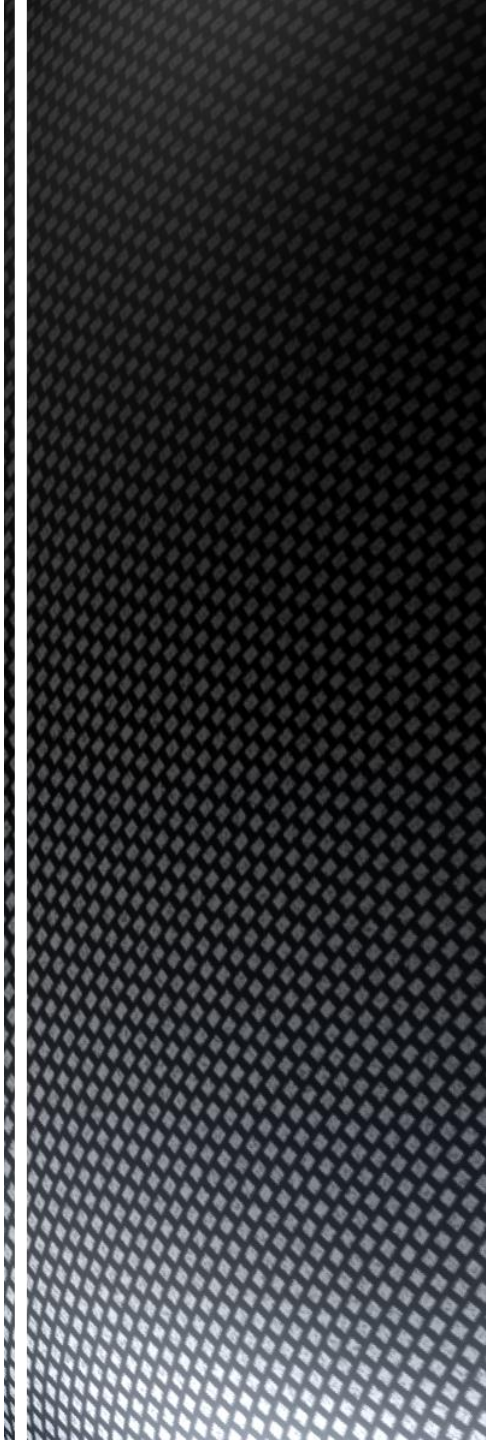
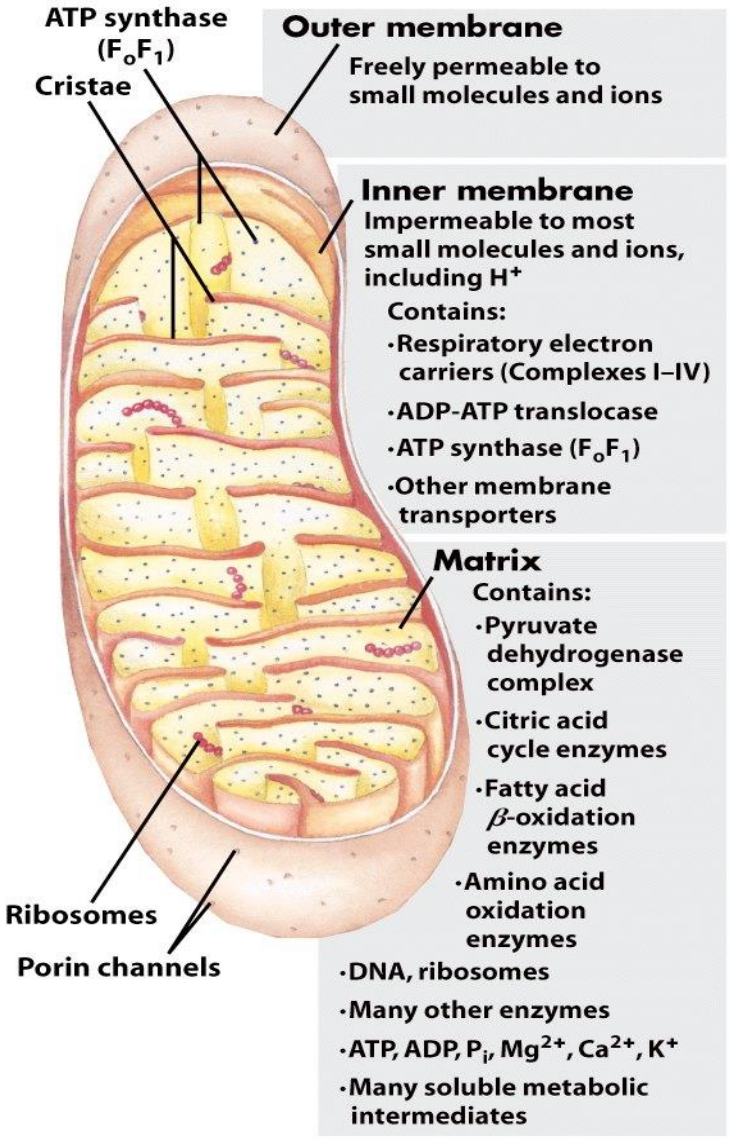
Mitochondrial respiration



