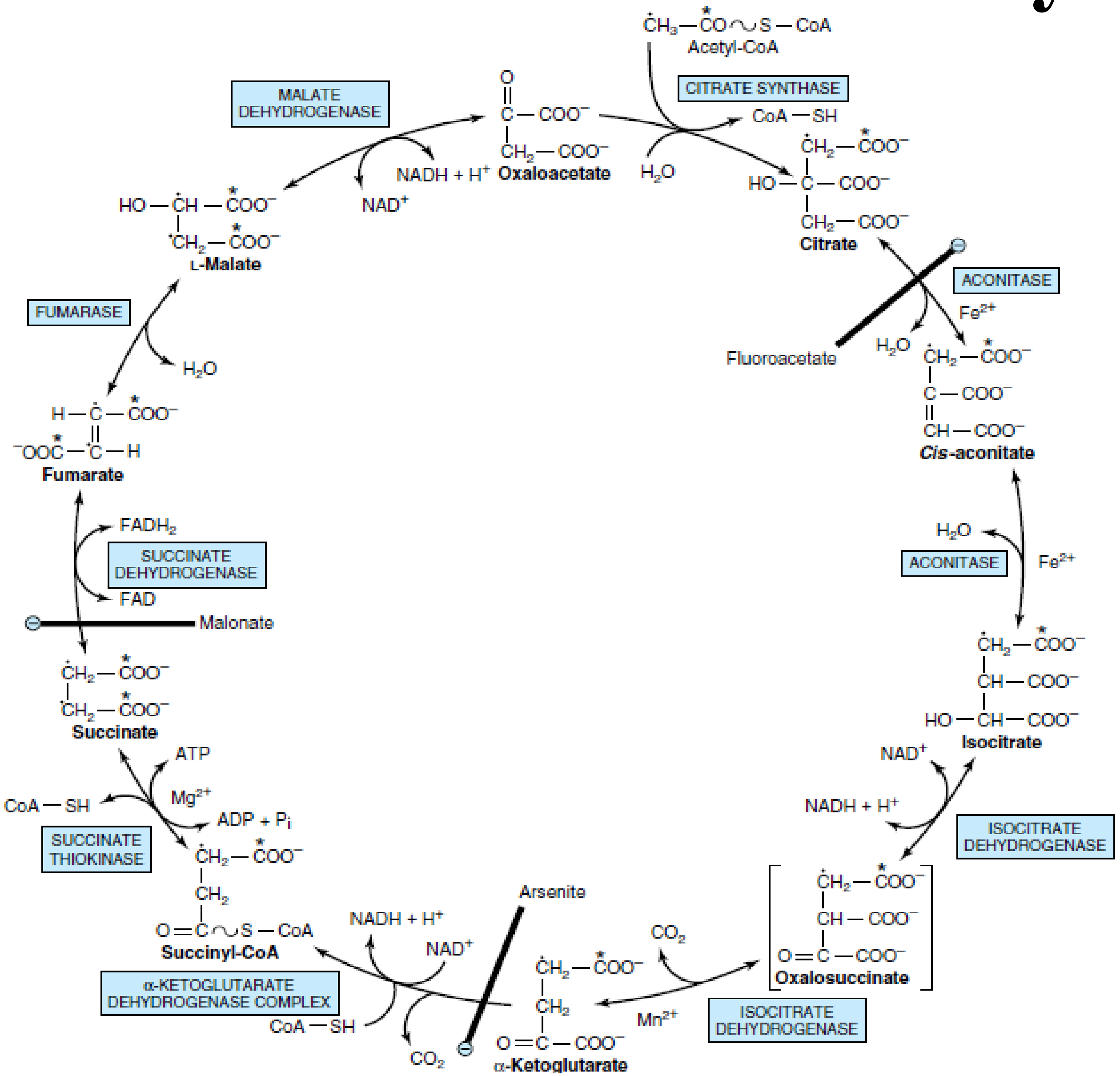
Citric acid cycle, terminal oxidation, oxidative phosphorylation

