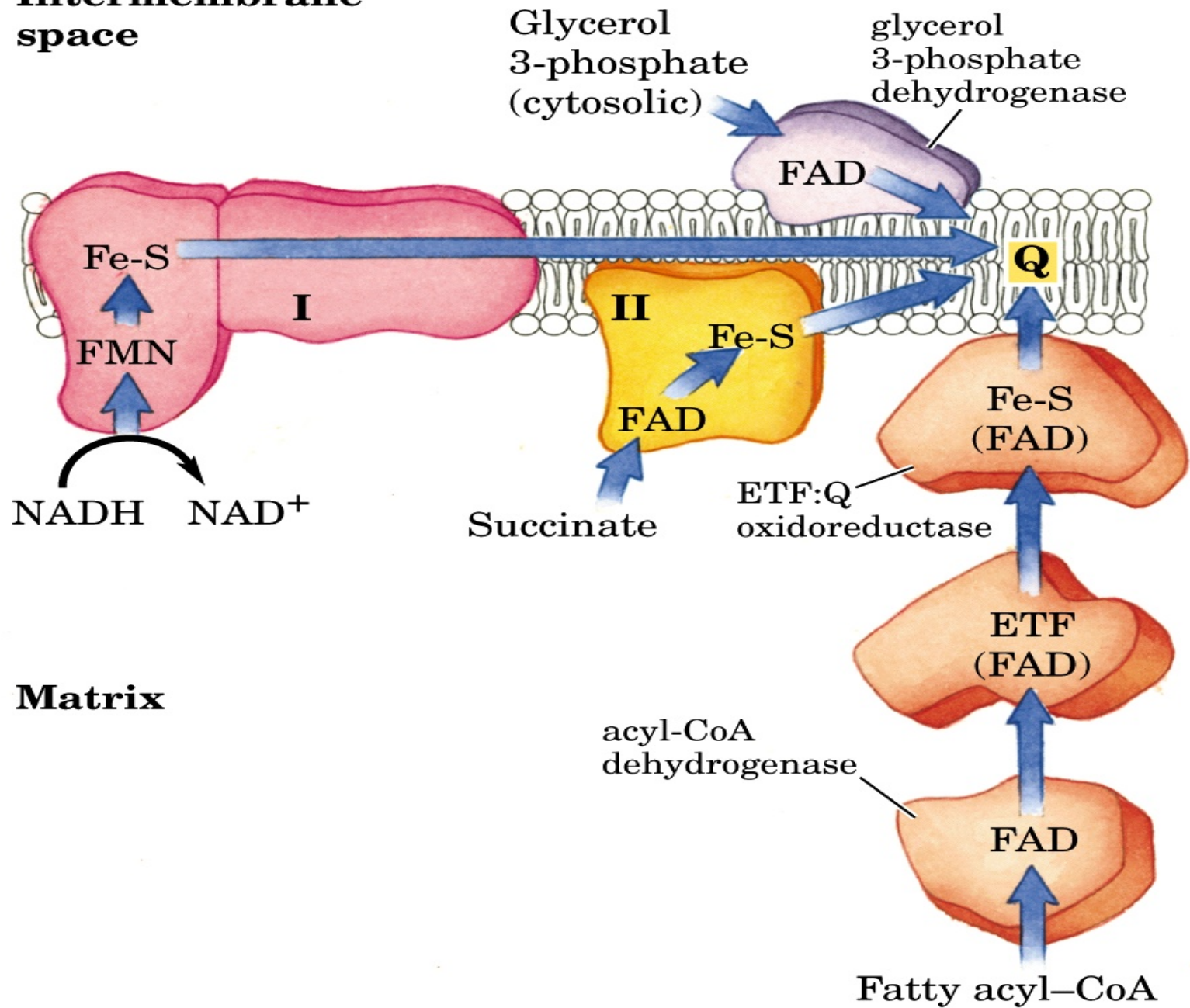
## Complesso I



## Complesso II

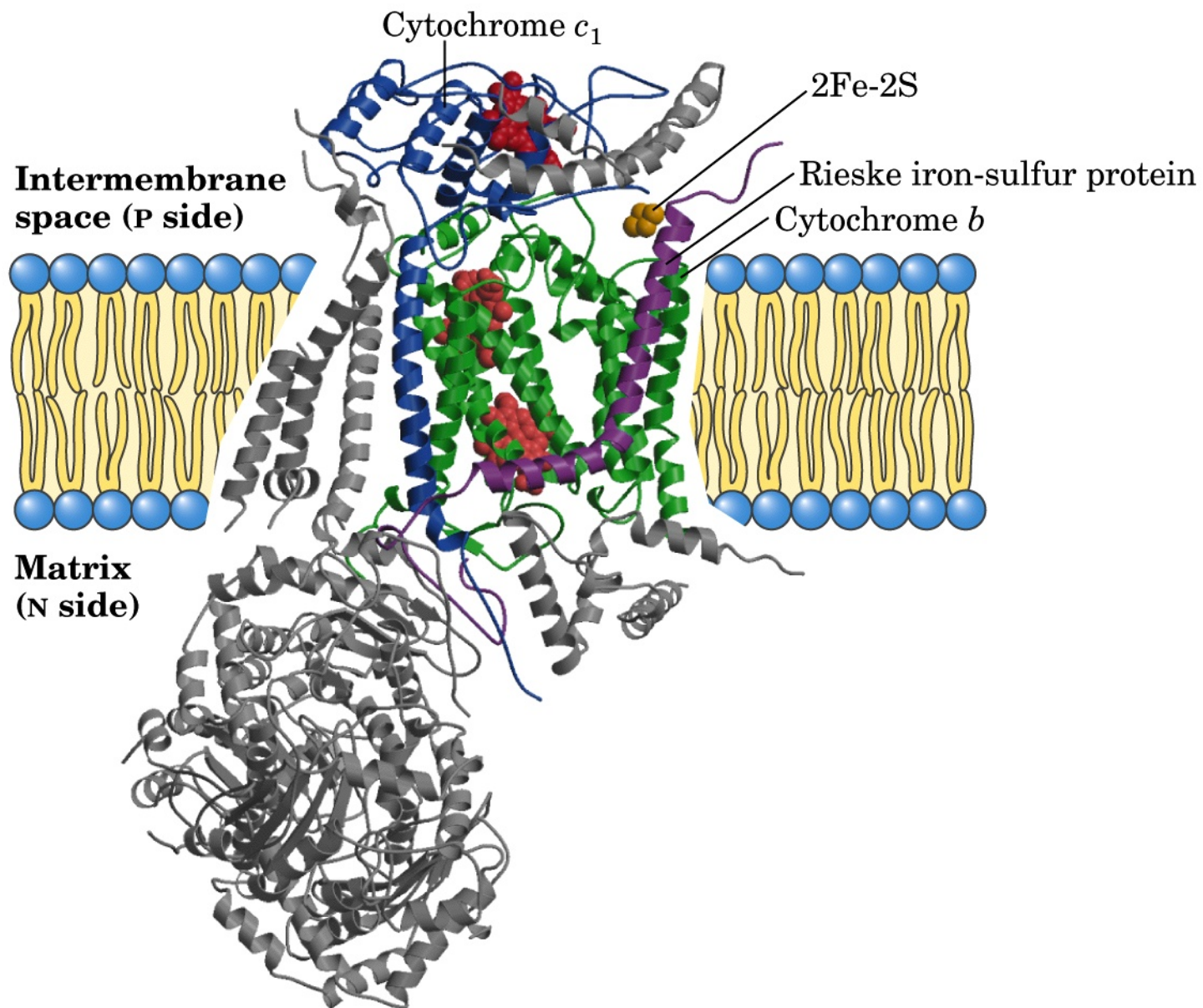
1. Contiene la succinato deidrogenasi (ciclo di Krebs) che contiene FAD
2. Passa gli elettroni dal succinato al CoQ
3. Contiene centri Fe-S
4. Contiene citocromo  $b_{560}$
5. NON opera dopo il Complesso I, ma fornisce un altro ingresso alla catena