Biochemical anatomy of a mitochondrion



Respiratory chain and ATP synthase

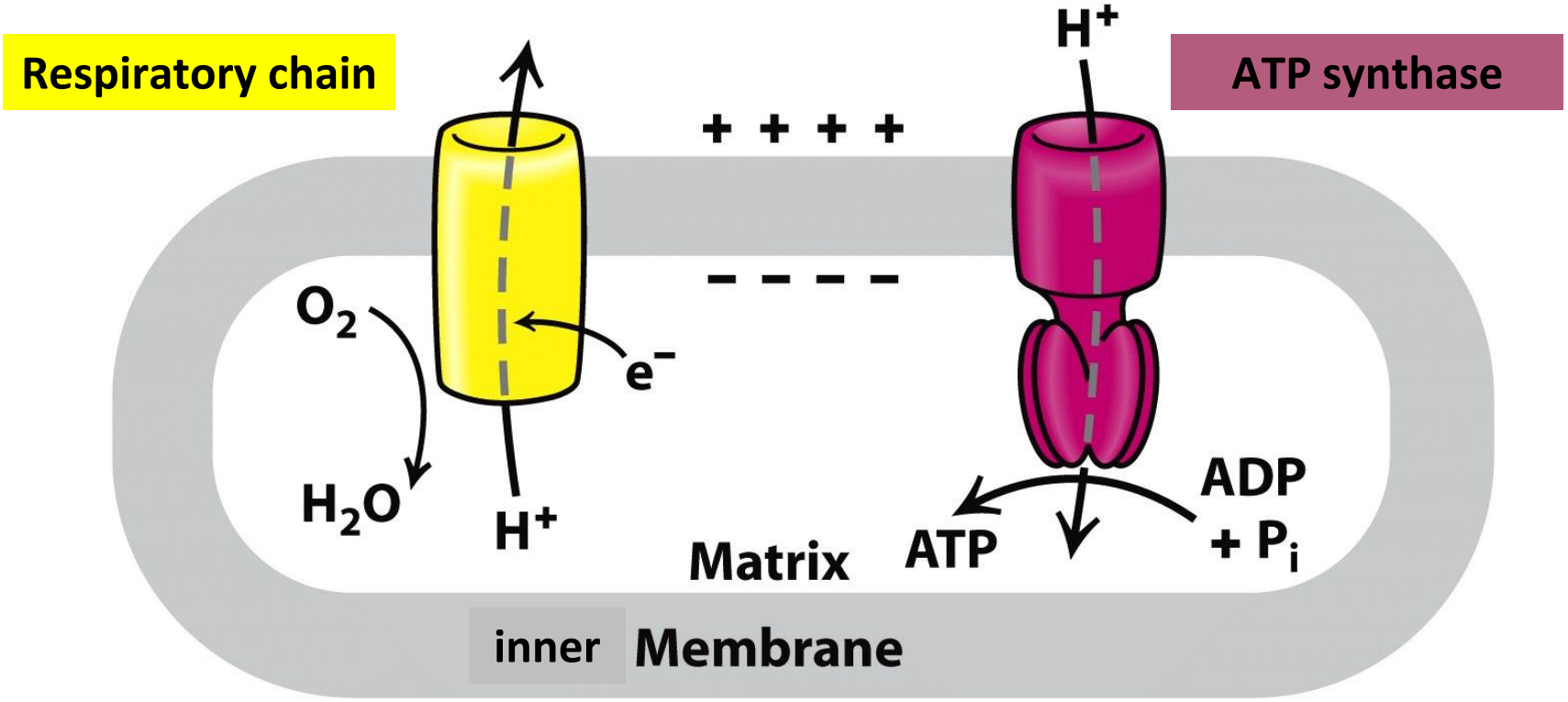


Figure 18-1
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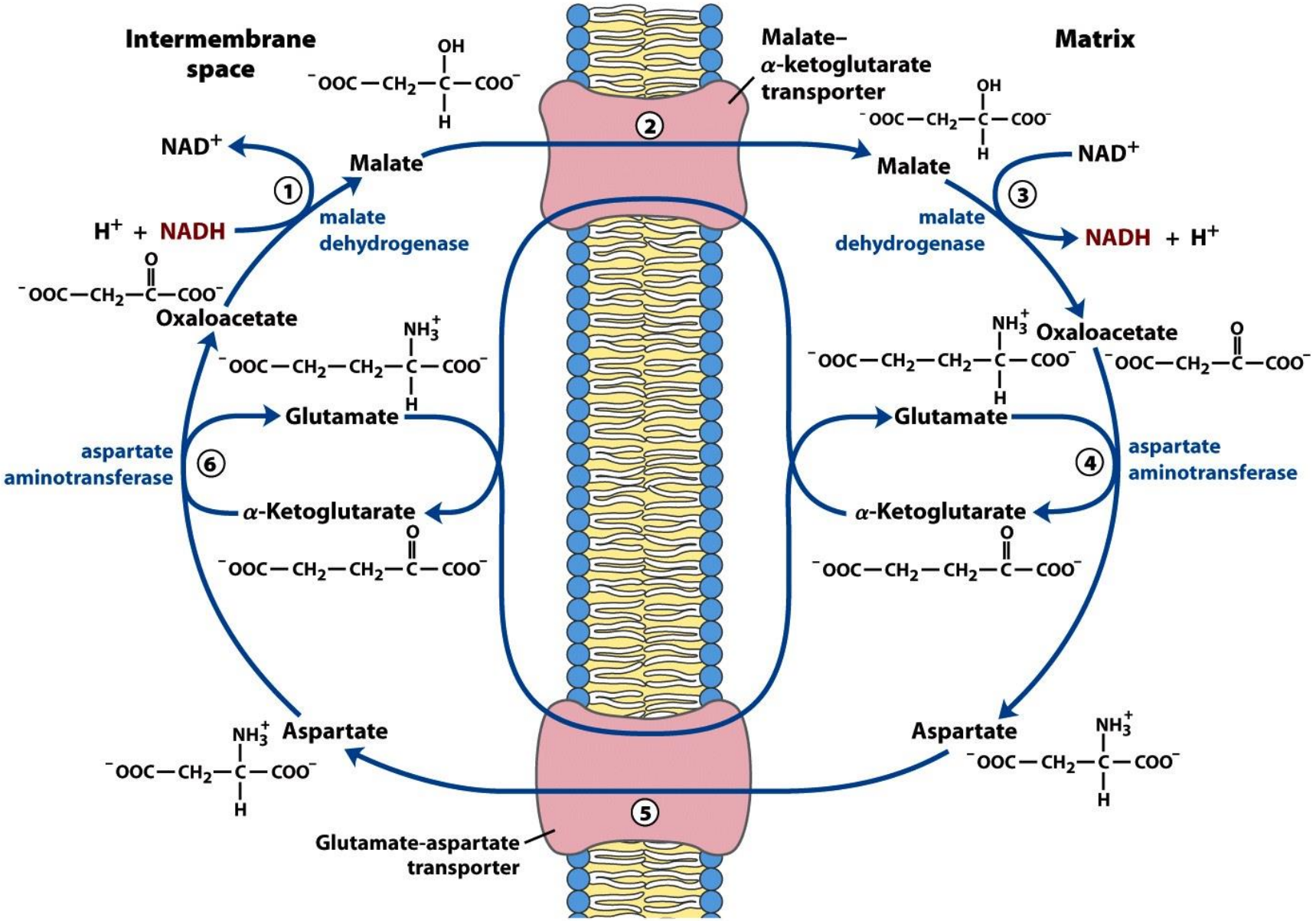
Mitochondrial transport of reducing equivalents:
1. Malate-aspartate shuttle



