LICENCIATURA EM BIOLOGIA

DISCIPLINA

BIOQUÍMICA

Ano Lectivo de 2013/2014

Aula nº 25

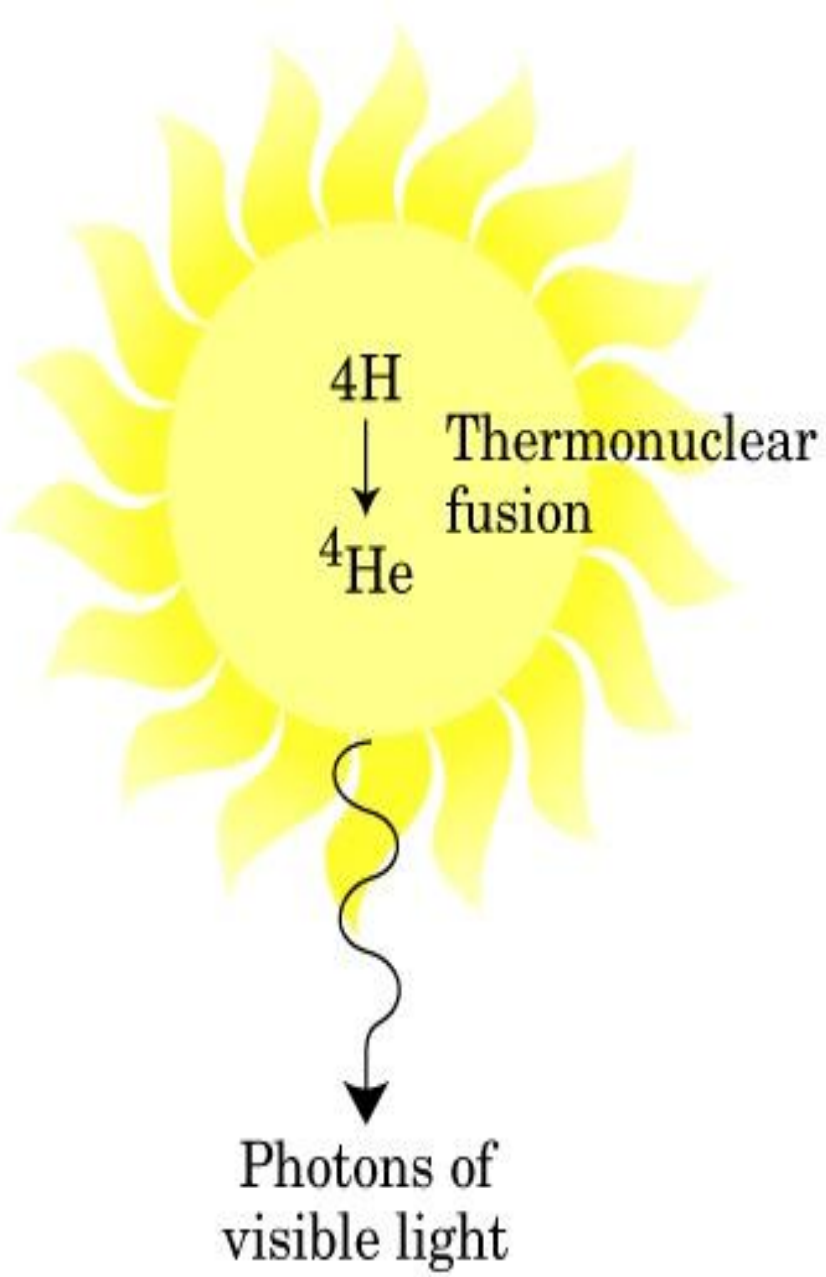
23 MAI 2014 **Ricardo Boavida Ferreira** **Lab 46**

O fluxo de energia nos seres vivos e a integração do metabolismo

Armazenamento de energia e de potencial redutor e formação de esqueletos carbonados. Sua inter-regulação: as proteínas desacopladoras das cadeias de transporte de electrões (ex. a termogenina) e as vias alternativas de transporte de electrões dos mitocôndrios vegetais (fundamentos). A bioenergética das cadeias de transporte de electrões: sua relação com as doenças neurodegenerativas do Homem e com a foto-oxidação nas plantas.
Material de estudo: diapositivos das aulas, bibliografia recomendada e textos de apoio.

Energy need of all organisms are provided directly or indirectly by solar energy

Conversion of mass into energy is a very large increase in disorder



First law of thermodynamics:

For any chemical or physical change, the total energy of the universe remains constant. In other words, energy may change form, or be transported from one place to another, but it cannot be destroyed or created.

Second law of thermodynamics:

Any spontaneous chemical or physical change is always accompanied by increase in the disorder of the universe. In other words, disorder or entropy of the universe increases in all natural processes.

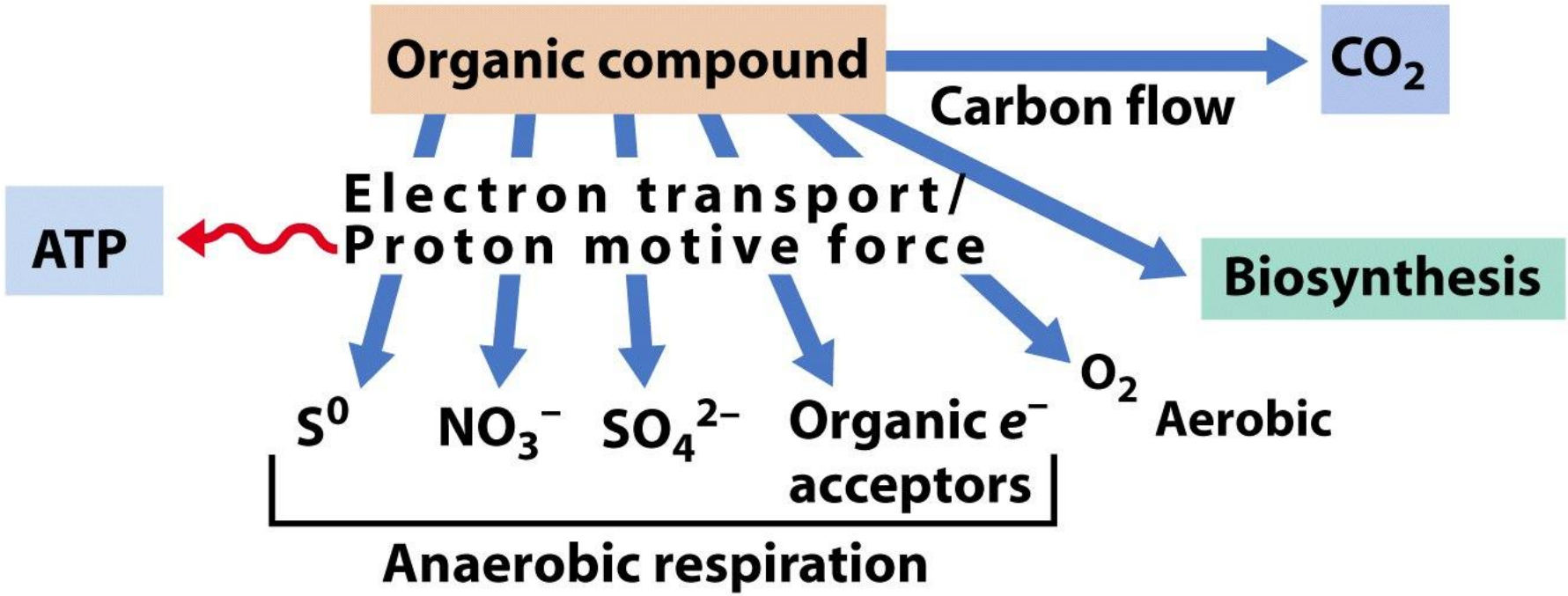
Organisms may be organized into groups based upon their nutritional and metabolic needs which are extremely diverse. Traditionally, these groupings have been based on two main criteria:

- The nature of the energy source;

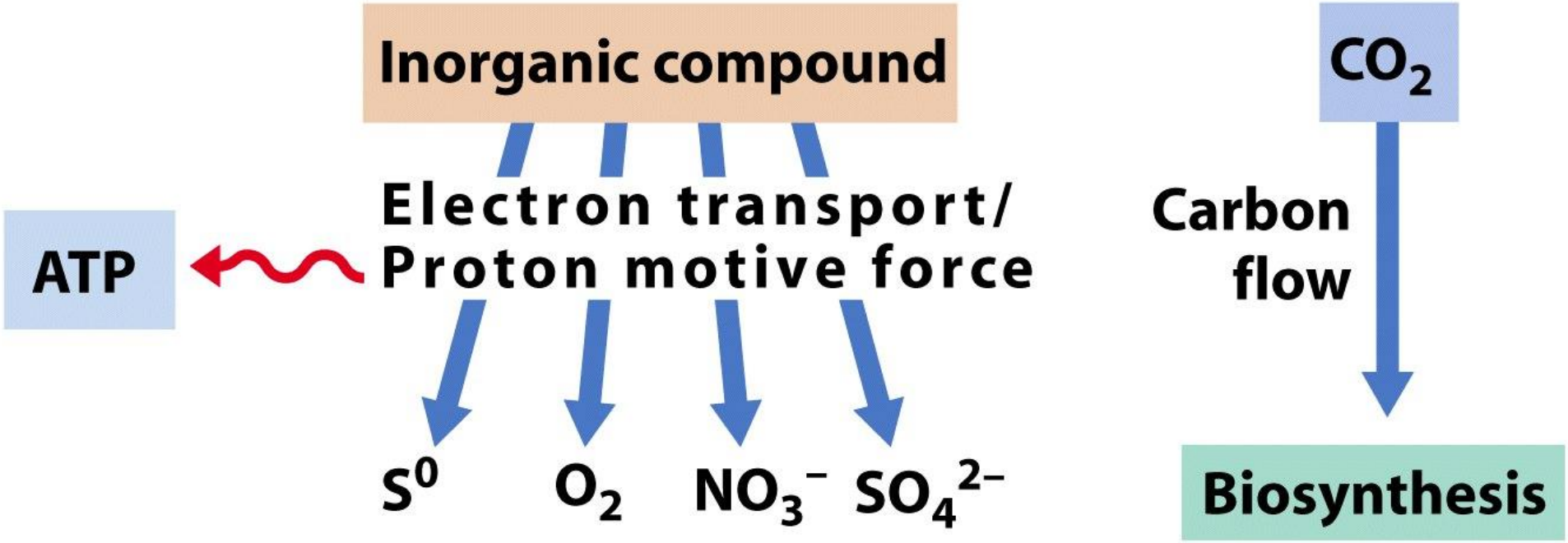
- The nature of the carbon source used for building organic, biological macromolecules.

Photoautotrophs	• Carbon source: CO_2 • Energy source: light • Examples: cyanobacteria, green and purple sulfur bacteria, algae, plants
Chemoautotrophs	• Carbon source: CO_2 • Energy source: oxidize inorganic compounds which are used to fix CO_2 • Examples: nitrifying, hydrogen, sulfur and iron-utilizing bacteria; Archaea which live among hydrothermal ocean vents
Photoheterotrophs	• Carbon source: from organic compounds made by other organisms • Energy source: light • Examples: green and purple nonsulfur bacteria
Chemoheterotrophs	• Carbon source: from organic compounds made by other organisms • Energy source: from oxidation of organic compounds • Examples: most bacteria, protozoa, all fungi and animals