The significance of citric acid cycle

- common phase of oxidation of nutrients
- center of material and energy turnover
- it operates as a distribution system in cell metabolism: its function changes according to the current energy conditions
- acetyl-CoA which formed through the various pathways is oxidized here and CO_2 is generated also
- NADH and FADH2 which generated during the cycle turns on the terminal oxidation
- catabolism of carbohydrates, fatty acids and amino acids turns on the cycle, but some intermediates of the cyle represent starting compounds of different biosyntheses also

Process of the citric acid cycle



Regulation of citric acid cycle

1. *Citrate synthase*: oxaloacetate $\longrightarrow$ citrate
   – allosteric inhibition: succinyl-CoA, ATP and NADH
   – allosteric activation: ADP

2. *Isocitrate dehydrogenase*: isocitrate $\longrightarrow$ α-ketoglutarate
   – allosteric inhibition: ATP and NADH
   – allosteric activation: ADP

3. *α-ketoglutarate dehydrogenase*: α-ketoglutarate $\longrightarrow$ succinyl-CoA
   – inhibition: ATP, NADH, GTP, succinyl-CoA

Anaplerotic reactions of the cycle

- citric acid cycle intermediates will rise and fall in concentration depending upon the needs of the cell
- it is essential for the cell to replenish citric cycle intermediates that are takenaway for metabolic synthesis reactions

1. *pyruvate carboxylase*: liver, kidneys (coenzyme: biotin)