Mitochondrial transport of reducing equivalents:
2. glycerol 3-phosphate shuttle

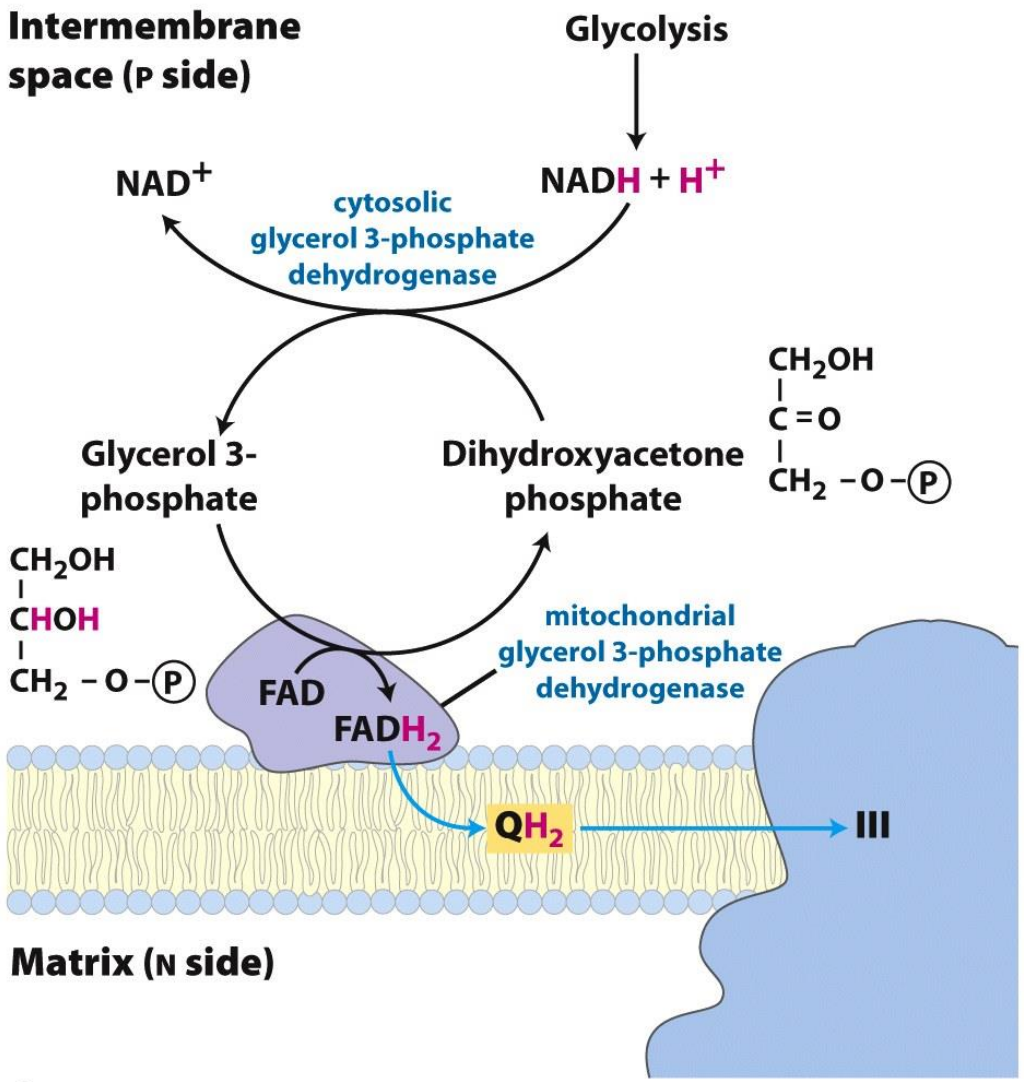


Figure 19-30
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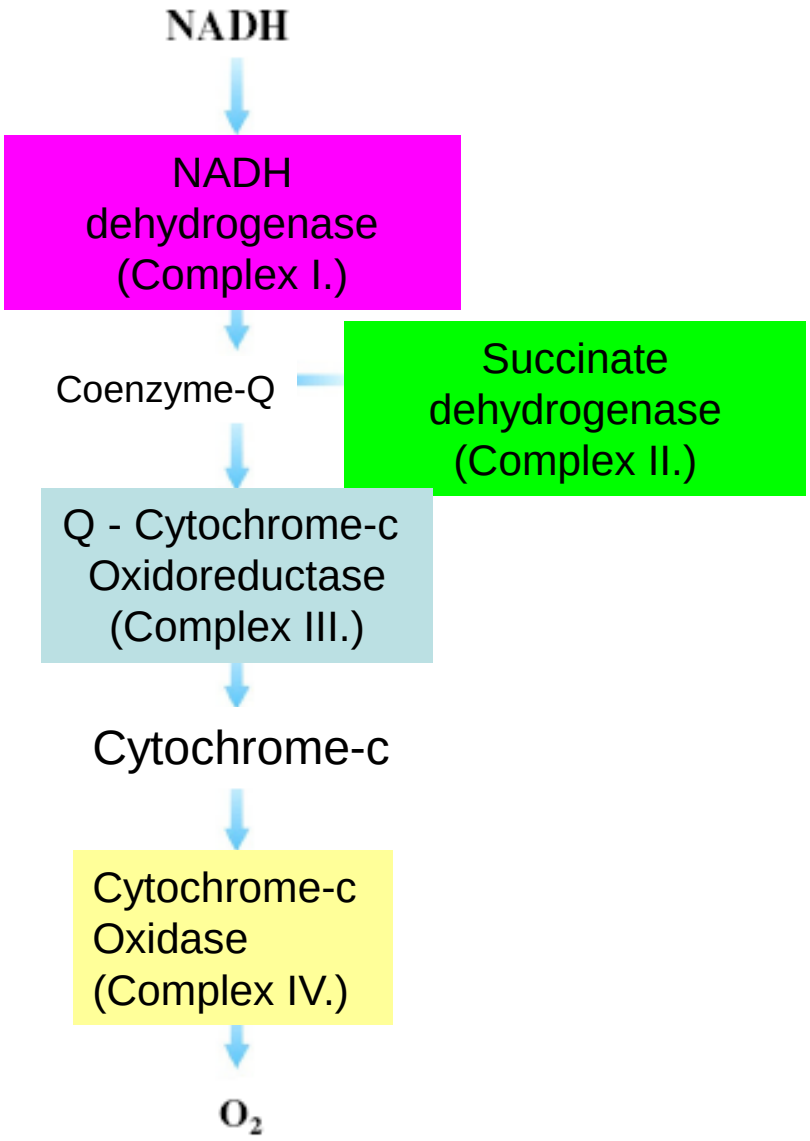
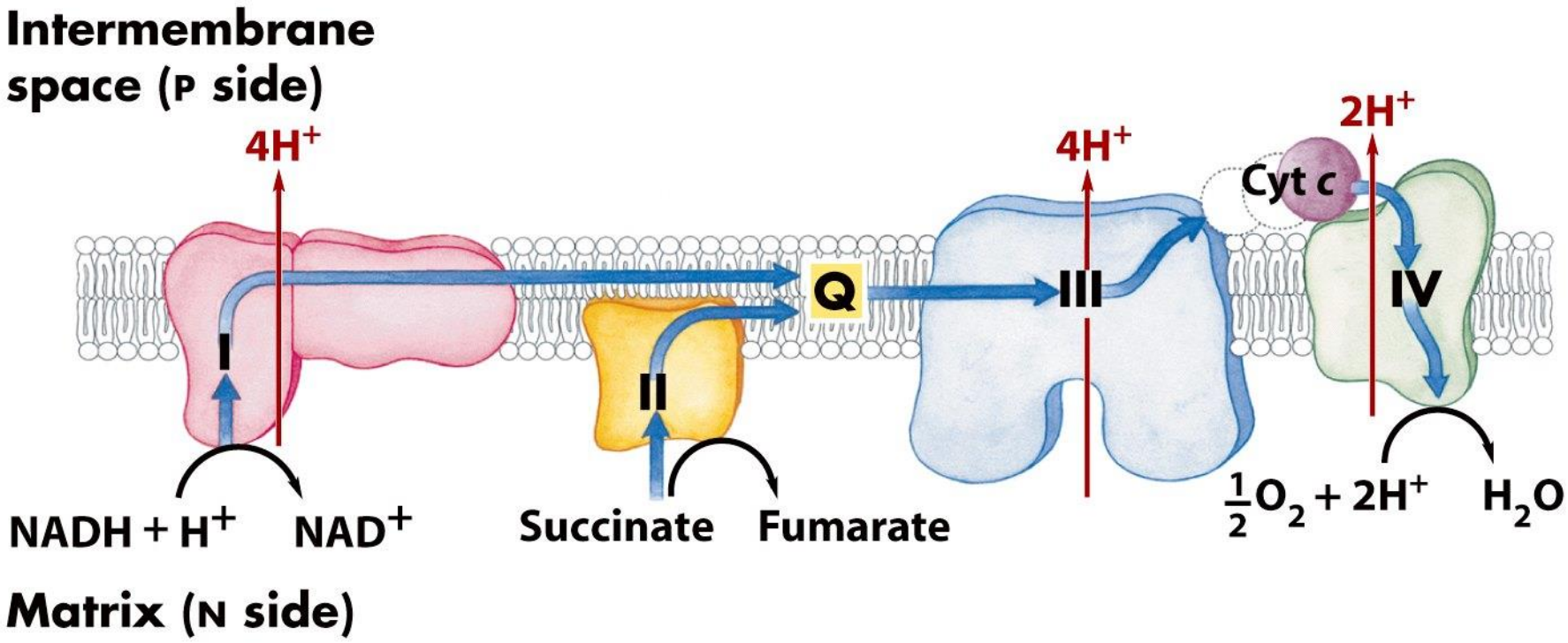
Oxidative phosphorylation

- Oxidative phosphorylation begins with the entry of electrons into the respiratory chain.
- Universal electron acceptors:
  - Nicotinamide nucleotides /NAD^+, $NADP^+$/
  - Flavin nucleotides /FMN, FAD/
- NAD linked dehydrogenases remove 2 ·H atoms from their substrates
  - :H^- hydride ion-NAD, released as H^+ in the medium
- Neither NADH nor NADPH can cross the inner mitoch. membrane, but the electrons they carry can be shuttled across indirectly.

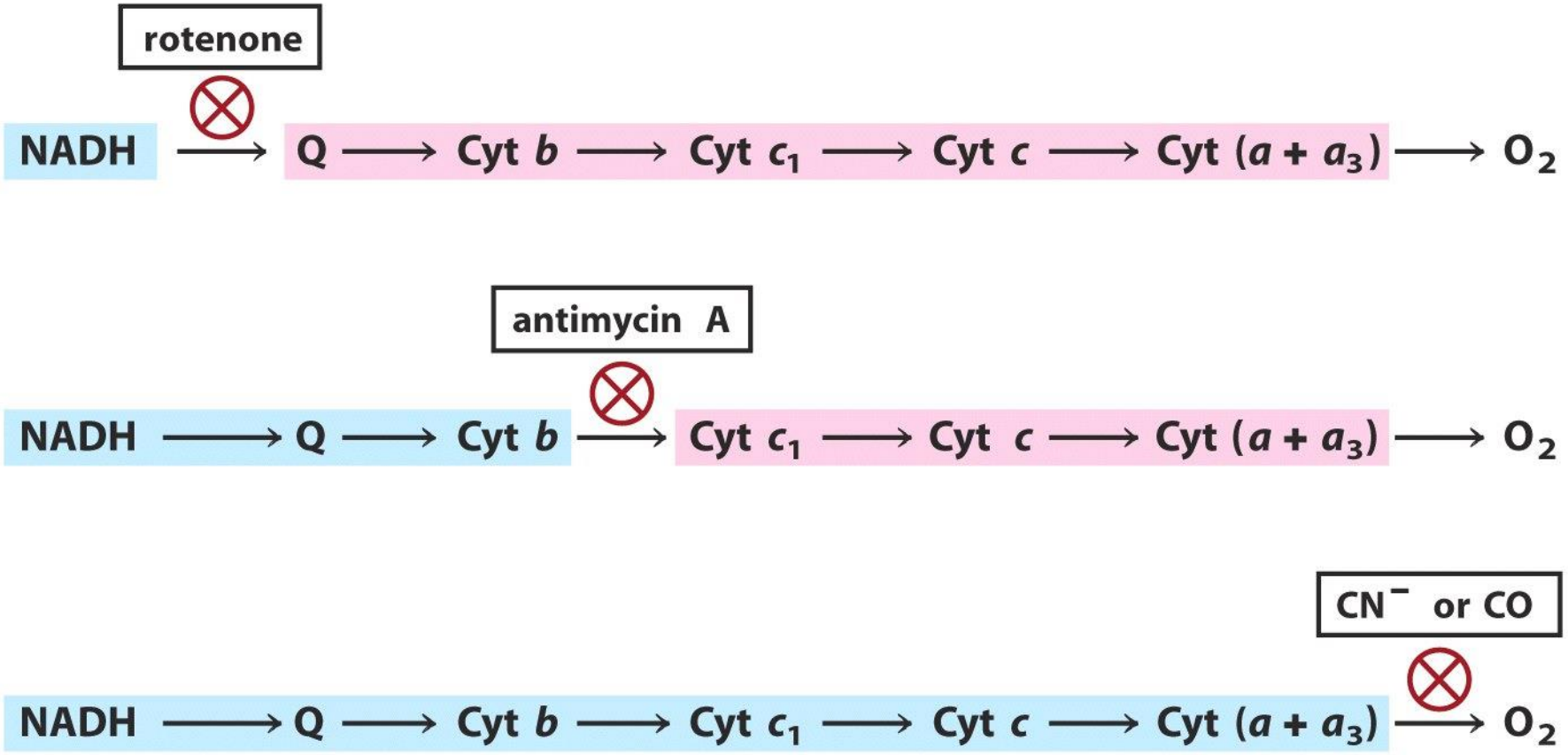
Overview of the respiratory chain



The mitochondrial respiratory chain consists of a series of sequentially acting electron carriers.
Most of them are capable of accepting and donating either 1 or 2 electrons.



