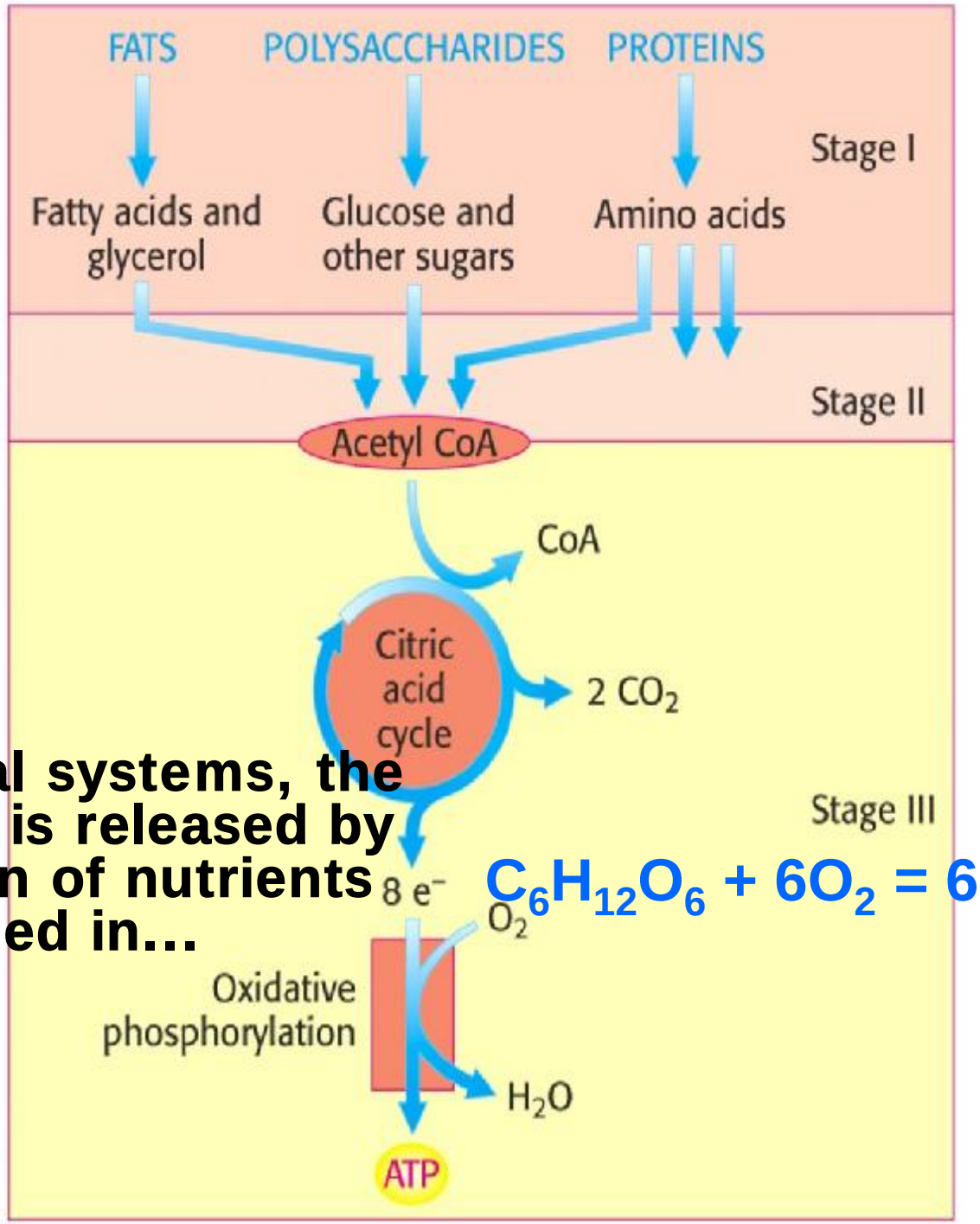
SB21

Regulation of Respiratory Chain



- **in biological systems, the energy that is released by the oxidation of nutrients is transformed in…**

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

- **Nutrients are first digested in the intestines from larger molecules into smaller**
- **Further decomposition (at the cellular level) is to C2-units (acetyl = CH_3COO-)**
- **Acetyl fragments are ultimately oxidized to CO_2 and H_2O**
- **One part of oxidation energy transits into heat and the second part is stored in the form of ATP**

Electron transport and oxidative phosphorylation

Complete oxidation of glucose by molecular oxygen can be described as:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Can be broken down into two half-reactions with the transfer of electrons:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O$$

Electrons from the oxidation of glucose are not transferred directly to O_2

go to NAD^+ and FAD

to form NADH and $FADH_2$

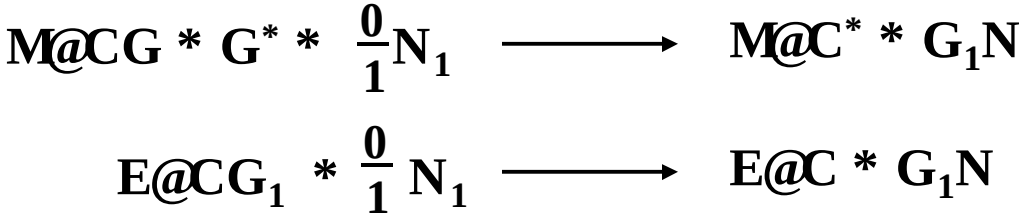
NADH and $FADH_2$ are reoxidized,
passing their electrons
to the electron-transport chain
to reduce O_2 to H_2O
causing the mitochondrion to create a proton gradient.

This pH gradient is used to drive
the synthesis of ATP via oxidative phosphorylation.

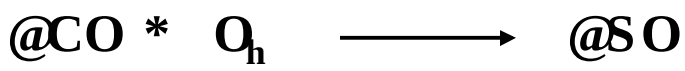
Oxidative Phosphorylation

Oxidative phosphorylation is the process by which the energy stored in NADH and $FADH_2$ is used to produce ATP

A. Oxidation step: electron transport chain



B. Phosphorylation step



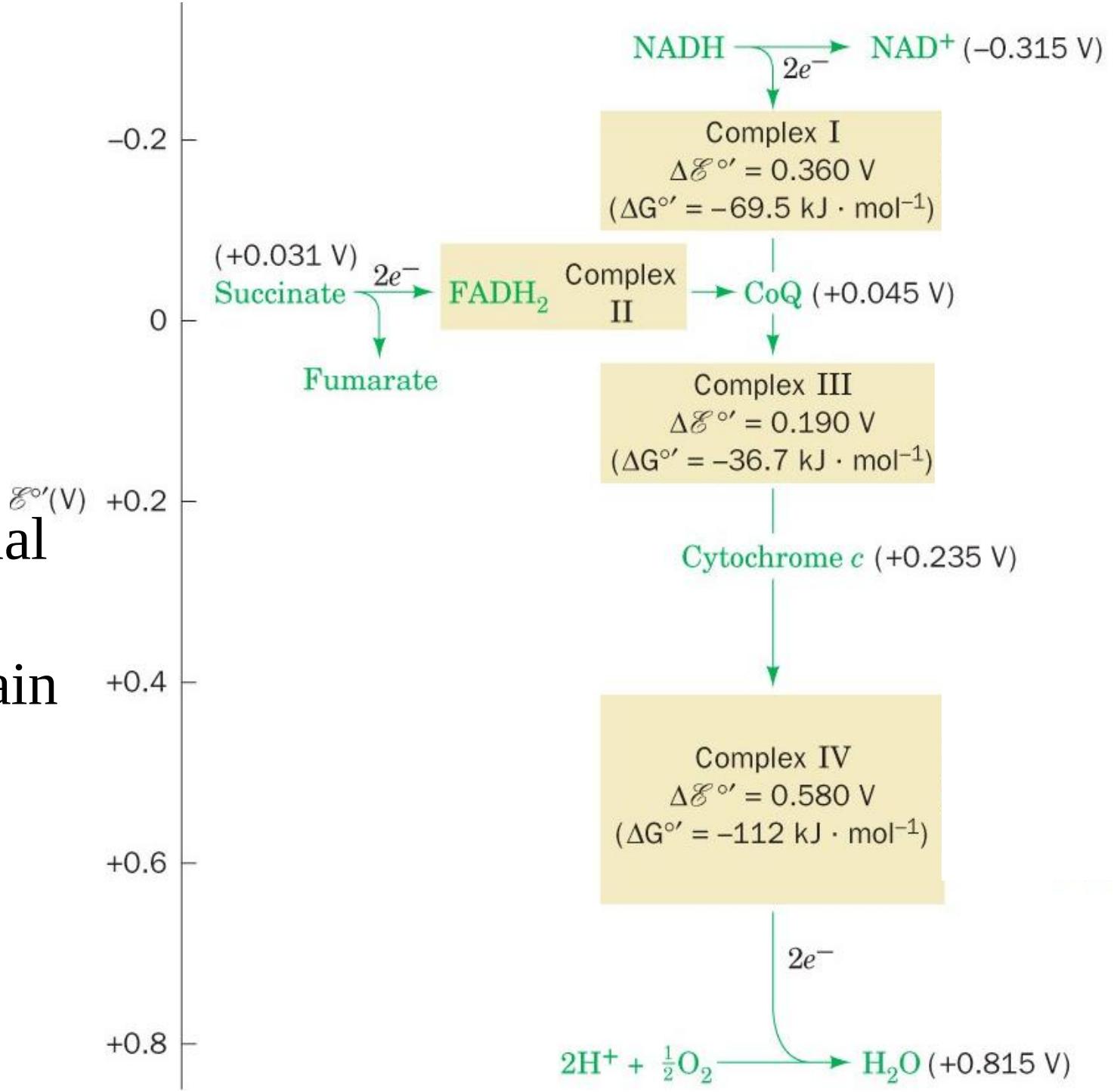
Important characteristic of the electron-transport chain

- Electron carriers are arranged in order of more negative E^o to less negative E^o
- This results in the spontaneous flow of electrons from carrier to carrier