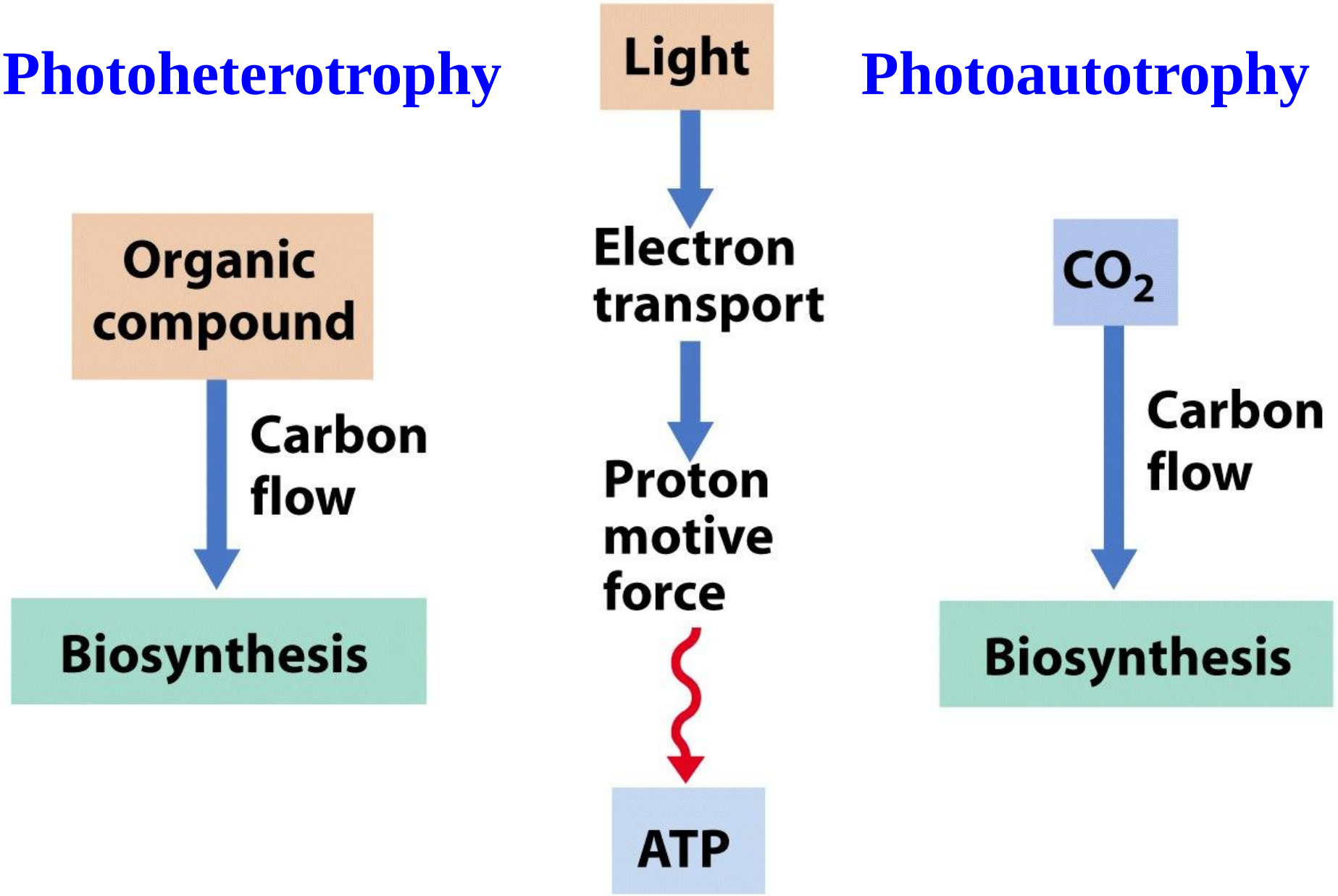
Energetics and carbon flow



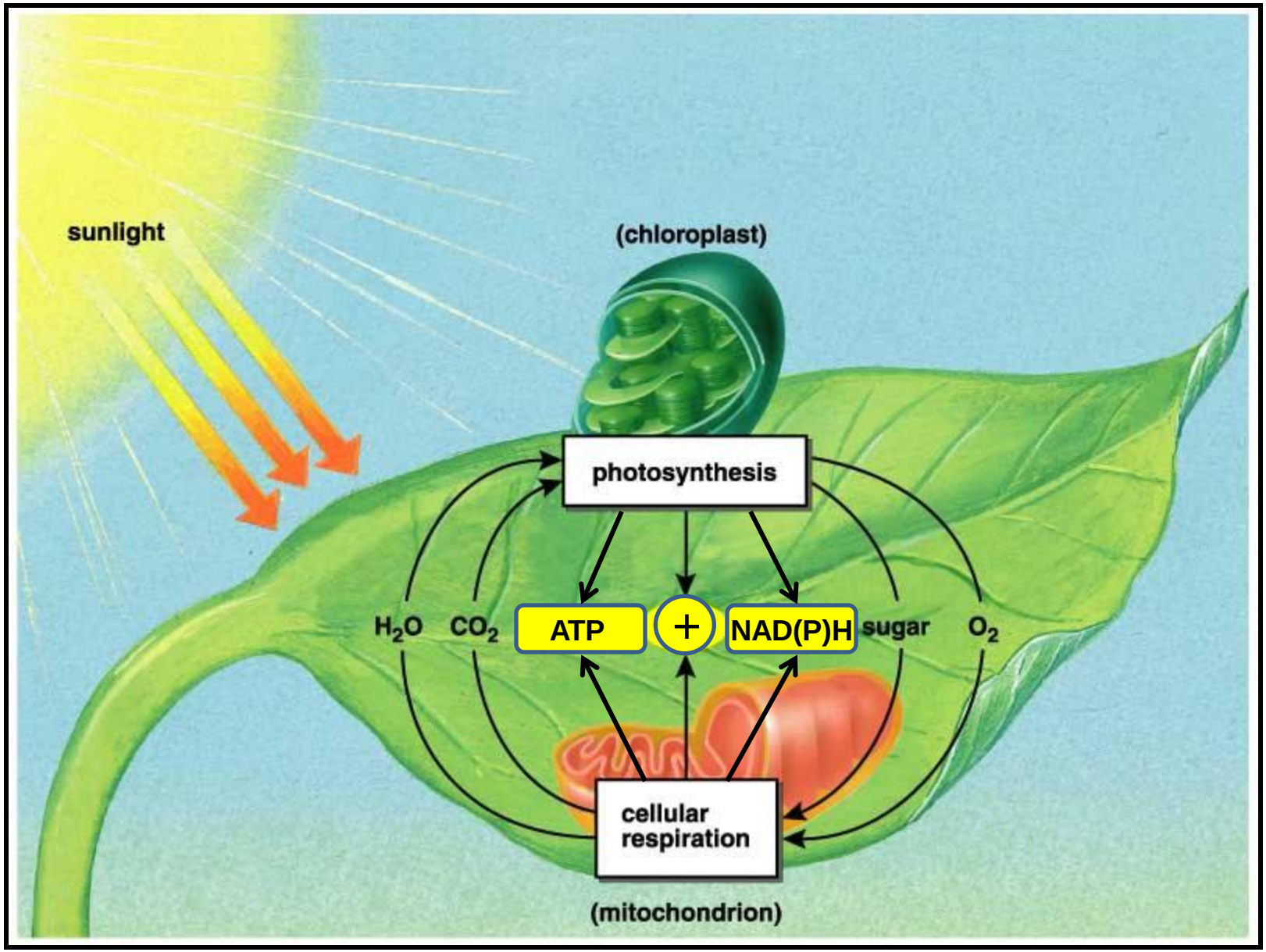
Chemoheterotrophic metabolism



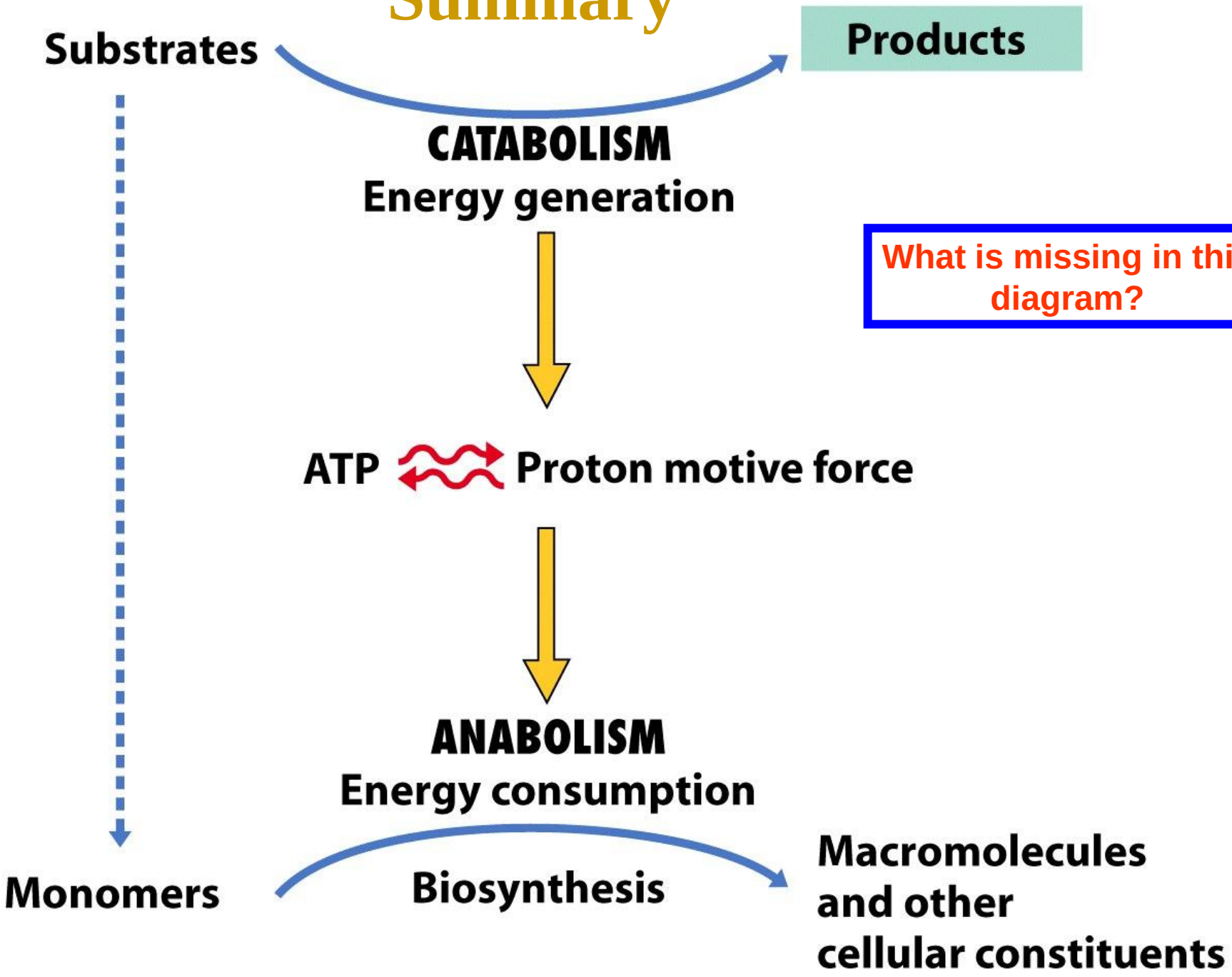
Chemoautotrophic metabolism



Phototrophic metabolism


sunlight
(chloroplast)
photosynthesis
H_2O
CO_2
ATP
+
NAD(P)H
sugar
O_2
cellular respiration
(mitochondrion)

Summary


Substrates
Products
CATABOLISM
Energy generation
What is missing in this diagram?
ATP
Proton motive force
ANABOLISM
Energy consumption
Monomers
Biosynthesis
Macromolecules and other cellular constituents

ORGANISMOS FOTOSSINTÉTICOS
(em geral, autotróficos)

↓

Fotossintetizam para quê?

Para obterem os materiais de que necessitam para crescer:
PRINCIPAIS FUNÇÕES DA FOTOSSÍNTESE:

I - Obter energia (ATP) a partir da luz do Sol;
II - Obter potencial redutor (NADPH) a partir da água e da luz do Sol;
III - Obter esqueletos carbonados (hidratos de carbono) sintetizados a partir do CO_2 atmosférico e do ATP e NADPH produzidos na fotossíntese.

→ Obtêm os hidratos de carbono directa ou indirectamente da ingestão de organismos fotossintéticos

ORGANISMOS NÃO-FOTOSSINTÉTICOS
(em geral, heterotróficos)

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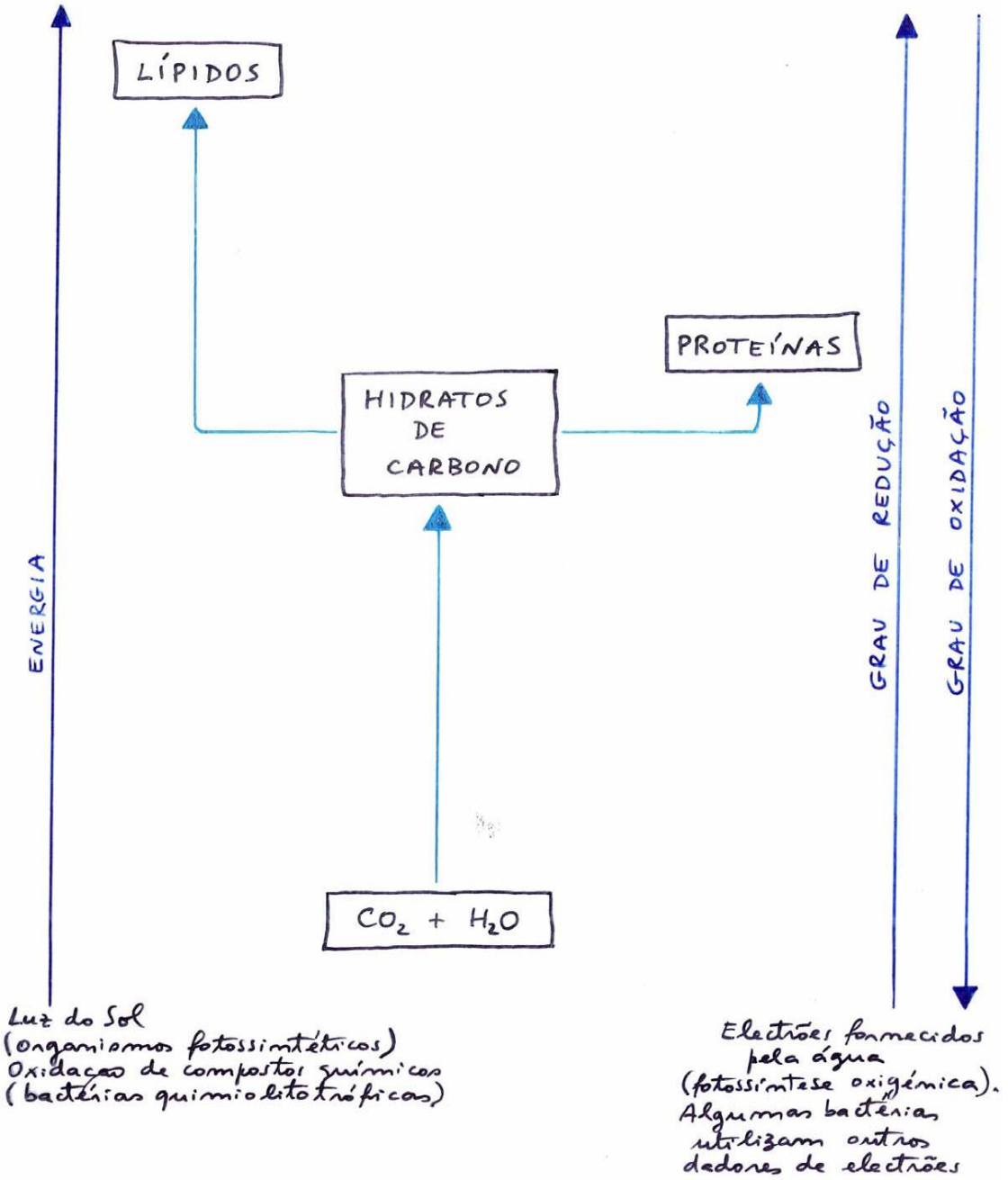
Respiram para quê?

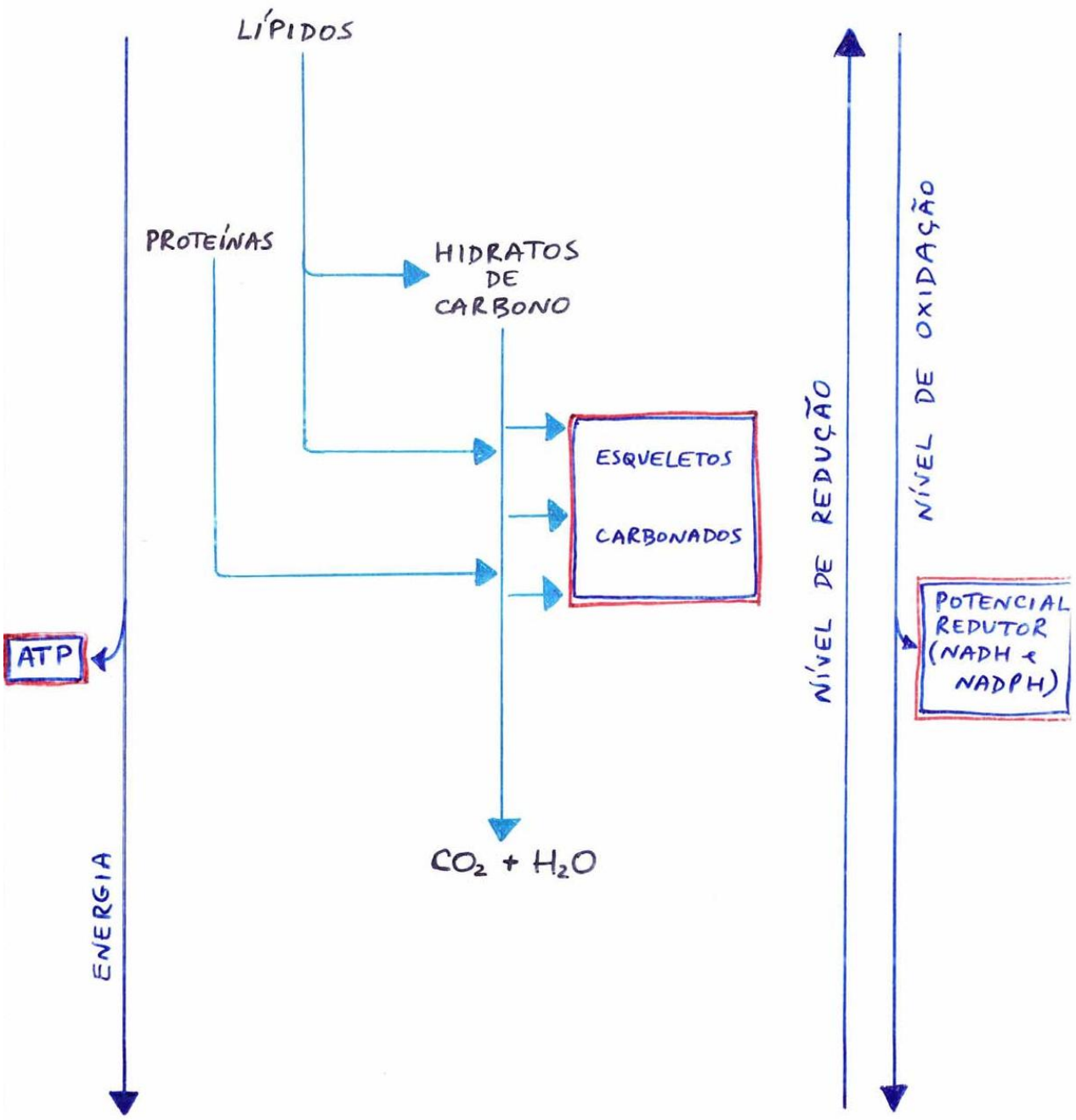
Para obterem os materiais de que necessitam para crescer:
PRINCIPAIS FUNÇÕES DA RESPIRAÇÃO

I - Obtenção de energia (ATP);
II - Obtenção de potencial redutor (NADH);
III - Obtenção de esqueletos carbonados.