Sequence of components in the respiratory chain determined by inhibitors



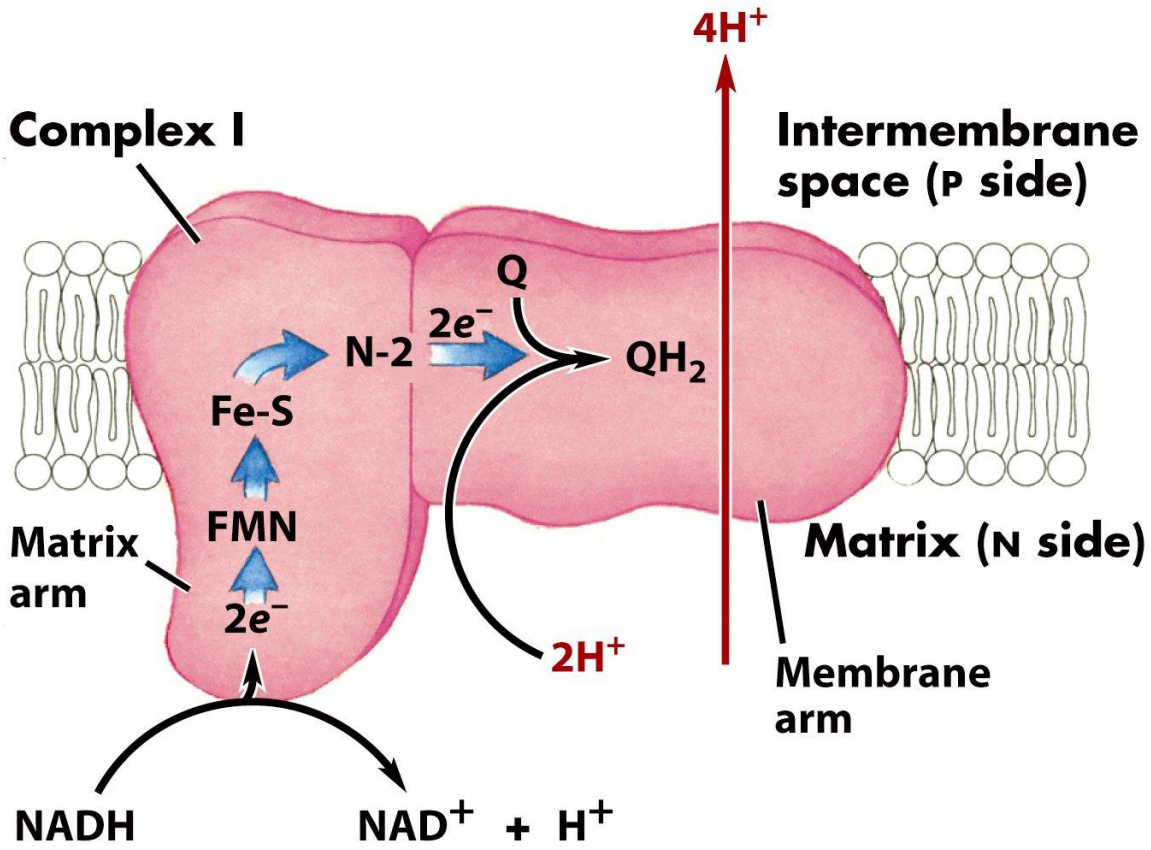
Complex I. Complex III. Complex IV.

Complex I. NADH-dehydrogenase

(NADH: ubiquinone oxidoreductase)