Standard potentials in biological systems

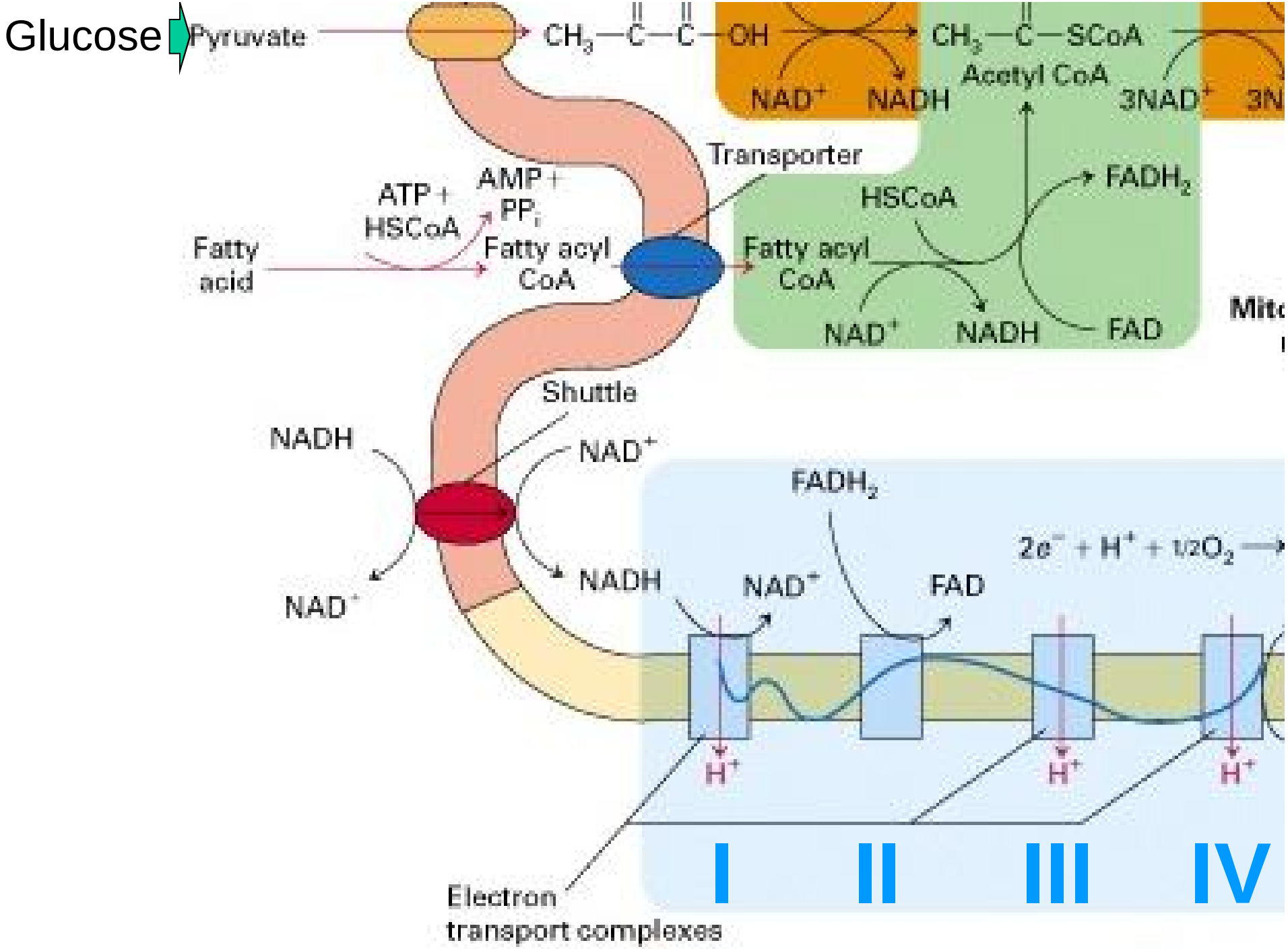
Oxidized form	Reduced form	z	E^o (V)
NAD^+	$NADH + H^+$	2	-0,32
FAD	$FADH_2$	2	-0,22
fumarat	sukcinat	2	0,03
citokrom b; Fe^{3+}	**citokrom b; Fe^{2+}**	**1**	**0,08**
ubikvinon (oks)	**ubikvinol (red)**	**2**	**0,10**
citokrom c_1; Fe^{3+}	**citokrom c_1; Fe^{2+}**	**1**	**0,22**
citokrom a; Fe^{3+}	**citokrom a; Fe^{2+}**	**1**	**0,77**
½ $O_2 + 2H^+$	H_2O	2	0,82

The mitochondrial electron-transport chain

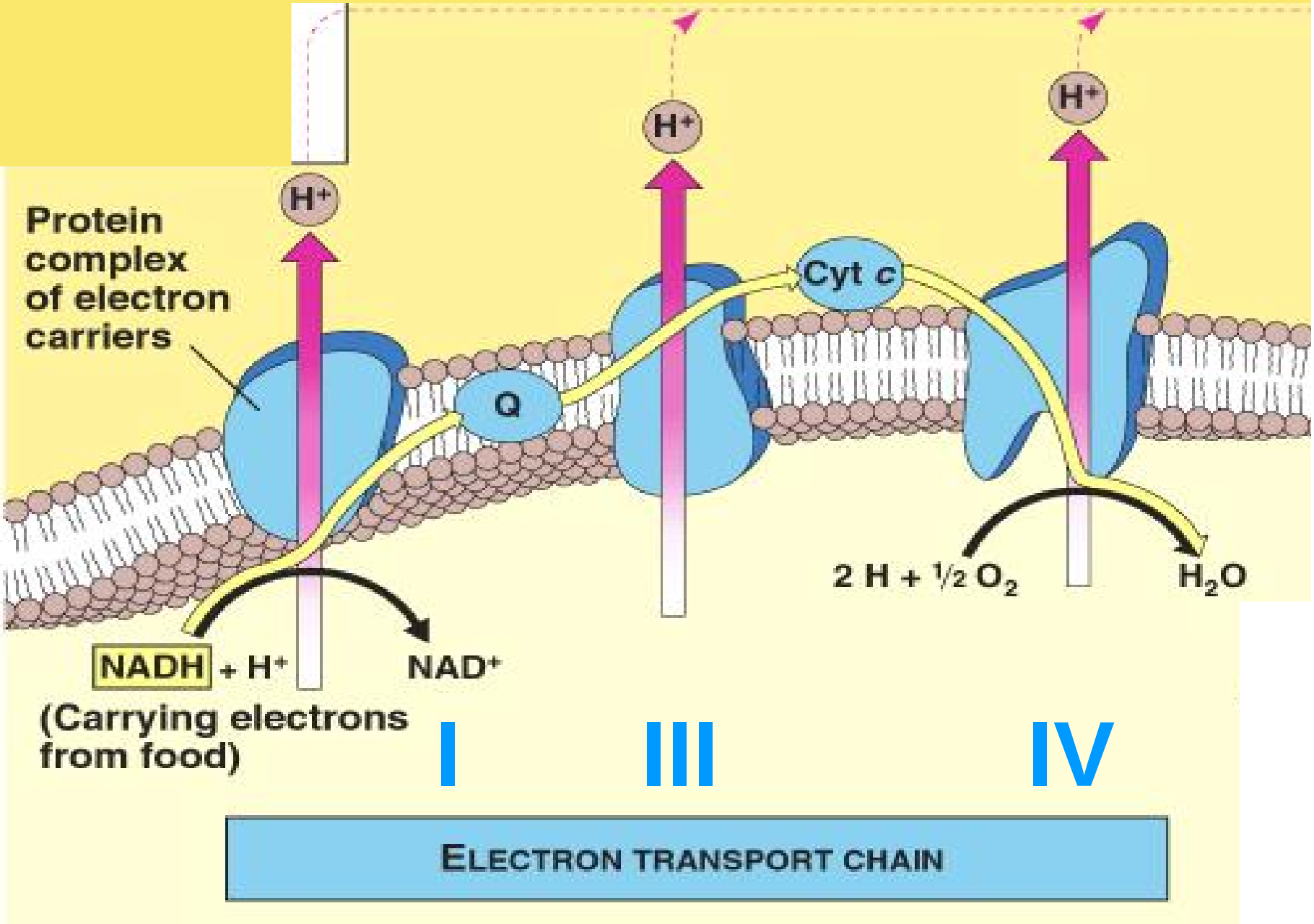


Composition of the Electron Transport Chain

- **Four large protein complexes.**
  - **Complex I**
  - **Complex II**
  - **Complex III**
  - **Complex IV**
- **Many of the components are proteins with prosthetic groups to move electrons.**

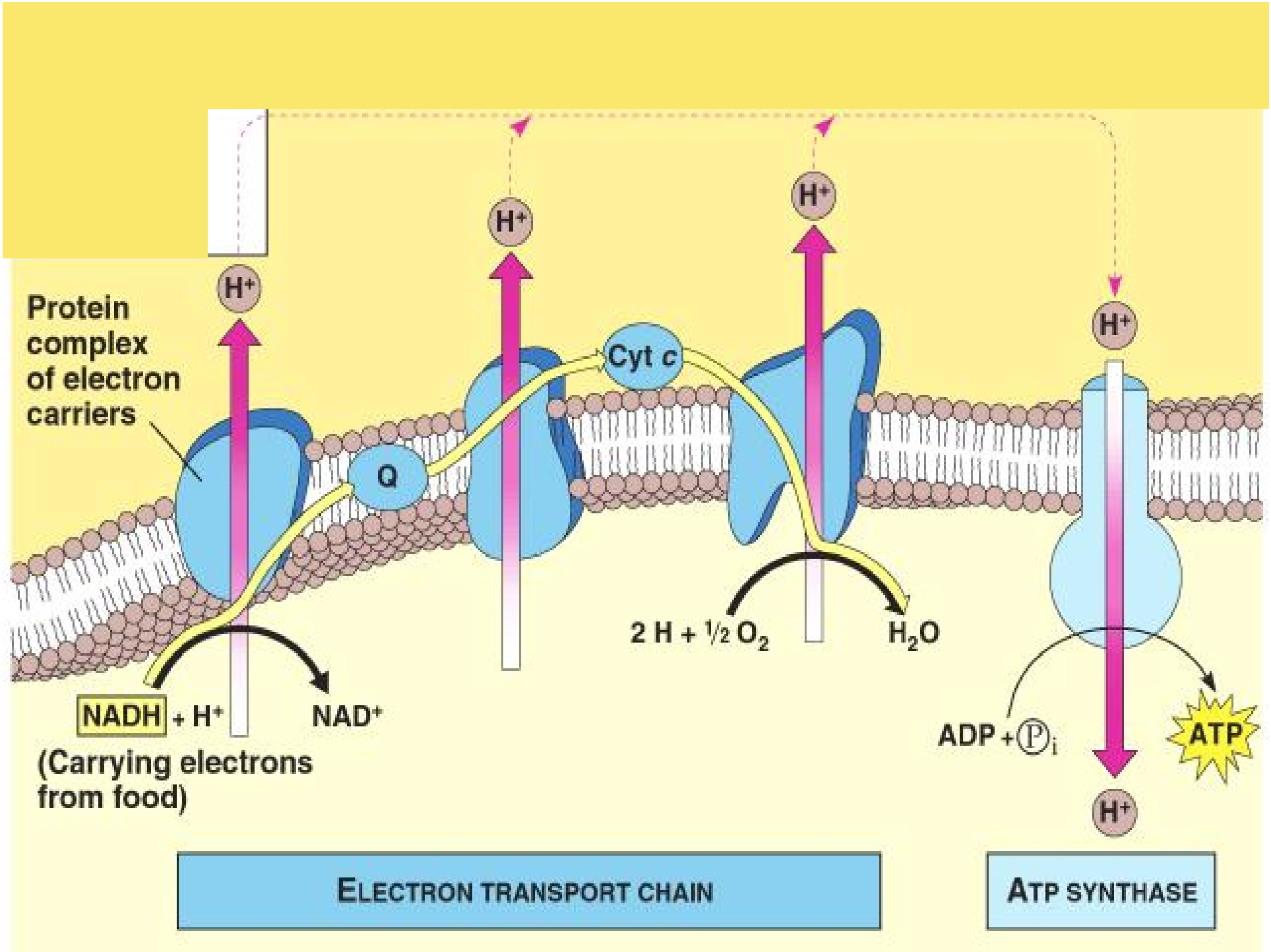

Glucose
Pyruvate
$CH_3-C(=O)-C(=O)-OH$
NAD+
NADH
$CH_3-C(=O)-SCoA$
Acetyl CoA
3NAD+
3N
Transporter
ATP + HSCoA
AMP + PP_i
Fatty acid
Fatty acyl CoA
HSCoA
$FADH_2$
Fatty acyl CoA
NAD+
NADH
FAD
Mito
Shuttle
NADH
NAD+
NAD+
NADH
$FADH_2$
$2e^- + H^+ + 1/2O_2$
NAD+
FAD
H^+
H^+
H^+
I
II
III
IV
Electron transport complexes