Vias redutivas - Fotossíntese e Quimiossíntese



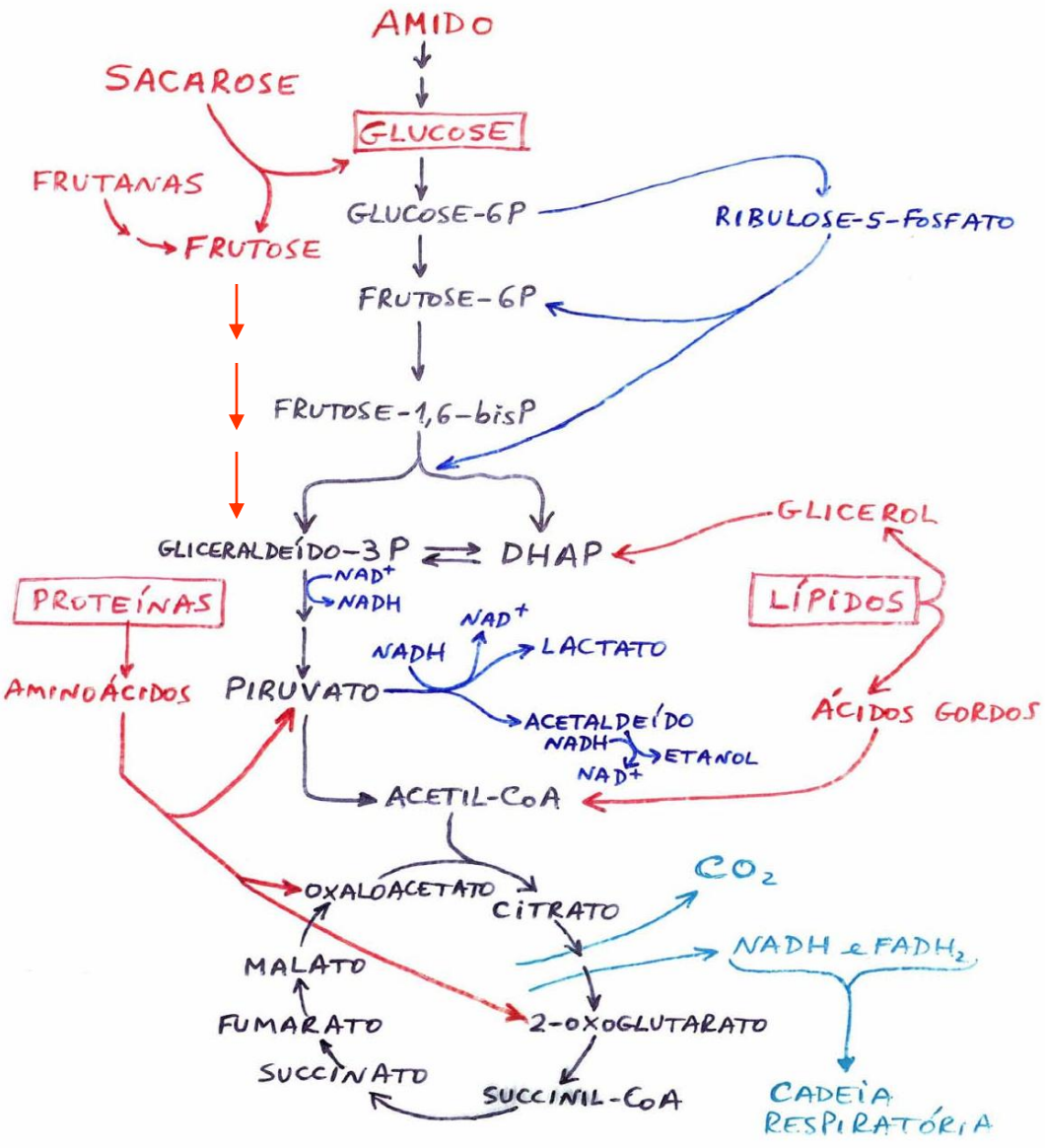
Vias Oxidativas - Respiração



As células utilizam três tipos principais de substratos respiratórios:

- **Hidratos de carbono**
- **Lípidos**
- **Proteínas**

AS VIAS DEGRADATIVAS DOS DIFERENTES SUBSTRATOS RESPIRATÓRIOS CONVERGEM NO CICLO DO ÁCIDO CÍTRICO

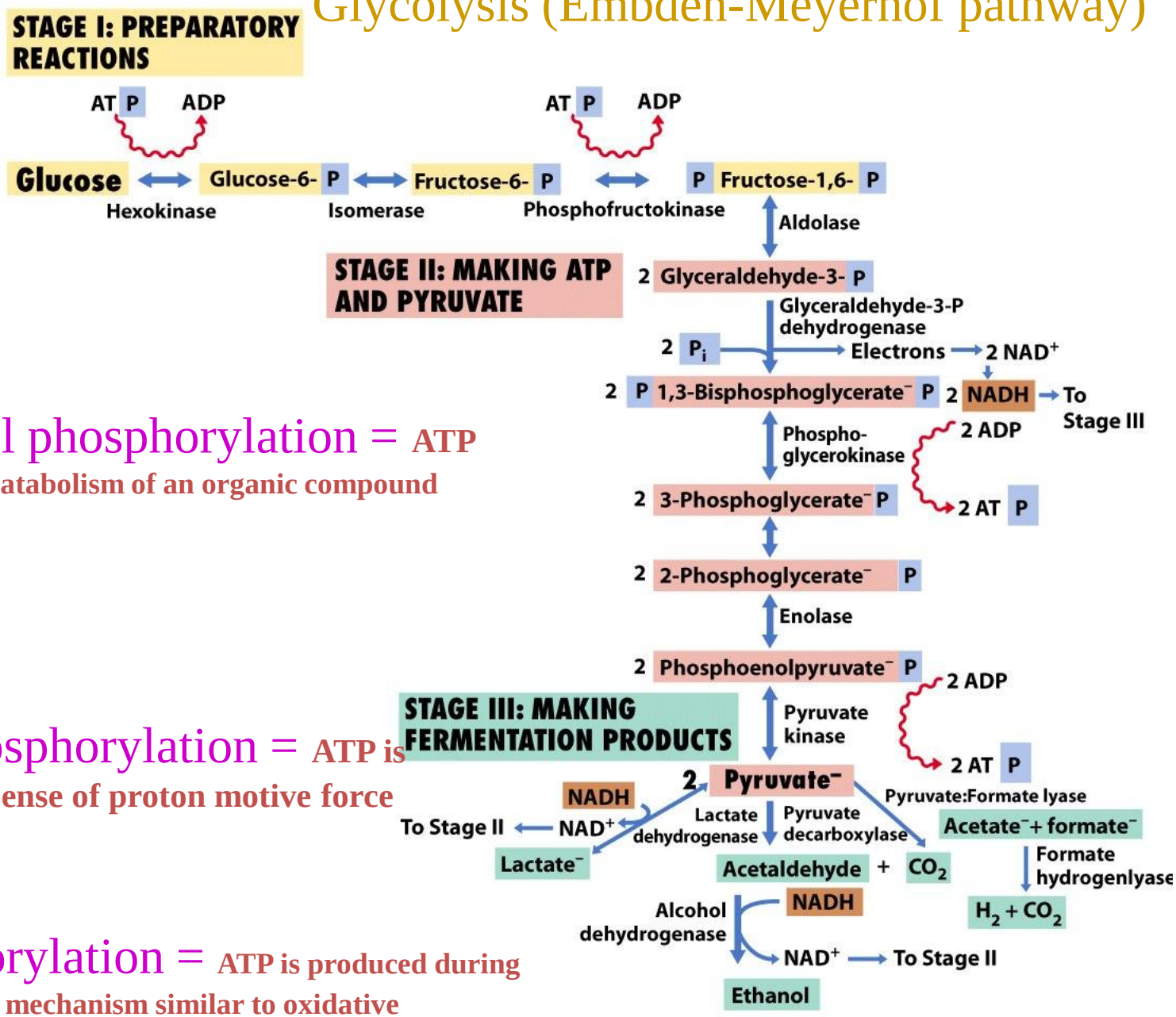


ATP

Há três processos de produzir ATP na natureza:

- Fosforilação a nível do substrato (glicólise e ciclo do ácido cítrico);
- Fosforilação oxidativa (cadeia mitocondrial de transporte de electrões);
- Fotofosforilação (cadeia de transporte de electrões do cloroplasto).

Glycolysis (Embden-Meyerhof pathway)



Substrate level phosphorylation = ATP

is synthesized during catabolism of an organic compound

Oxidative phosphorylation = ATP is

produced at the expense of proton motive force

Photophosphorylation = ATP is produced during

photosynthesis using a mechanism similar to oxidative phosphorylation

Potencial redutor

NADH:

É produzido durante a respiração (glicólise, oxidação β dos ácidos gordos, ciclo do ácido cítrico e ciclo do glioxilato);

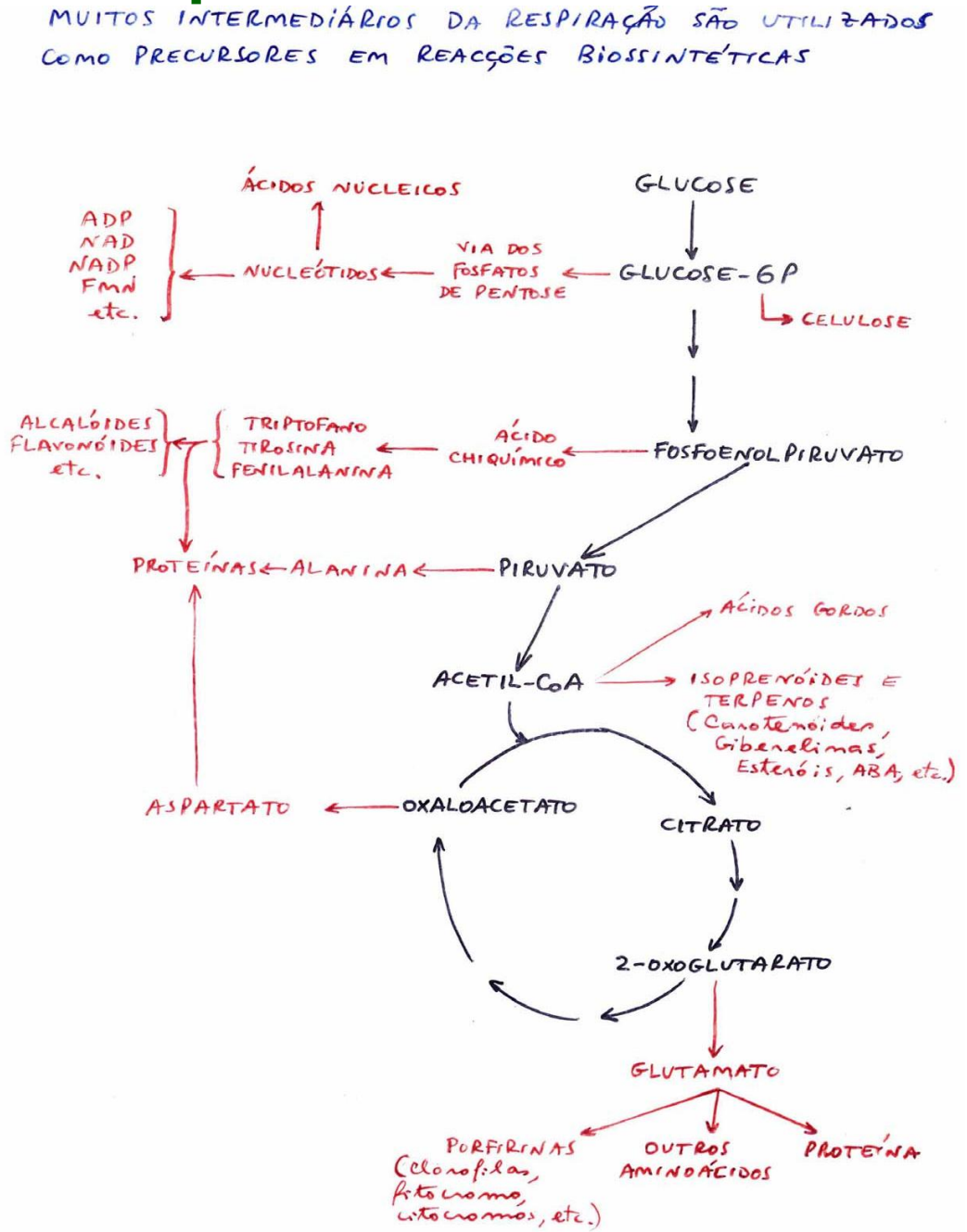
É oxidado na cadeia mitocondrial de transporte de electrões, com formação de ATP (fosforilação oxidativa).

NADPH:

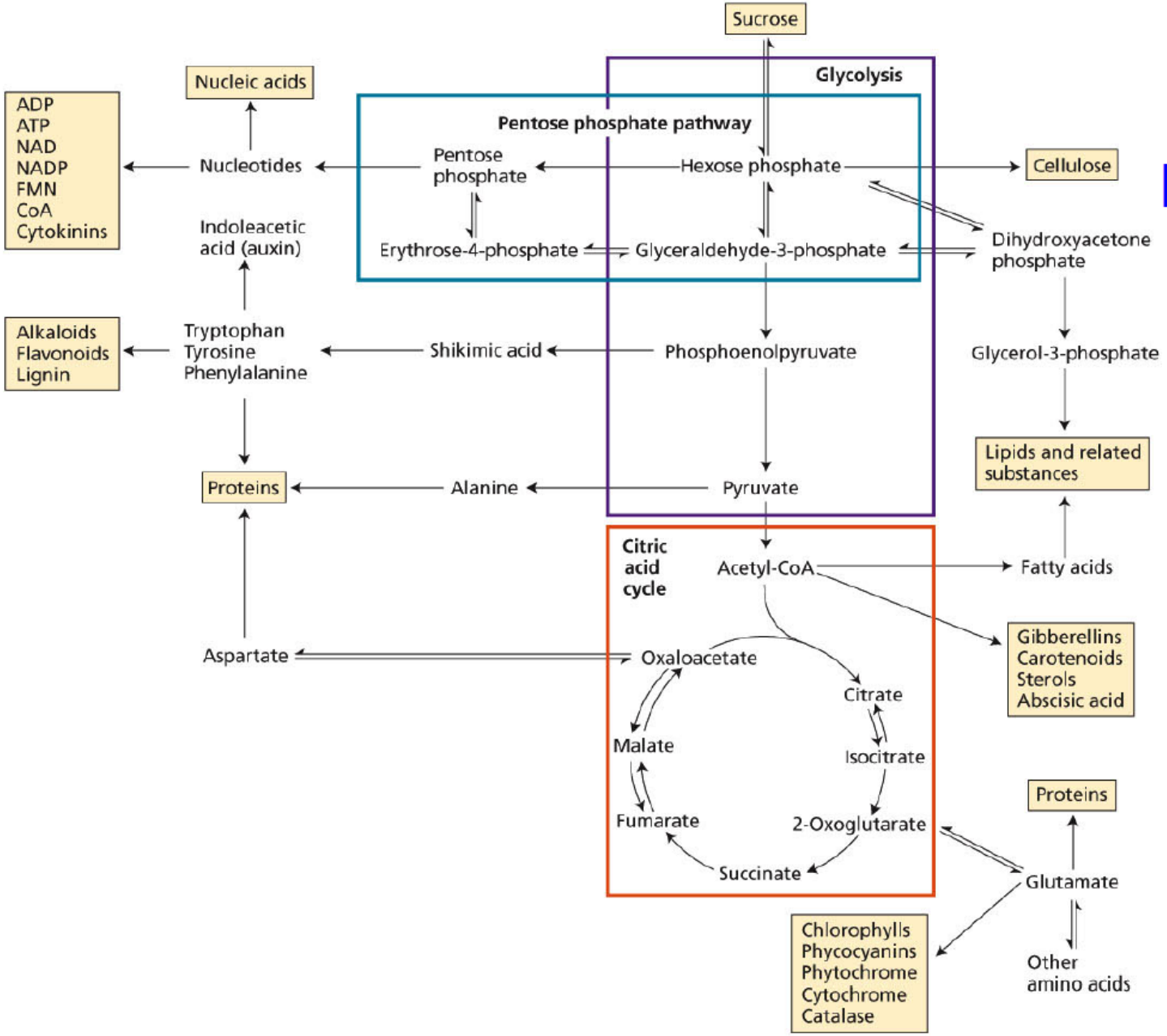
É produzido pela via dos fosfatos de pentose e pela cadeia de transporte de electrões do cloroplasto;

É consumido pelas reacções biossintéticas ou oxidado pelos mitocôndrios vegetais.