$$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$$

inhibitors: amytal, rotenone, piericidin A



Complex II. Succinate-dehydrogenase

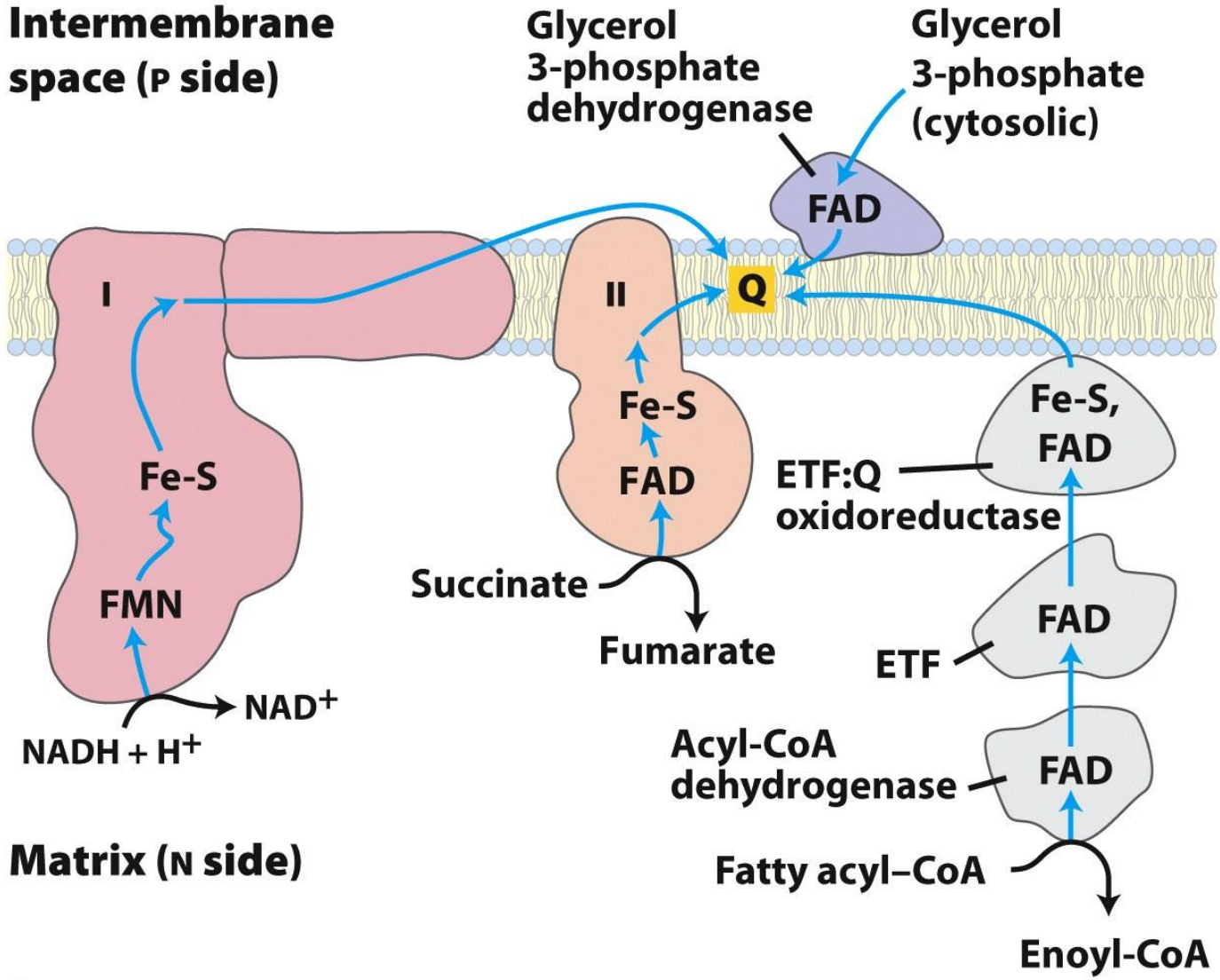


Figure 19-8
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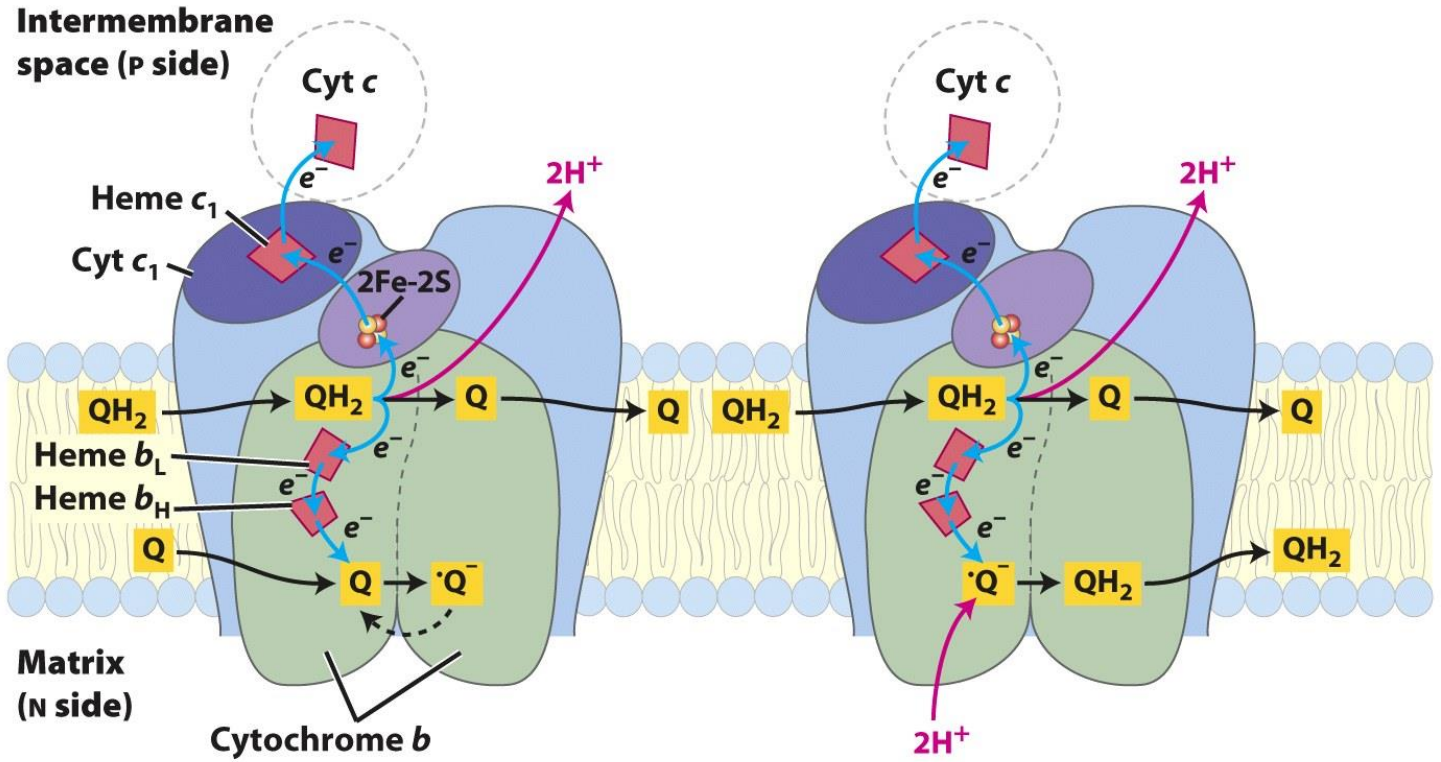
Complex III. Ubiquinone-cytochrome c-oxidoreductase

Inhibitor: antimycin-A, myxothiazol



$$QH_2 + Q + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow \cdot Q^- + Q + 2H_P^+ + \text{cyt } c_1 \text{ (reduced)}$$

$$QH_2 + \cdot Q^- + 2H_N^+ + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow QH_2 + 2H_P^+ + Q + \text{cyt } c_1 \text{ (reduced)}$$

$$\text{Net equation: } QH_2 + 2 \text{ cyt } c_1 \text{ (oxidized)} + 2H_N^+ \longrightarrow Q + 2 \text{ cyt } c_1 \text{ (reduced)} + 4H_P^+$$

Figure 19-12
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Complex IV. Cytochrome c-oxidase

Inhibitors: cyanide, CO, hydrogen sulfide, azides

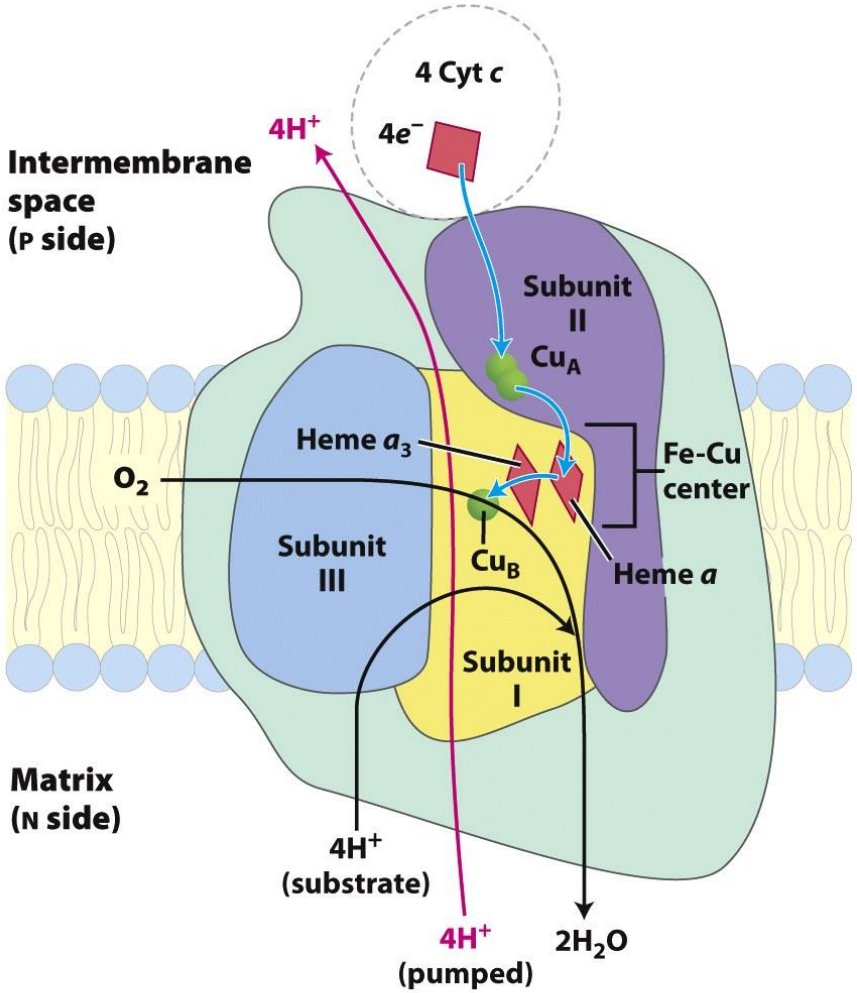

Figure 19-14
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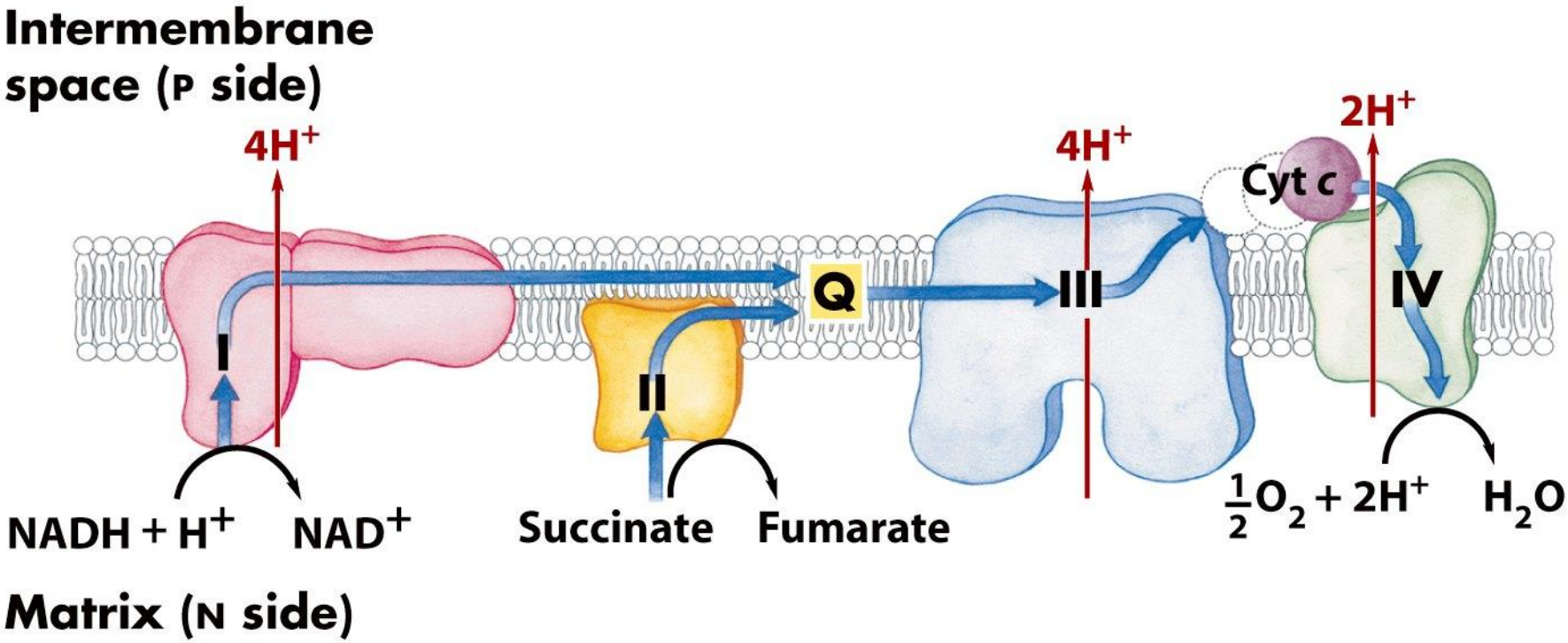
Summary