**Intermembrane  
space**



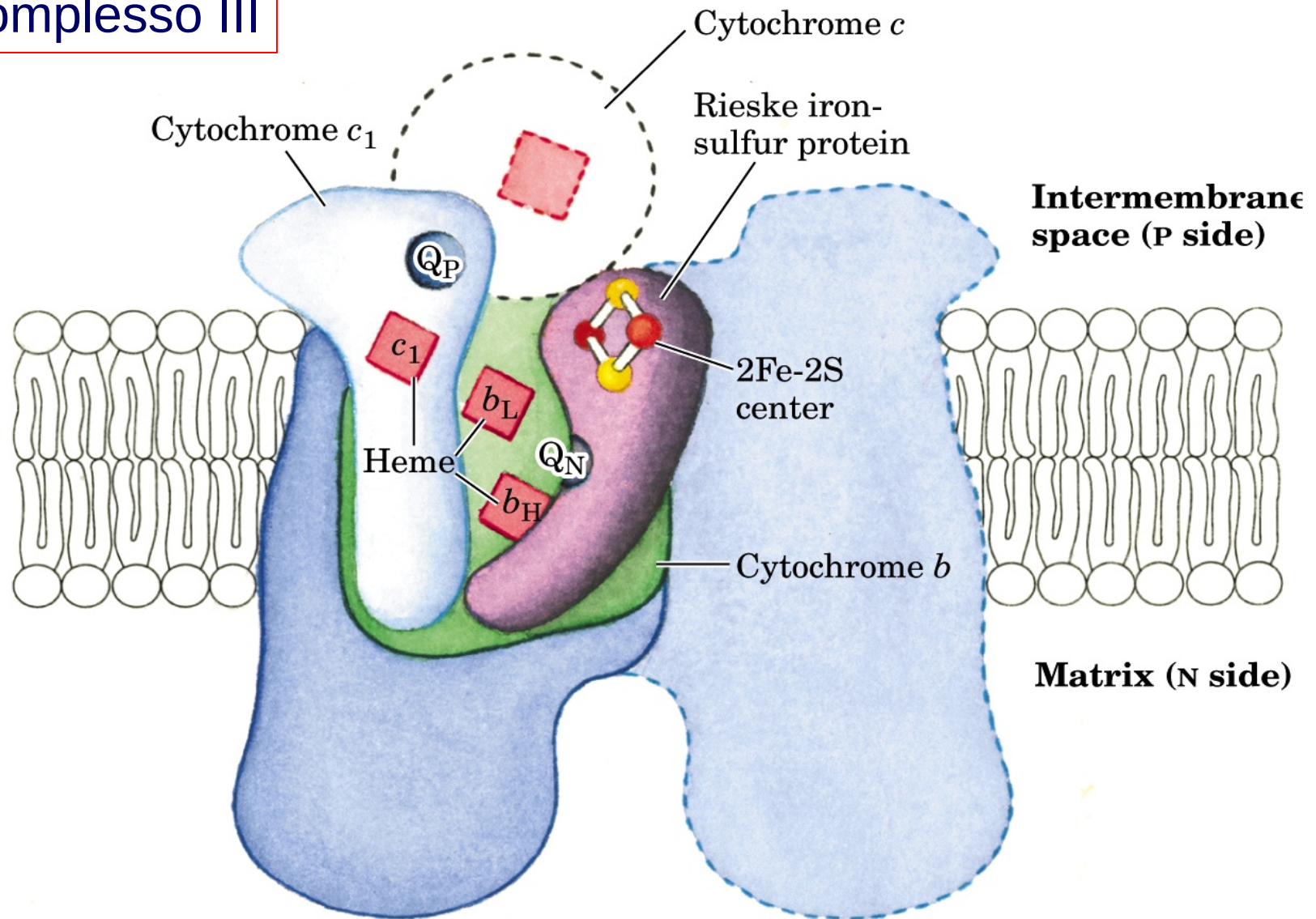
## Complesso III

1. Contiene 2 x 11 subunità (PM : 2 x 248 kD)
2. Contiene 2 citocromi b, 1 citocromo  $c_1$ , 1 centro [2Fe-2S]
3. Consente al trasportatore a 2  $e^-$  (CoQH<sub>2</sub>) di ridurre due molecole di citocromo c (trasportatore a un  $e^-$ ) mediante il **Ciclo Q**



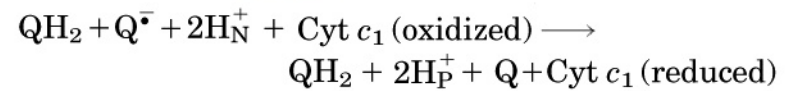
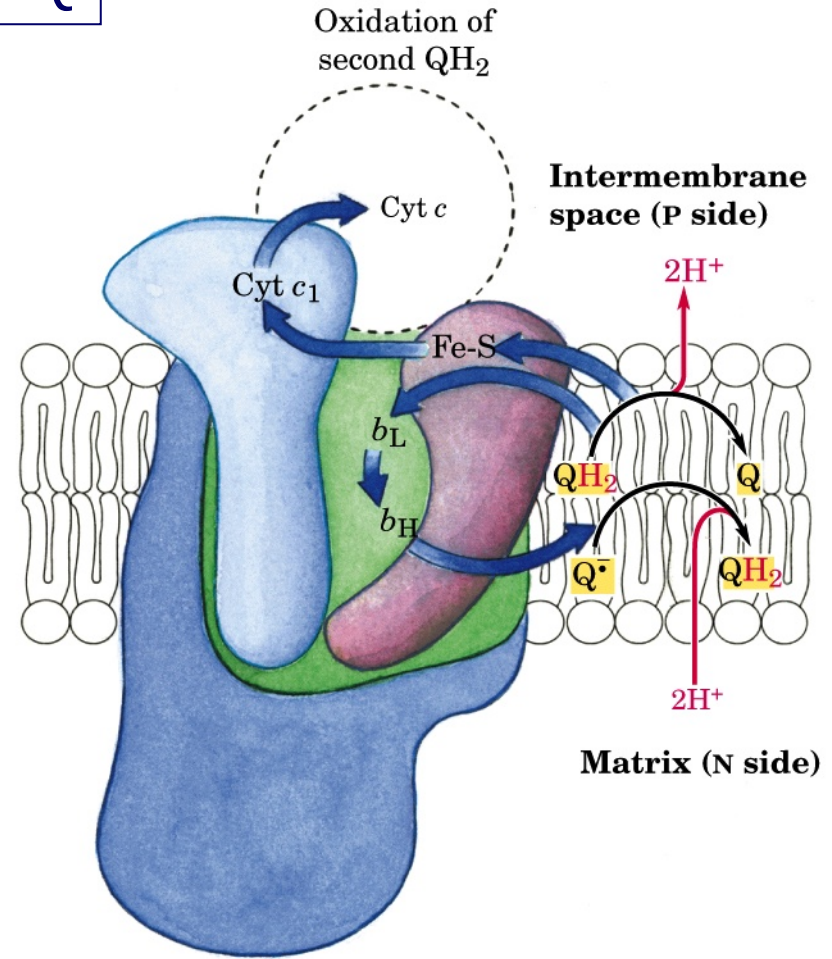
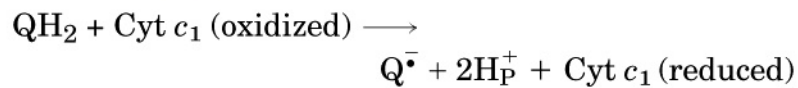
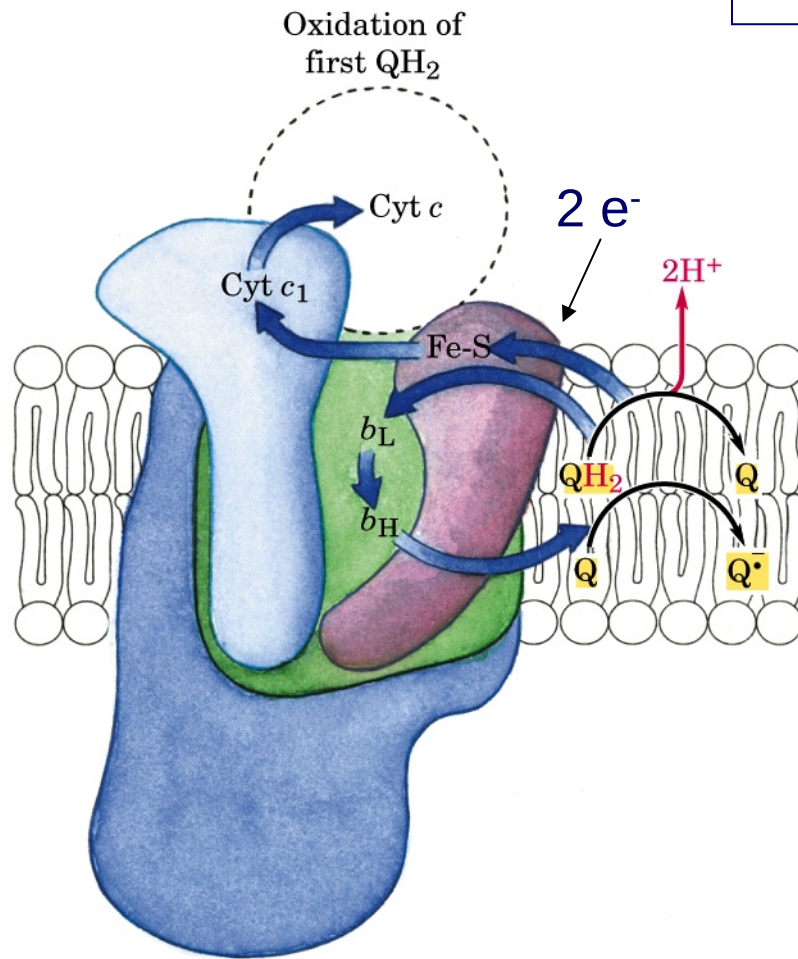
**(a)**

## Complesso III

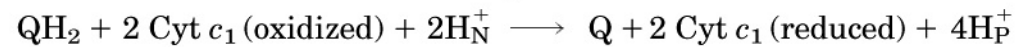


(b)

# CICLO Q



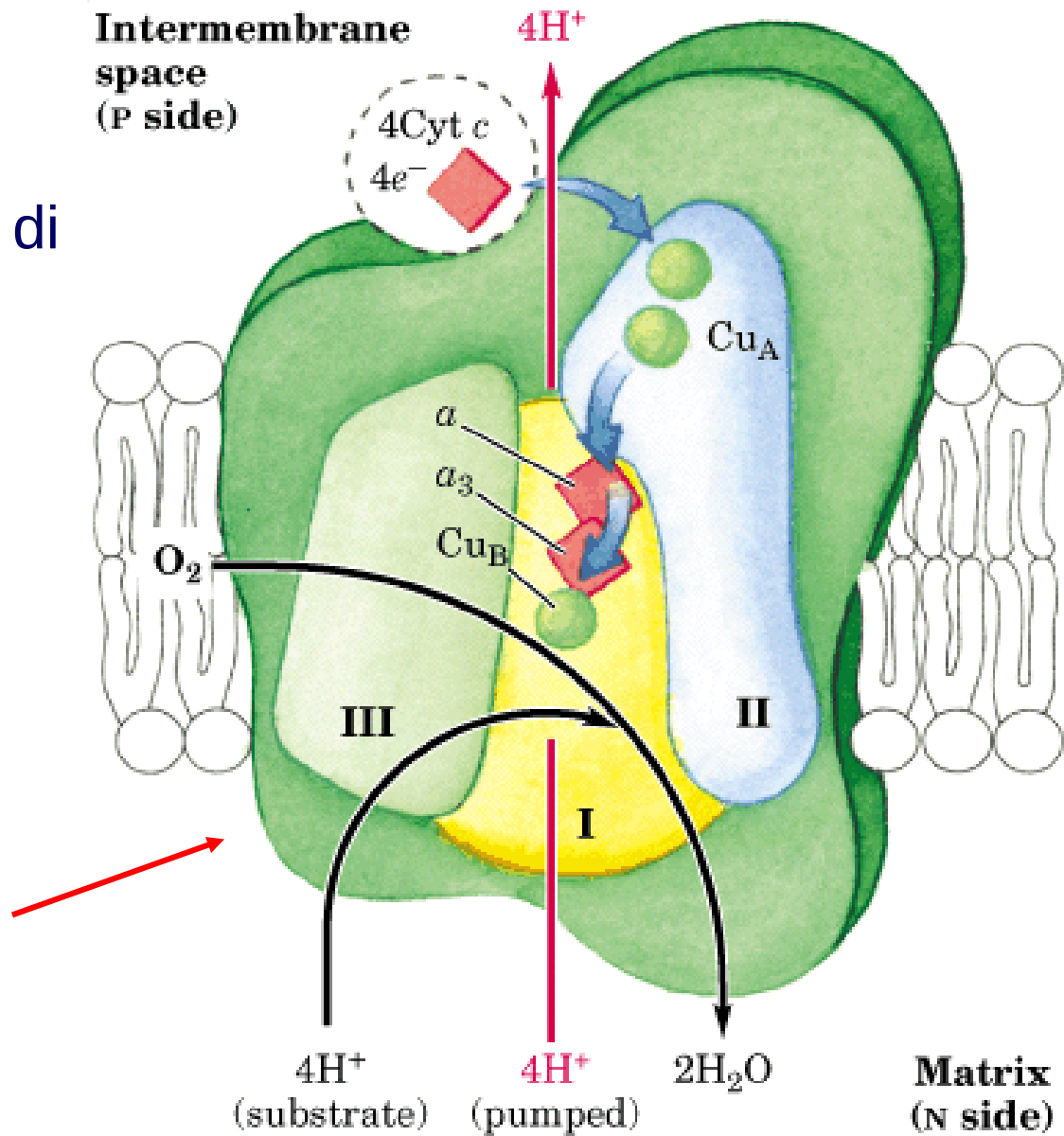
Net equation:



## Citocromo c

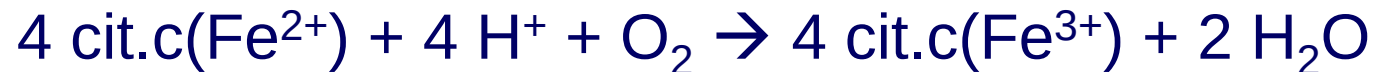
E' un trasportatore di  $e^-$  solubile

Complesso IV



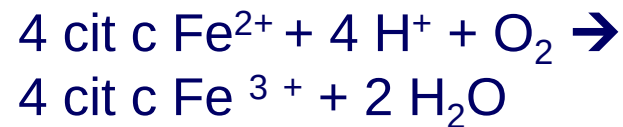
## Complesso IV (citocromo ossidasi)

Catalizza le ossidazioni (con acquisto di 1 e<sup>-</sup>) di **4 molecole consecutive** di citocromo c ridotto e la contemporanea **riduzione di una molecola di O<sub>2</sub>**

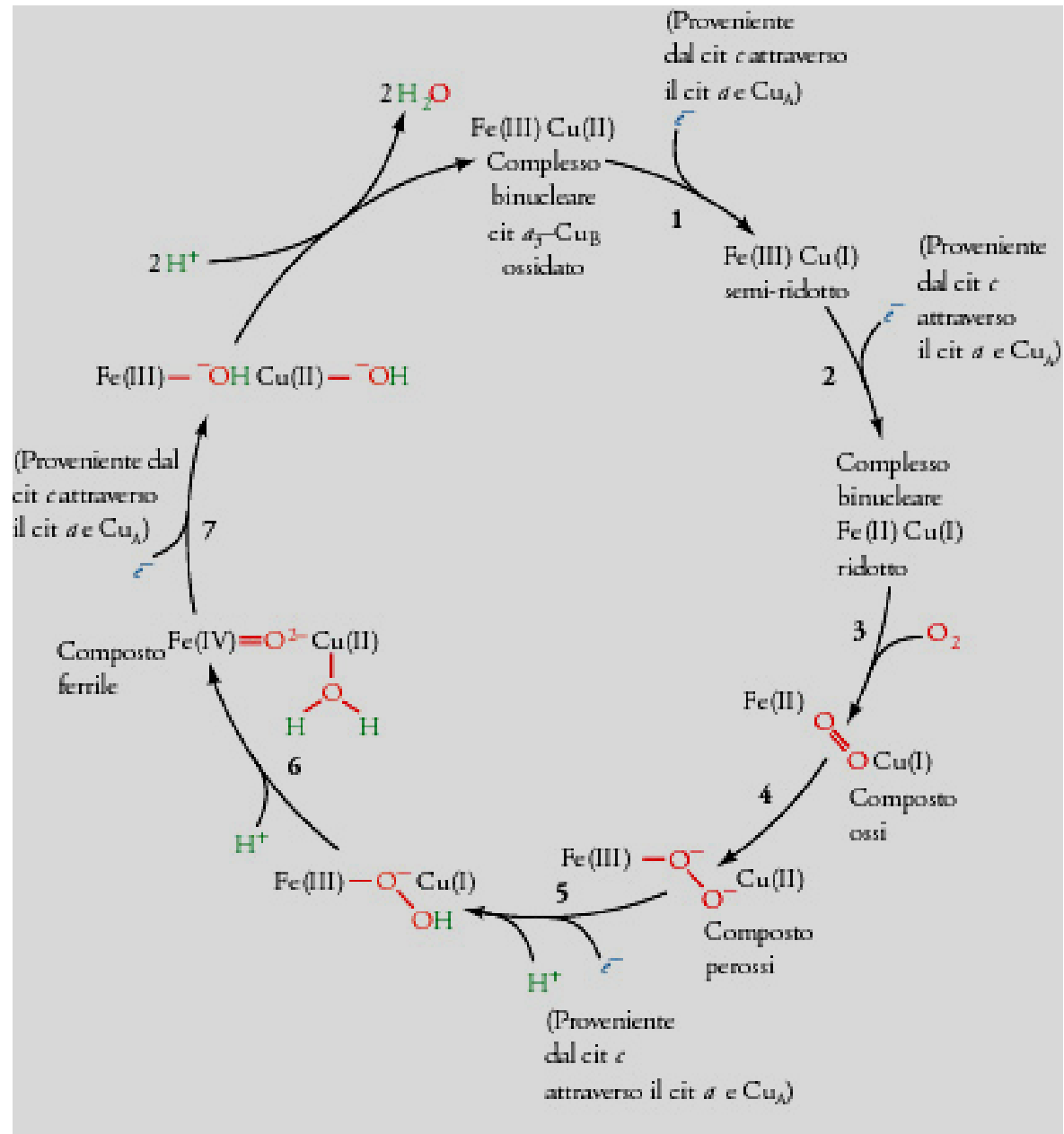


- E' un dimero 2 x 200 kD, 2 x 13 subunità
- Lega 4 centri redox :
  1. cit.a
  2. cit a<sub>3</sub>
  3. 1 x Cu<sub>B</sub>
  4. centro 2 x Cu<sub>A</sub>

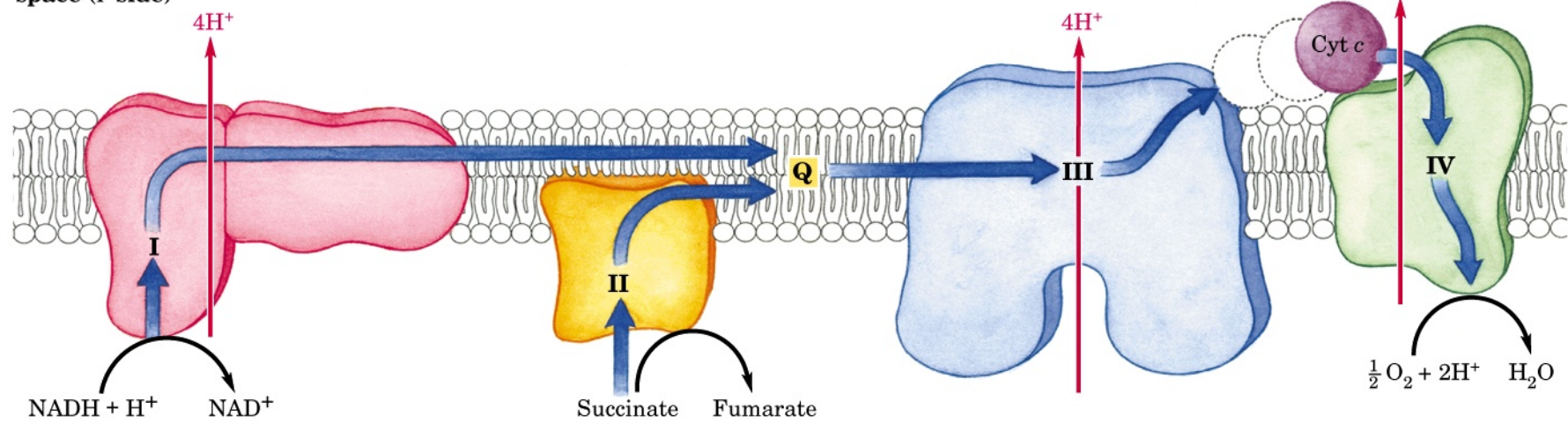
# MECCANISMO DI REAZIONE DELLA CITOCROMO C OSSIDASI



Oltre ai 4 H<sup>+</sup> utilizzati per ridurre l'O<sub>2</sub>, 4 H<sup>+</sup> vengono traslocati nello spazio intermembrane.



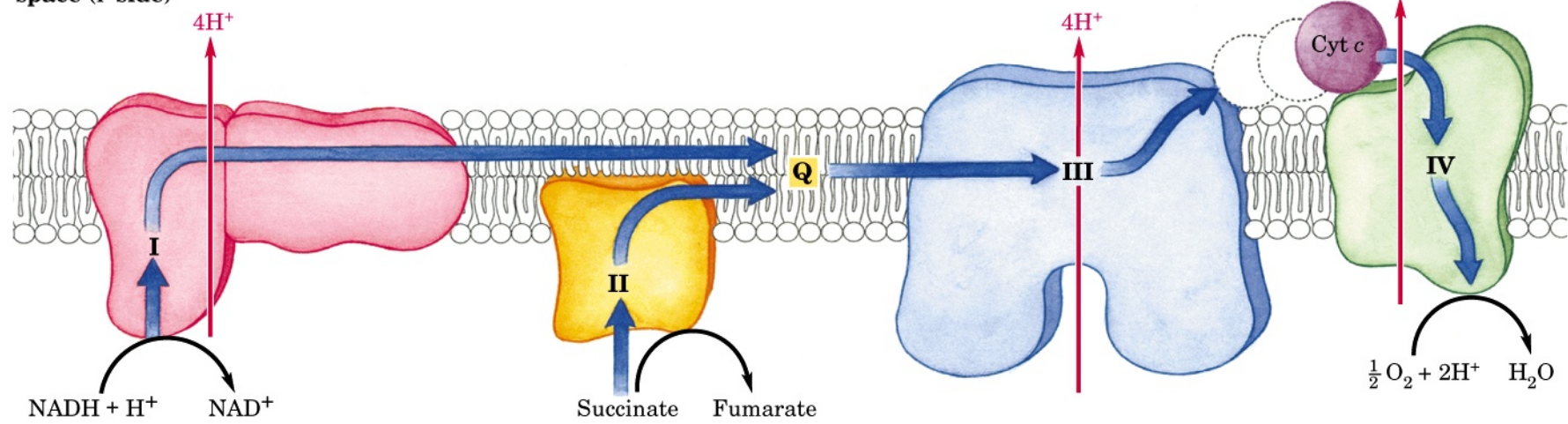
Intermembrane  
space (P side)



Matrix (N side)