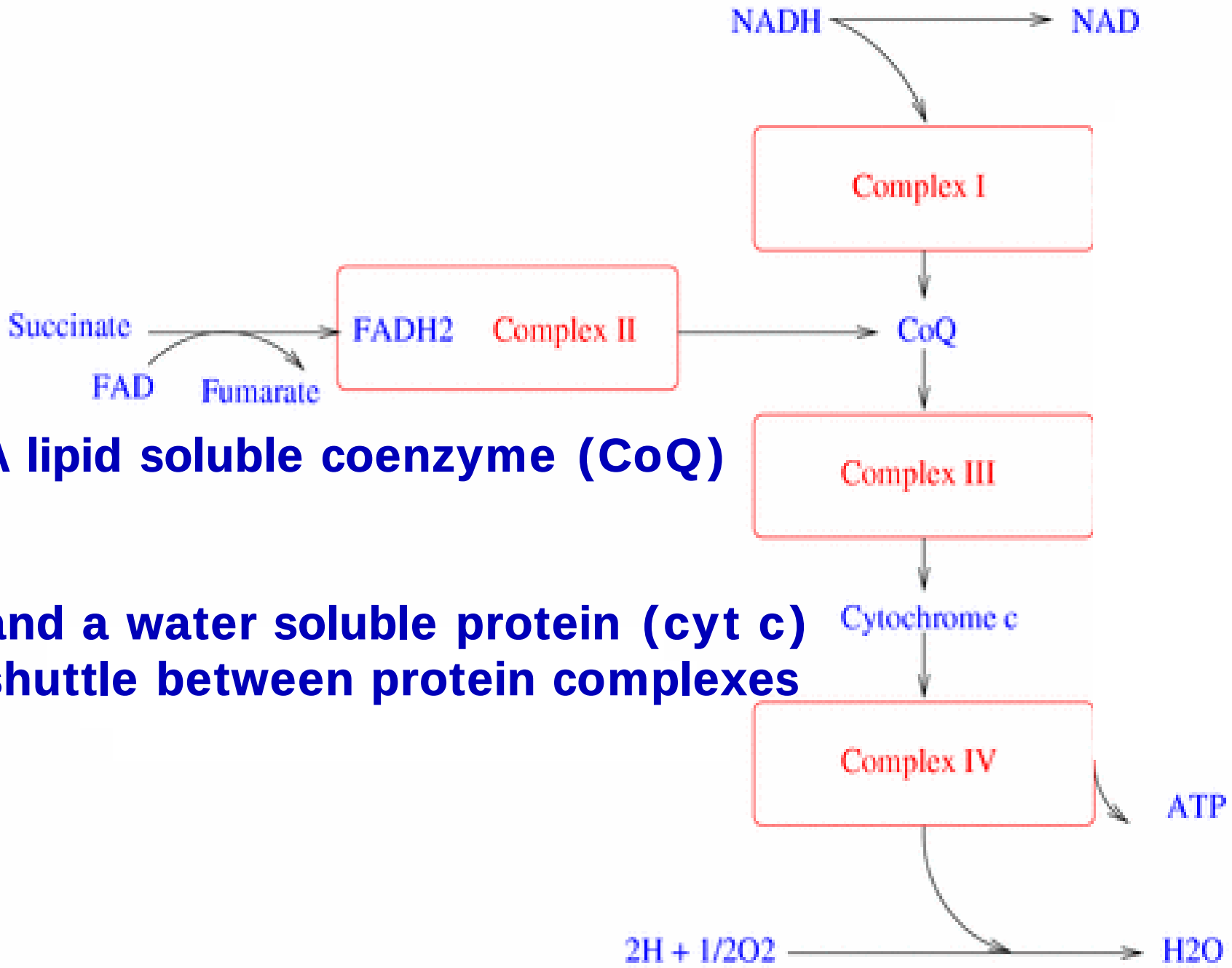
During electron transport, energy released is used to transport H^+ across the inner mitochondrial membrane to create an electrochemical gradient



Chemiosmotic Theory

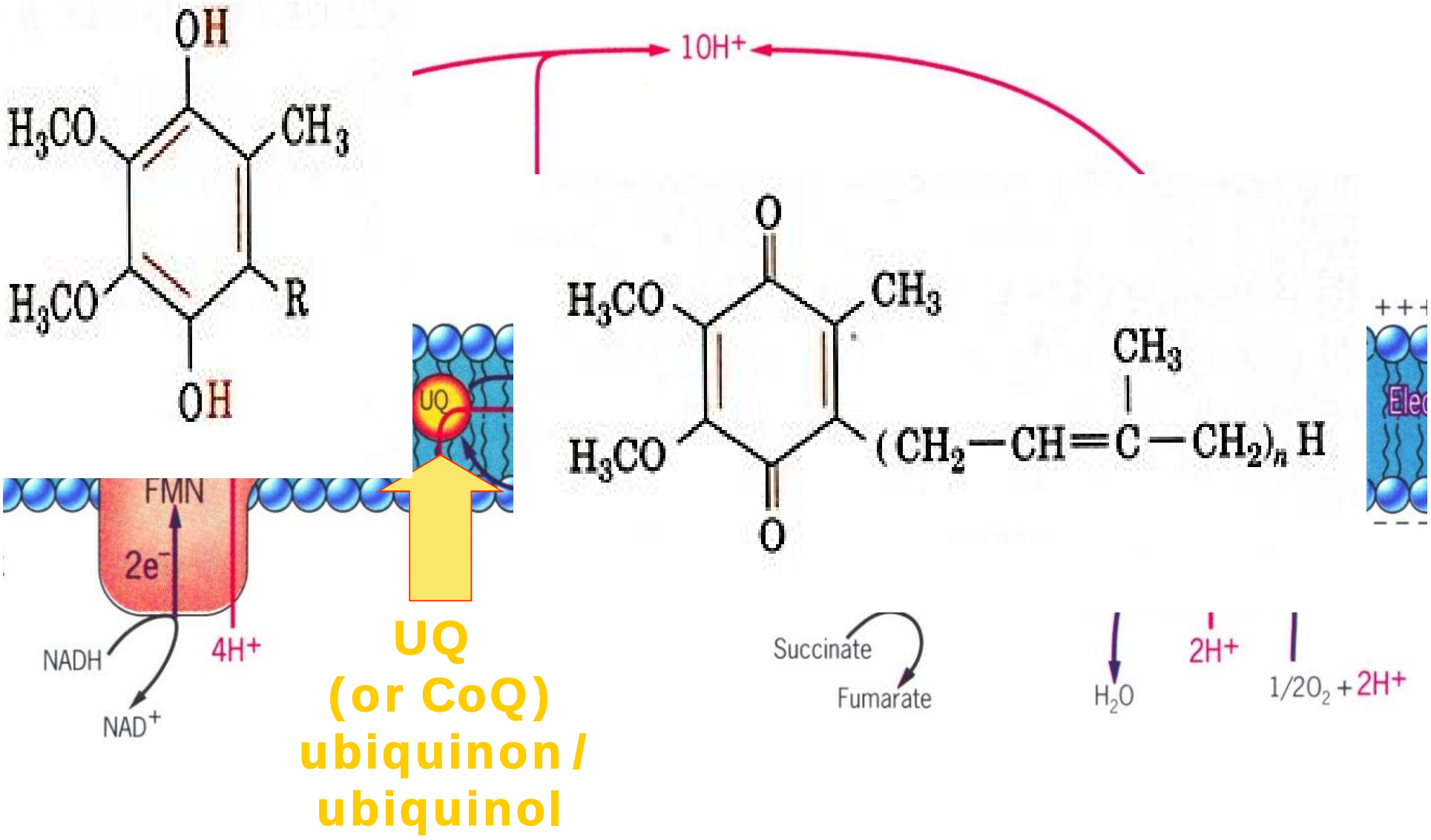
- **Electron Transport**: Electrons carried by reduced coenzymes are passed through a chain of proteins and coenzymes to drive the generation of a proton gradient across the inner mitochondrial membrane
- **Oxidative Phosphorylation**: The proton gradient runs downhill to drive the synthesis of ATP
- **Electron transport is coupled with oxidative phosphorylation**


Protein complex of electron carriers
H+
H+
H+
H+
Cyt c
Q
2 H + ½ O2
H2O
NADH + H+
NAD+
(Carrying electrons from food)
ADP + Pi
ATP
H+
ELECTRON TRANSPORT CHAIN
ATP SYNTHASE



A lipid soluble coenzyme (CoQ)