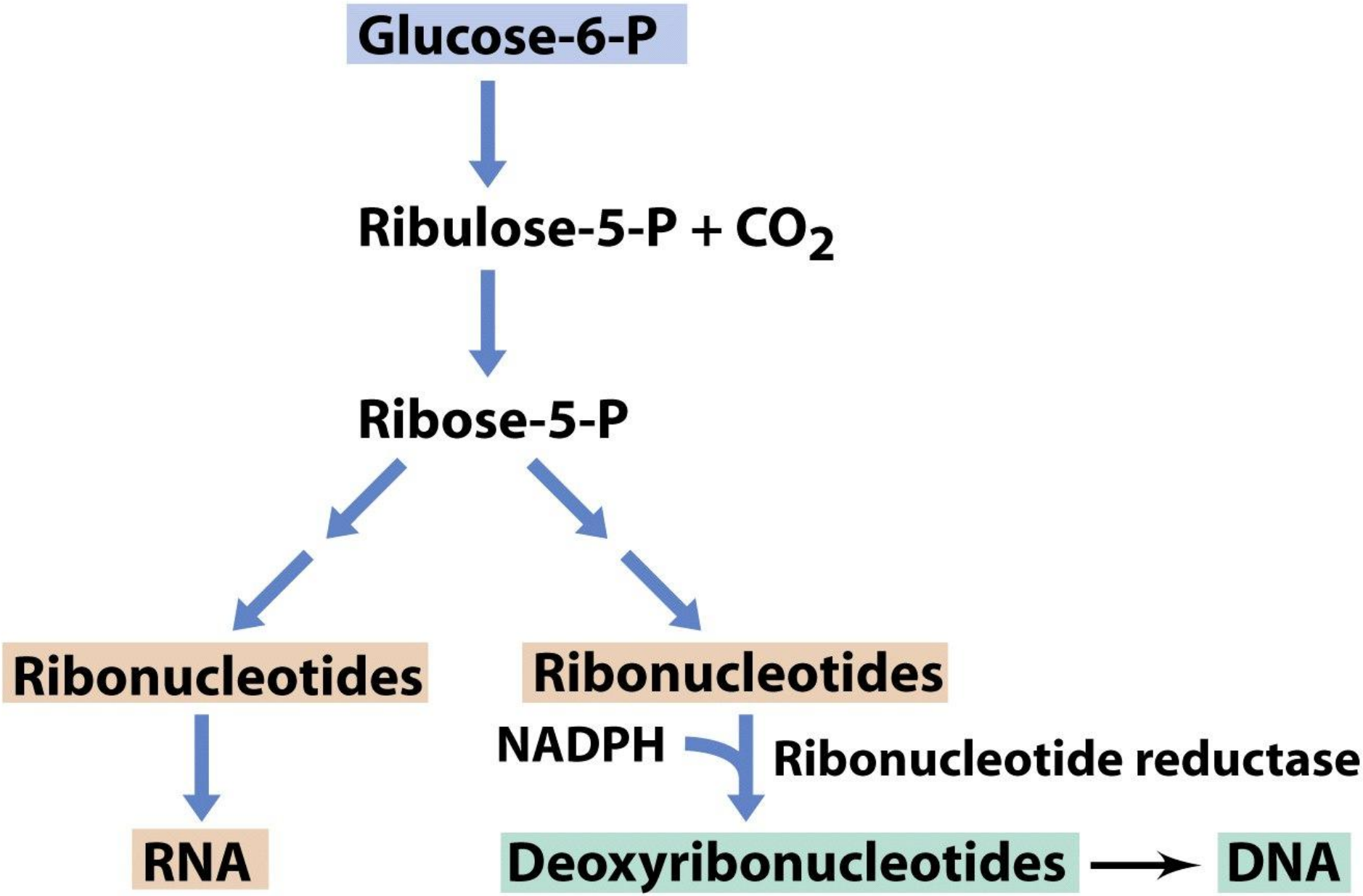
Esqueletos carbonados



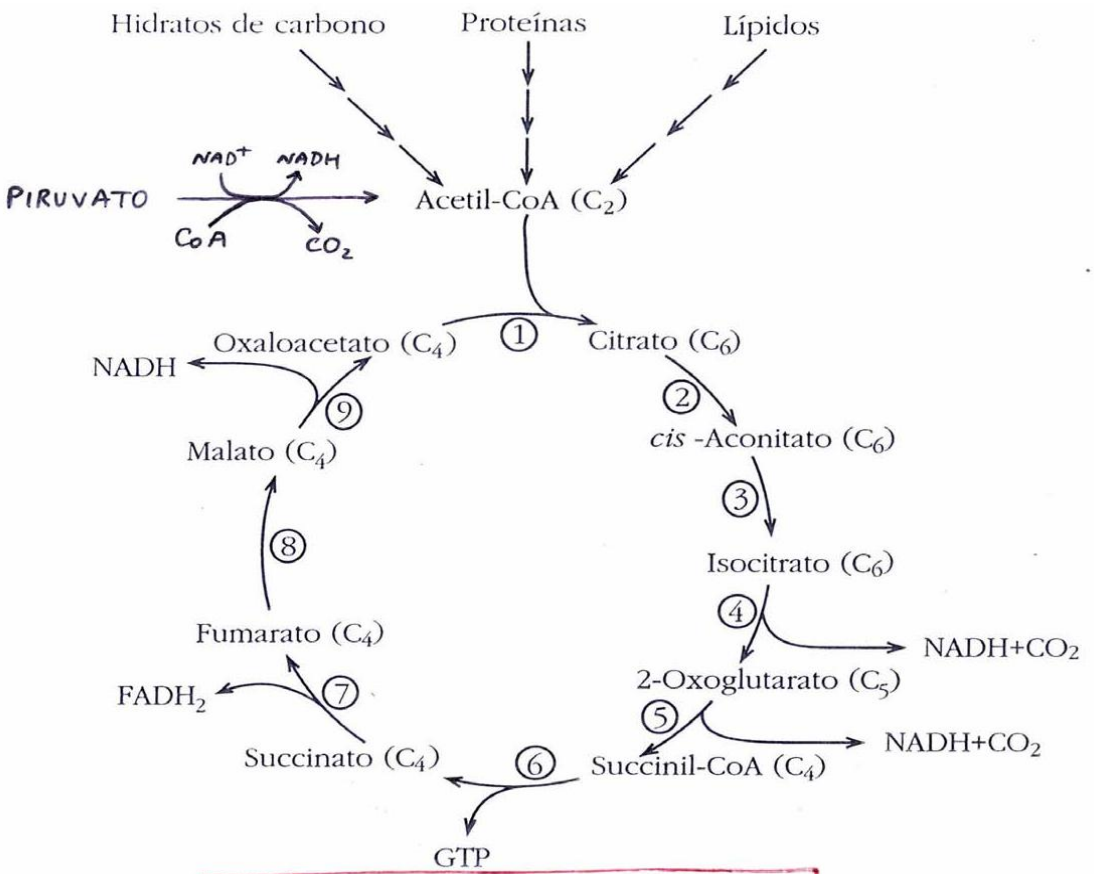
Glycolysis, pentose phosphate pathway, and citric acid cycle contribute precursors



Pentoses for nucleic acid synthesis



Ciclo do ácido cítrico

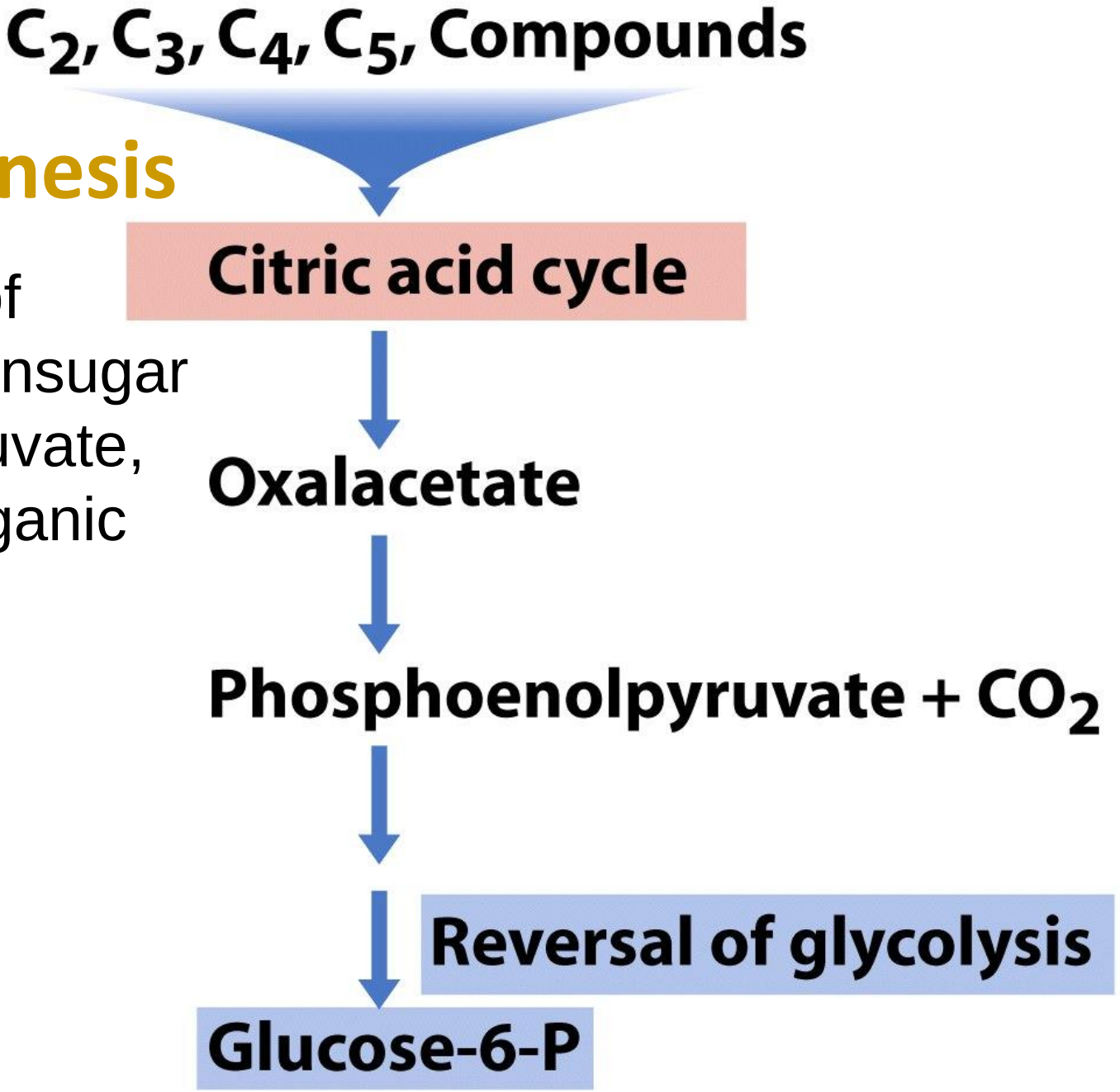


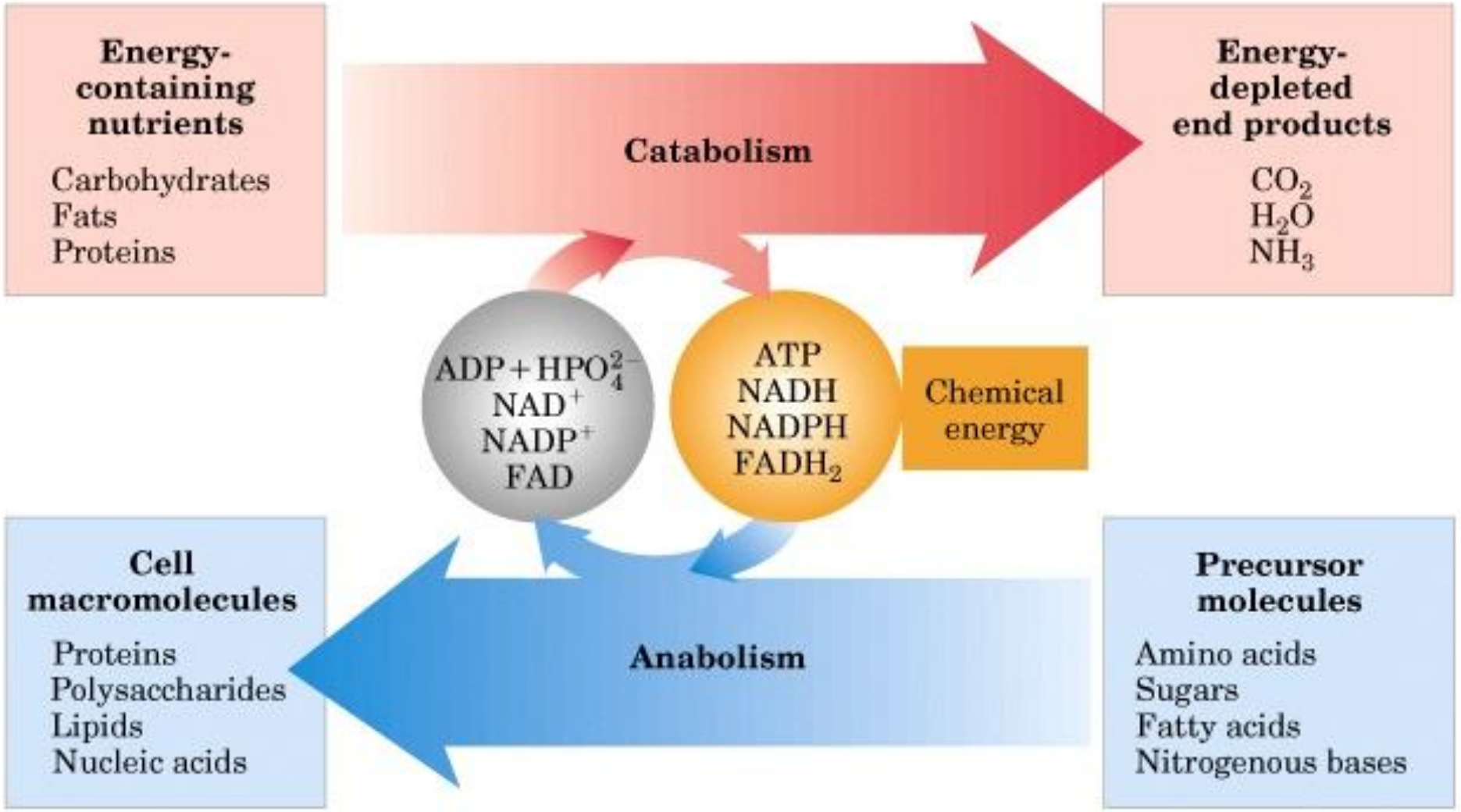
Esquema simplificado do ciclo do ácido cítrico