Intermembrane  
space (P side)



Matrix (N side)

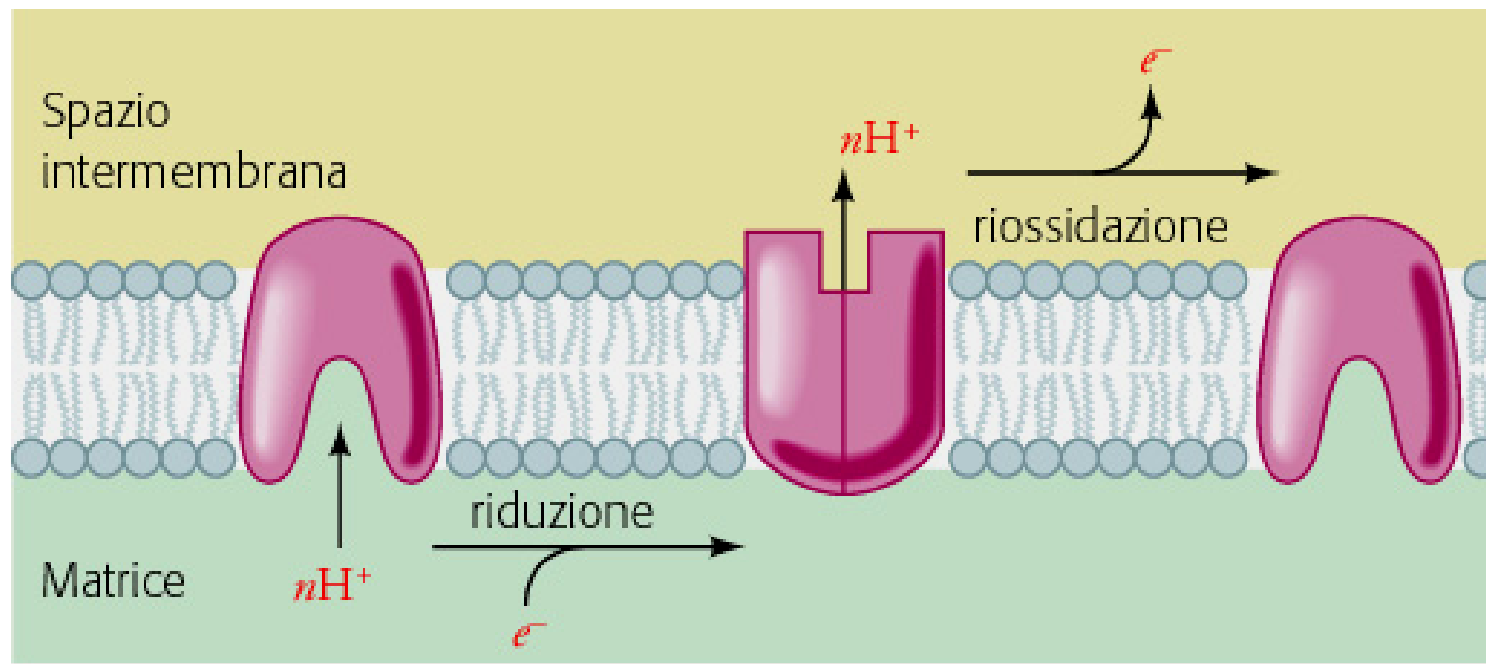
**table 19-4**

**Some Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation**

| Type of interference                                 | Compound*       | Target/mode of action                                                                 |
|------------------------------------------------------|-----------------|---------------------------------------------------------------------------------------|
| Inhibition of electron transfer                      | Cyanide         | Inhibit cytochrome oxidase                                                            |
|                                                      | Carbon monoxide |                                                                                       |
|                                                      | Antimycin A     | Blocks electron transfer from cytochrome <i>b</i> to cytochrome <i>c</i> <sub>1</sub> |
|                                                      | Myxothiazol     |                                                                                       |
|                                                      | Rotenone        | Prevent electron transfer from Fe-S center to ubiquinone                              |
|                                                      | Amytal          |                                                                                       |
|                                                      | Piericidin A    |                                                                                       |
| Inhibition of ATP synthase                           | DCMU            | Competes with Q <sub>B</sub> for binding site in PSII                                 |
|                                                      | Aurovertin      | Inhibits F <sub>1</sub>                                                               |
|                                                      | Oligomycin      | Inhibit F <sub>o</sub> and CF <sub>o</sub>                                            |
|                                                      | Venturicidin    |                                                                                       |
| Uncoupling of phosphorylation from electron transfer | DCCD            | Blocks proton flow through F <sub>o</sub> and CF <sub>o</sub>                         |
|                                                      | FCCP            | Hydrophobic proton carriers                                                           |
|                                                      | DNP             |                                                                                       |
|                                                      | Valinomycin     | K <sup>+</sup> ionophore                                                              |
|                                                      | Thermogenin     | Forms proton-conducting pores in inner membrane of brown fat mitochondria             |
| Inhibition of ATP-ADP exchange                       | Attractyloside  | Inhibits adenine nucleotide translocase                                               |

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

## Modello del trasporto di elettroni legato alla pompa protonica



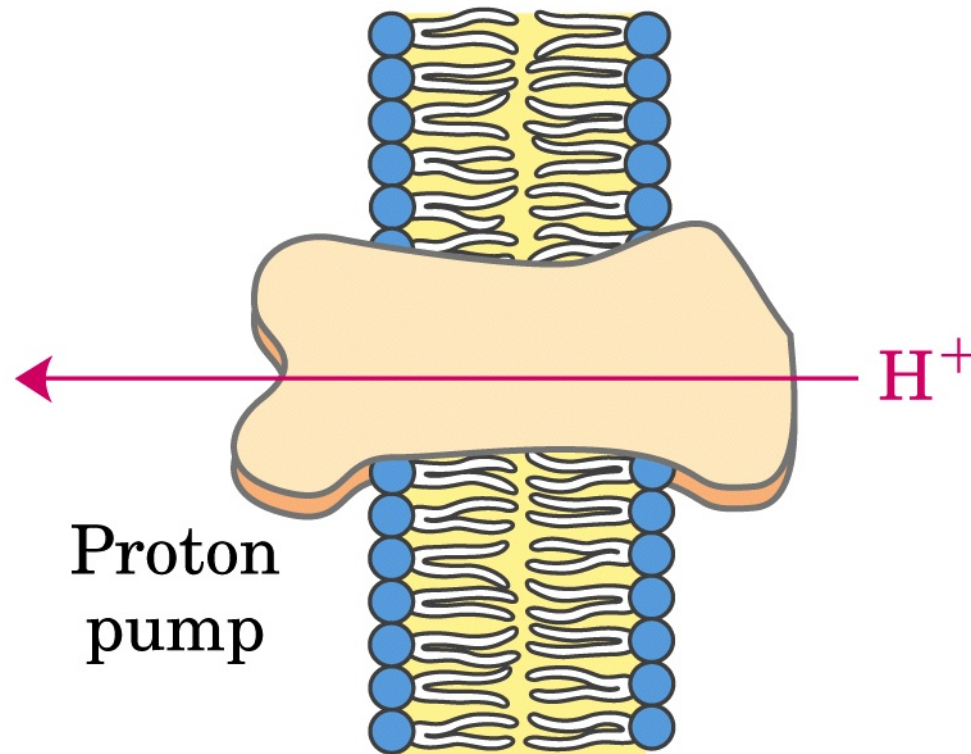
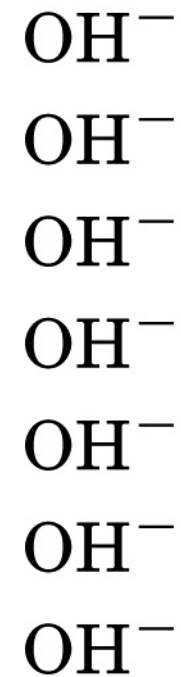
**P side**

$$[\text{H}^+]_{\text{P}} = C_2$$



**N side**

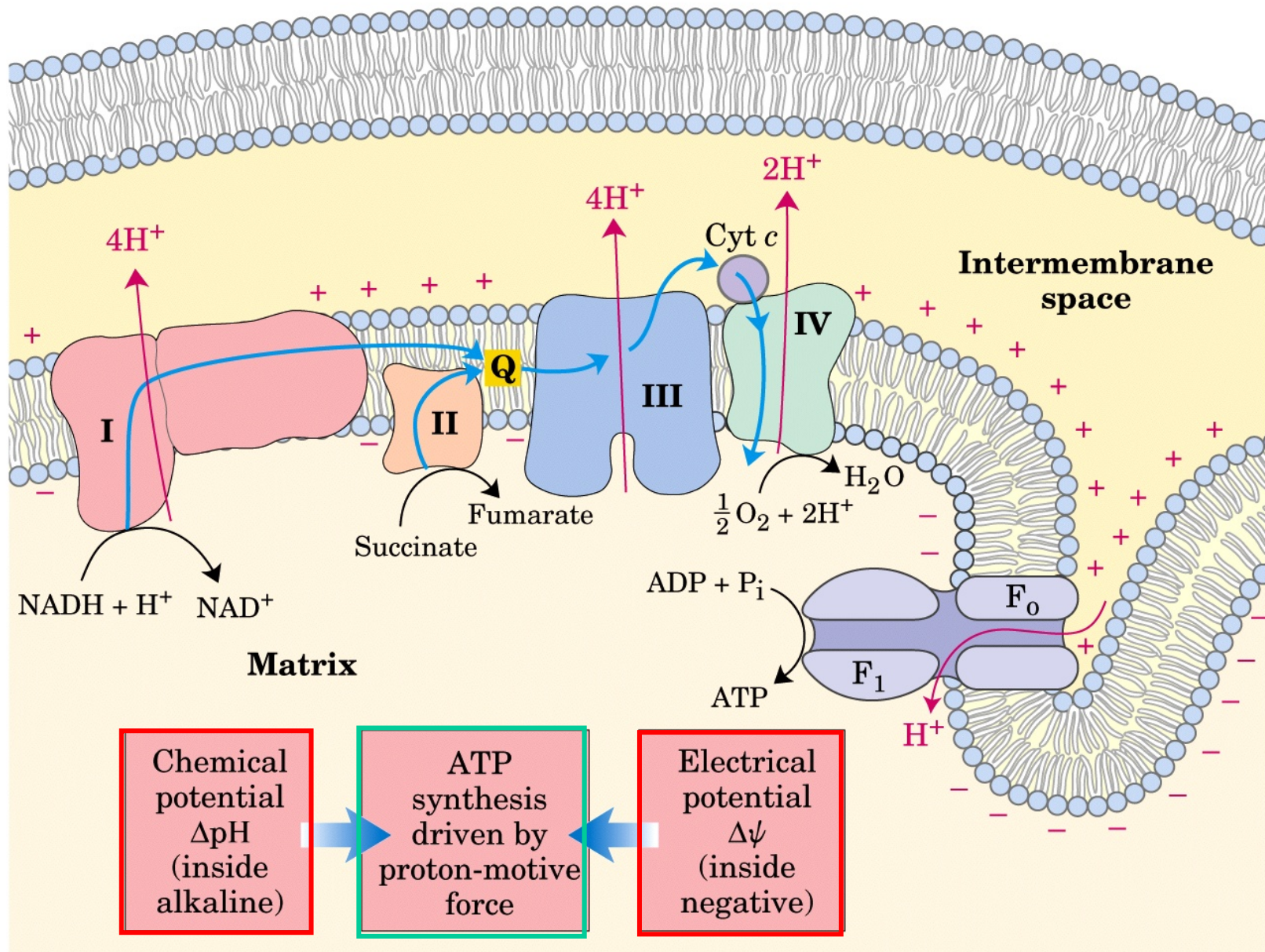
$$[\text{H}^+]_{\text{N}} = C_1$$



$$\begin{aligned}\Delta G &= RT \ln (C_2/C_1) + Z \mathcal{F} \Delta \psi \\ &= 2.3RT \Delta \text{pH} + \underline{\mathcal{F} \Delta \psi}\end{aligned}$$