and a water soluble protein (cyt c) shuttle between protein complexes



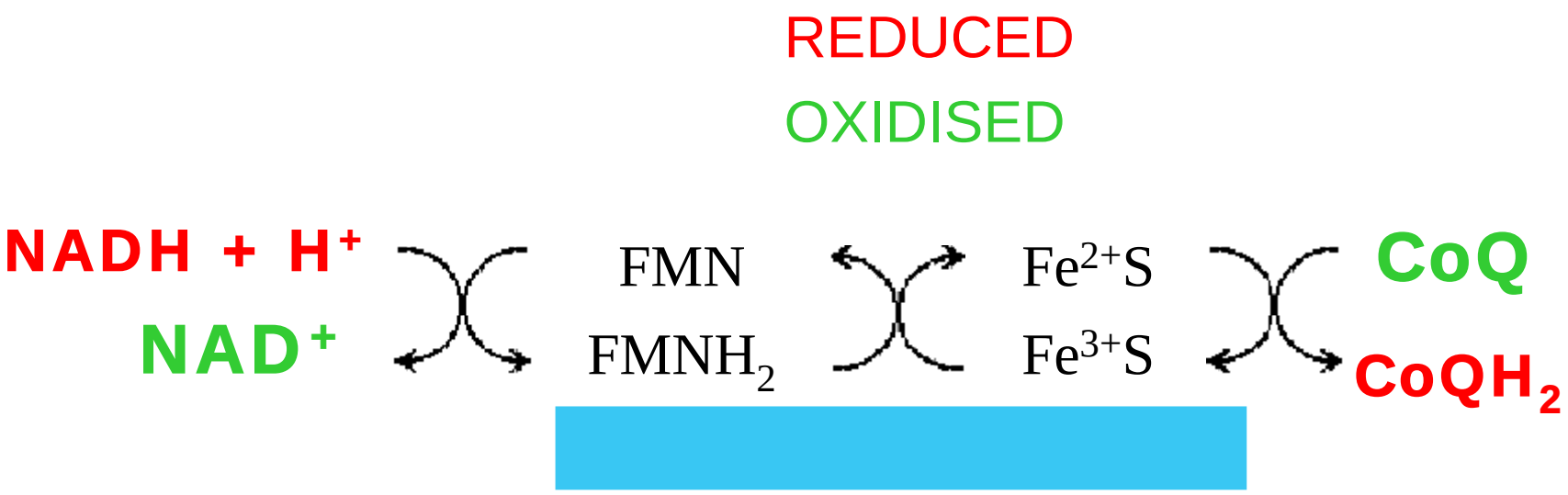
UQ (or CoQ) ubiquinon / ubiquinol

Complex I

- Electron transfer from NADH to CoQ

$NADH + H^+ + CoQ \rightarrow NAD^+ + CoQH_2$

- ENTRANCE: reactants EXIT: products



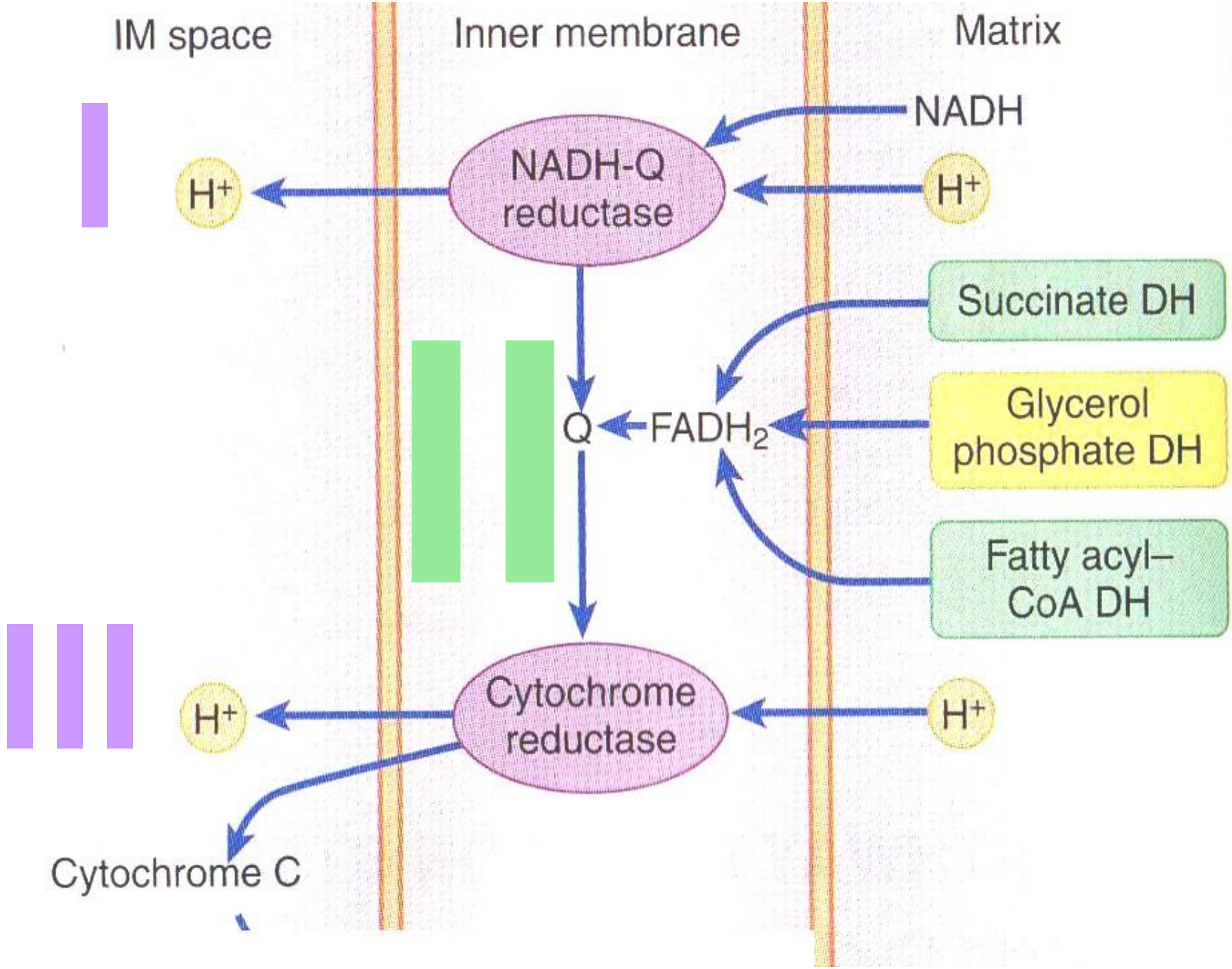
- **Complex II**

- **Entry point for $FADH_2$**

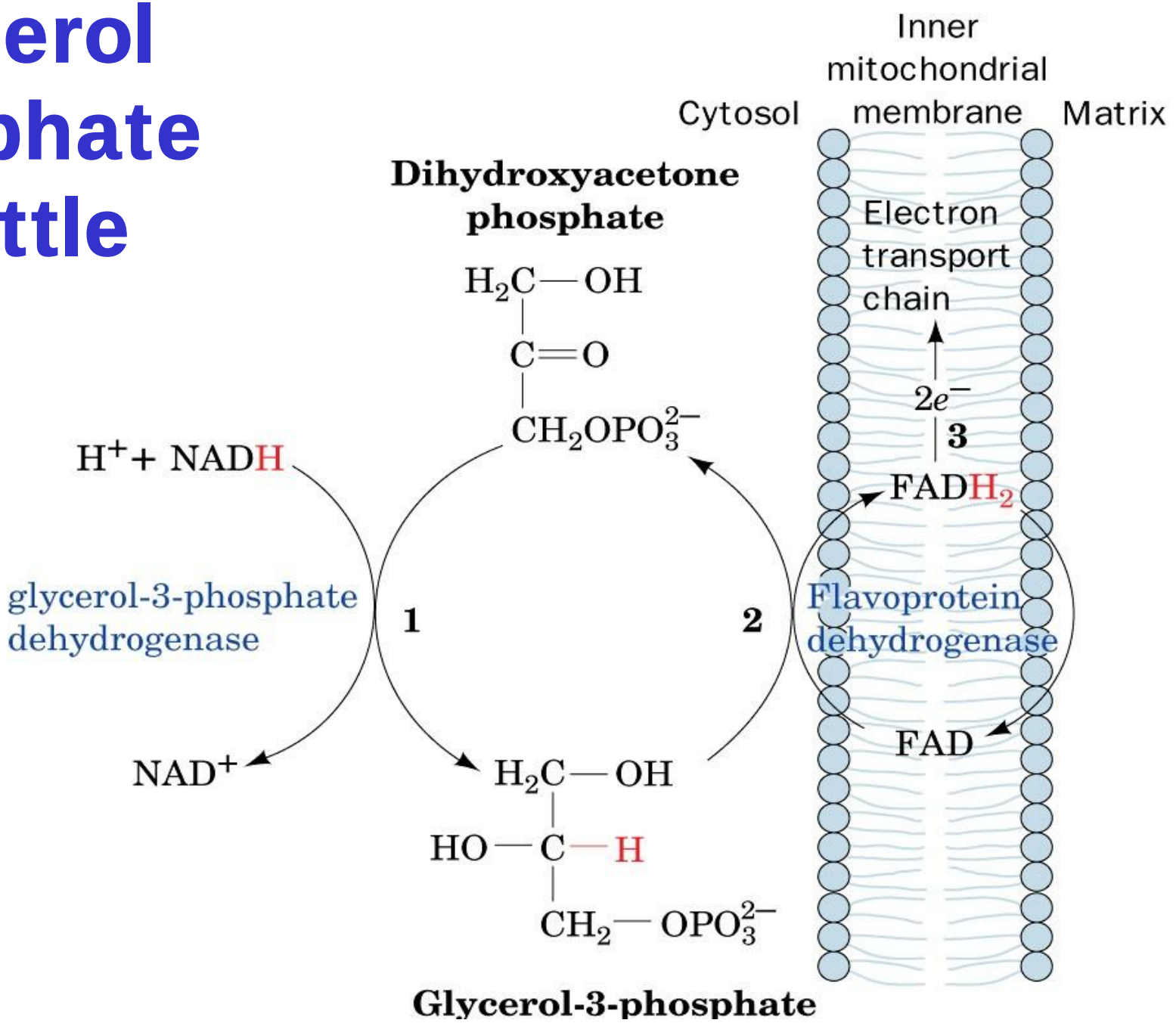
- **Succinate dehydrogenase** (from the citric acid cycle) directs transfer of electrons from succinate to CoQ via $FADH_2$

- **Acyl-CoA dehydrogenase** (from beta-oxidation of fatty acids) also transfers electrons to CoQ via $FADH_2$

- **Glycerol-phosphate dehydrogenase**

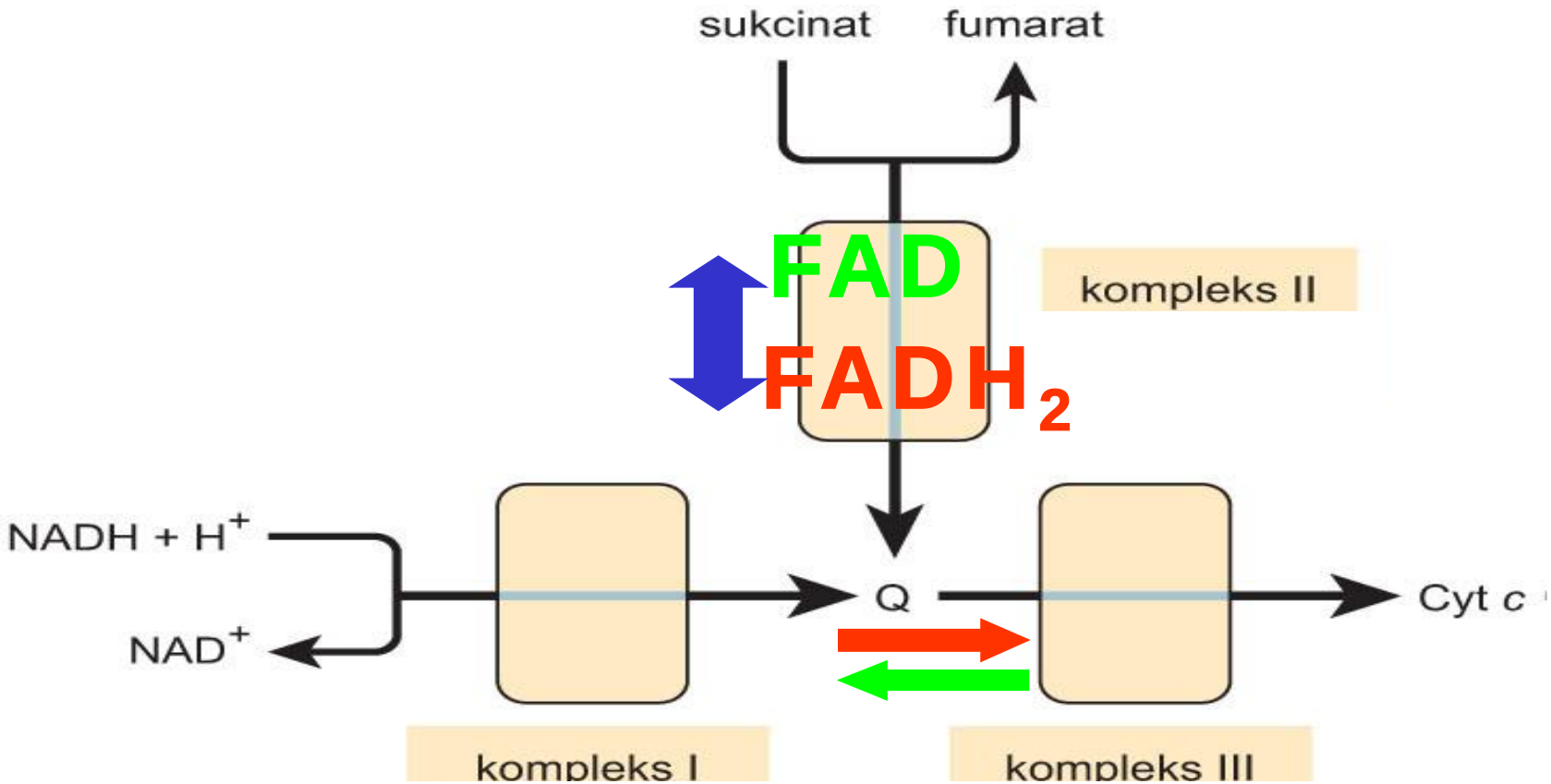

IM space
Inner membrane
Matrix
NADH
NADH-Q reductase
H+
H+
Succinate DH
Glycerol phosphate DH
Fatty acyl–CoA DH
Q
FADH2
Cytochrome reductase
H+
H+
Cytochrome C