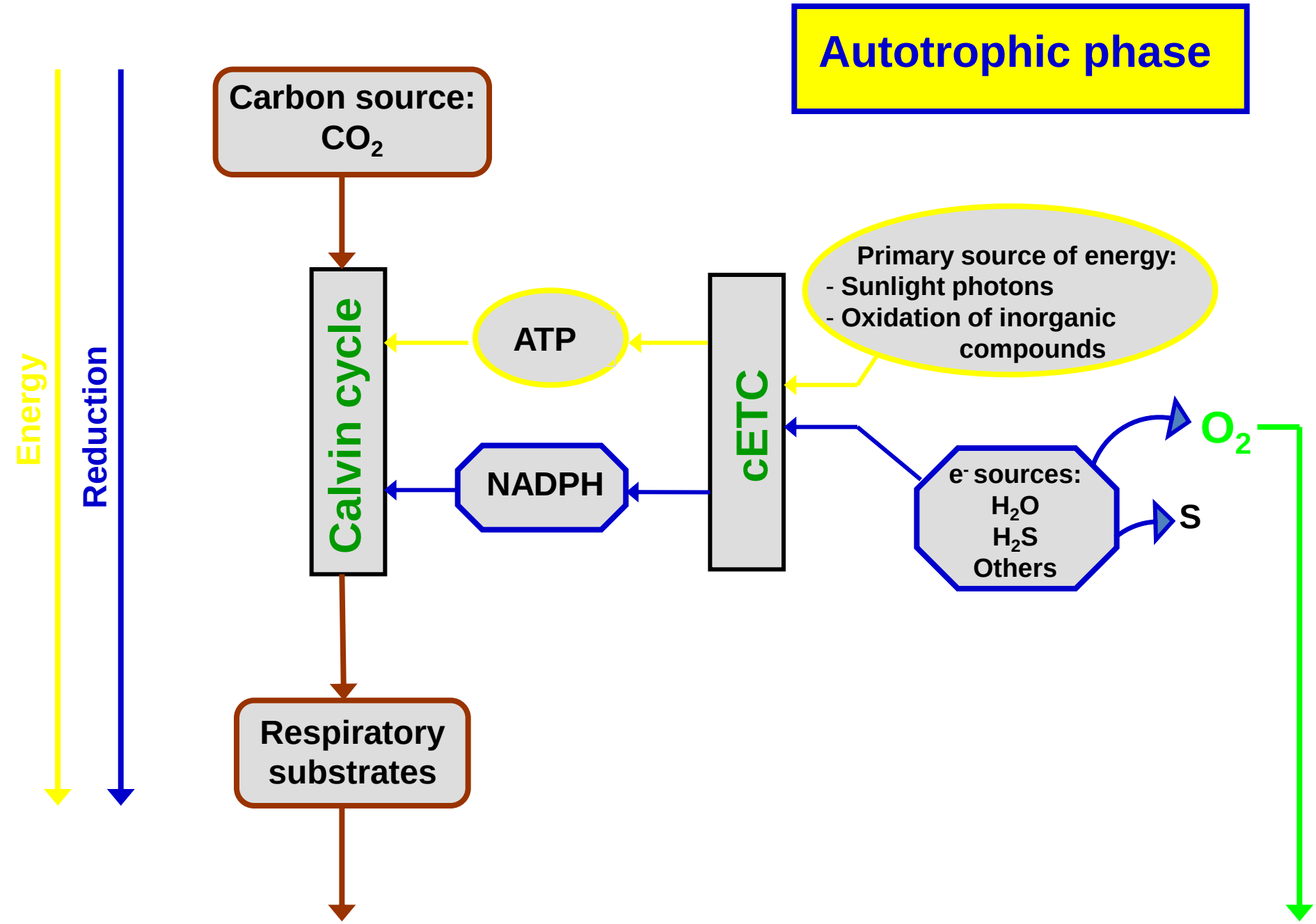
1 – Citrato sintase (EC 4.1.3.7)
2 – Aconitato hidratase ou aconitase (EC 4.2.1.3)
3 – Aconitato hidratase ou aconitase (EC 4.2.1.3)
4 – Isocitrato desidrogenase (EC 1.1.1.41-42)
5 – Complexo multienzimático 2-oxoglutarato desidrogenase (EC 1.2.4.2)
6 – Succinato-CoA ligase ou succinil-CoA sintetase (EC 6.2.1.4-5)
7 – Succinato desidrogenase (EC 1.3.99.1)
8 – Fumarato hidratase ou fumarase (EC 4.2.1.2)
9 – Malato desidrogenase (EC 1.1.1.37)

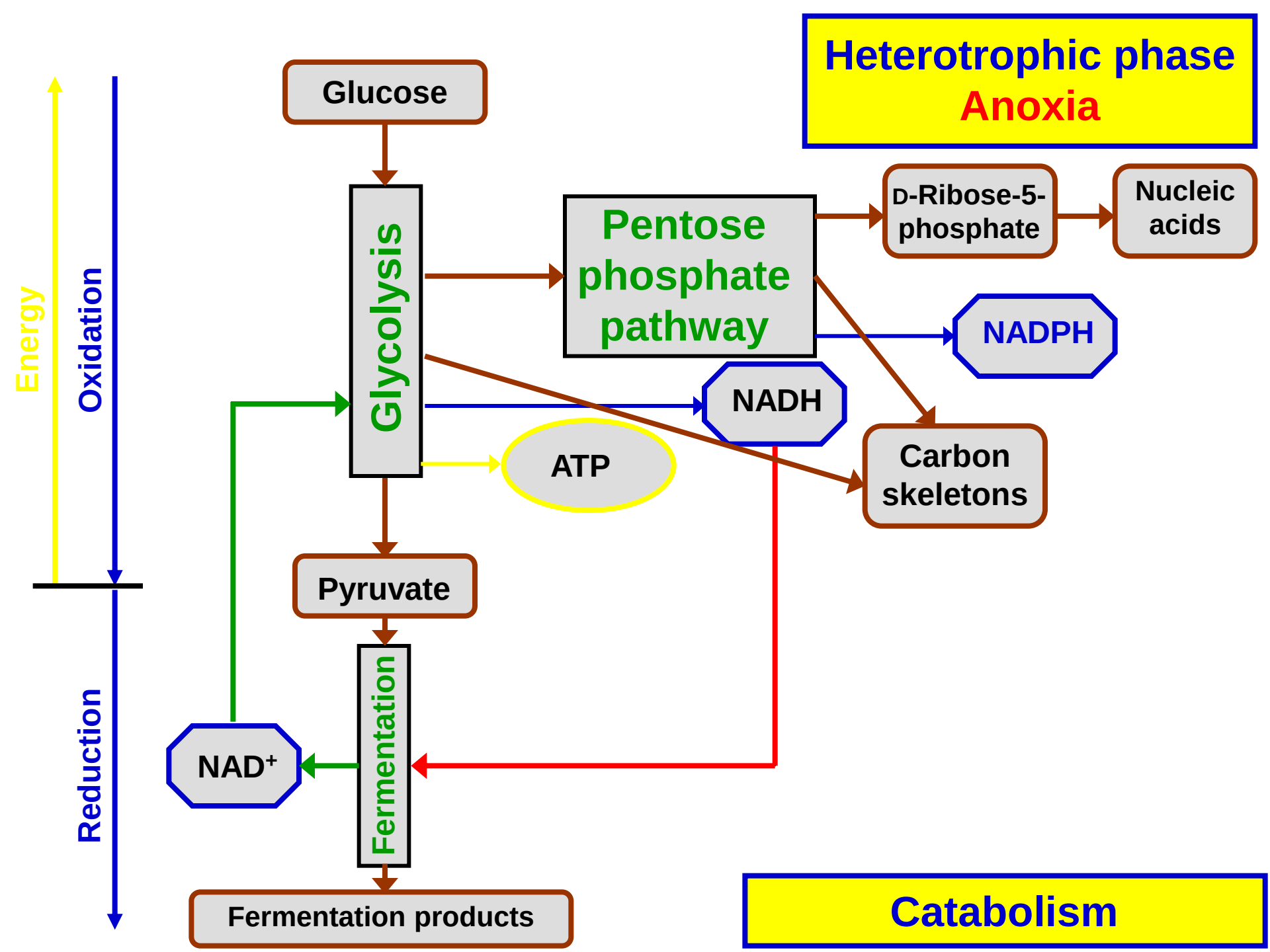
Gluconeogenesis

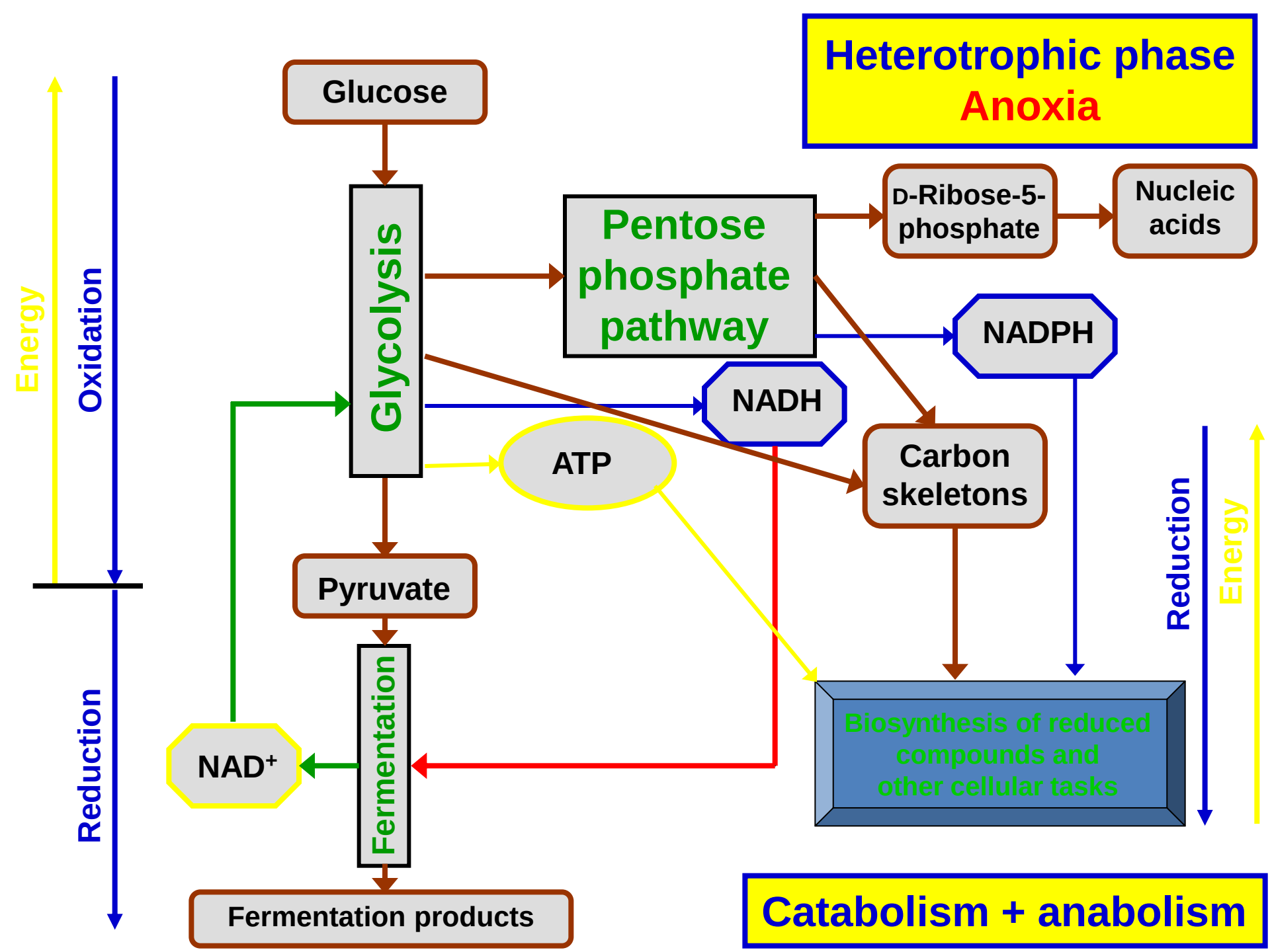
the production of glucose from nonsugar precursors (pyruvate, amino acids, organic acids, etc.) in heterotrophic organisms




Energy-containing nutrients
Carbohydrates
Fats
Proteins
Catabolism
Energy-depleted end products
CO_2
H_2O
NH_3
$ADP + HPO_4^{2-}$
NAD^+
$NADP^+$
FAD
ATP
NADH
NADPH
$FADH_2$
Chemical energy
Cell macromolecules
Proteins
Polysaccharides
Lipids
Nucleic acids
Anabolism
Precursor molecules
Amino acids
Sugars
Fatty acids
Nitrogenous bases


Autotrophic phase
Carbon source:
CO_2
Calvin cycle
ATP
NADPH
cETC
Primary source of energy:
- Sunlight photons
- Oxidation of inorganic compounds
e^- sources:
H_2O
H_2S
Others
O_2
S
Respiratory substrates
Energy
Reduction


Heterotrophic phase
Anoxia
Glucose
Glycolysis
Pentose phosphate pathway
D-Ribose-5-phosphate
Nucleic acids
NADPH
NADH
ATP
Carbon skeletons
Pyruvate
Fermentation
NAD+
Fermentation products
Energy
Oxidation
Reduction
Catabolism


Heterotrophic phase
Anoxia
Glucose
Glycolysis
Pentose phosphate pathway
D-Ribose-5-phosphate
Nucleic acids
NADPH
NADH
ATP
Carbon skeletons
Pyruvate
Fermentation
NAD+
Fermentation products
Biosynthesis of reduced compounds and other cellular tasks
Energy
Oxidation
Reduction
Reduction
Energy
Catabolism + anabolism

Condições de anaerobiose

FERMENTATION

Fermentation can have a variety of meanings, ranging from informal to more scientific definitions. The various meanings of fermentation may be summarized as follows:

- Any spoilage of food by microbes. For example, the spoilage of wine to vinegar. This is a very general usage of fermentation;
- Any process that produces alcoholic beverages or acidic dairy products (again general use);
- Any large scale microbial process occurring with or without air (industrial use);
- Any energy-releasing process that occurs only under anaerobic conditions (more scientific).

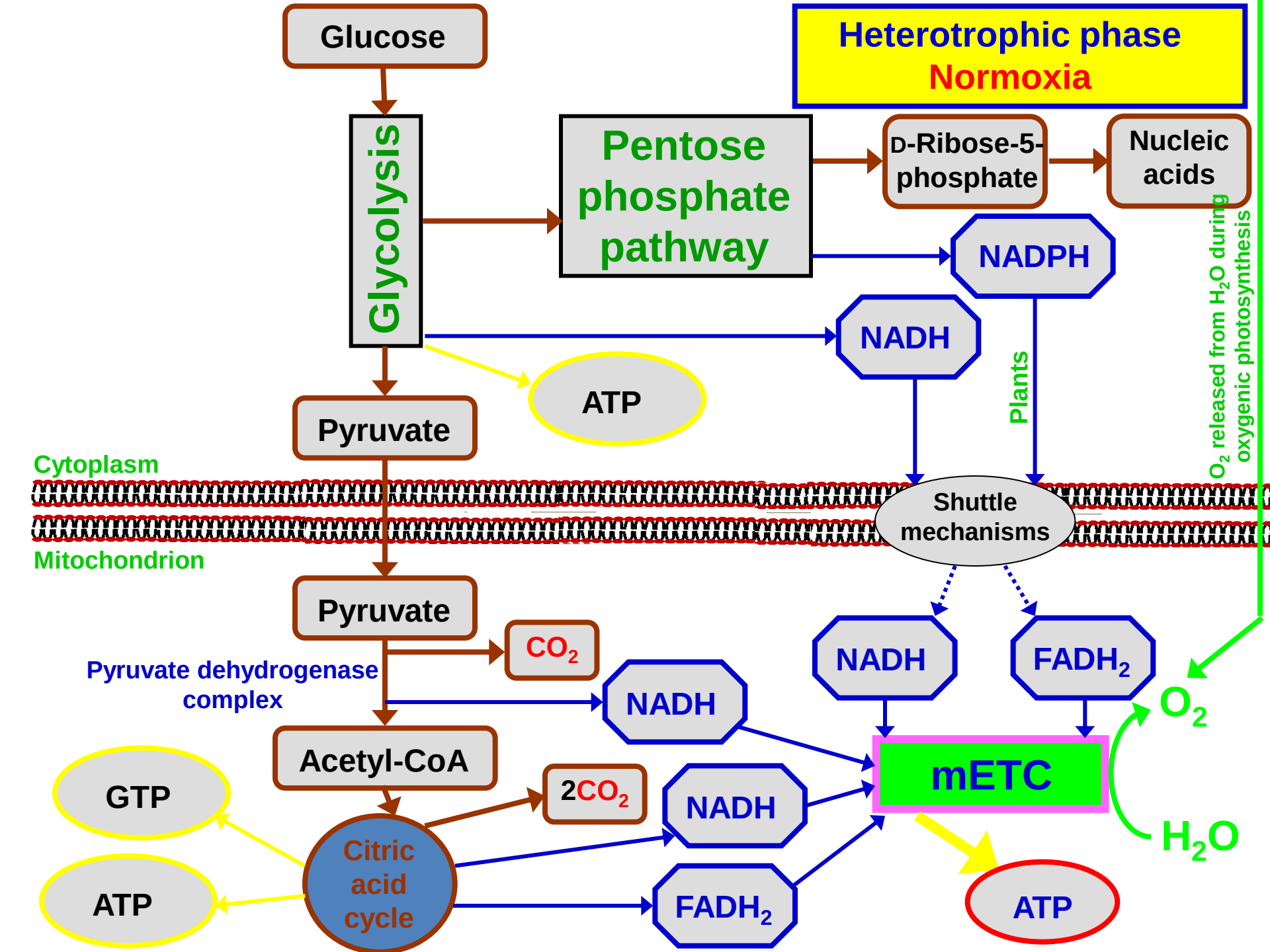
Any metabolic process that releases energy from a sugar or other organic molecule, does not need oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor. It is this last definition that we will use.