   pyruvate + HCO_3^- + ATP ↔ oxaloacetate + ADP + P_i

2. *phosphoenolpyruvate carboxylase*: heart, skeletal muscle

   phosphoenolpyruvate + CO_2 + GDP ↔ oxaloacetate + GTP

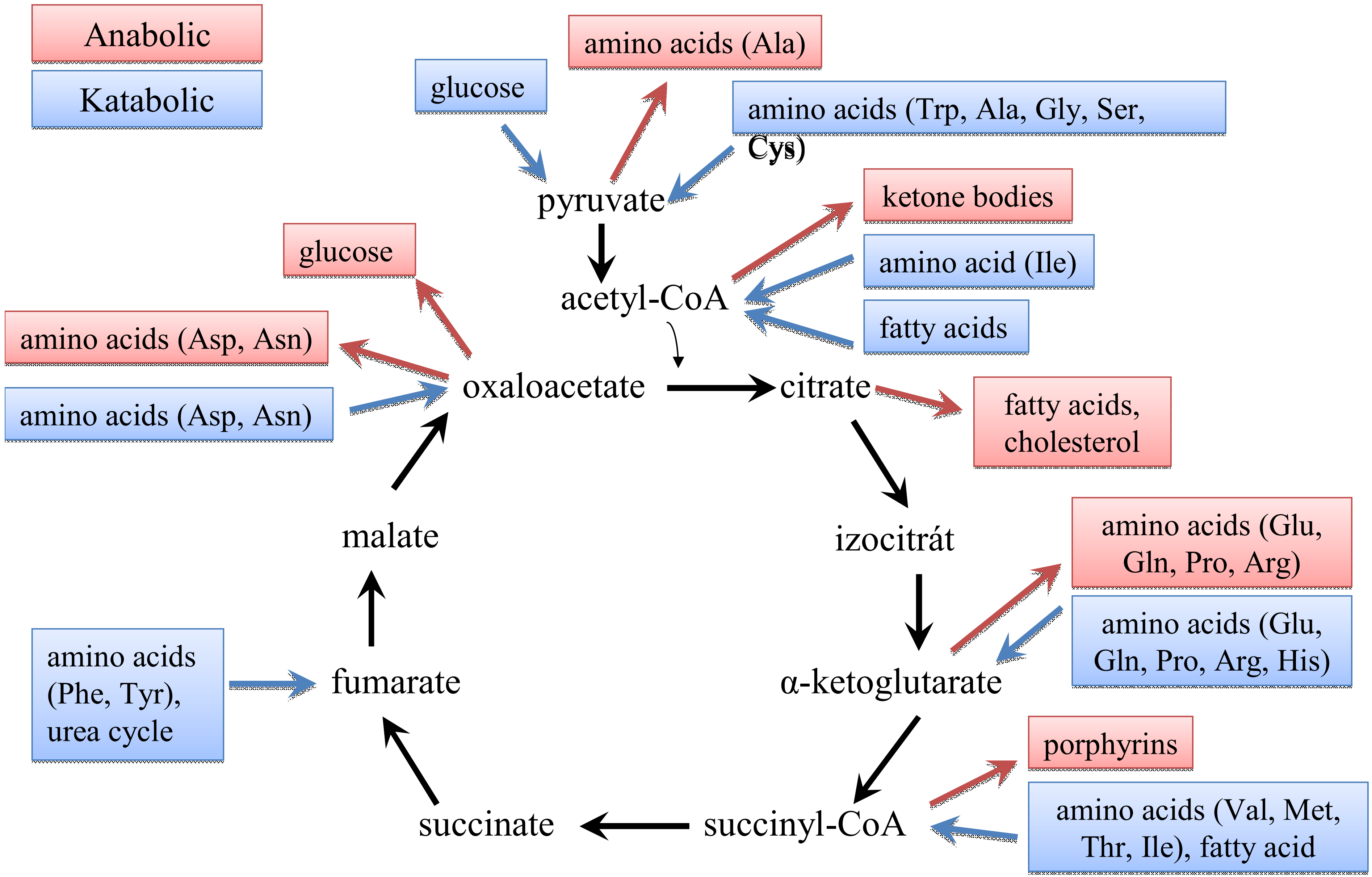
3. *malic enzyme:*

   pyruvate + HCO_3^- + NAD(P)H ↔ malate + $NAD(P)^+$

4. *glutamate dehydrogenase:*

   glutamate + $NAD(P)^+$ ↔ α-ketoglutarate + NAD(P)H + H^+ + NH_4^+

Connection with other metabolic pathways



Energy production of citric acid cycle

- one GDP is phosphorylated to GTP during one cycle
- two CO_2 are generated and four pairs of hydrogen get on the electron careers
- two water molecules are necessary in one cycle

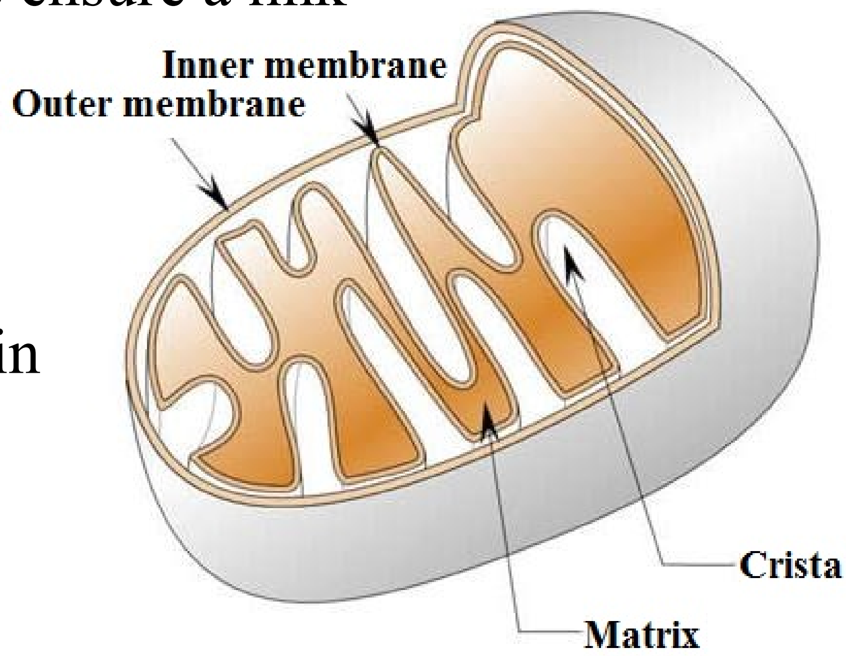
$$\text{acetyl-CoA} + 3NAD^+ + FAD + GDP + P_i + 2H_20 \longrightarrow 2C0_2 + 3NADH + 3H^+ + FADH_2 + GTP + CoA$$