# TEORIA CHEMIOSMOTICA: Peter Mitchell, 1961 (Nobel nel 1978)

## Gradiente elettrochimico di $H^+$



La variazione di energia libera che serve a traslocare un protone verso lo spazio intermembrana è formata da **due componenti**:

$$\Delta G = 2,3 RT [\text{pH dentro} - \text{pH fuori}] + ZF \Delta \Psi$$

(dove  $\Delta \Psi$  è il potenziale di membrana)

La formazione del gradiente protonico è un processo **endoergonico**

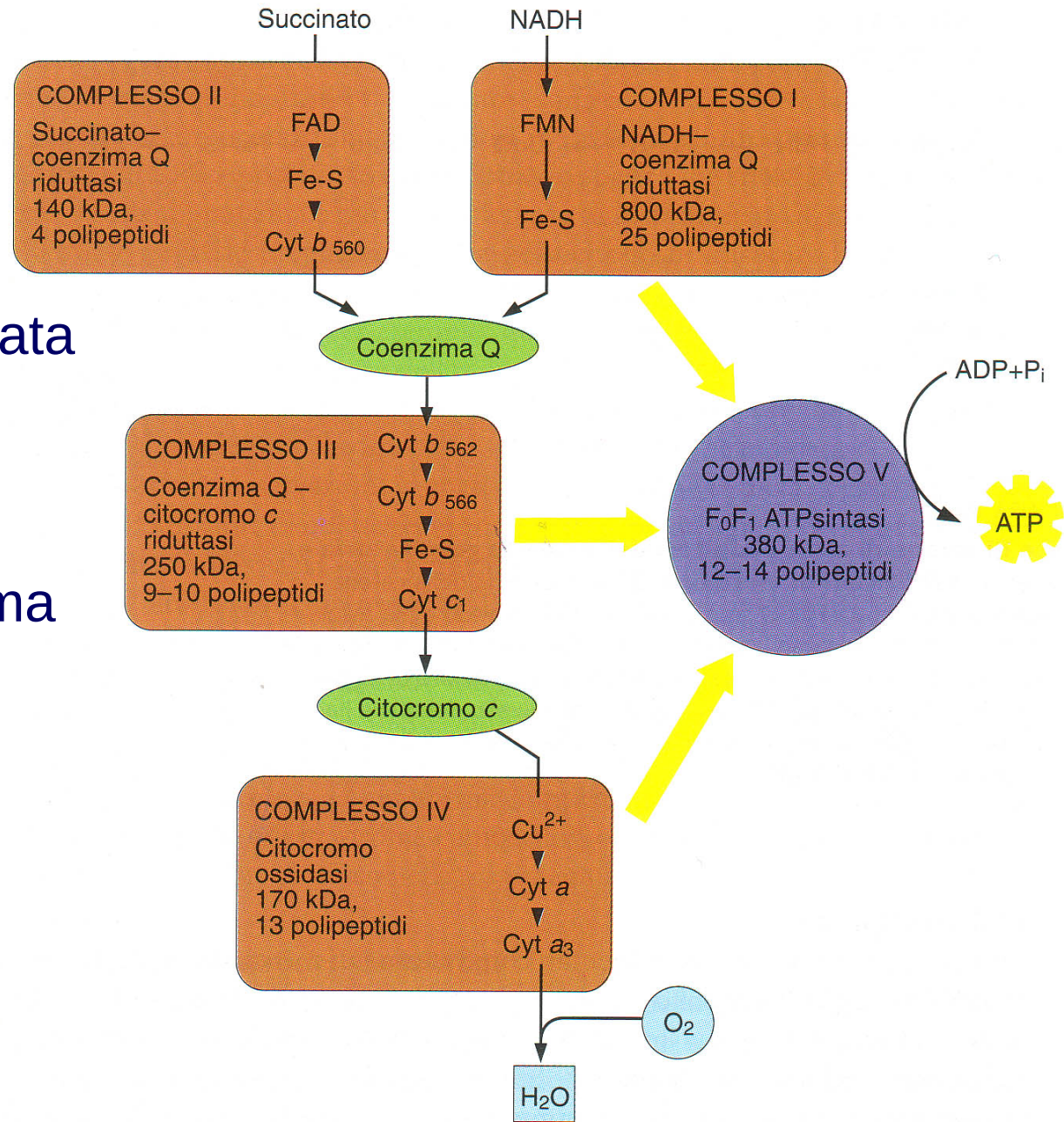
Quindi

Lo scaricamento del gradiente protonico è un processo **esoergonico**  
**accoppiato** a sintesi di ATP

# PROVE SPERIMENTALI DELLA TEORIA CHEMIOSMOTICA DELLA FOSFORILAZIONE OSSIDATIVA

1. è necessaria una membrana interna intatta
2. Le proteine del trasporto sono proteine transmembrana
3. Uso di agenti che dissipano il gradiente:  
disaccoppianti (2, 4 dinitrofenolo)  
ionofori (valinomicina)
4. inibitori della pompa protonica (oligomicina B)

L'energia libera rilasciata dal passaggio di  $e^-$  attraverso i complessi I-IV deve essere conservata in una forma utilizzabile dall'ATP sintasi (complesso V)



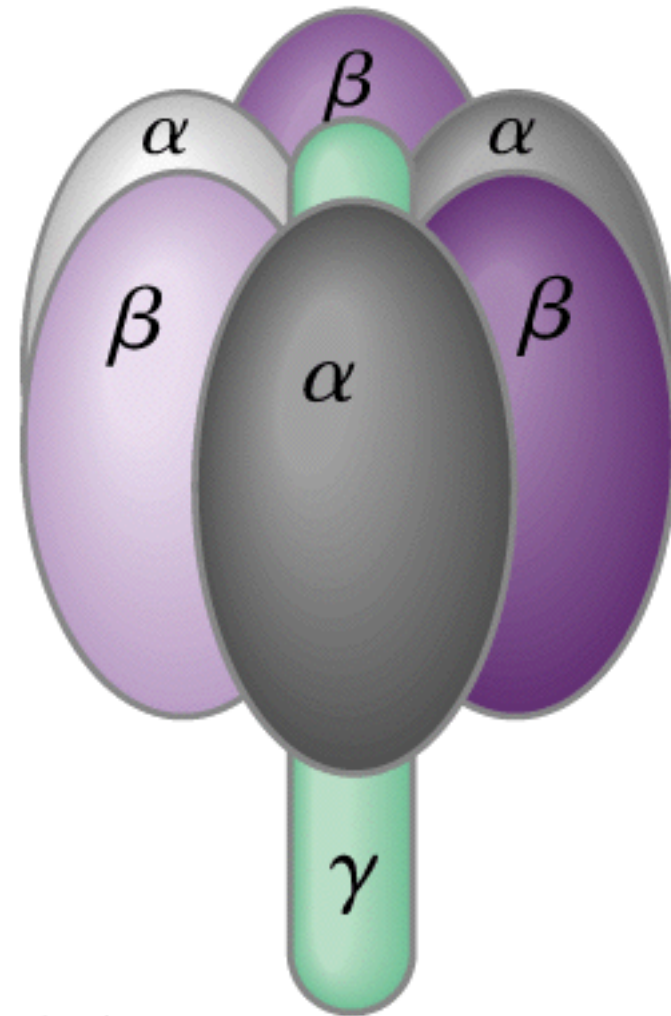
## F<sub>0</sub> – F<sub>1</sub> ATPasi ( complesso V)