$$NADH + H^+ + 1/2O_2 \longrightarrow NAD^+ + H_2O$$

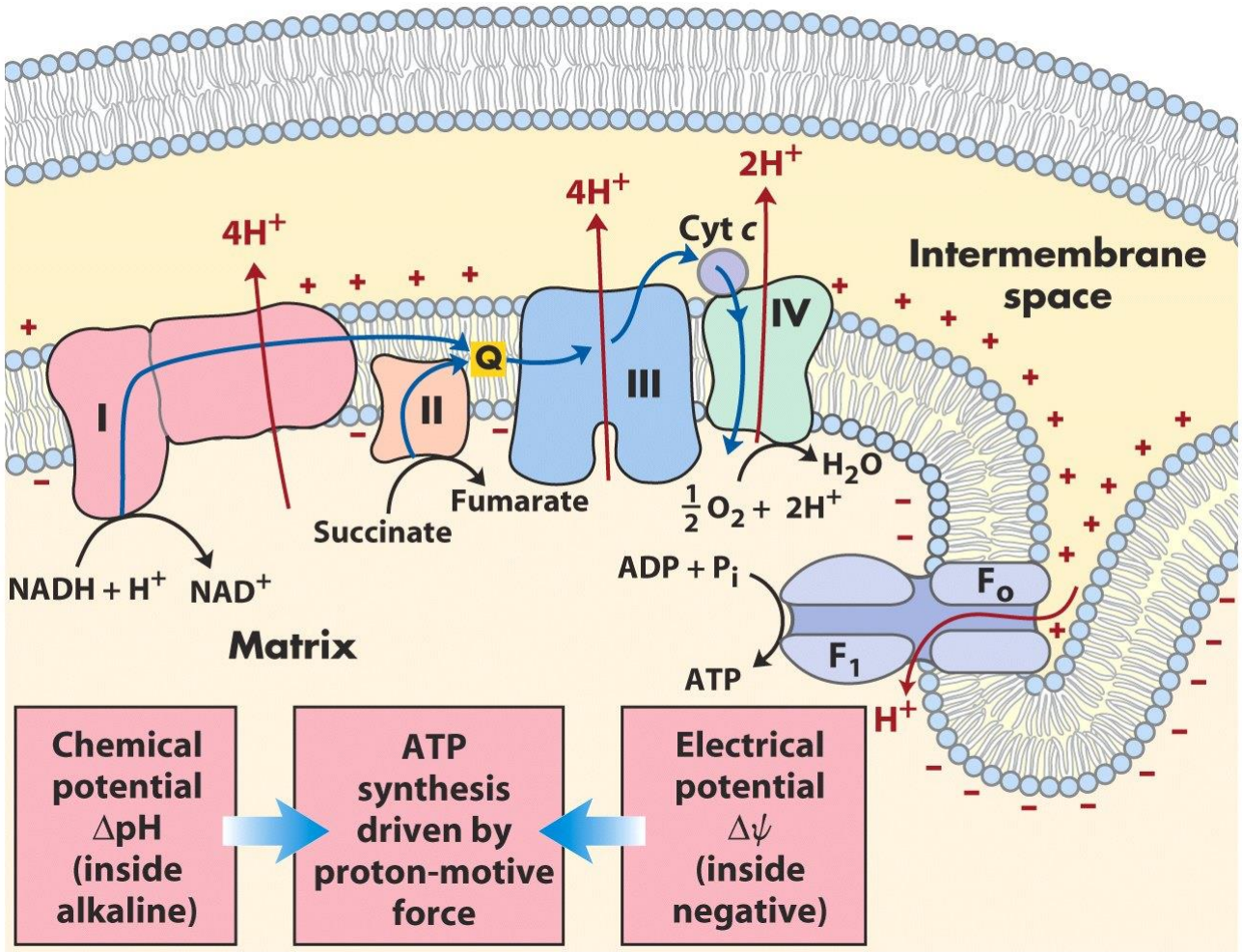
$$NADH + \sim 11H^+_N + 1/2O_2 \longrightarrow NAD^+ + H_2O + \sim 10H^+_P$$



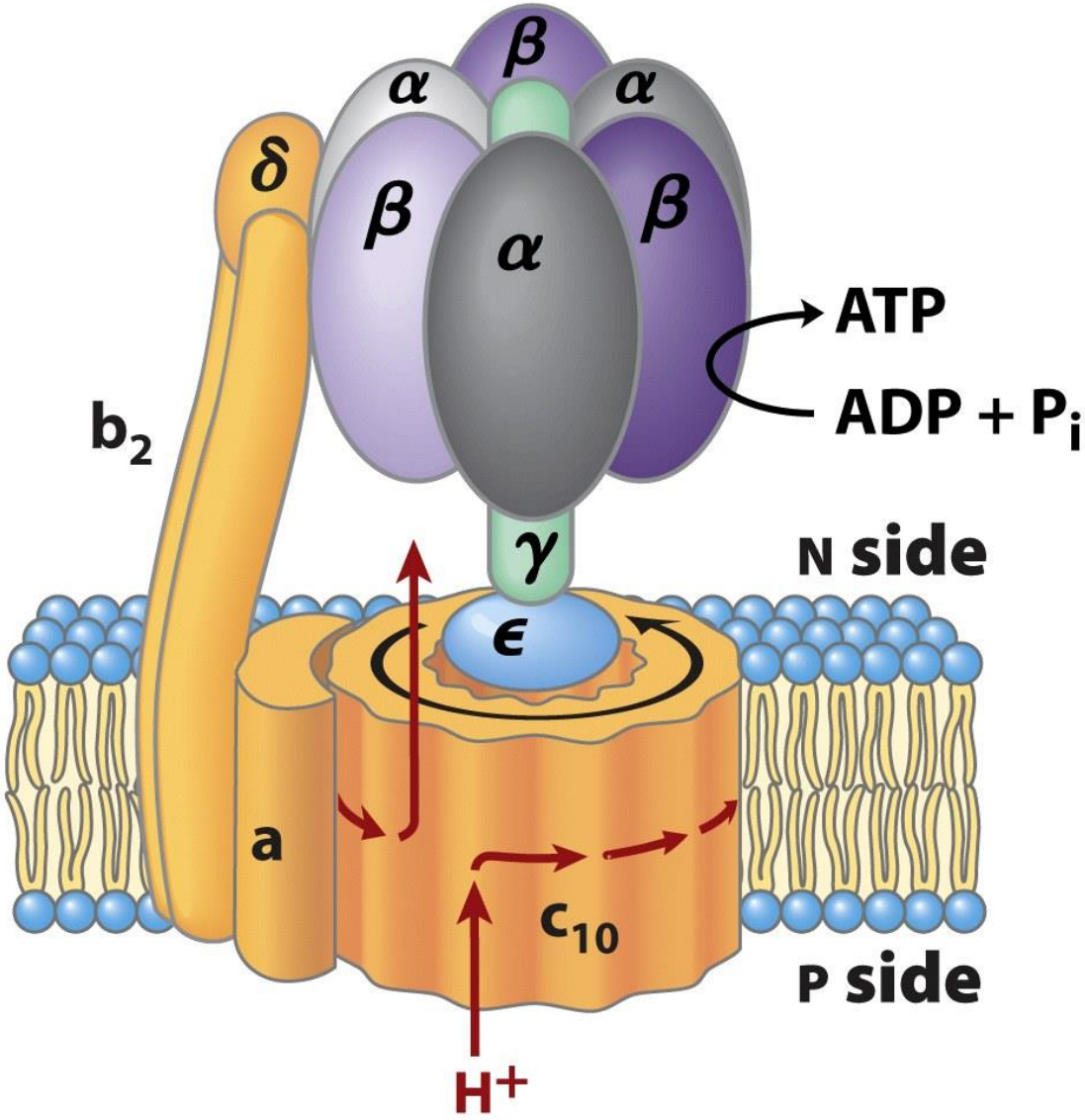
Transformation of proton gradient into ATP