Terminal oxidation

- mitochondria contains the respiratory chain: take place in inner membrane
- the last step in the catabolism: hydrogen is oxidized to water
- interconnected process in space and time with oxidative phosphorylation
- flow of electrons is associated with release of energy, which is devoted to the synthesis of ATP
- 95% of energy which released during the biological oxidation is due to the terminal oxidation

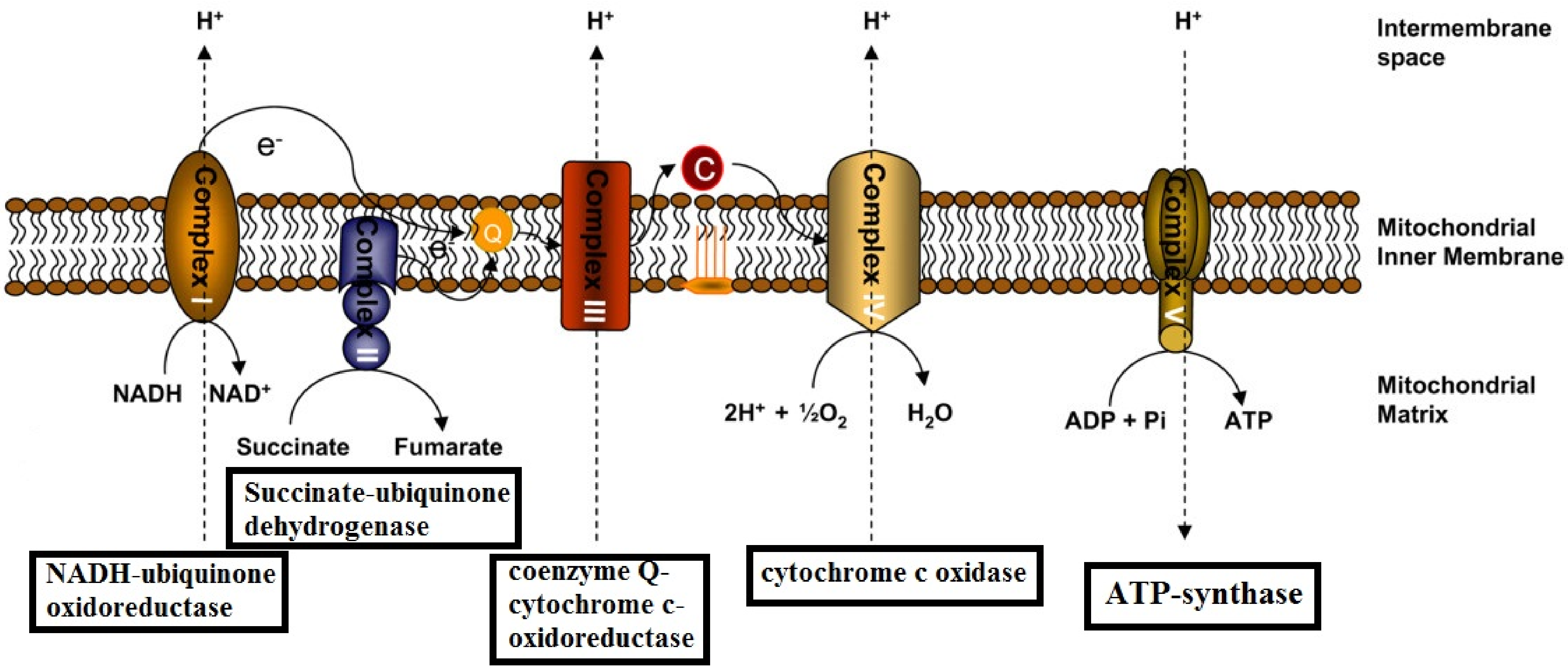
The structure of mitochondria

- bounded by a double membrane:
  - outer membrane: 50% lipid 50% protein, porin: permeable to the metabolic intermediates
  - inner membrane: 75% protein, impermeable to all ions almost, transporters ensure a link between the matrix and the cytosol, the respiratory chain electron transfer system and ATP synthase are found here in



The mitochondrial respiratory chain

- four membrane-bound complexes have been identified in mitochondria + ATP-synthase



Respiratory chain complexes

Complex I:

- it contains a FMN prosthetic group. It accepts electrons from NADH and it translocates four protons across the membrane, thus producing a proton gradient; proton pump activation

Complex II:

- additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via FAD) to Q

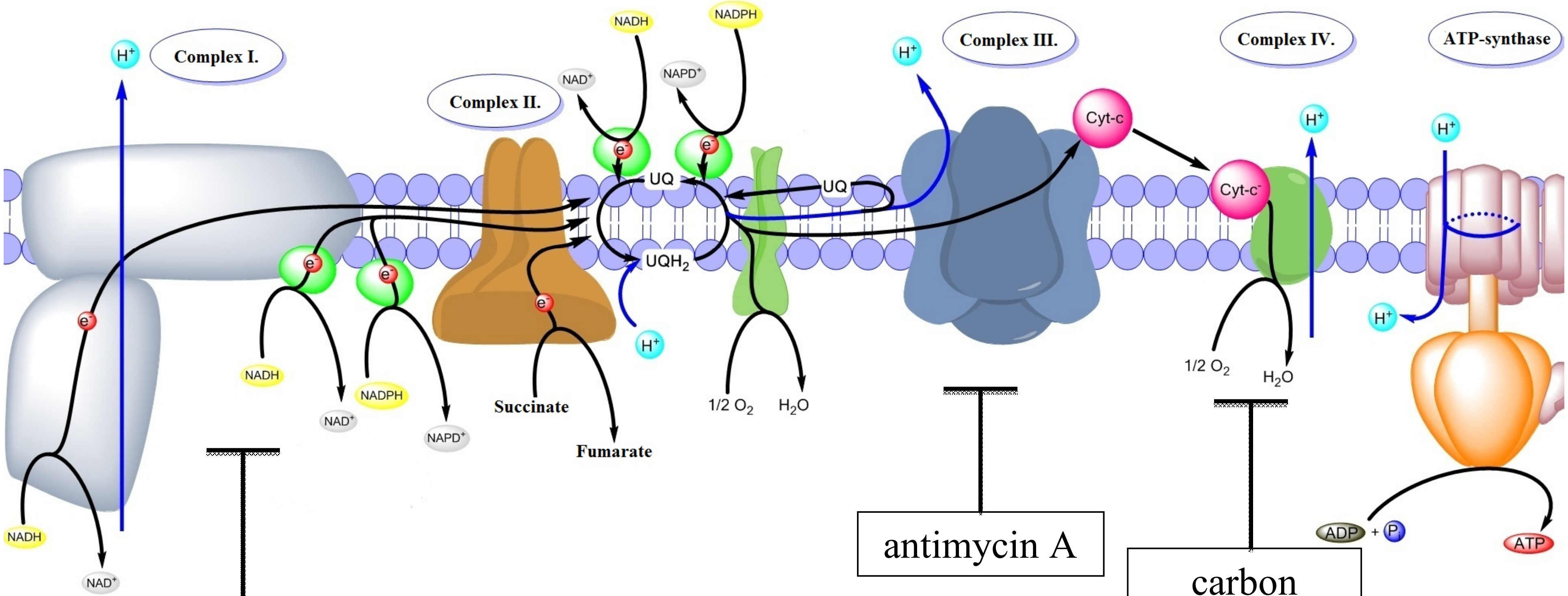
Complex III:

- transfers the electrons from CoQH2 to cytochrome c which is substrate for Complex IV; proton pump activation

Complex IV:

- four electrons are removed from four molecules of cytochrome c and transferred to molecular oxygen, producing two molecules of water; proton pump activation

Inhibiton of the respiratory chain



rotenone
amobarbital
(Amytal®)

antimycin A

carbon
monoxide
azides
cyanide, NO

Oxidative phosphorylation

- the purpose of the terminal oxidation: energy service with burning of hydrogen
- this energy is stored in ATP during parallel running oxidative phosphorylation
- feature of OF: P / O ratio: the number of ATPs produced per pair of electrons traveling through the electron transport system

$$P/O = \frac{\text{number of built-in inorganic phosphate}}{\text{number of consumed oxygen}}$$