**F<sub>1</sub>**

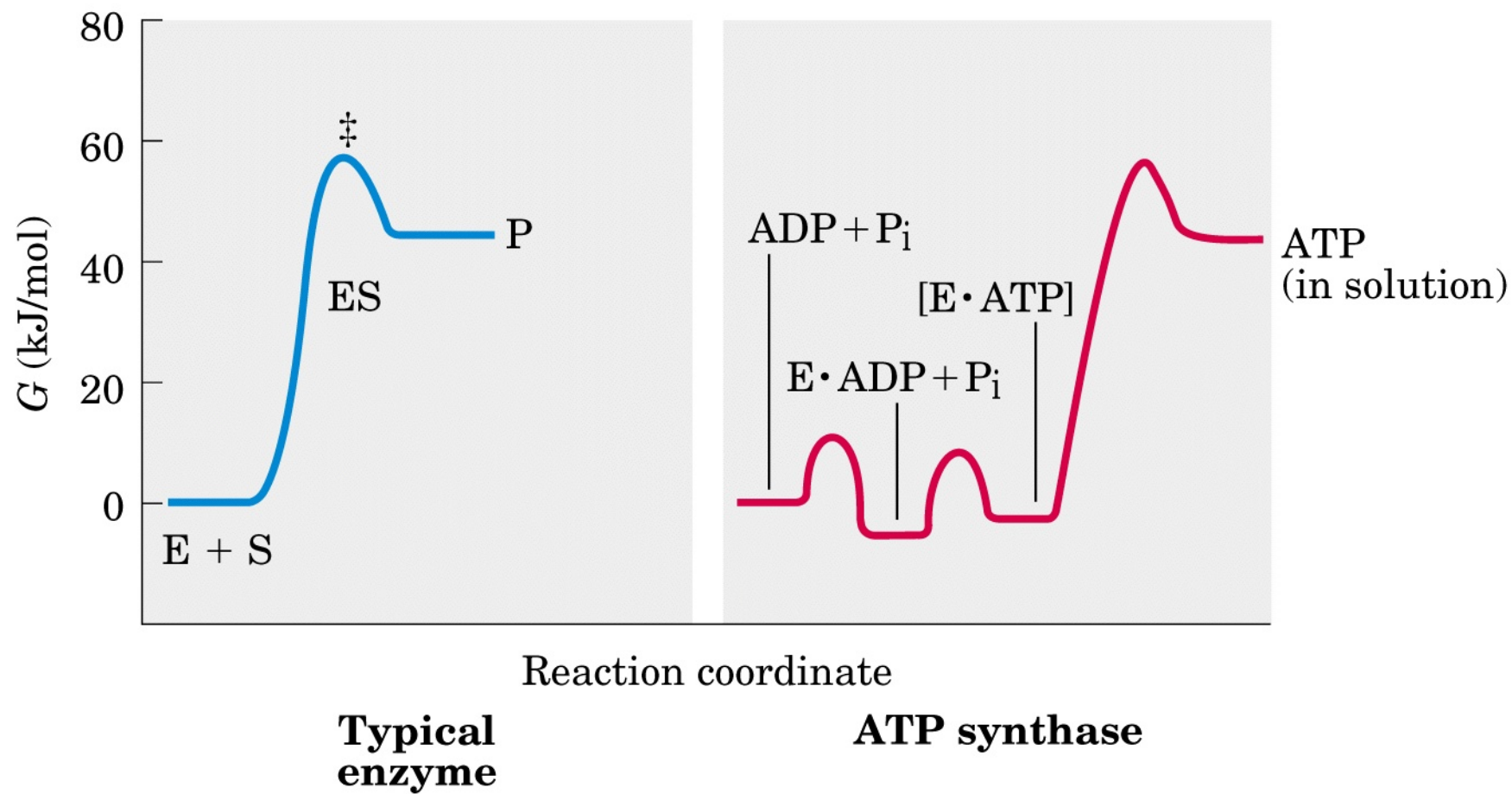
$\alpha_3\beta_3\gamma\delta\epsilon$

Boyer e Walker, Nobel 1997

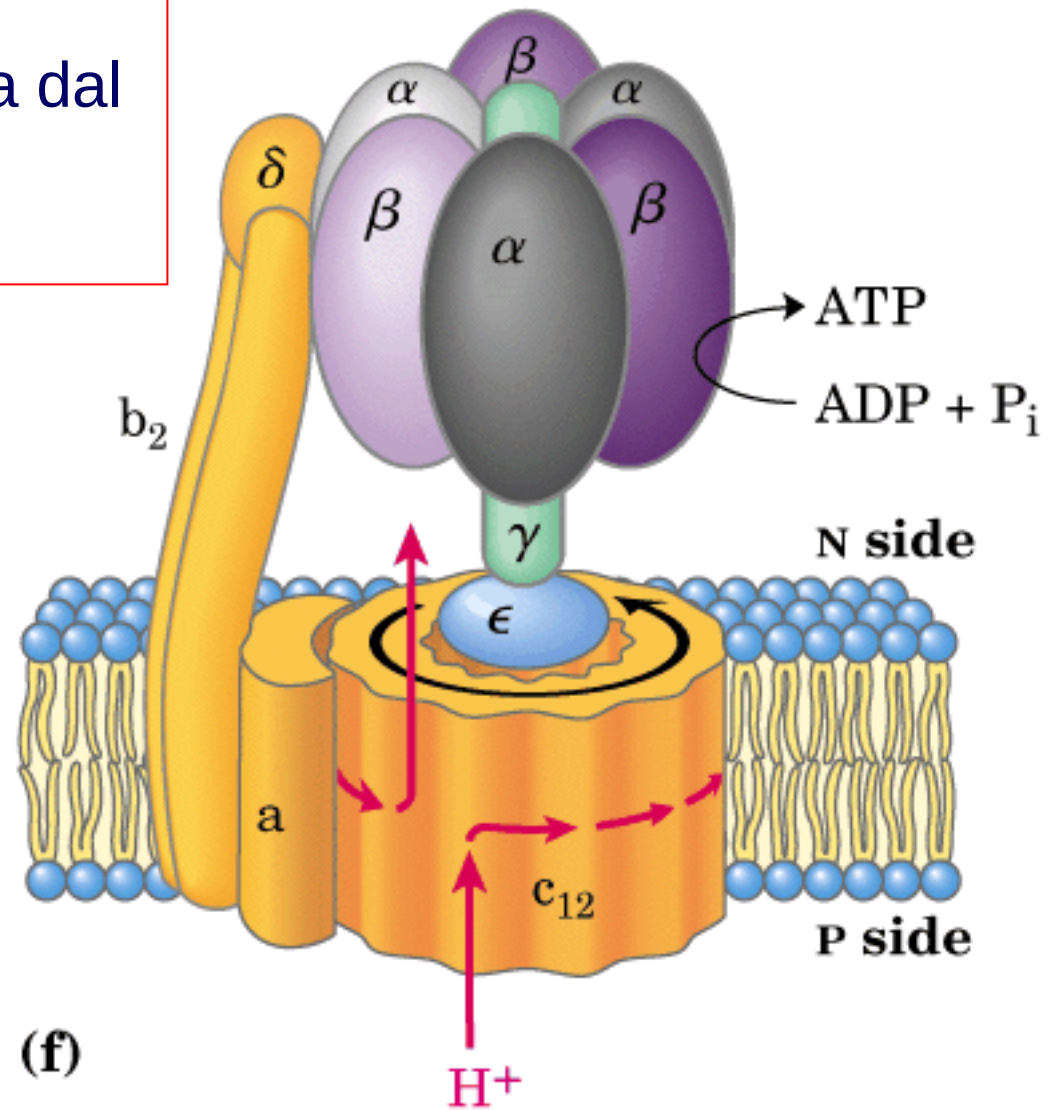
$\beta$  catalizza la formazione di ATP  
 $\alpha$  lega ATP

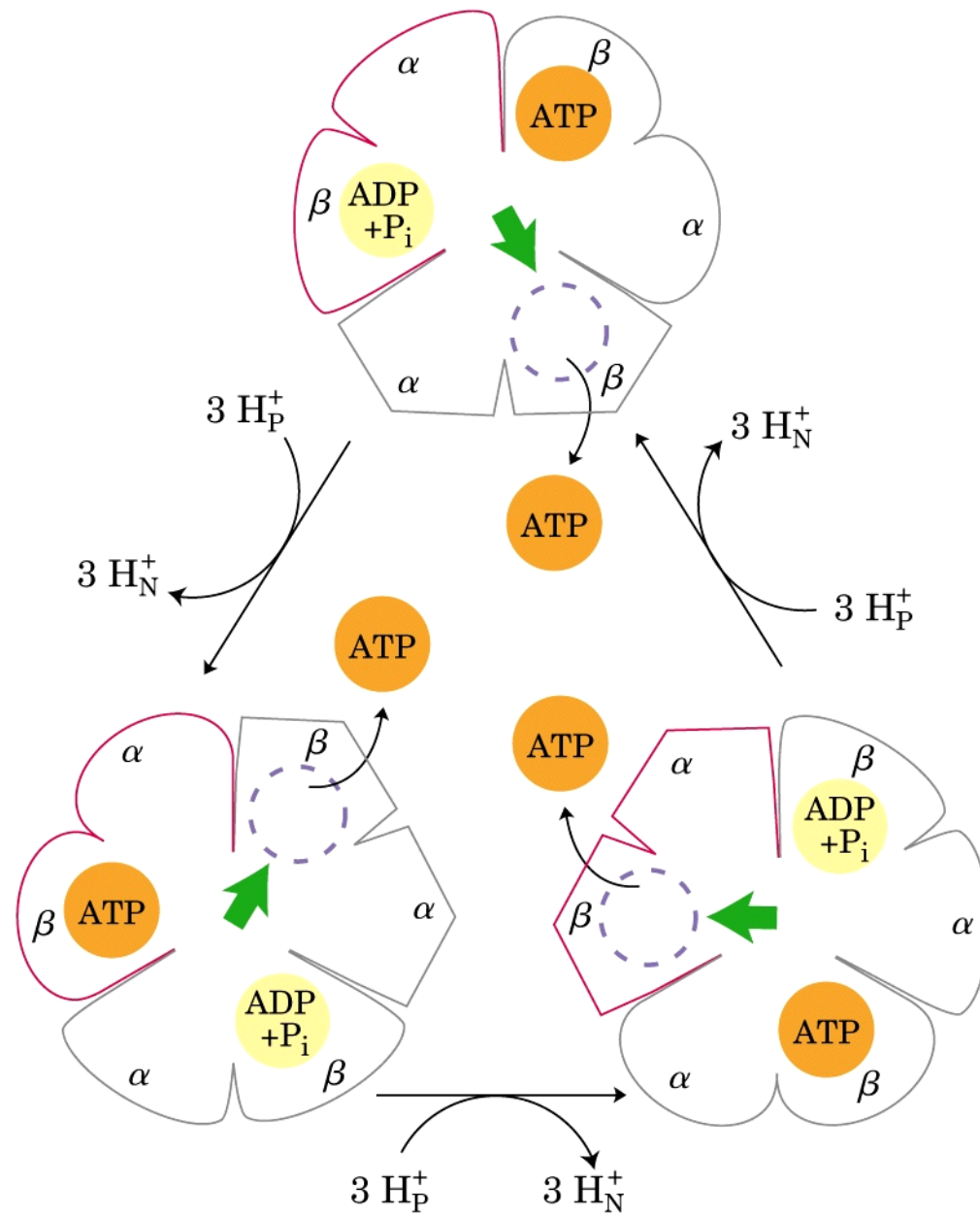


**(a)**



La rotazione della parte catalitica  $\alpha_3\beta_3$  viene indotta dal trasferimento di protoni e consente la sintesi di ATP