Some other key points that we need to keep in mind are:

- A complete fermentation pathway begins with a substrate, includes glycolysis and results in various end-products. The different fermentation pathways typically are named for the end products that are formed;.
- As far as an energy is concerned, fermentation does not generate ATP directly but recycles a limited amount of NAD^+ back into glycolysis to keep glycolysis going. Recall that each pass through glycolysis generates 2 ATP molecules by substrate level phosphorylation;
- All fermentation pathways are anaerobic;
- Cells that are capable of both respiration and fermentation will typically use respiration when possible. Respiration yields more energy from a lot less substrate.

TYPES OF FERMENTATION PATHWAYS

PATHWAY	END PRODUCTS	EXAMPLES
Lactic acid (Homolactic)	lactic acid (2 molecules)	*Lactobacillus*, *Enterococcus*, *Streptococcus* spp. Pathway can result in food spoilage.
Heterolactic	lactic acid, ethanol and CO_2	*Leuconostoc.* Used in sauerkraut production.
Alcohol	ethanol and CO_2	*Saccharomyces* (yeast). Important in production of alcoholic beverages, bread and gasohol.
Proprionic acid	proprionic acid and CO_2	*Proprionibacterium acnes*: metabolizes fatty acids in oil glands to proprionic acid. *Proprionibacterium freudenreichii* gives flavor to and produces holes in Swiss cheese.
Butyric acid	Butyric acid, butanol, acetone, isopropyl alcohol and CO_2	*Clostridium* spp. produce butyric acid that causes butter and cheese spoilage. Butanol and acetone are important organic solvents.
Butanediol	Butanediol and CO_2	Butanediol produced by *Enterobacter*, *Serratia*, *Erwinia* and *Klebsiella*. The intermediate, acetoin, is detected by the VP test. This test is used together with the MR test often to distinguish *Enterobacter* from *Escherichia coli* (VP-). *E.coli* is an important indicator organism of fecal contamination.
Mixed acid	ethanol, acetic acid, lactic acid, succinic acid, formic acid and CO_2	Variety of acid products. Typically carried out by members of the Enterobacteriaceae including *E. coli*, *Salmonella* and *Shigella* pathogens. Products detected by reaction with methyl red pH indicator.
Methanogenesis	methane and CO_2	Certain Archaea. Majority of earth's methane production.


Heterotrophic phase
Normoxia
Glucose
Glycolysis
Pentose phosphate pathway
D-Ribose-5-phosphate
Nucleic acids
NADPH
NADH
ATP
Pyruvate
Plants
O2 released from H2O during oxygenic photosynthesis
Cytoplasm
Mitochondrion
Shuttle mechanisms
Pyruvate
CO2
Pyruvate dehydrogenase complex
NADH
NADH
FADH2
O2
Acetyl-CoA
mETC
GTP
2CO2
NADH
Citric acid cycle
H2O
ATP
FADH2
ATP

ATP yield during respiration

Total ATP molecules from respiration of one molecule sucrose = 60

About 52% of energy is released from one sucrose molecule by oxidation

Only about 4% from the fermentation process

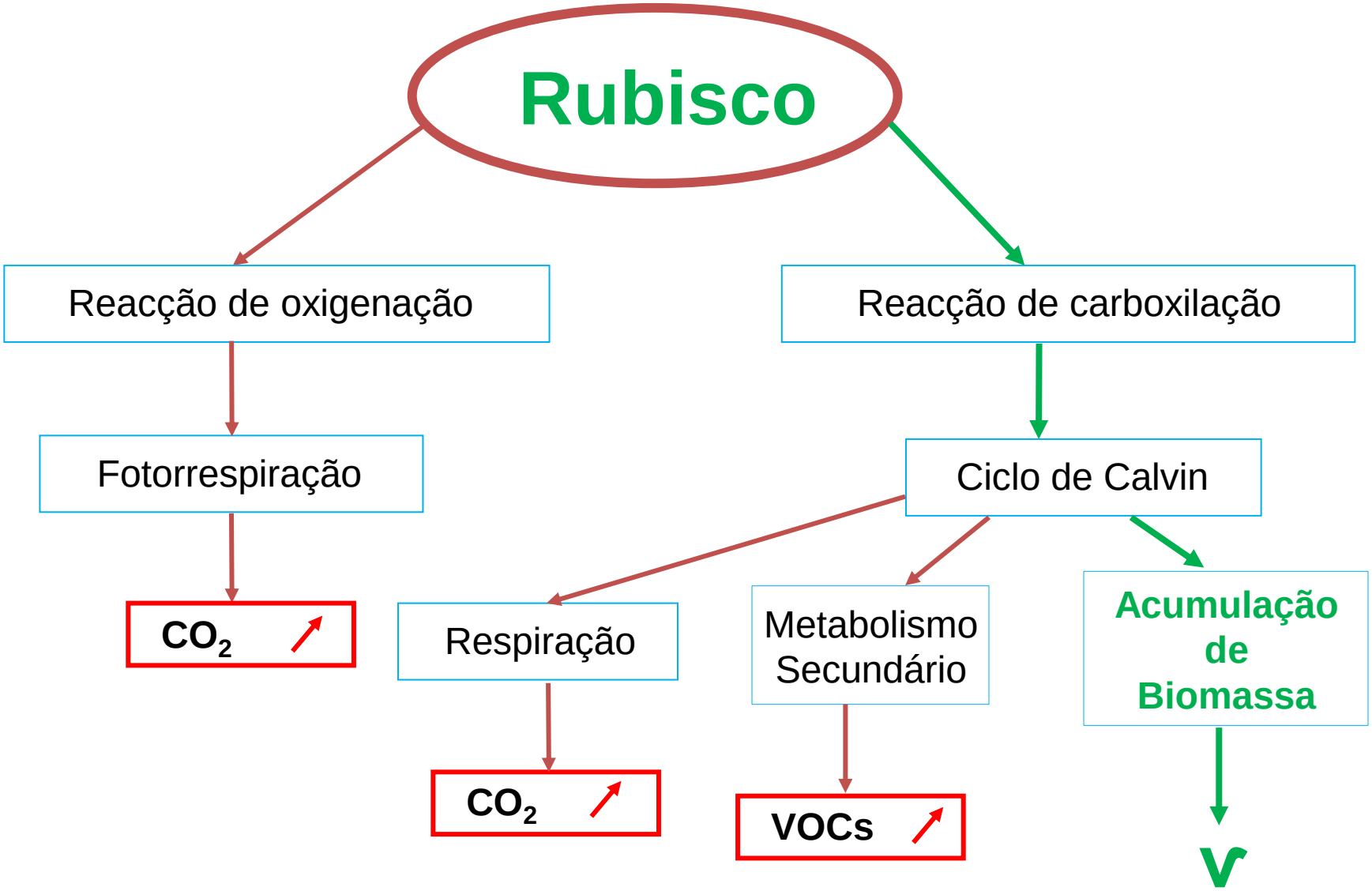
Efficiency comparisons

Total oxidation of glucose: -2870 kJ/mol

- Cellular oxidation of glucose yields about 30 ATP:
  [(30 x 30.5 kJ/mol)/2870 kJ/mol] x 100 = 31.9%

- Fermentation yields 2 ATP:
  [(2 x 30.5 kJ/mol)/2870 kJ/mol] x 100 = 2.1%

Actividade catalítica da Rubisco versus acumulação de biomassa



Electrons and Electronegativity

Most often, cell metabolites must be “prepared” by cellular metabolism, most notably by enzymes, to perform their specific role.

Recall glycolysis, in which an energy input must be made throughout the first part of this pathway, so that energy may be extracted a later stage.

Oxidation-Reduction and Energy-Rich Compounds

Oxidation - removal of electron(s) from a substance

Reduction – addition of electron(s) to a substance

Energy (ATP) is released or consumed during oxidation or reduction reactions, respectively

Reduction Potential Difference = $\Delta E^{o\prime}$

$$\Delta E^{o\prime} = E^{\circ}\text{ (acceptor)} - E^{\circ}\text{ (donor)}$$

- measured in volts
- The more *positive* the reduction potential difference is, the easier the redox reaction
- Energy can be derived from the spontaneous transfer of electrons

♦ The standard reduction potential can be related to standard free energy change by:

$$\Delta G^{o\prime} = - n F \Delta E^{o\prime}$$

where n = # electrons transferred = 1,2,3

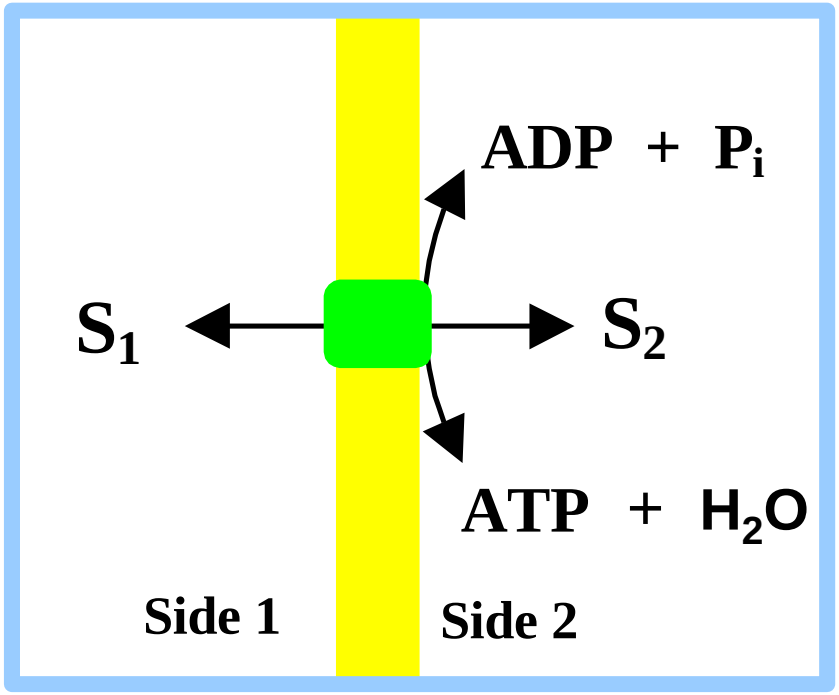
F = 96.5 kJ/V, called the Faraday constant

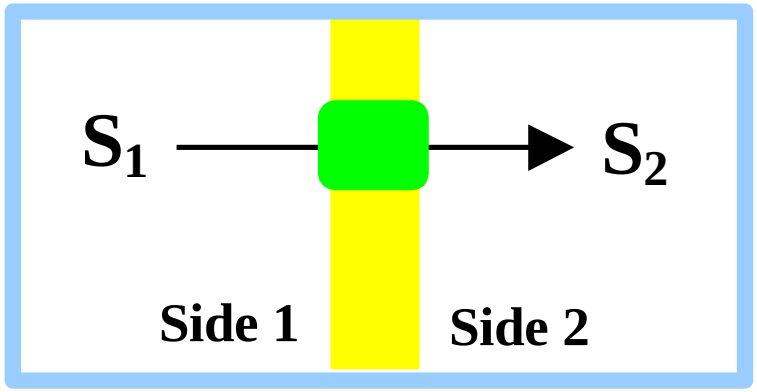
Energy coupling in ion transport

Ion Transport may be coupled to a chemical reaction, e.g., hydrolysis or synthesis of ATP.

It should be recalled that the ATP hydrolysis/synthesis reaction is:

$$ATP + H_2O \leftrightarrow ADP + P_i$$



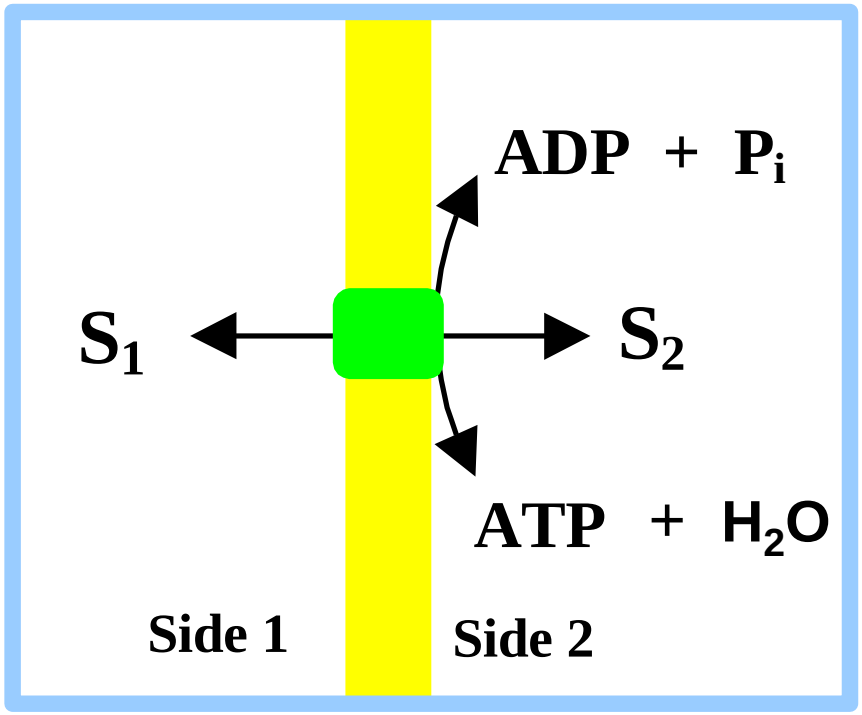


The free energy change (electrochemical potential difference) associated with transport of an ion **S** across a membrane from side 1 to side 2 is:

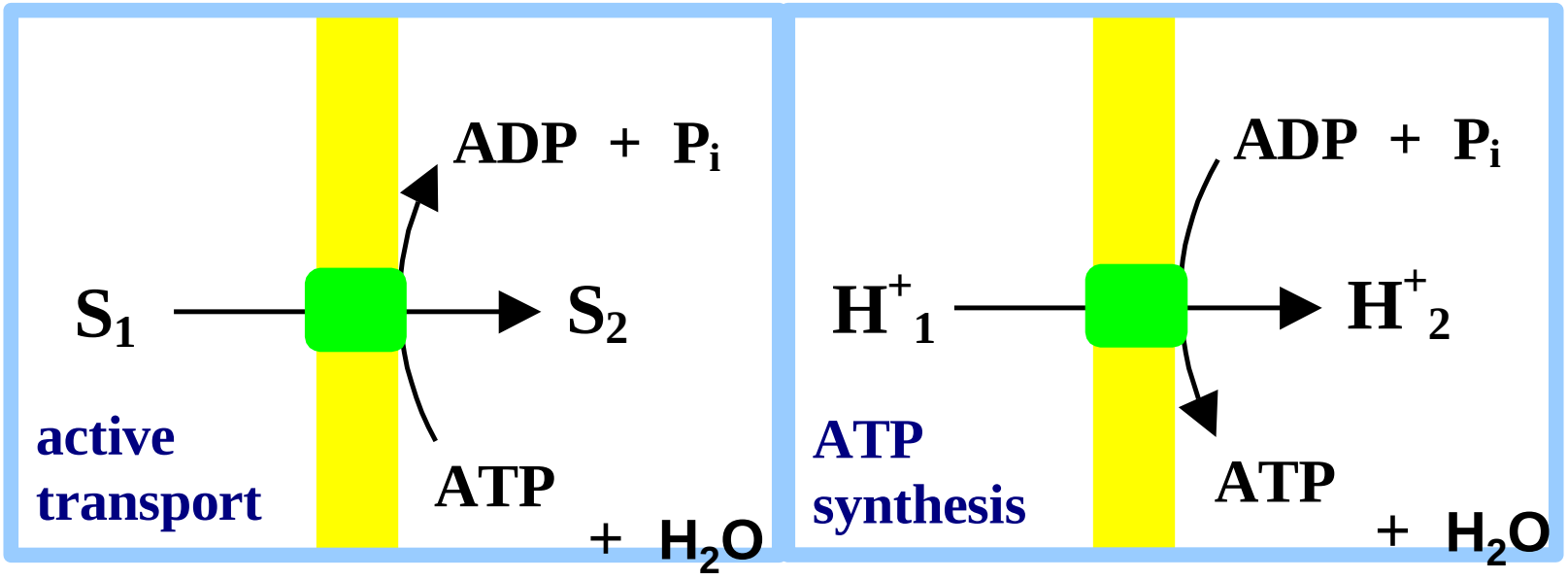
$$\Delta G = R\,T\,\ln\left(\frac{[S]_2}{[S]_1}\right) + n\,F\,\Delta E'^{\circ}$$

R = gas constant, **T** = temperature, **n** = charge on the ion, **F** = Faraday constant, E'° = voltage.