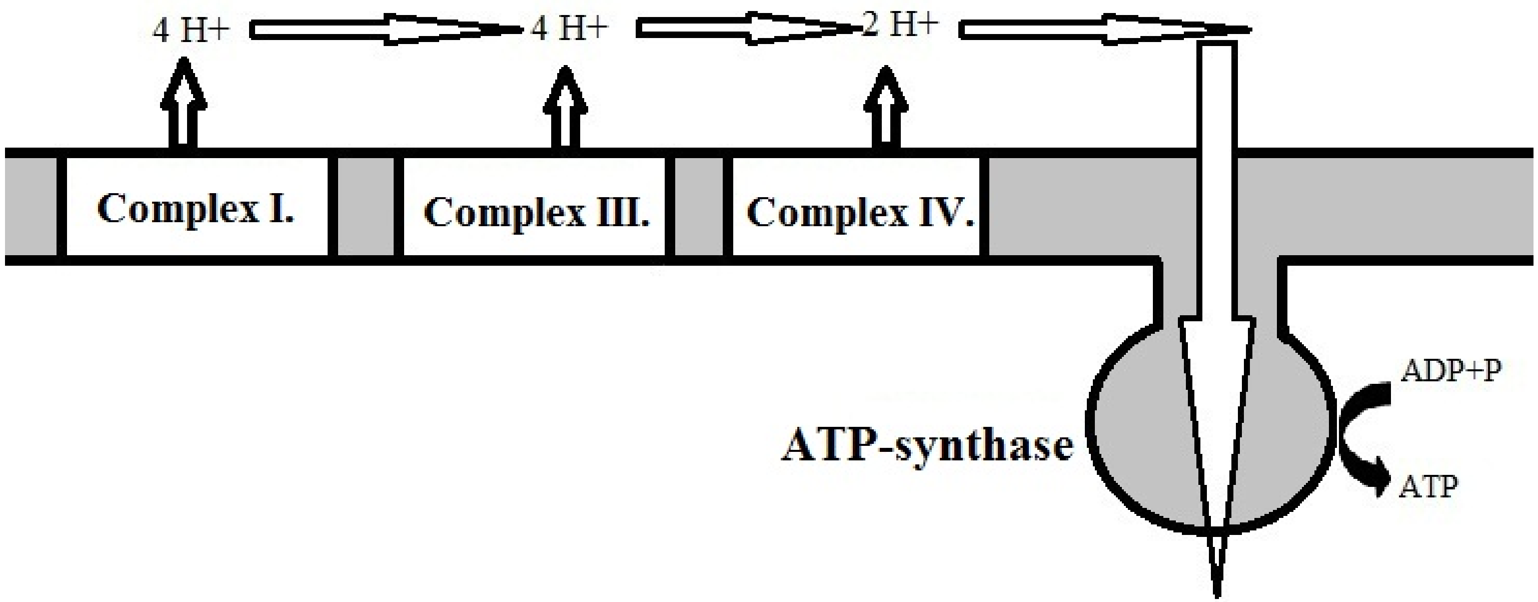
oxidation of NADH: 3
oxidation of $FADH_2$: 2

Structure and regulation of ATP-synthase

- universal enzyme, it consists of two parts: F_1 and F_0
  - F_1: it converts a molecule of ADP and P_i into a molecule of ATP
  - F_0: proton channel
- the rate of oxidative phosphorylation depends primarily on the concentration of ADP $\longrightarrow$ acceptor control
- oxidative phosphorylation works with high intensity in the presence of ADP
- ADP level determines not only the rate of oxidative phosphorylation, but also the rate of terminal oxidation

Mitchell's chemiosmotic theory

- the energy from oxidation of components in the respiratory chain is coupled to the translocation of hydrogen ions from the inside to the outside of the inner mitochondrial membrane
- the electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP



Inhibitors of ATP-synthase

- *2,4-dinitrophenol:* uncoupler $\longrightarrow$ it incorporates into the membrane and stops the gradient $\longrightarrow$ turn off the functioning of ATP-synthase

- *oligomycin*: inhibition of F_0-subunit (proton channel)

- *thermogenin:* uncoupling protein found in brown adipose tissue which decrease the proton gradient $\longrightarrow$ important role in the regulation of thermogenesis