Glycerol phosphate shuttle



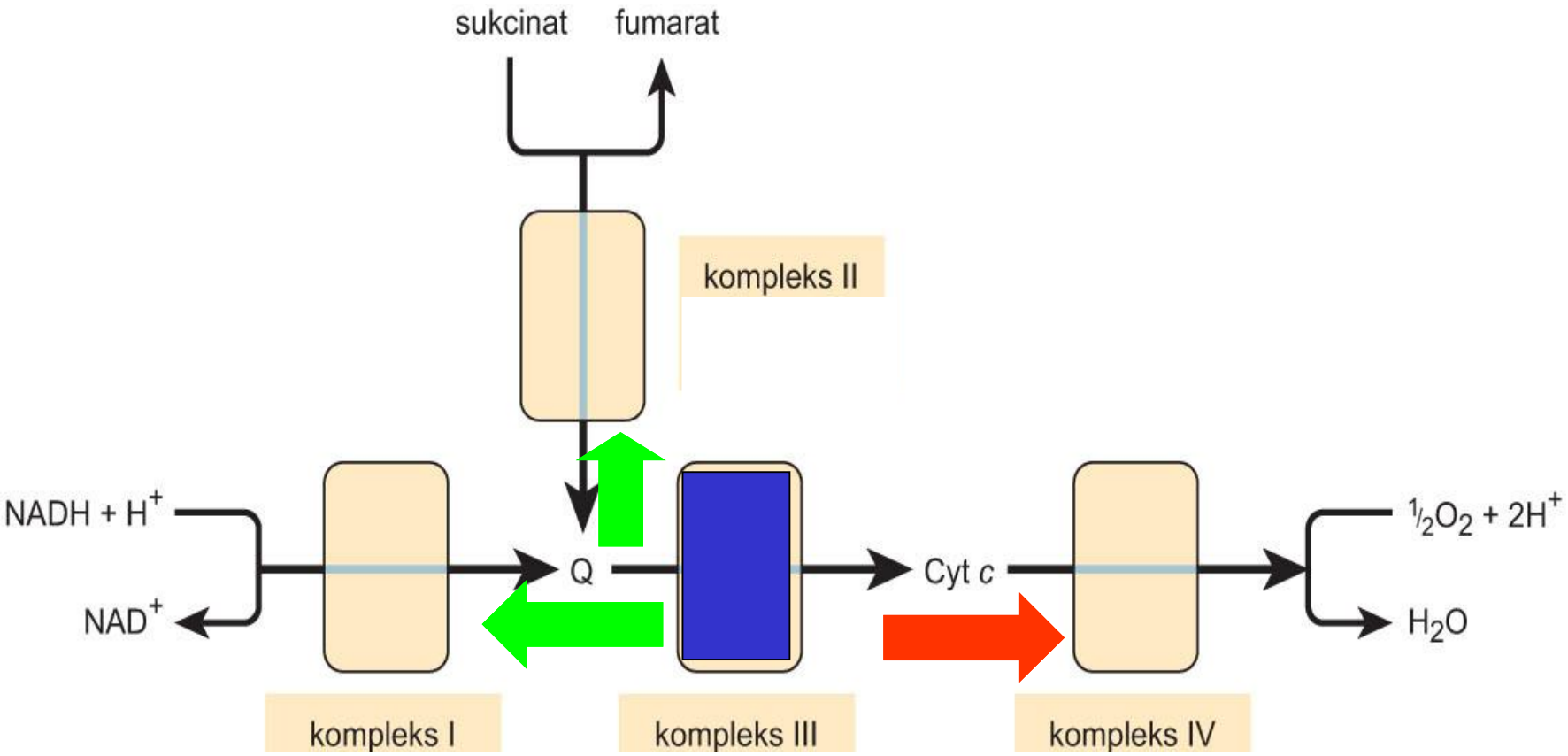
Complex II

$$FADH_2 + CoQ \rightarrow FAD + CoQH_2$$



- **Complex III**

$CoQH_2 + 2\ cyt\ c_{ox} \rightarrow CoQ + 2\ cyt\ c_{red}$.



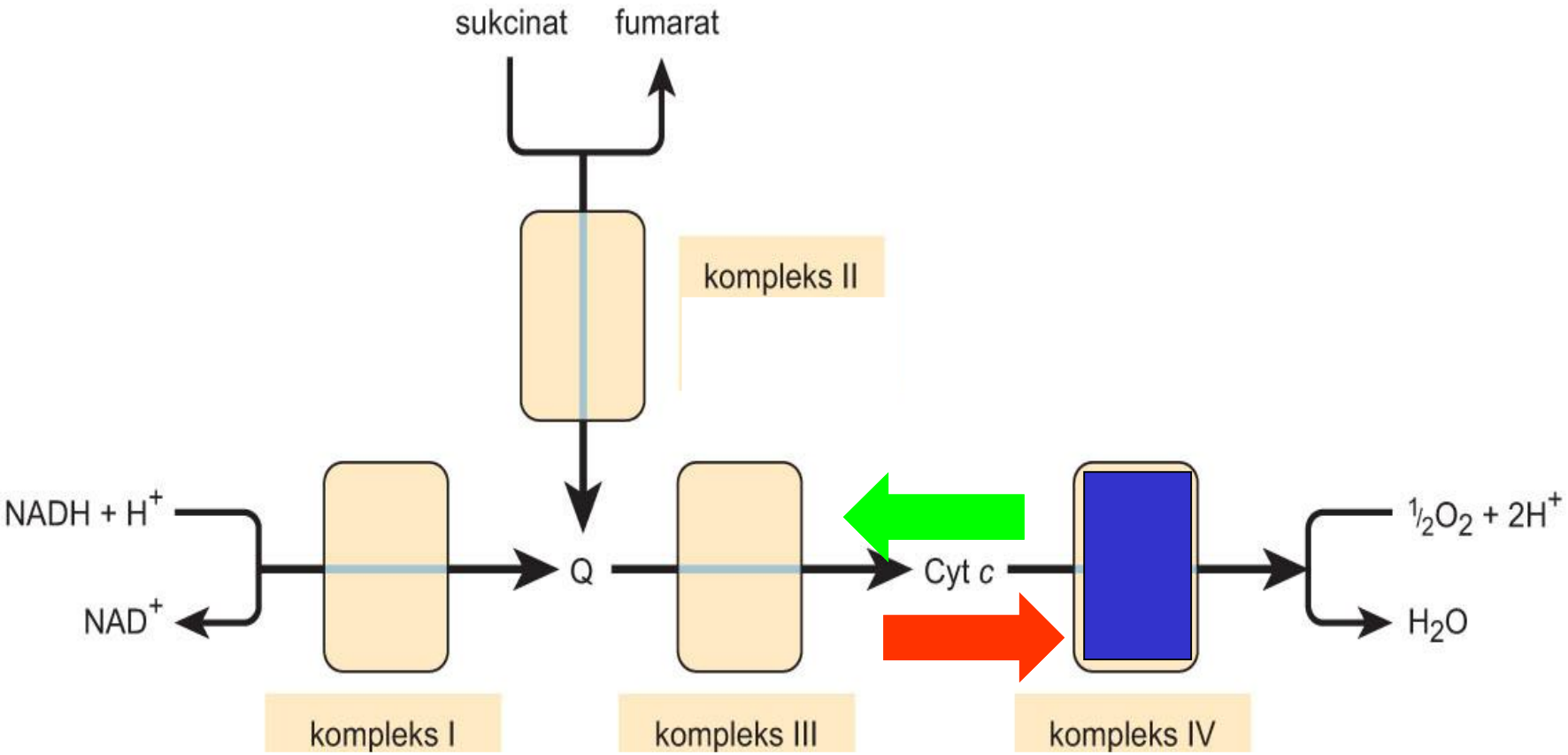
Cytochromes are electron-transfer proteins that contain a heme prosthetic group

The iron atom in heme also cycles through reduced form (Fe^{2+}) and the oxidized form (Fe^{3+}).

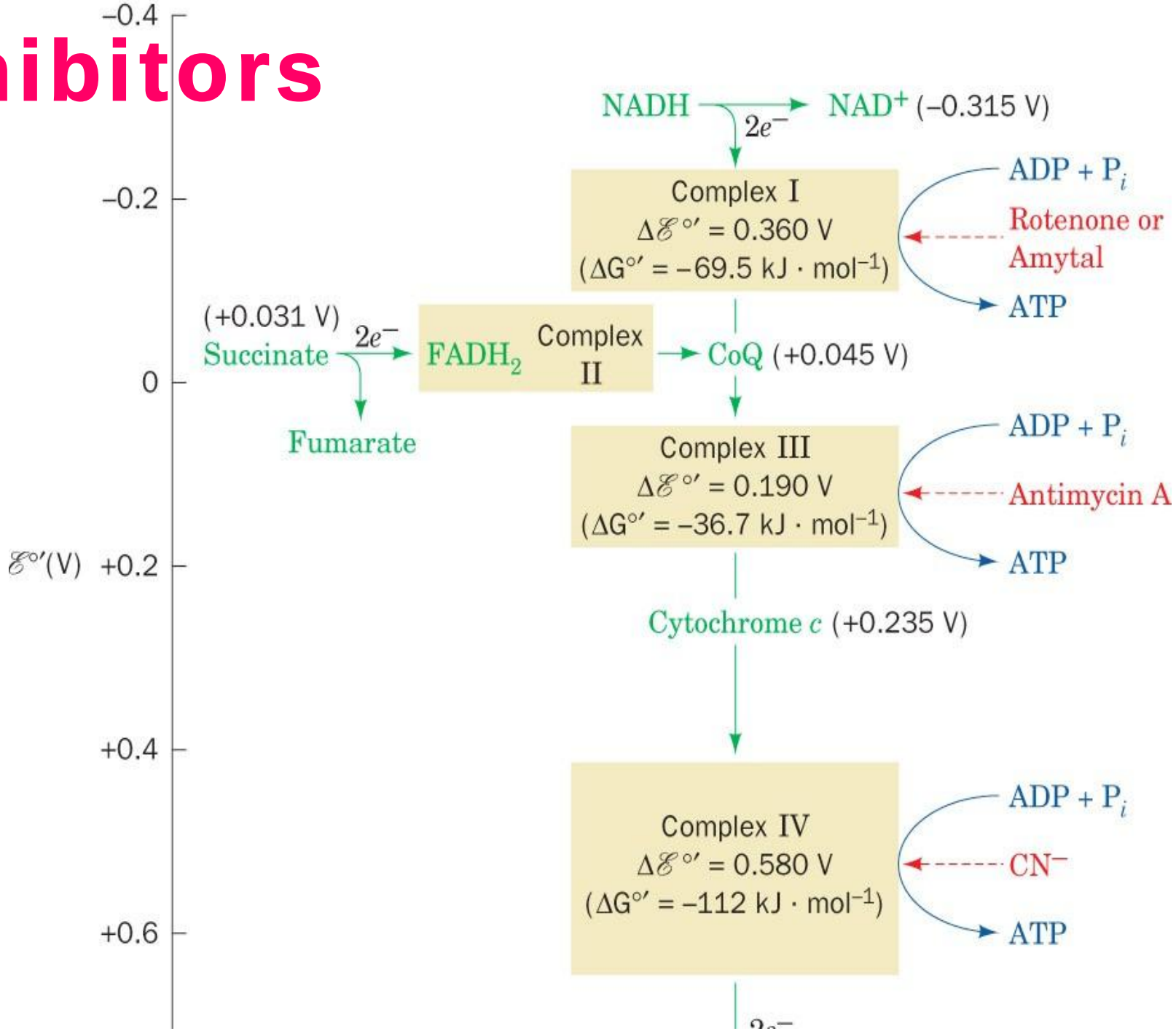
Red muscles are rich in mitochondria, which contains electron transport system and cytochromes.