## RAPPORTO FOSFORO / OSSIGENO

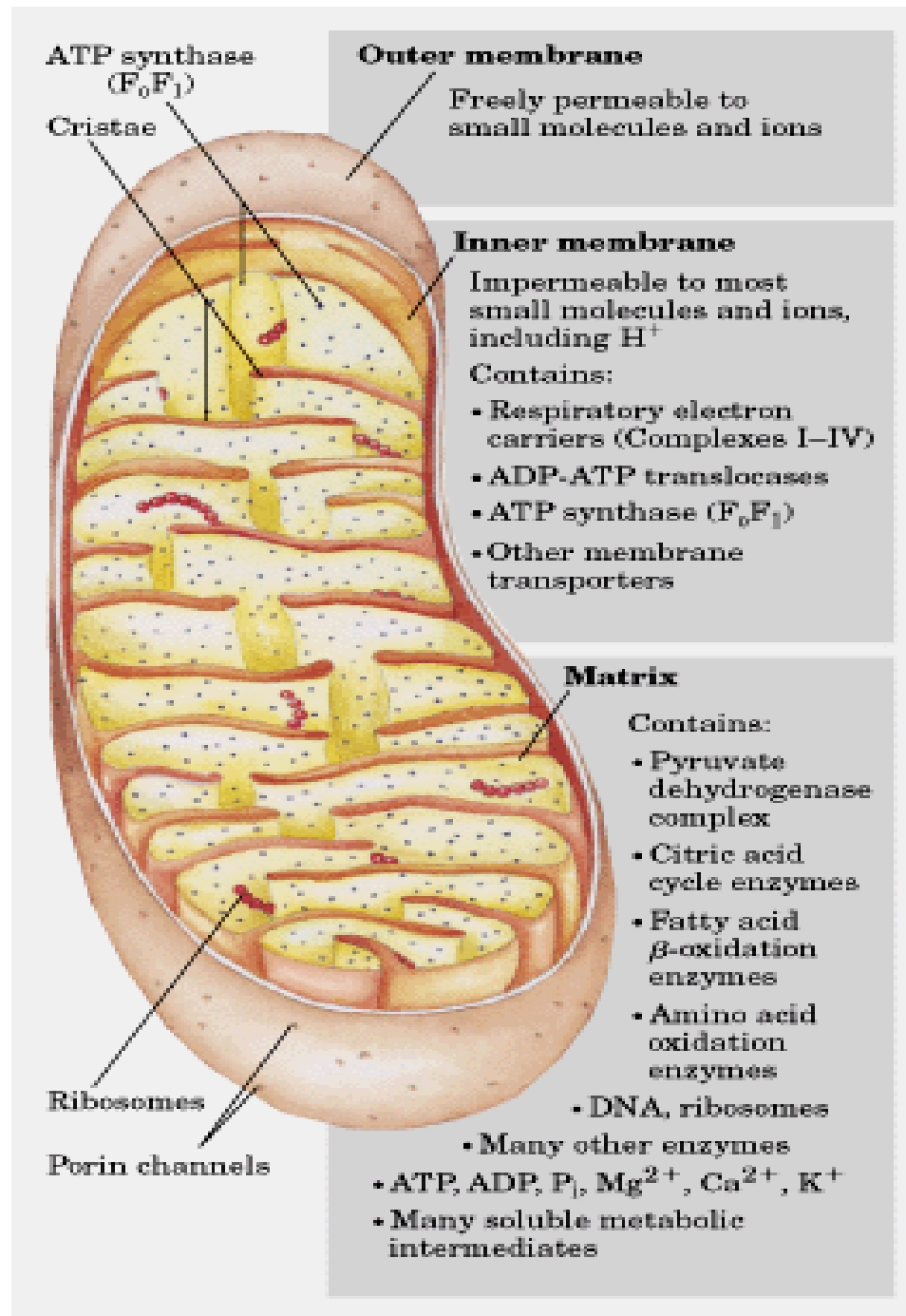
(quantità di ATP sintetizzato / O<sub>2</sub> ridotto)

Il passaggio di *circa* un H<sup>+</sup> a livello della subunità F<sub>0</sub> produce un solo cambiamento conformazionale (rotazione di 120°).

Quindi **per sintetizzare 1 ATP occorrono *circa* 3 H<sup>+</sup>**

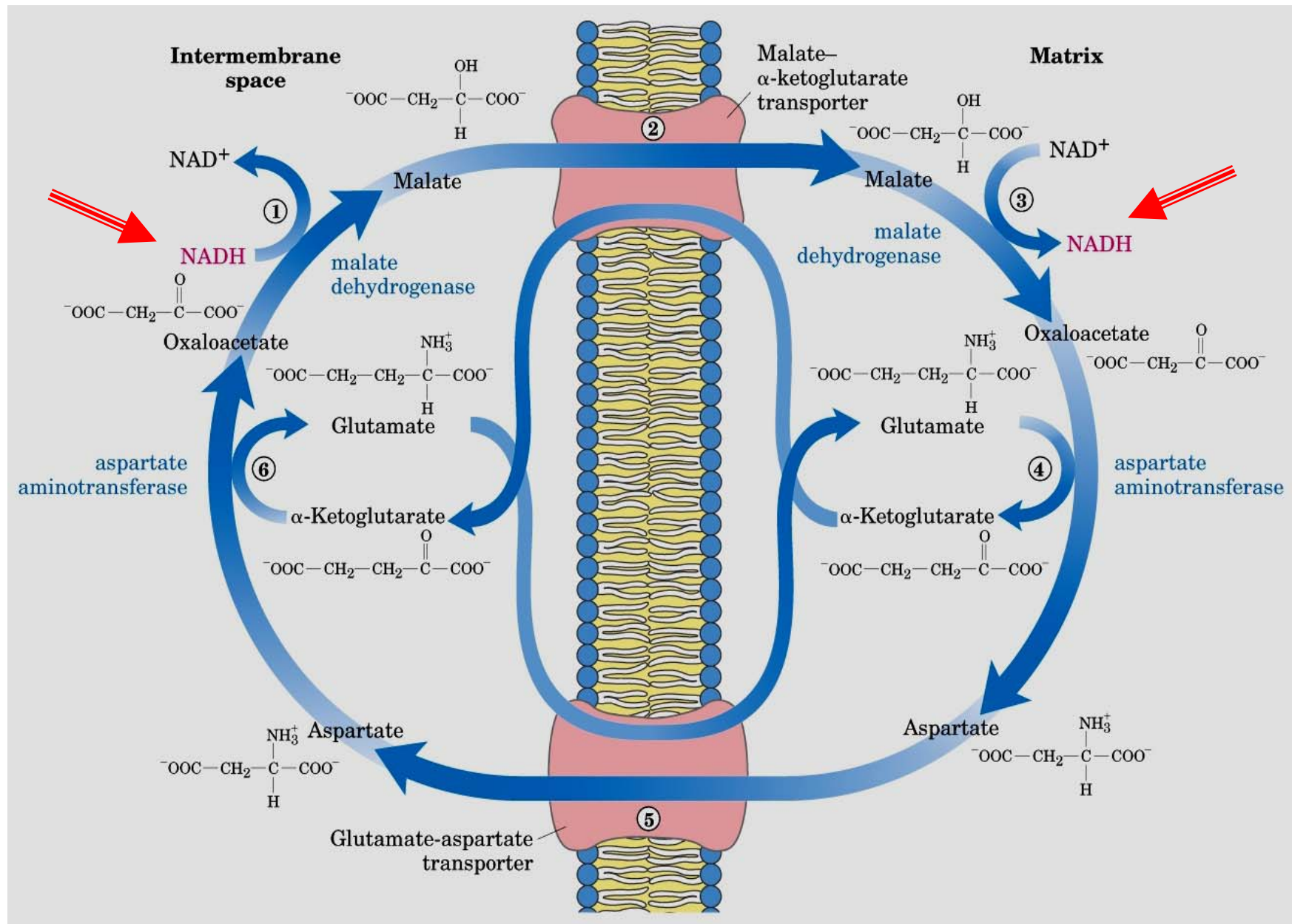
Il flusso di 2 elettroni nella catena respiratoria induce il passaggio di 10 H<sup>+</sup>

Quindi dal NADH si possono produrre *circa* 3 ATP,  
dal FADH<sub>2</sub> *circa* 2 ATP



Gli **equivalenti riducenti** vengono trasportati all'interno dei mitocondri attraverso dei sistemi navetta (Shuttle)