The electrochemical energy due to the H^+ concentration gradient and charge separation drives the ATP synthesis *(coupled mitochondrion).*

$$ADP + Pi + nH^+_p \longrightarrow ATP + H_2O + nH^+_n$$



Structure of ATP synthase



1 turnaround

translocation of 10 protons

synthesis of 3 ATP

Figure 19-25f
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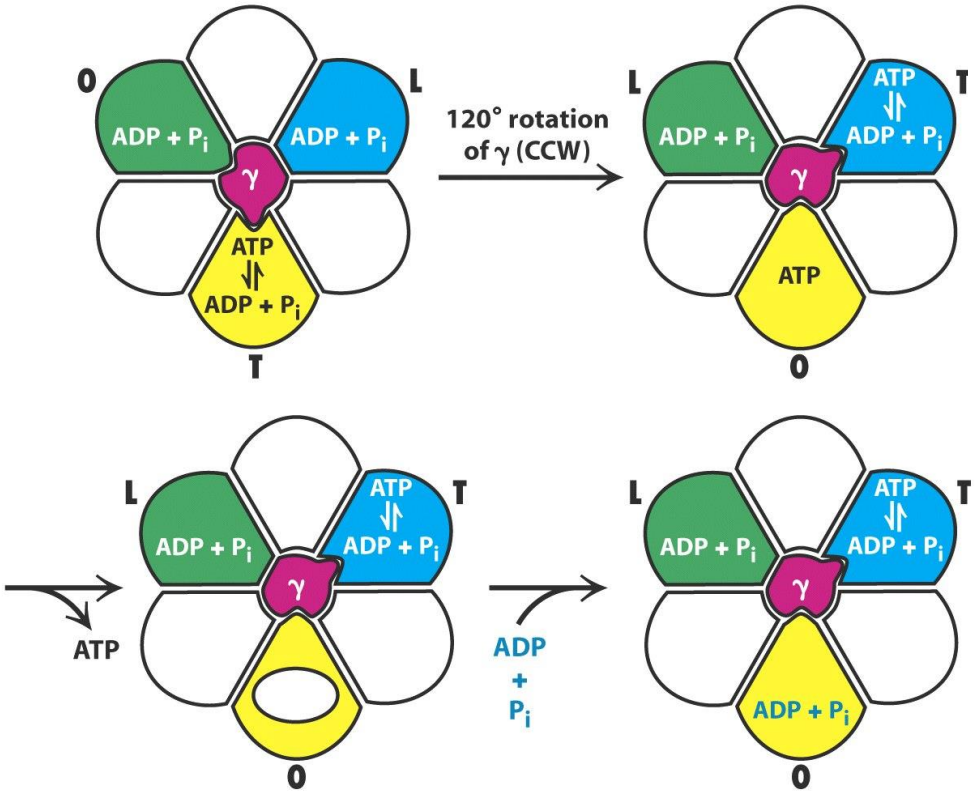
Cyclic conformation changes of the nucleotide binding sites on the F_1 subunit of ATP synthase



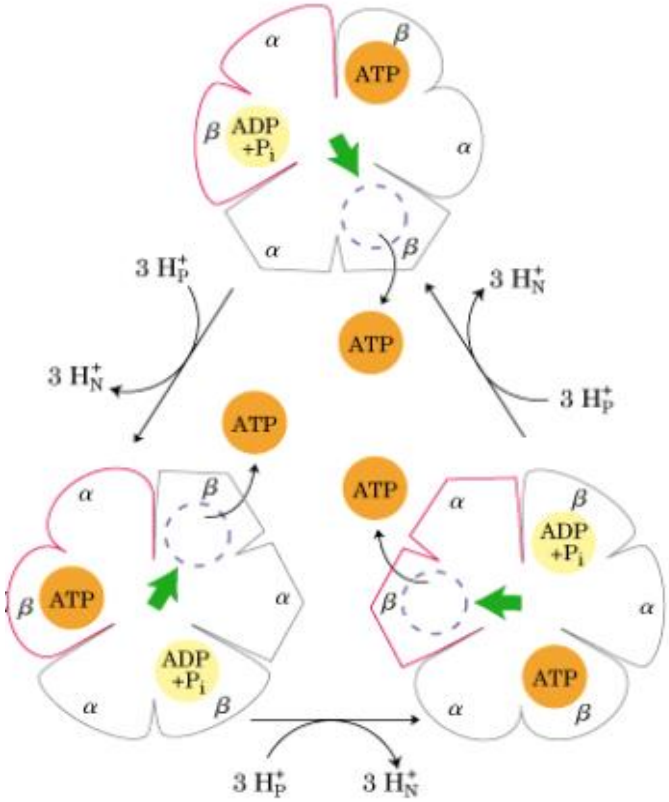


Figure 18-29
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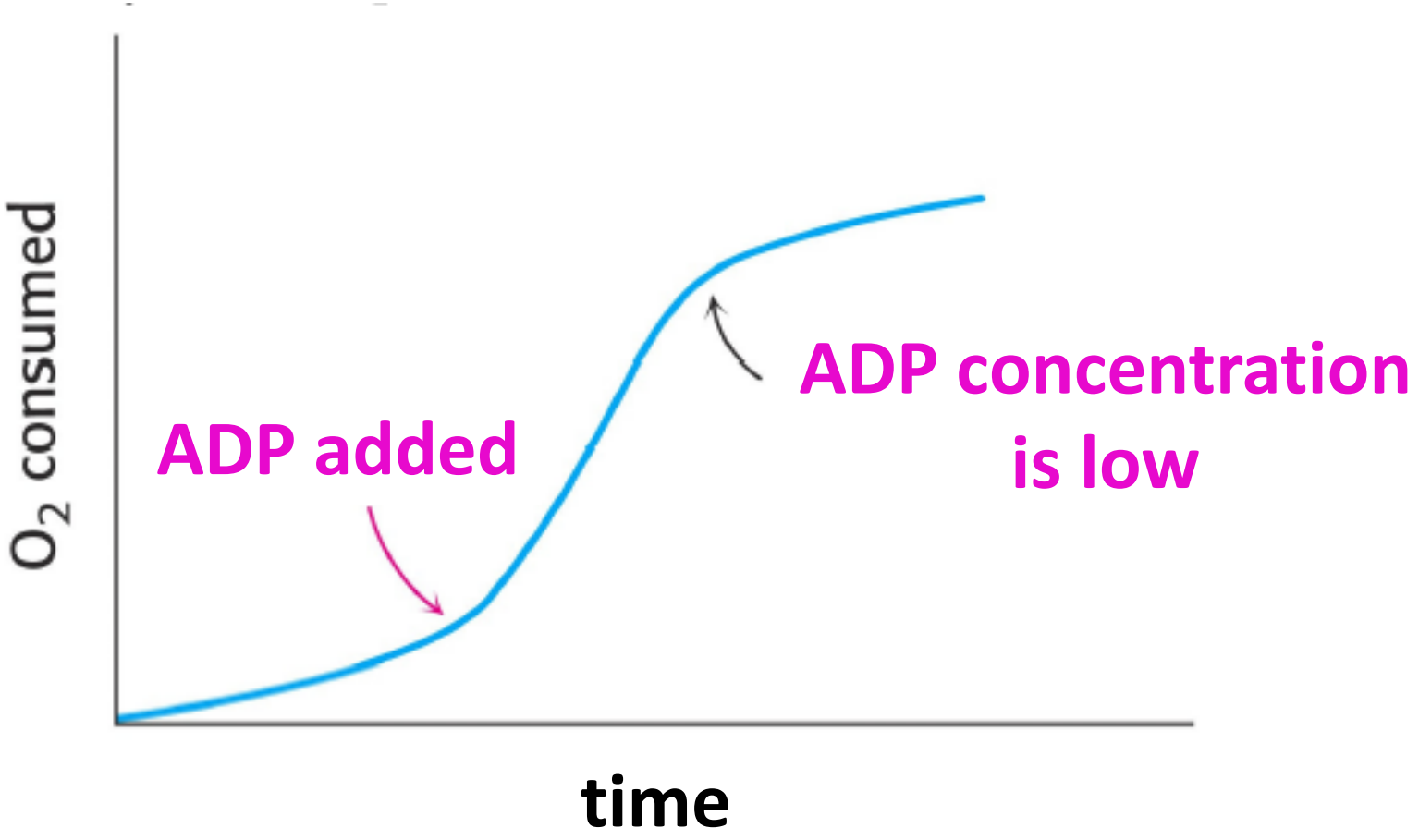
ADP regulates the electrontransport chain



The P/O ratio

- The ATP/O ratio (how many ATP will be produced by the oxidation of NADH or succinate ($FADH_2$)) was thought to be 3 ATP / NADH and 2 ATP / $FADH_2$ (outdated!!!).

- Today we have experimentally determined results, which show ~**2.5** ATP / NADH and ~**1.5** ATP / $FADH_2$.

ATP synthesis is coupled with the redoxreactions of the respiratory chain (electrontransfer).
In uncoupled mitochondrium, oxidation of NADH or succinate (without oxidative phophorylation [ATP synthesis]) leads to heat production.
e.g.: **thermogenin** (physiologic uncoupling protein).