- **Complex IV**

2 cyt c_{red} + 1/2O_2 ® 2 cyt c_{ox} + H_2O

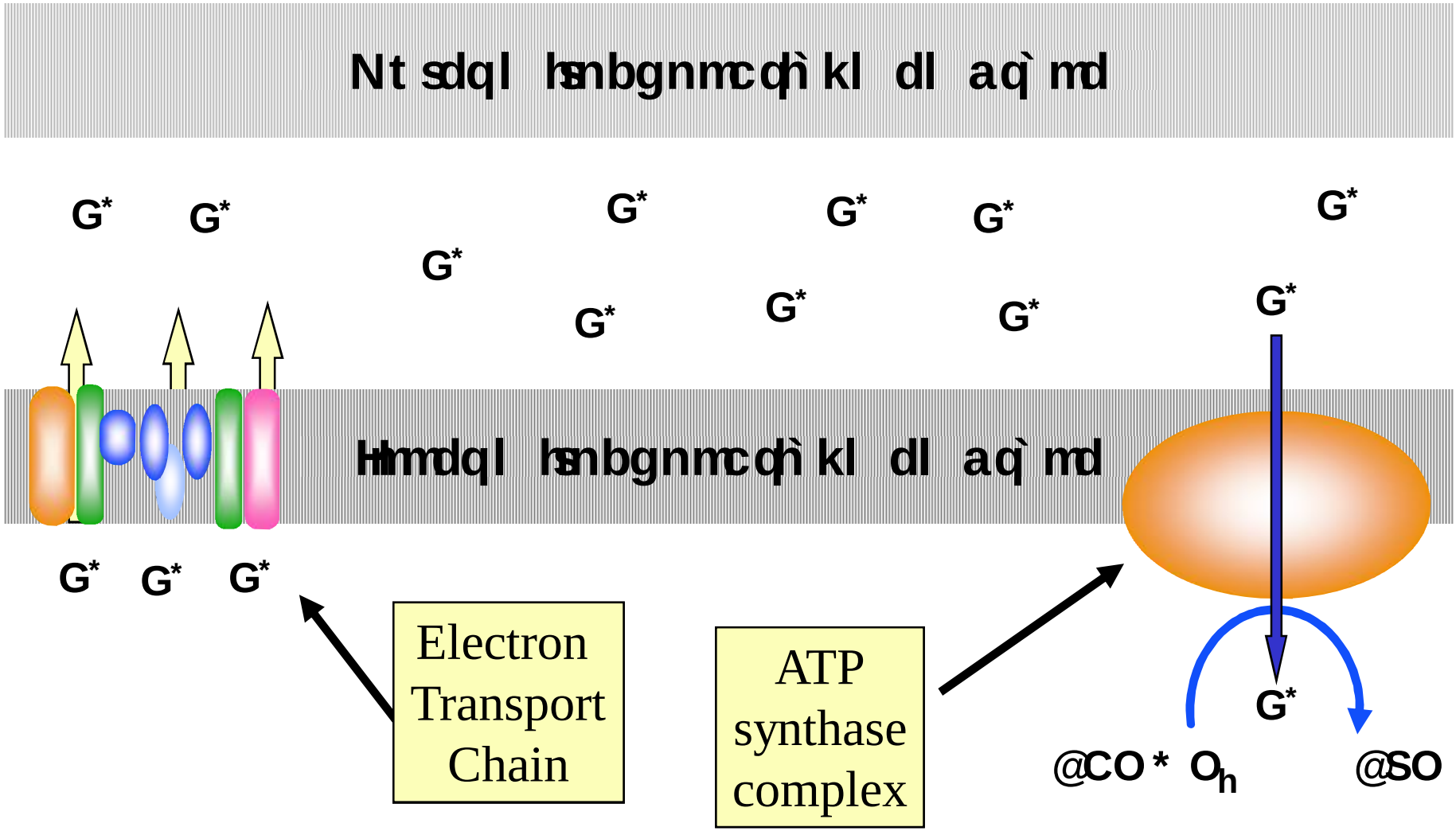


Inhibitors


−0.4
−0.2
0
+0.2
+0.4
+0.6
$\mathscr{E}^{\circ\prime}$(V)
NADH
$2e^-$
NAD^+ (−0.315 V)
Complex I
$\Delta\mathscr{E}^{\circ\prime}$ = 0.360 V
($\Delta G^{\circ\prime}$ = −69.5 kJ · mol^{-1})
ADP + P_i
Rotenone or Amytal
ATP
(+0.031 V)
Succinate
$2e^-$
$FADH_2$
Complex II
CoQ (+0.045 V)
Fumarate
Complex III
$\Delta\mathscr{E}^{\circ\prime}$ = 0.190 V
($\Delta G^{\circ\prime}$ = −36.7 kJ · mol^{-1})
ADP + P_i
Antimycin A
ATP
Cytochrome *c* (+0.235 V)
Complex IV
$\Delta\mathscr{E}^{\circ\prime}$ = 0.580 V
($\Delta G^{\circ\prime}$ = −112 kJ · mol^{-1})
ADP + P_i
CN^-
ATP

Coupling of electron-transport with ATP synthesis

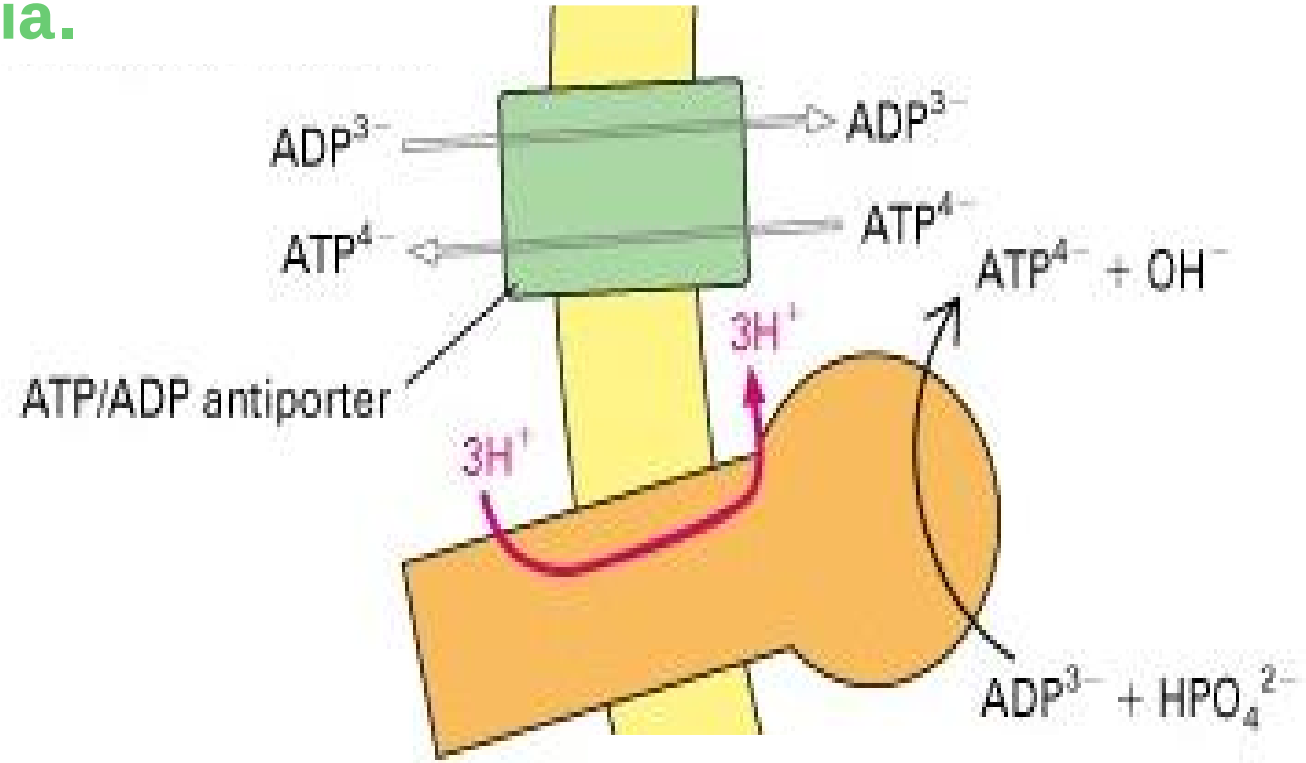
- **Chemiosmotic coupling mechanism**
- **Electron-transport causes unidirectional movement of H^+ into the intermembrane space.**
- **The results in a H^+ gradient being produced.**
- **The gradient then drives the synthesis of ATP.**


G*
G*
G*
G*
G*
G*
G*
G*
G*
G*
G*
G*
G*
G*
Electron Transport Chain
ATP synthase complex

ATP is transported from the matrix of mitochondria to cytosole by ATP-ADP translocase.

ATP and ADP cannot diffuse through the mitochondria membrane freely.

The exit of ATP is coupled with the entry of ADP into mitochondria.



Regulation of oxidative phosphorylation

- **Electrons do not flow unless ADP is present for phosphorylation**

- **Increased ADP levels cause an increase in the activity of various enzymes including:**
  - **glycogen phosphorylase**
  - **phosphofructokinase**
  - **citrate synthase**

Uncoupling of electron-transport and oxidative phosphorylation

- **In some special cases, the coupling of the two processes can be disrupted.**

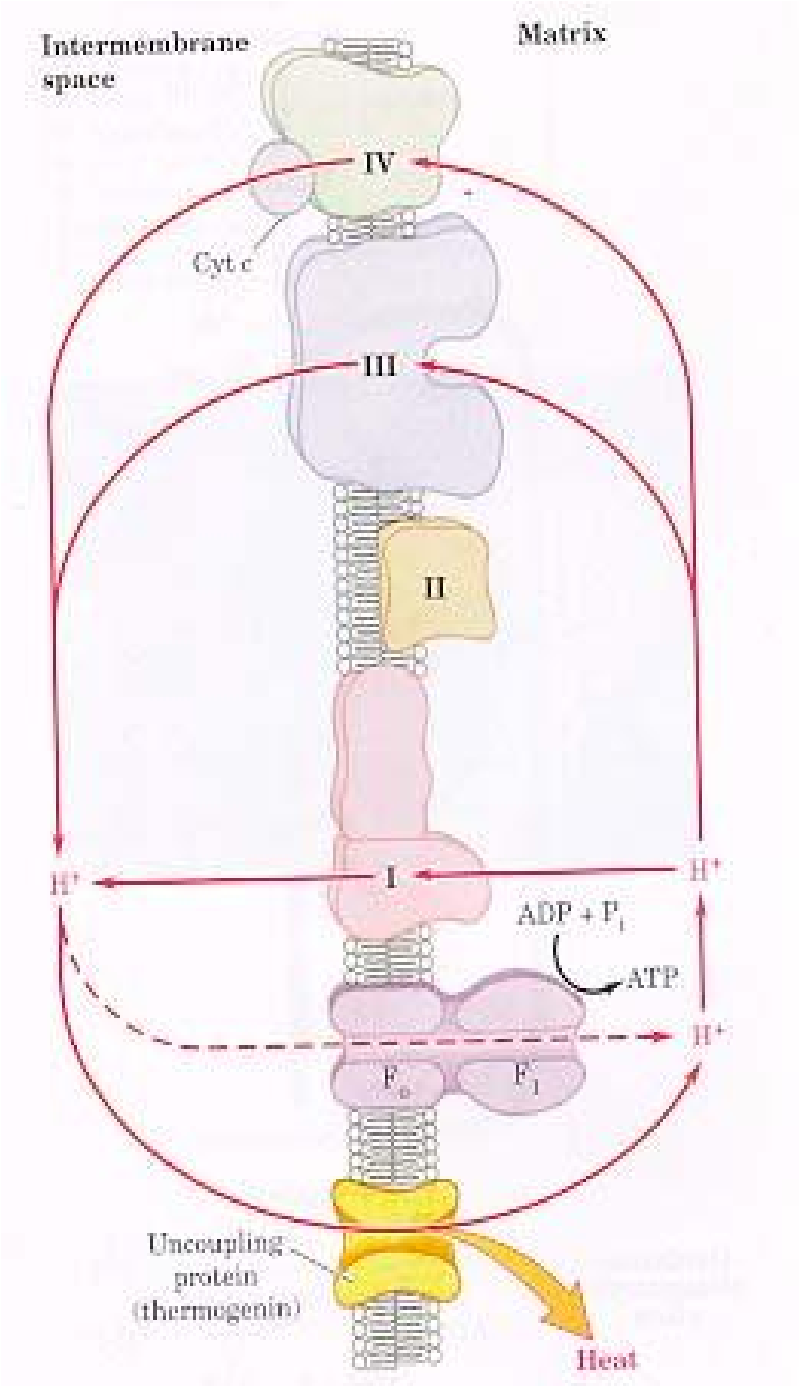
- **Large amounts of O_2 are consumed but no ATP is produced.**

- **Used by newborn animals and hibernating mammals.**

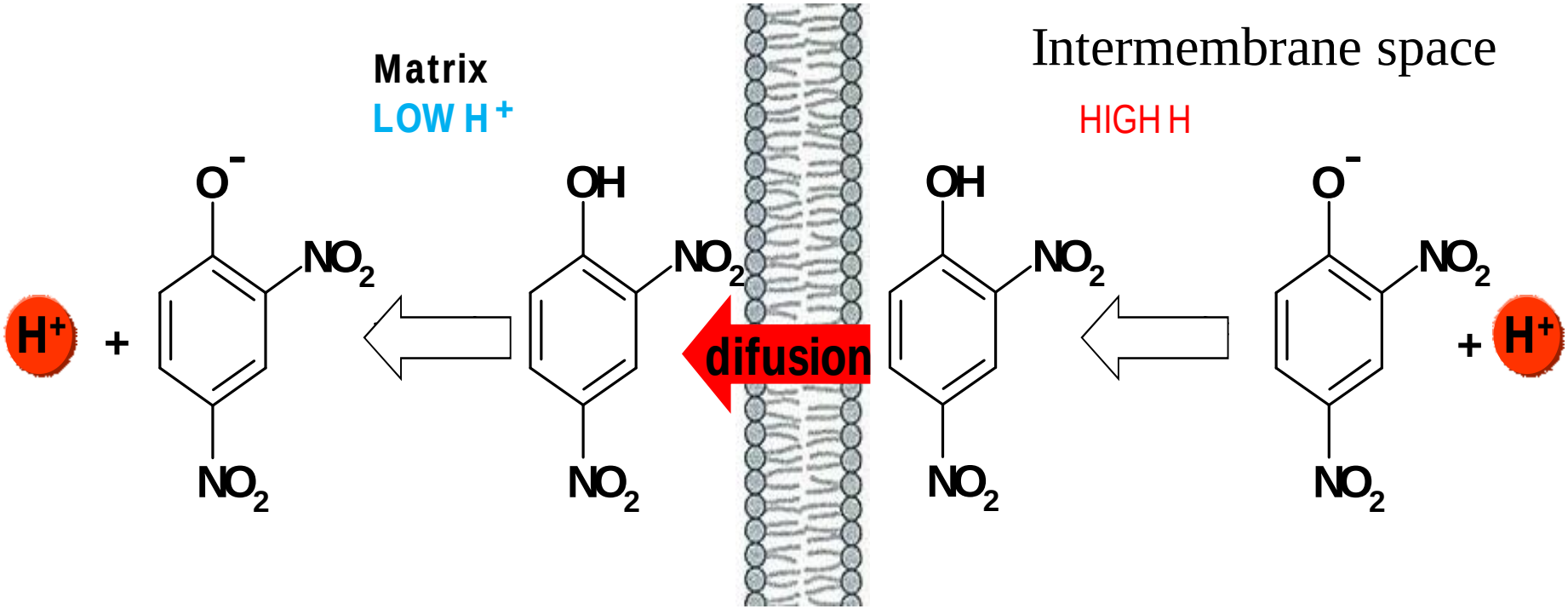
Uncoupling of electron-transport and oxidative phosphorylation