# Shuttle del malato-aspartato

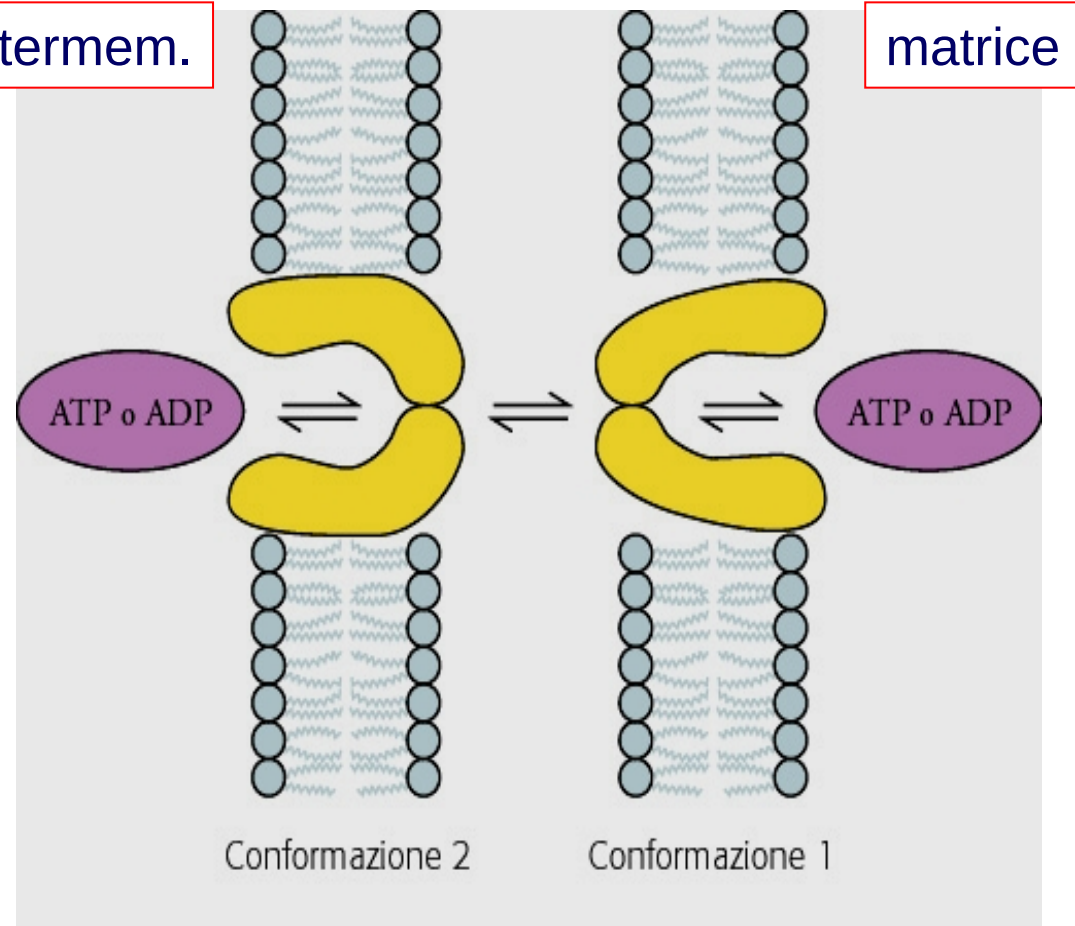


## Traslocatore ADP/ATP

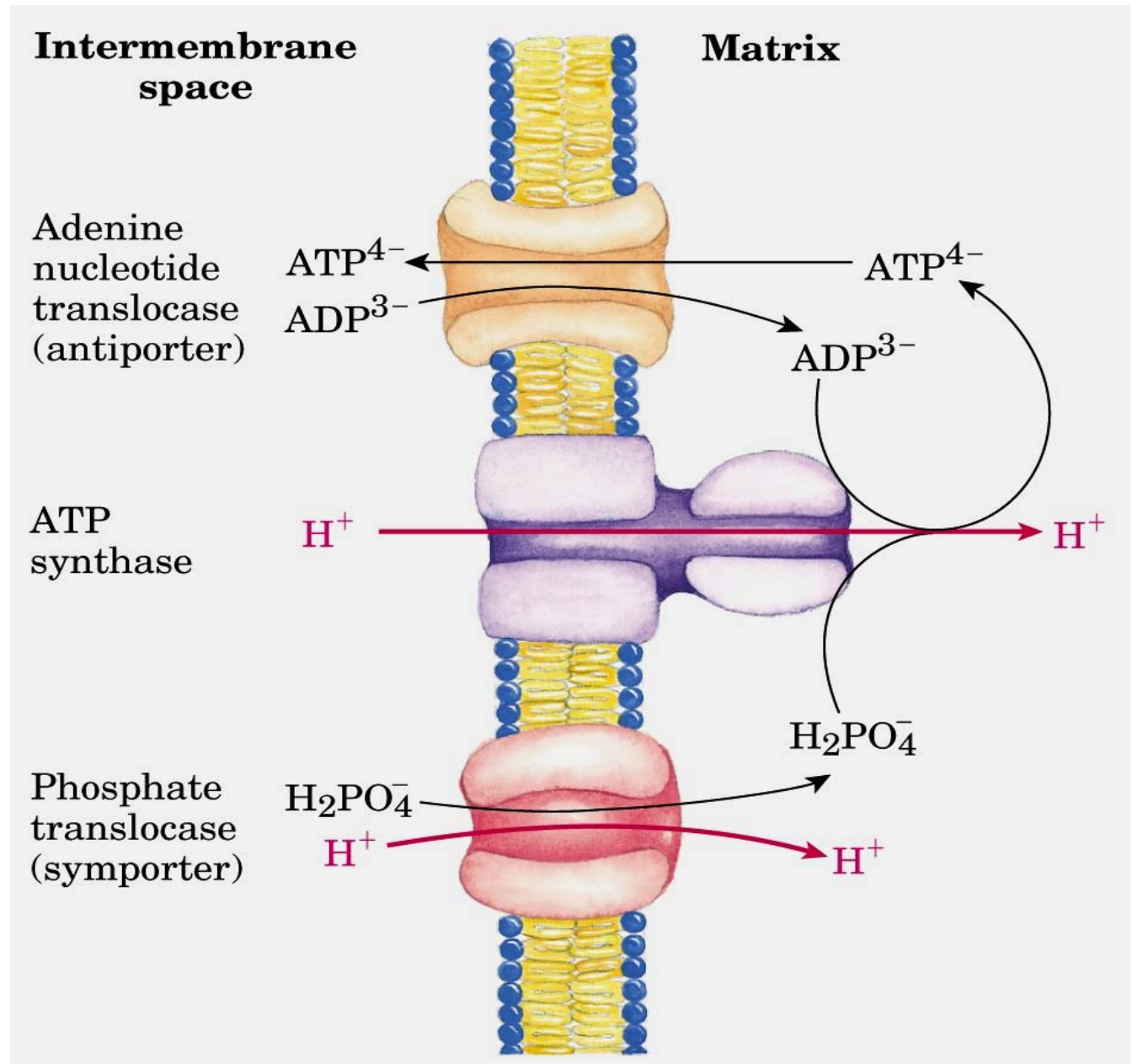
Sp. intermem.

matrice

Cambia conformazione solo se legato.  
Ad ogni esportazione di ATP (-4) ed importazione di ADP (-3) corrisponde il trasferimento di una carica, che è guidato dalla differenza di potenziale  $\Delta\Psi$  attraverso la membrana mitocondriale interna (+ all'esterno), che guida anche l'entrata di  $\text{Ca}^{2+}$



## Traslocatore ADP/ATP



**table 19–5**

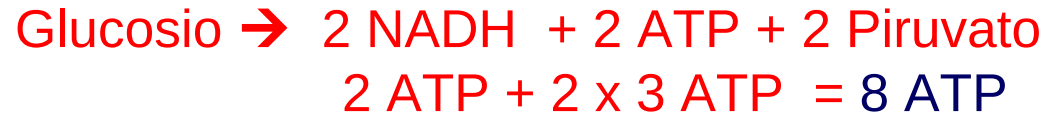
**ATP Yield from Complete Oxidation of Glucose**

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

\*The number depends on which shuttle system transfers reducing equivalents into mitochondria.

# RESA ENERGETICA DAL METABOLISMO AEROBIO DEL GLUCOSIO

## GLICOLISI



## PIRUVATO DEIDROGENASI

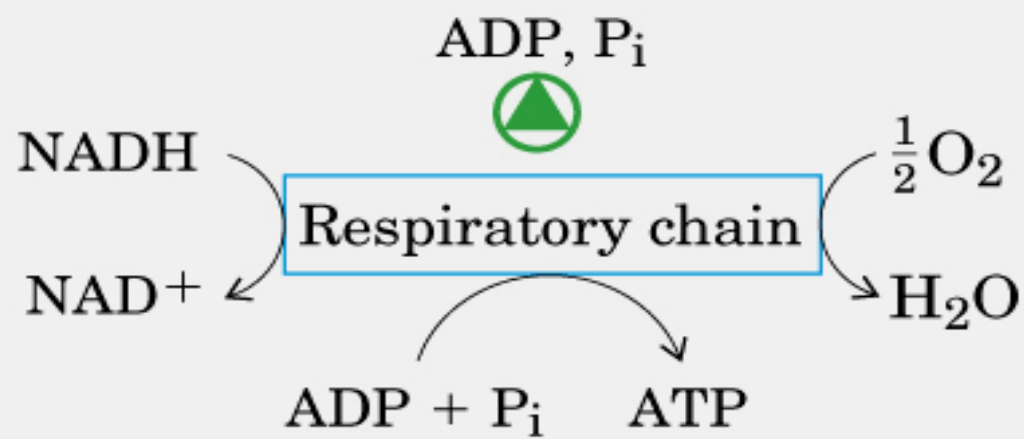


## CICLO DI KREBS



TOTALE 38 ATP (CIRCA)

**Oxidative  
phosphoryl-  
ation**



The diagram illustrates the metabolic pathways of cellular respiration, organized into three main stages:

- Glycolysis:**
  - Glucose** is converted to **Glucose 6-phosphate** by the enzyme **hexokinase**. This step is inhibited by **P<sub>i</sub>** (green circle with a red X) and produces **P<sub>i</sub>** (green circle with a green checkmark).
  - Glucose 6-phosphate** is converted to **Fructose 1,6-bisphosphate** by the enzyme **phosphofructokinase-1**. This step is inhibited by **ATP** and **citrate** (red circles with red Xs) and produces **AMP** (green circle with a green checkmark).
  - Fructose 1,6-bisphosphate** is converted to **Phosphoenolpyruvate** by the enzyme **pyruvate kinase**. This step is inhibited by **ATP** and **NADH** (red circles with red Xs) and produces **ADP** (green circle with a green checkmark).
- Krebs cycle:**
  - Phosphoenolpyruvate** is converted to **Pyruvate** by the enzyme **pyruvate dehydrogenase complex**. This step is inhibited by **ATP** and **NADH** (red circles with red Xs) and produces **AMP**, **ADP**, and **NAD<sup>+</sup>** (green circles with green checkmarks).
  - Pyruvate** is converted to **Acetyl-CoA** by the enzyme **citrate synthase**. This step is inhibited by **ATP** and **NADH** (red circles with red Xs) and produces **ADP** (green circle with a green checkmark).
  - Acetyl-CoA** enters the cycle and is converted to **Citrate**. This step is inhibited by **ATP** (red circle with a red X) and produces **ADP** (green circle with a green checkmark).
  - Citrate** is converted to **α-Ketoglutarate** by the enzyme **isocitrate dehydrogenase**. This step is inhibited by **ATP** (red circle with a red X) and produces **ADP** (green circle with a green checkmark).
  - α-Ketoglutarate** is converted to **Succinyl-CoA** by the enzyme **α-ketoglutarate dehydrogenase**. This step is inhibited by **ATP** and **NADH** (red circles with red Xs).
  - Succinyl-CoA** is converted to **Oxaloacetate**. This step produces **ADP** and **P<sub>i</sub>** (green circles with green checkmarks).
  - Oxaloacetate** combines with **Acetyl-CoA** to restart the cycle, a step inhibited by **citrate** (red circle with a red X).
- Oxidative phosphorylation:**
  - Succinyl-CoA** enters the **Respiratory chain**.
  - The **Respiratory chain** uses **NADH** and **1/2 O<sub>2</sub>** to produce **NAD<sup>+</sup>** and **H<sub>2</sub>O**.
  - The **Respiratory chain** also uses **ADP + P<sub>i</sub>** to produce **ATP**.

citrate synthase

isocitrate dehydrogenase

$\alpha$ -ketoglutarate dehydrogenase

The diagram illustrates the Respiratory chain. On the left, NADH is oxidized to NAD<sup>+</sup>. On the right,  $\frac{1}{2} \text{O}_2$  is reduced to H<sub>2</sub>O. The chain is represented by a central box labeled "Respiratory chain". Above the box, ADP and P<sub>i</sub> are shown entering, and ATP is shown leaving. Below the box, ADP + P<sub>i</sub> is shown entering, and ATP is shown leaving. The diagram also shows the flow of electrons and protons through the chain.

Nel tessuto adiposo bruno (nei neonati e negli animali in letargo), anche in presenza di alte concentrazioni di ATP, la respirazione continua, ma l'energia prodotta viene utilizzata per produrre calore anziché ATP attraverso la termogenina, una proteina disaccoppiante.