Adenine nucletide and phosphate translocases

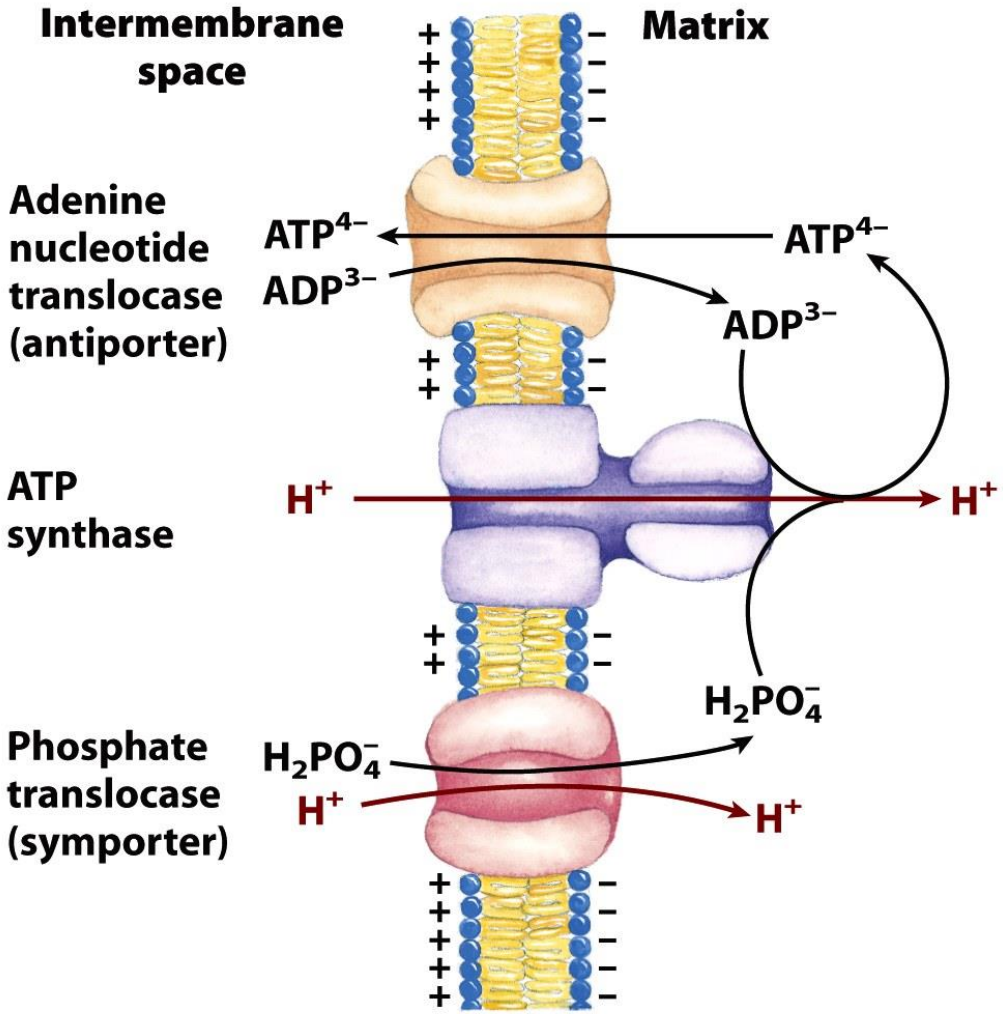


Figure 19-28
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Summary I.

- Oxidative phosphorylation occurs in the inner membrane of eukaryotic mitochondrion. Protonpumps of the respiratory chain and the F_OF_1 ATP synthase work in this process together, they are coupled.

- 4 complexes of the respiratory chain (many subunits and redoxcenters) transfer electrons from reduced coenzymes to the terminal electron acceptor O_2, which will be reduced to water.

- In this process, protongradient arises between the outer and inner sides of the membrane. Protons reenter the matrix through the F_OF_1 ATP synthase and drive ATP synthesis.

- Protongradient arose by the oxidation of NADH, produces ~2,5 mol ATP, in the case of $FADH_2$ ~1,5 mol ATP. This is the P/O-ratio.

Summary II.

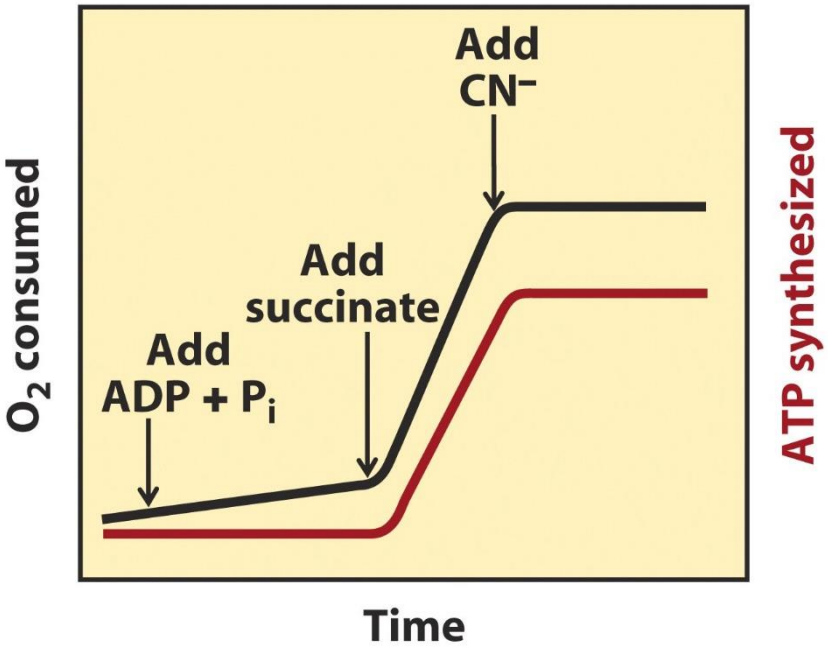
- Oxidation and phosphorylation can be uncoupled! The uncoupling protein thermogenin induces heat production instead of ATP synthesis.

- Oxidative phosphorylation is regulated by ADP level.

- Communication between cytosol and mitochondrium is fullfilled by several transporters located in the inner membrane.

Isolated mitochondria + ADP + Pi + substrate (succinate) + buffer

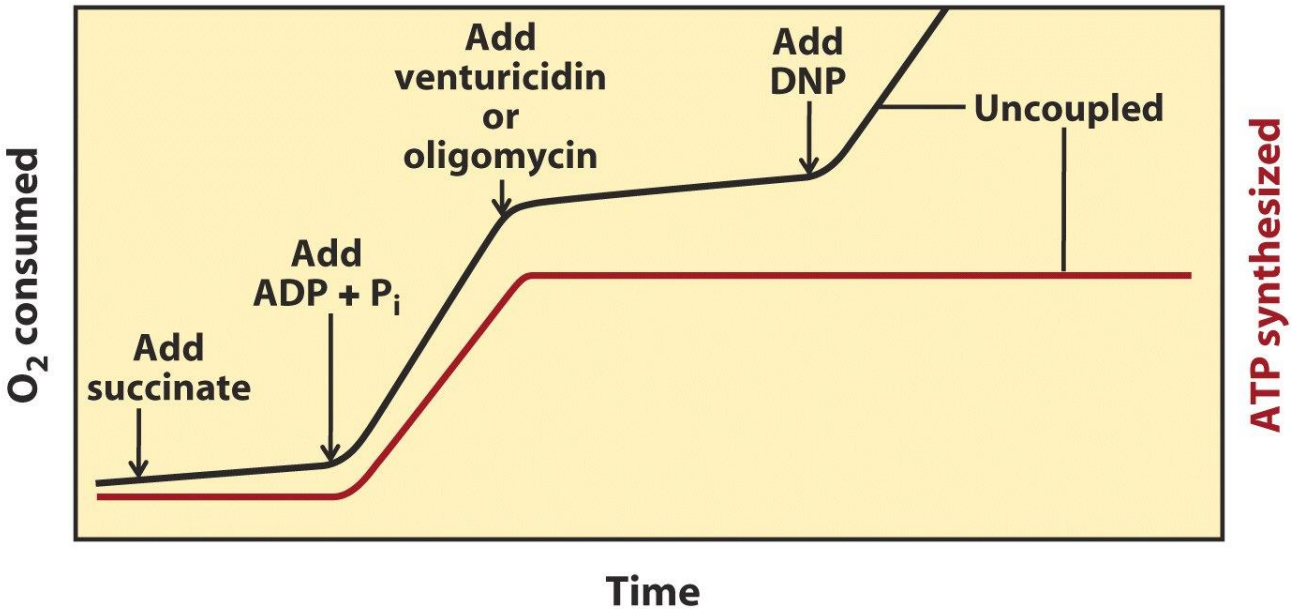
- Substrate (succinate) is oxidized
- O_2 is consumed
- ATP is synthesized



O_2 consumption in coupled mitochondria can be inhibited by CIII and CIV inhibitors:

Cyanid, CO, Antimycin A

Isolated mitochondria + ADP + Pi + substrate (succinate) + buffer + inhibitors



- **In intact (coupled) mitochondria the inhibition of ATP synthesis *(Fo-F1)* blocks electron transfer.**
  Inhibitors: Venturicidin, Oligomycin, Aurovertin
- **Uncoupling of oxidation and phosphorylation can also be demonstrated using chemical compounds. Respiration increases, but no ATP is produced.**
  Uncoupling with chemicals: 2,4 Dinitrophenol (DNP), FCCP