Since free energy changes are additive, the **spontaneous direction** for the coupled reaction will depend on **relative magnitudes** of:



- **ΔG for ion flux** - varies with ion gradient & voltage.
- **ΔG for chemical reaction** - negative $\Delta G^{o'}$ for ATP hydrolysis; ΔG depends also on [ATP], [ADP], [P_i].

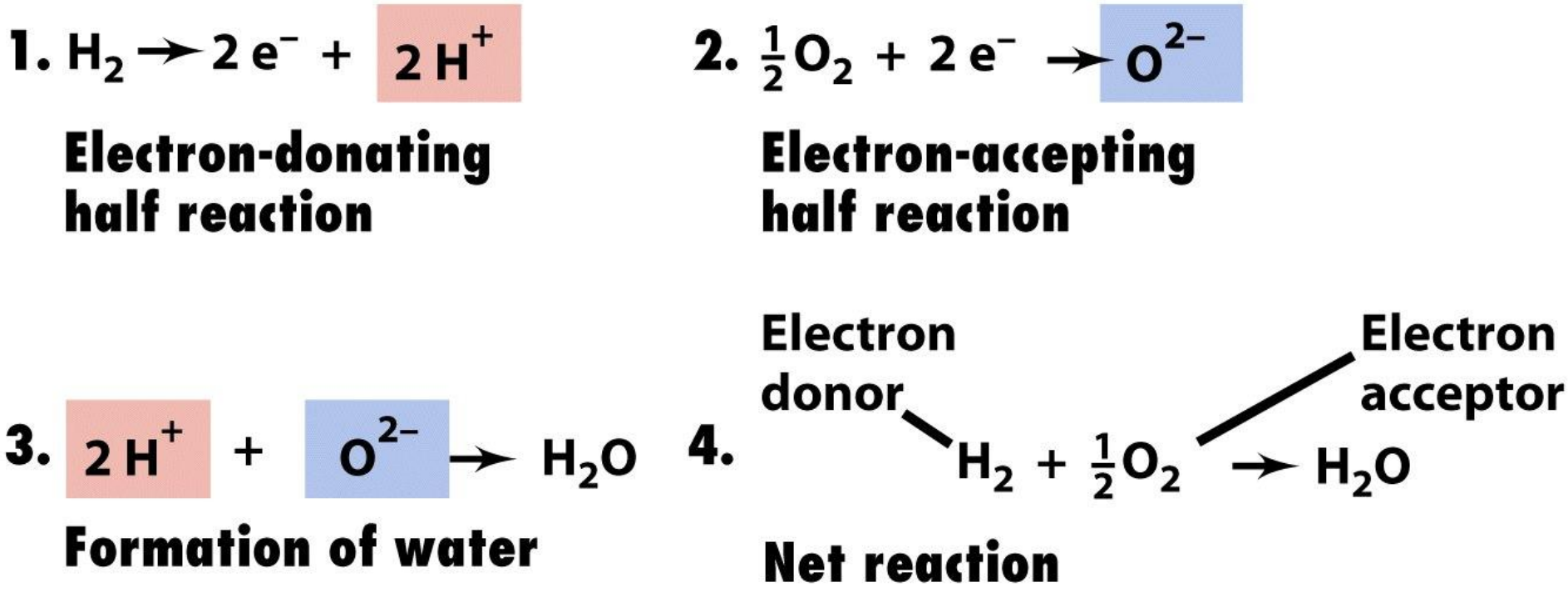


Two examples:

Spontaneous Active Transport: spontaneous ATP hydrolysis (negative ΔG_1) is coupled to (drives) ion flux against a gradient (positive ΔG_2) if $\Delta G_1 + \Delta G_2 < 0$.

Spontaneous ATP synthesis: spontaneous H^+ flux (negative ΔG_1) is coupled to (drives) ATP synthesis (positive ΔG_2) if $\Delta G_1 + \Delta G_2 < 0$.

- Oxidation-reduction (redox) reactions involve the transfer of electrons from electron donor to electron acceptor:



Most of the chemistry in living system is the result of interplay of electrons in the outer orbits of H, C, N, O, P and S atoms.

Electronegativity: It is the tendency of an atom to attract an electron. F is the most electronegative element, but in biological system, O is the most electronegative atom.

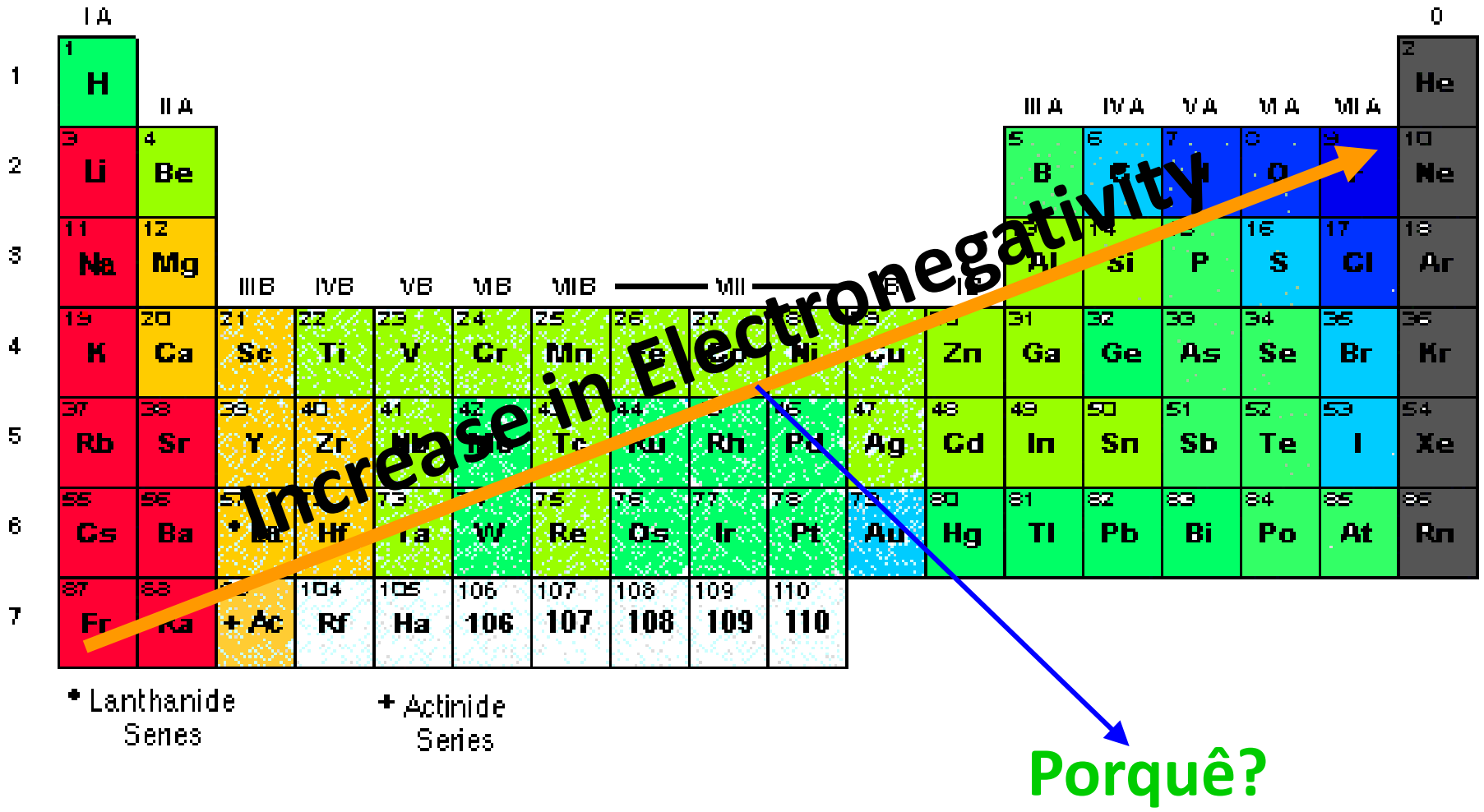
Many of the reactions in biological systems are catalysed by enzymes and involve the lone pair of electrons in N, O, S and P.

In many complex reactions, particular groups of atoms (as part of compounds) are actively involved in chemical reactions. They are called functional groups.

The Electronegativities of Some Elements

Element	Electronegativity*
F	4.0
O	3.5
Cl	3.0
N	3.0
Br	2.8
S	2.5
C	2.5
I	2.5
Se	2.4
P	2.1
H	2.1
Cu	1.9
Fe	1.8
Co	1.8
Ni	1.8
Mo	1.8
Zn	1.6
Mn	1.5
Mg	1.2
Ca	1.0
Li	1.0
Na	0.9
K	0.8

*The higher the number, the more electronegative (the greater the electron affinity of) the element.


IA
0
1 H
II A
III A
IV A
V A
VI A
VII A
2 He
3 Li
4 Be
5 B
6 C
7 N
8 O
9 F
10 Ne
11 Na
12 Mg
III B
IV B
V B
VI B
VII B
VIII
I B
II B
13 Al
14 Si
15 P
16 S
17 Cl
18 Ar
19 K
20 Ca
21 Sc
22 Ti
23 V
24 Cr
25 Mn
26 Fe
27 Co
28 Ni
29 Cu
30 Zn
31 Ga
32 Ge
33 As
34 Se
35 Br
36 Kr
37 Rb
38 Sr
39 Y
40 Zr
41 Nb
42 Mo
43 Tc
44 Ru
45 Rh
46 Pd
47 Ag
48 Cd
49 In
50 Sn
51 Sb
52 Te
53 I
54 Xe
55 Cs
56 Ba
57 *La
72 Hf
73 Ta
74 W
75 Re
76 Os
77 Ir
78 Pt
79 Au
80 Hg
81 Tl
82 Pb
83 Bi
84 Po
85 At
86 Rn
87 Fr
88 Ra
89 +Ac
104 Rf
105 Ha
106 106
107 107
108 108
109 109
110 110
Increase in Electronegativity
* Lanthanide Series
+ Actinide Series
Porquê?

Electrons, Chemical Bonds and Periodic Table

The chemical properties of an atom are determined largely by how full or empty the outer electron shell is. For example, atoms of fluorine (F), chlorine (Cl) and the other elements in that second from the last column of the periodic table need only one electron to fill the outer shell. These atoms have a very strong tendency to steal electrons from other atoms. Oxygen and sulphur have 6 electrons in their outer shell which again holds 8 maximum. Thus these elements tend to steal electrons.

Elements such as Lithium (Li) Sodium (Na) and Potassium (K) on the left hand side of the periodic table have an almost empty shell and these elements readily give up those outer shell electrons to atoms such as oxygen and chlorine. Elements that tend to give up electrons to other atoms are called metals.

Elements in the middle of the periodic table tend to share electrons rather than give them up or take them entirely. Many of these such as iron, copper or gold are also considered metals.

The elements at the far right: Helium, Neon, Argon etc... are chemically inert because they have a full outer shell. They will only react with other chemicals under very special conditions. These elements are sometimes called the 'noble' or inert gases because it is so difficult to get them to form chemical bonds.

Electronegativity. The tendency of atoms to grab electrons is called **electronegativity**. The previous periodic table shows relative electronegativities. Higher electronegativites are shown in blue. Notice Fluorine (F) is the most electronegative element. Oxygen and chlorine less so.

Note that many of the elements we think of as metals iron (Fe), nickel (Ni), Copper (Cu), Silver (Ag) are intermediate in electronegativity between the metals in red on the far left of the table (low electronegativity) and the column near the right that contains fluorine (F). But real strong metals such as sodium or potassium ready give up electrons.

So the rule of thumb is atoms of the elements on the left side of the periodic table have a tendency to give up their electrons to atoms of the elements in the row beginning with chlorine. This is important because it dicatates the kinds of chemical bonds there elements will form with each other.

- **The tendency of a compound to accept or release electrons is expressed quantitatively by its reduction potential, E_0'.**

- In a redox reaction

The substance oxidized is the **electron donor**

The substance reduced is the **electron acceptor**

Oxidation-reduction reactions in biological systems

As we have discussed so far transfer of phosphoryl groups as a central feature of metabolism and in energy transfer (due to the tendency of ATP to get hydrolyzed desperately). An equally important reaction mechanism to transfer free energy in biological systems is the transfer of electrons in oxidation-reduction reactions (due to tendency of some atoms to accept electron desperately).

Oxygen is one of the strongest electron acceptors in biological systems, due to it very high electronegativity and hence to it's strong oxidizing capacity. Fluorine is the strongest oxidizing agent but it is present in trace amount in living system.

Oxidizing ability: capacity to accept electrons depends on the electro-negativity of the atom.

Flow of electrons can be used to do useful work as is done in battery operated motors, the electromotive force (EMF). In living systems, electron flow from various electron carriers to oxygen and the EMF generated is utilized for various energy transduction reactions.

Standard Reduction Potentials of Some Biologically Important Half-Reactions, at 25 °C and pH 7

Half-reaction	$E'°$ (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome *f* $(Fe^{3+}) + e^- \longrightarrow$ cytochrome *f* (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) $+ e^- \longrightarrow Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome *a* $(Fe^{3+}) + e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* $(Fe^{3+}) + e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome c_1 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* $(Fe^{3+}) + e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Ubiquinone $+ 2H^+ + 2e^- \longrightarrow$ ubiquinol $+ H_2$	0.045
Fumarate$^{2-} + 2H^+ + 2e^- \longrightarrow$ succinate^{2-}	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA $+ 2H^+ + 2e^- \longrightarrow$ butyryl-CoA	−0.015
Oxaloacetate$^{2-} + 2H^+ + 2e^- \longrightarrow$ malate^{2-}	−0.166
Pyruvate$^- + 2H^+ + 2e^- \longrightarrow$ lactate$^-$	−0.185
Acetaldehyde $+ 2H^+ + 2e^- \longrightarrow$ ethanol	−0.197
FAD $+ 2H^+ + 2e^- \longrightarrow FADH_2$	−0.219*
Glutathione $+ 2H^+ + 2e^- \longrightarrow$ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid $+ 2H^+ + 2e^- \longrightarrow$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow$ NADH	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow$ NADPH	−0.324
Acetoacetate $+ 2H^+ + 2e^- \longrightarrow$ β-hydroxybutyrate	−0.346
α-Ketoglutarate $+ CO_2 + 2H^+ + 2e^- \longrightarrow$ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin $(Fe^{3+}) + e^- \longrightarrow$ ferredoxin (Fe^{2+})	−0.432

• One way to view electron transfer reactions in biological systems is to imagine a vertical tower. The tower represents the range of reduction potentials possible for redox couples in nature, from those with the most negative E^{0}'s on the top to those with the most positive at E^{0}'s on the bottom.