- **Occurs in ‘brown fat’- which contain thermogenin (uncoupling protein).**

- **Thermogenin allows the release of energy as heat instead of ATP.**



Uncouplers (eg, dinitrophenol)



Dinitrophenol was used as a drug for weight loss (1920s):

- lowers the proton gradient,
- decreases ATP
- increases ADP
- the rate of respiratory chain?
- Increased
- but again does not generate sufficient proton gradient to synthesize the necessary amounts of ATP

Dinitrophenol

- The result: the consumption of fat,
- and death due to liver dysfunction (liver is rich in mitochondria): insufficient production of ATP for the liver

Hypoxia

- **The MELAS syndrome (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes)**

- **is a mitochondrial disease with heart manifestations**
- **in which hypertrophic cardiomyopathy is the most outstanding.**

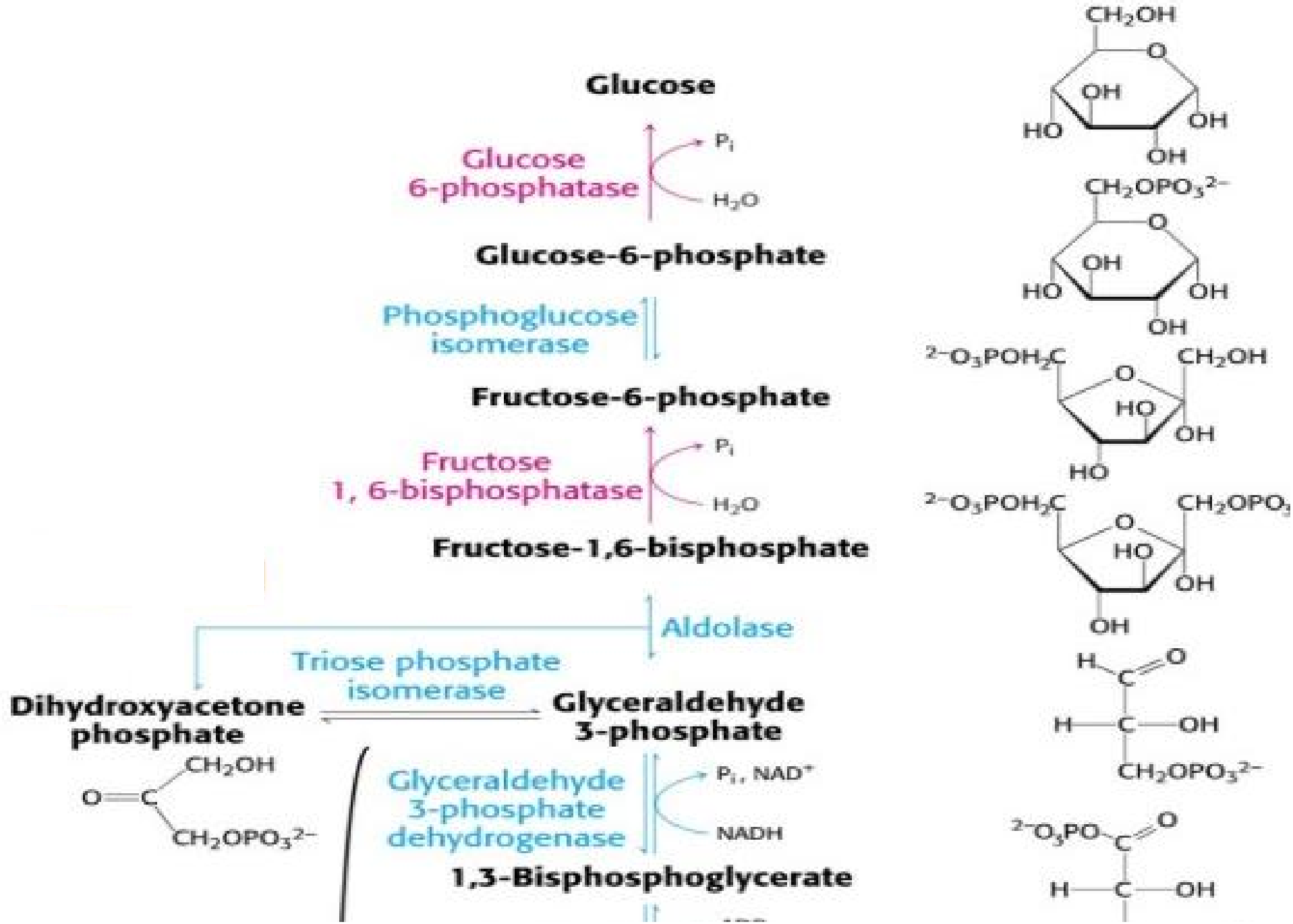
- **A pregnant 23-year-old female undergoes neurology and cardiology consultations for possible mitochondrial disease**

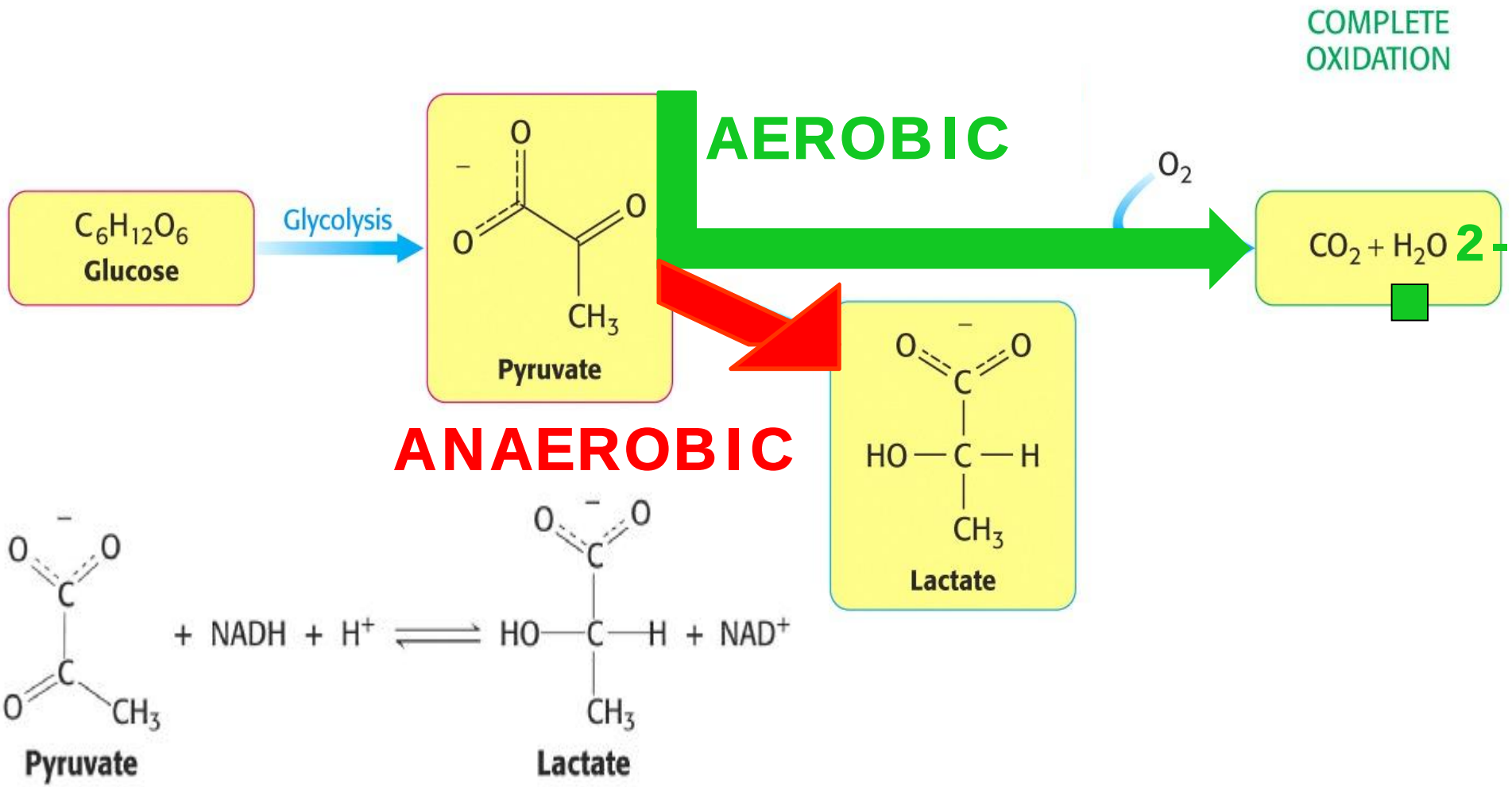
- **On ECG concentric ventricular hypertrophy and normal ventricular function were found, with no other cardiovascular history**

- **Her mother had died at 42 years of age diagnosed with MELAS syndrome, with hypertrophic cardiomyopathy, multiple episodes of acute lung oedema, deafness and repeat ictus.**

- **Her grandmother had died in the fourth decade of life due to non-specified heart causes.**

- **From the 20th week of gestation on she began to have episodes in which she had difficulty breathing accompanied occasionally by typical chest pain.**


Glucose
Glucose 6-phosphatase
P_i
H_2O
Glucose-6-phosphate
Phosphoglucose isomerase
Fructose-6-phosphate
Fructose 1, 6-bisphosphatase
P_i
H_2O
Fructose-1,6-bisphosphate
Aldolase
Triose phosphate isomerase
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
Glyceraldehyde 3-phosphate dehydrogenase
P_i, NAD^+
NADH
1,3-Bisphosphoglycerate

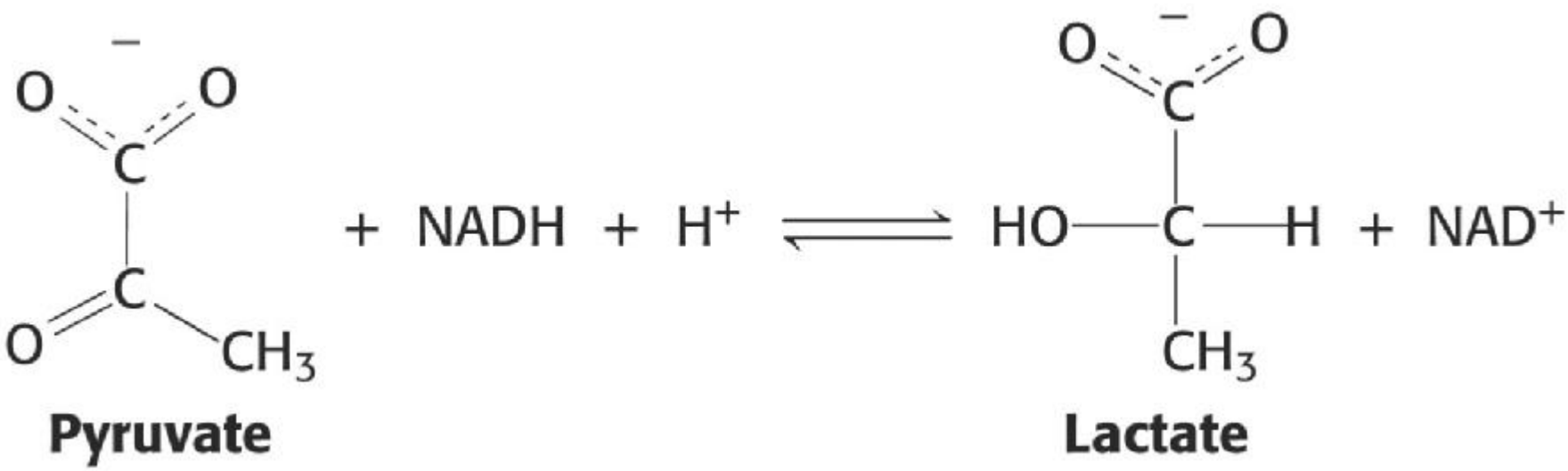

COMPLETE OXIDATION
AEROBIC
$C_6H_{12}O_6$
Glucose
Glycolysis
Pyruvate
O_2
$CO_2 + H_2O$
2
ANAEROBIC
Lactate
+ NADH + H^+
NAD^+
Pyruvate
Lactate

- **Which substrates are oxidized in the respiratory chain?**

- **What will be the ratio of NADH and NAD^+ concentrations in hypoxia?**

- **How increased concentrations of NADH will affect upon concentration of lactate?**

- **Lactate can be elevated up to 30 fold**



Respiratory chain (RCH)

a) is found in all cells

b) is located in a mitochondrion

c) includes enzymes integrated in the inner mitochondrial membrane

d) produces reducing equivalents ($NADH+H^+$, $FADH_2$)

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Respiratory chain (RCH)

a) belongs among oxidative pathways

b) can proceed under both aerobic and anaerobic conditions

c) is a reversible pathway

d) needs oxygen (O_2) for its function

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b) ~~can proceed under both aerobic~~ and ~~anaerobic conditions~~

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Enzymes of the RCH

a) belongs among oxidoreductases

b) can transfer either H or electrons

c) are called Complex I, II, III and IV

d) transfer protons and electrons in the same direction

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The function of the RCH

a) is to regenerate NAD^+ from NADH

b) is to regenerate $NADP^+$ from NADPH

c) is to regenerate FAD from $FADH_2$

d) is to finish oxidation of energy substrates and conserve their energy in a form of ATP

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In reactions of the RCH

a) oxygen is reduced to H_2O

b) protons (H^+) are transfered into an intermembrane space

c) ATP is produced by the Complex I

d) all reduced coenzymes ($NADH+H^+$ and $FADH_2$) are reoxidized by the same mechanism

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Choose correct statement

a) Complex I transfers H^+ into an intermembrane space

b) Complex II transfers H^+ into an intermembrane space

c) Coenzyme Q accepts e- from both Complex I and Complex II

d) Complex IV transfers electrones to oxygen

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b) Complex II transfers H^+ into an intermembrane space

c) Coenzyme Q accepts e- from both Complex I and Complex II

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Citrate cycle (CC) and the RCH are interconnected

a) by CO_2 (produced by CC, used by RCH)

b) by NADH (produced by CC, used by RCH)

c) an enzyme succinate dehydrogenase

d) ATP (produced by RCH, used by CC)

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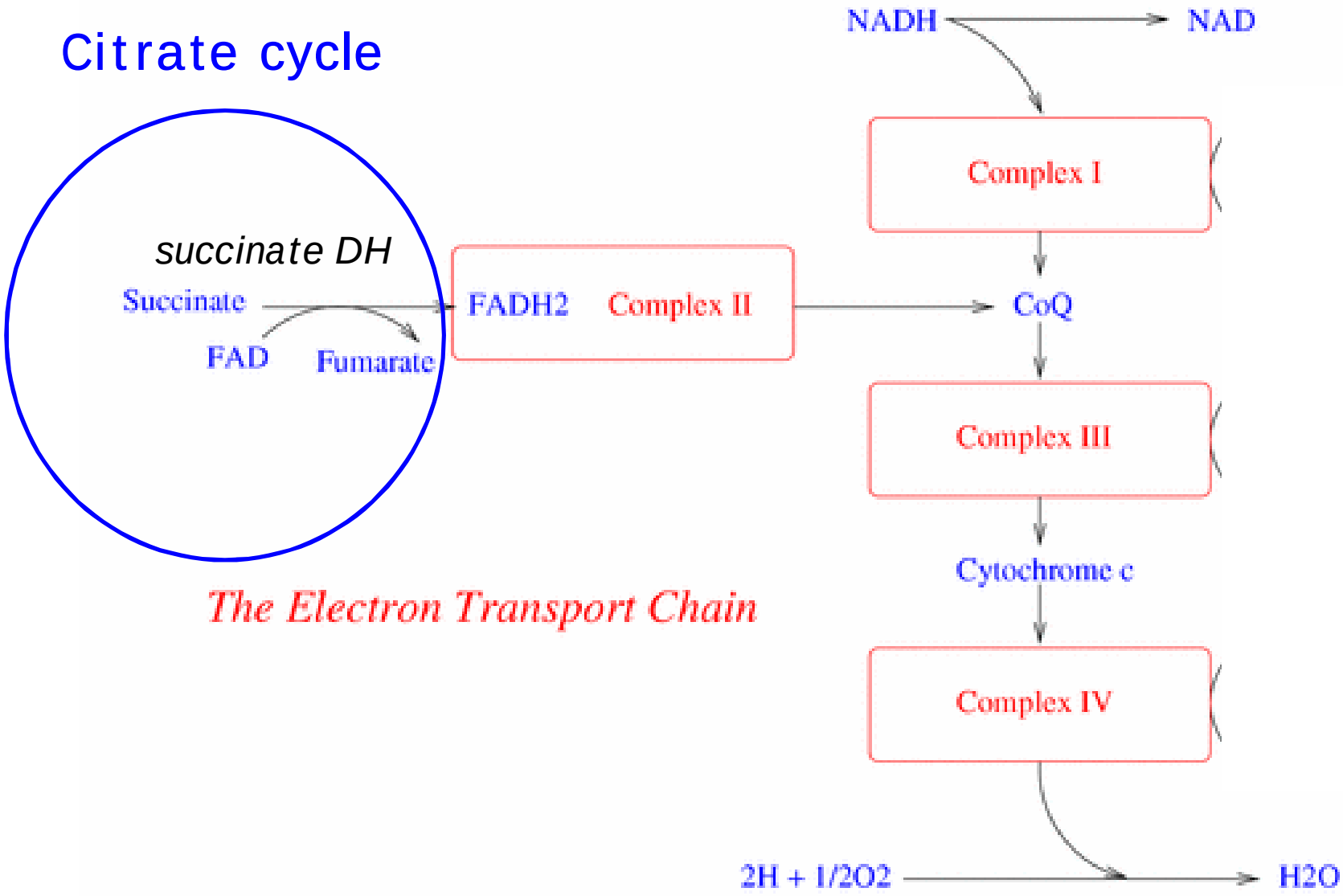
Citrate cycle



The Electron Transport Chain

The figure is found at http://www.cellml.org/examples/images/metabolic_models/the_electron_transport_chain.gif (December 2006)

Adenosine triphosphate (ATP)

a) can be produced only in a cooperation with the RCH

b) can be synthesized only under aerobic conditions

c) is formed from ADP by addition of one phosphate

d) is transported from a mitochondrion into a cytoplasm by exchange with ADP

Oxidative phosphorylation

a) needs proton gradient on the inner mitochondrial membrane

b) is catalyzed by ATP synthase

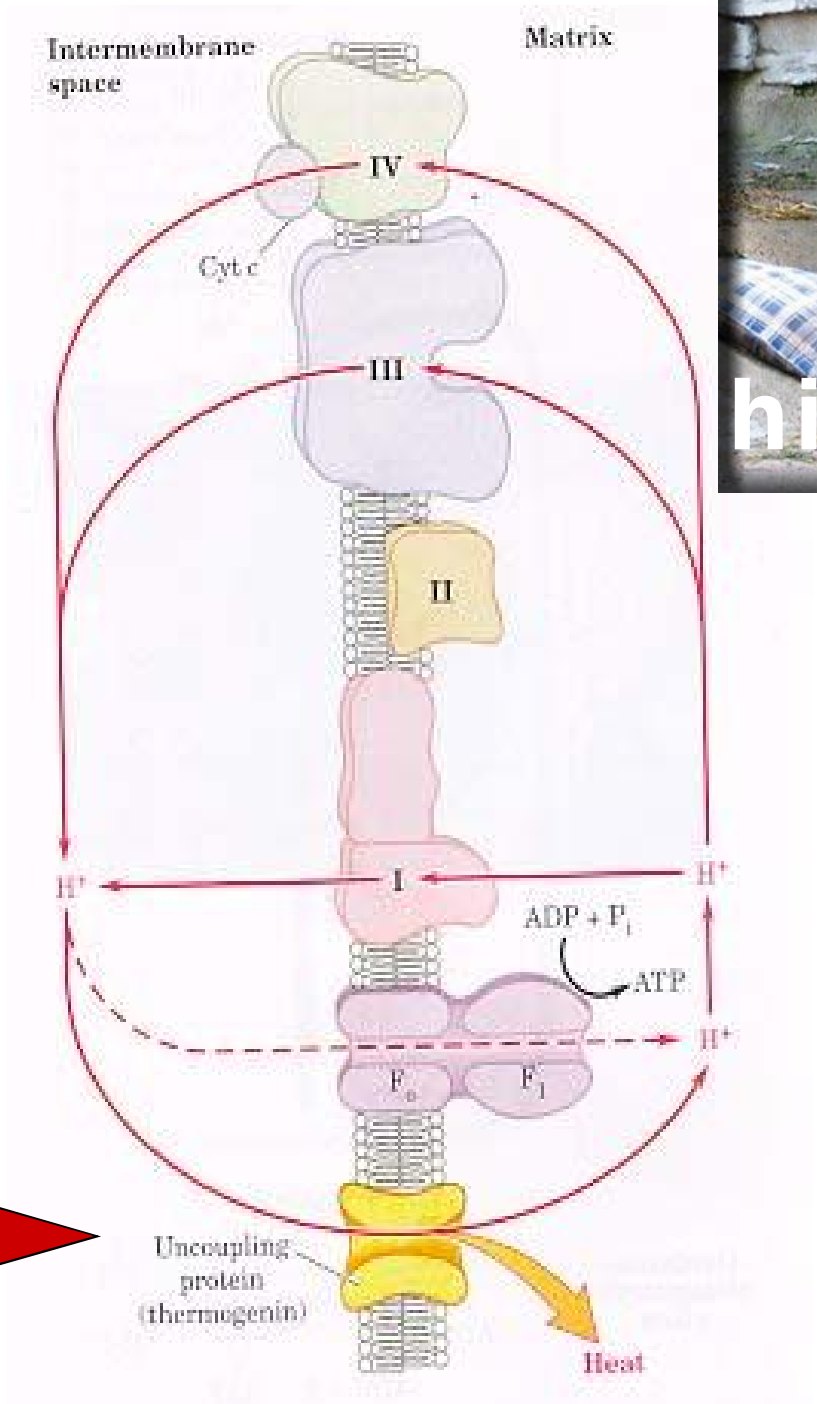
c) can be interrupted by uncoupling proteins (UCP)

d) means ATP synthesis in any oxidative metabolic pathway

Uncoupling proteins (UCP)

= separate RCH from ATP synthesis

(the synthesis is interrupted)



energy from H^+ gradient is released as a heat

Choose correct statement(s) about regulation of RCH and ATP synthesis

a) $^{-}O_2$ decreases the pathways

b) uncoupling proteins increases ATP synthesis

c) - $NADH+H^+/NAD^+$ increases the pathways

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