• Anaerobic (anoxic)

H_2 + fumarate (e^- acceptor) ⟶ succinate

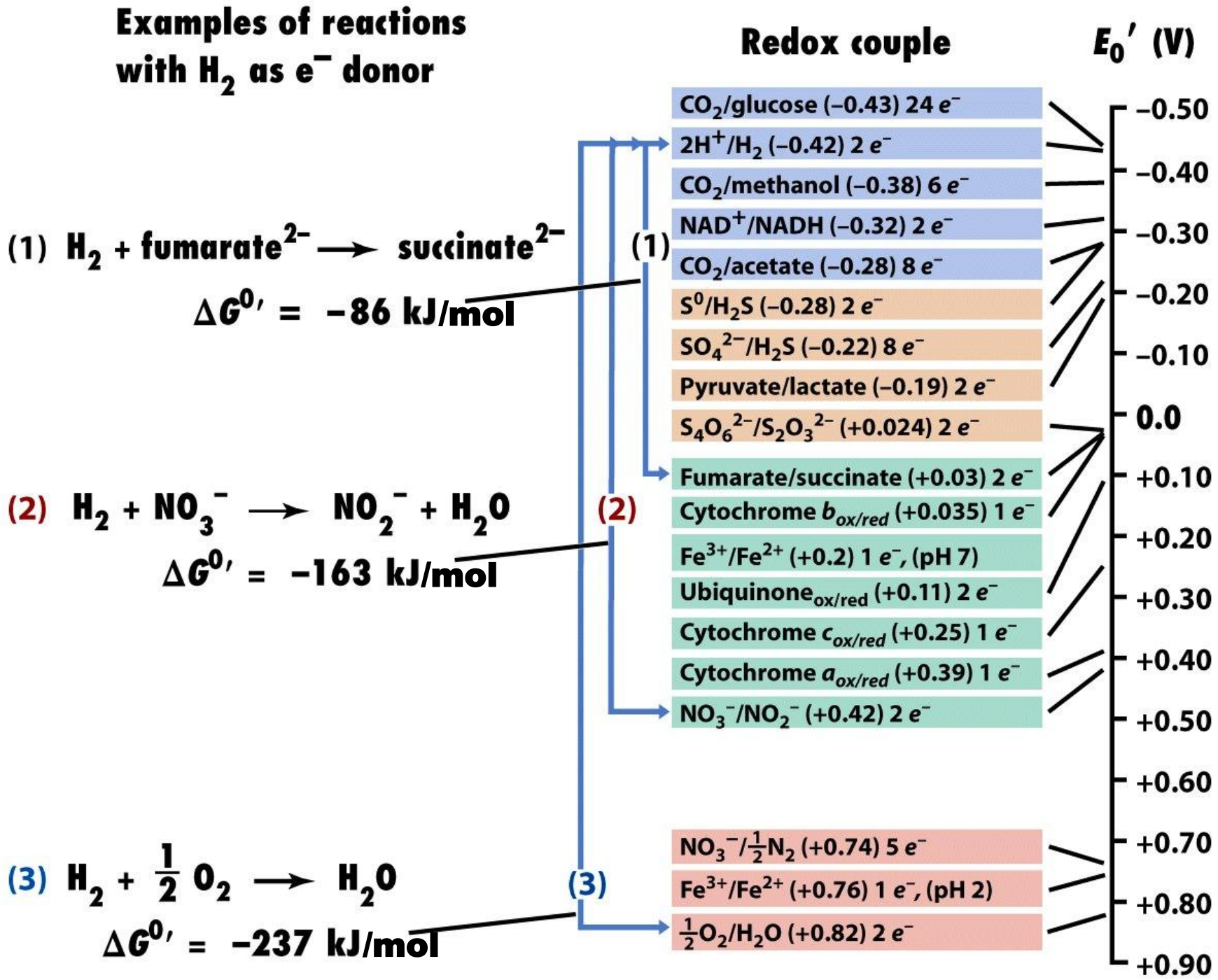
• Aerobic (normoxic)

Succinate + ½ O_2 ⟶ fumarate + H_2O

(e^- donor)

• Others

Succinate + NO_3^- ⟶ fumarate + NO_2^- + H_2O


Examples of reactions with H_2 as e^- donor
Redox couple
E_0' (V)
(1) H_2 + fumarate^{2-} → succinate^{2-}
$\Delta G^{0\prime}$ = −86 kJ/mol
(2) $H_2 + NO_3^- \rightarrow NO_2^- + H_2O$
$\Delta G^{0\prime}$ = −163 kJ/mol
(3) $H_2 + \frac{1}{2} O_2 \rightarrow H_2O$
$\Delta G^{0\prime}$ = −237 kJ/mol
CO_2/glucose (−0.43) 24 e^-
$2H^+/H_2$ (−0.42) 2 e^-
CO_2/methanol (−0.38) 6 e^-
NAD^+/NADH (−0.32) 2 e^-
CO_2/acetate (−0.28) 8 e^-
S^0/H_2S (−0.28) 2 e^-
SO_4^{2-}/H_2S (−0.22) 8 e^-
Pyruvate/lactate (−0.19) 2 e^-
$S_4O_6^{2-}/S_2O_3^{2-}$ (+0.024) 2 e^-
Fumarate/succinate (+0.03) 2 e^-
Cytochrome $b_{ox/red}$ (+0.035) 1 e^-
Fe^{3+}/Fe^{2+} (+0.2) 1 e^-, (pH 7)
Ubiquinone$_{ox/red}$ (+0.11) 2 e^-
Cytochrome $c_{ox/red}$ (+0.25) 1 e^-
Cytochrome $a_{ox/red}$ (+0.39) 1 e^-
NO_3^-/NO_2^- (+0.42) 2 e^-
$NO_3^-/\frac{1}{2}N_2$ (+0.74) 5 e^-
Fe^{3+}/Fe^{2+} (+0.76) 1 e^-, (pH 2)
$\frac{1}{2}O_2/H_2O$ (+0.82) 2 e^-
(1)
(2)
(3)
−0.50
−0.40
−0.30
−0.20
−0.10
0.0
+0.10
+0.20
+0.30
+0.40
+0.50
+0.60
+0.70
+0.80
+0.90

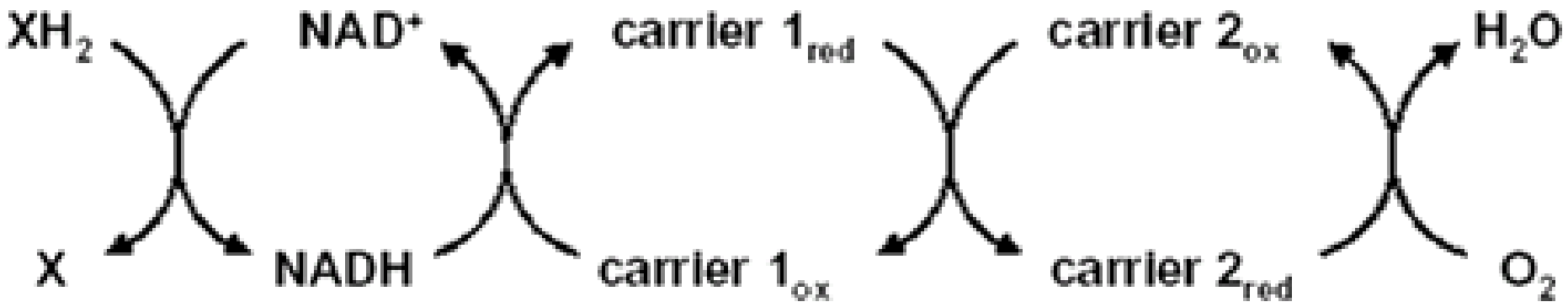
ELECTRON TRANSPORT CHAINS

Electron transport systems (ETCs) consist of a series of membrane-associated electron carriers that function in an integrated way to carry electrons from the primary electron donor ($NADH/FADH_2$ in the mETC or H_2O in the cETC) to the terminal electron acceptor (molecular oxygen in the mETC or $NADP^+$ in the cETC).

- Electrons can move through a chain of donors and acceptors.

- In the electron transport chain, electrons flow down a gradient.

- Electron transport movement **ALWAYS TAKE PLACE** from a carrier with low reduction potential (higher tendency to donate electrons or lower affinity towards electrons) toward carriers with higher reduction potential (higher tendency to accept electrons or higher affinity towards electrons).



- The overall voltage drop from NADH to O_2 is

$E^{O\prime}$ (NADH) = -0.32 V

$E^{O\prime}$ (O_2) = +0.82 V

$\Delta E^{O\prime}$ = +0.82 V – (-0.32 V)

$\Delta E^{O\prime}$ = 1.14 V

$$\tfrac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O \qquad E^{o\prime} = +0.82\ V$$

$$NADH \longrightarrow NAD^+ + H^+ + 2e^- \qquad E^{o\prime} = +0.32\ V$$

$$\tfrac{1}{2}O_2 + 2H^+ + NADH \longrightarrow H_2O + NAD^+ + H^+ \qquad \Delta E^{o\prime} = +1.14\ V$$

- This corresponds to a large free energy change of

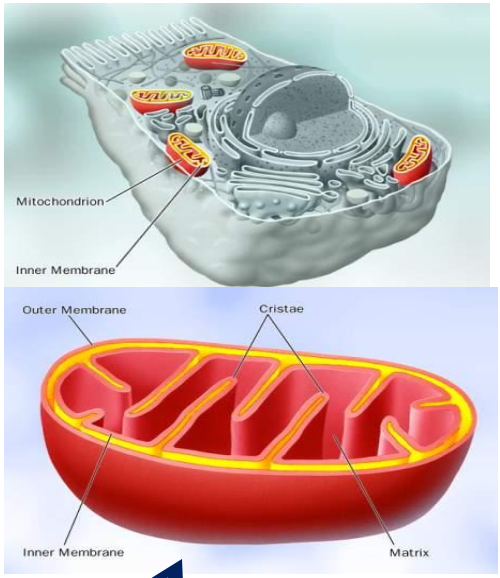
$\Delta G^{\circ\prime} = - n F \Delta E^{\circ\prime} = -220$ kJ/mol (n =2)

- Since ATP requires 30.5 kJ/mol to form from ADP, more than enough energy is available to synthesize 3 ATPs when the electrons move from NADH to O_2.

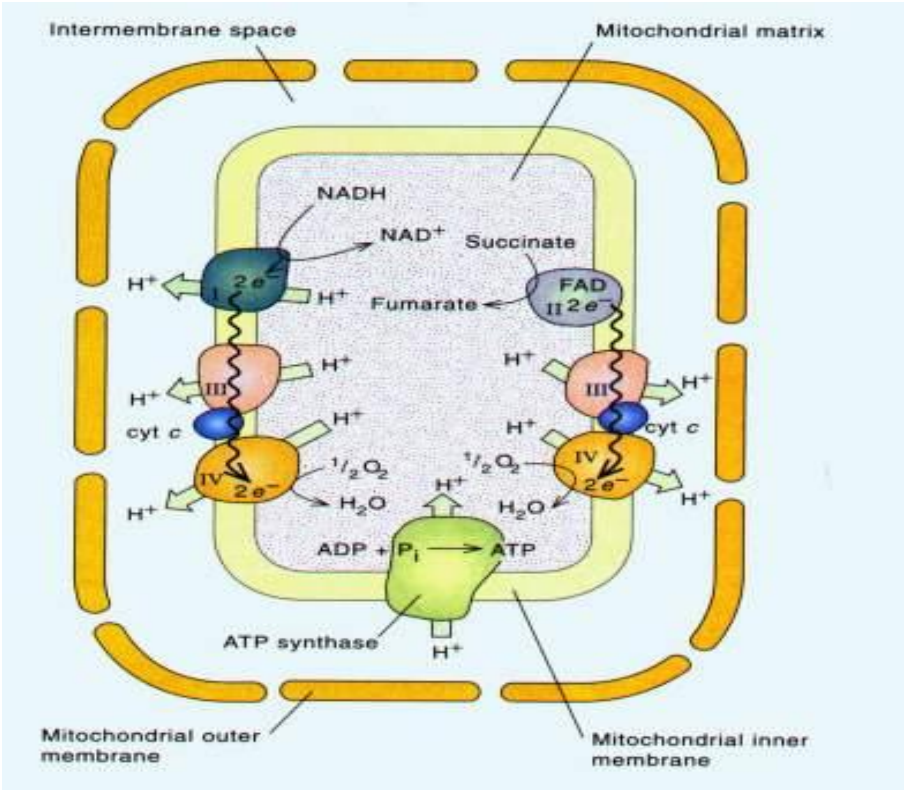
The mitochondrial electron transport chain (mETC)

Localização da cadeia de transporte de electrões (CTE) nas células eucariotas

A CTE está localizado na **membrana interna** dos mitocôndrios:



mitocôndrio



A cadeia mitocondrial de transporte de electrões ou cadeia respiratória das células vegetais é mais complexa do que a das células animais.

Podemos, assim, considerar:

- A via principal de transporte de electrões, que ocorre nos mitocôndrios vegetais e animais, dita via citocrómica ou via sensível ao cianeto;

- As vias alternativas de transporte de electrões, que ocorrem exclusivamente nos mitocôndrios vegetais.

A via principal de transporte de electrões, via citocrómica ou via sensível ao cianeto

Electron transport chain

- **Catalyzes a flow of electrons from NADH to O_2**
- **Electron transport is coupled with formation of proton gradient → used for ATP synthesis**
- **Consists of 5 complexes:**
  - Complex I (NADH dehydrogenase)
  - Complex II (Succinate dehydrogenase)
  - Complex III (Cytochrome bc_1 complex)
  - Complex IV (Cytochrome c oxidase)
  - Complex V (ATP synthase)

O NADH e a $FADH_2$ são moléculas ricas em energia, porque cada uma delas possui um par de electões com um elevado potencial de transferência:

- NADH $E'_0 = -0,32$ V
- $FADH_2$ $E'_0 = -0,04$ V

Têm, por isso, potenciais de redução negativos, ao passo que o O_2 tem um potencial de redução fortemente positivo ($E'_0 = +0,82$ V).

Como o NADH tem um potencial de redução mais negativo que o $FADH_2$, a sua oxidação pelo O_2 liberta mais energia do que a do $FADH_2$:

$NADH + H^+ + 1/2O_2 \rightarrow NAD^+ + H_2O$ $\quad \Delta G^{o'} = -218$ kJ.mol^{-1}

$FADH_2 + 1/2O_2 \rightarrow FAD + H_2O$ $\quad \Delta G^{o'} = -166$ kJ.mol^{-1}

Por este motivo, na cadeia de transporte de electrões, a oxidação de uma molécula de NADH dá origem à síntese de 2,5 a 3 moléculas de ATP, enquanto que a oxidação de uma molécula de $FADH_2$ fornece apenas 1,5 a 2 moléculas de ATP.

Os electrões do NADH e a $FADH_2$ não são transferidos directamente para o O_2. Eles são transferidos através de uma série de moléculas transportadoras, cujos potenciais de redução vão aumentando sucessivamente até ao O_2.

Isto permite libertar a energia em pequenas porções, tornando termodinamicamente mais eficiente a sua conservação sob a forma de ATP.

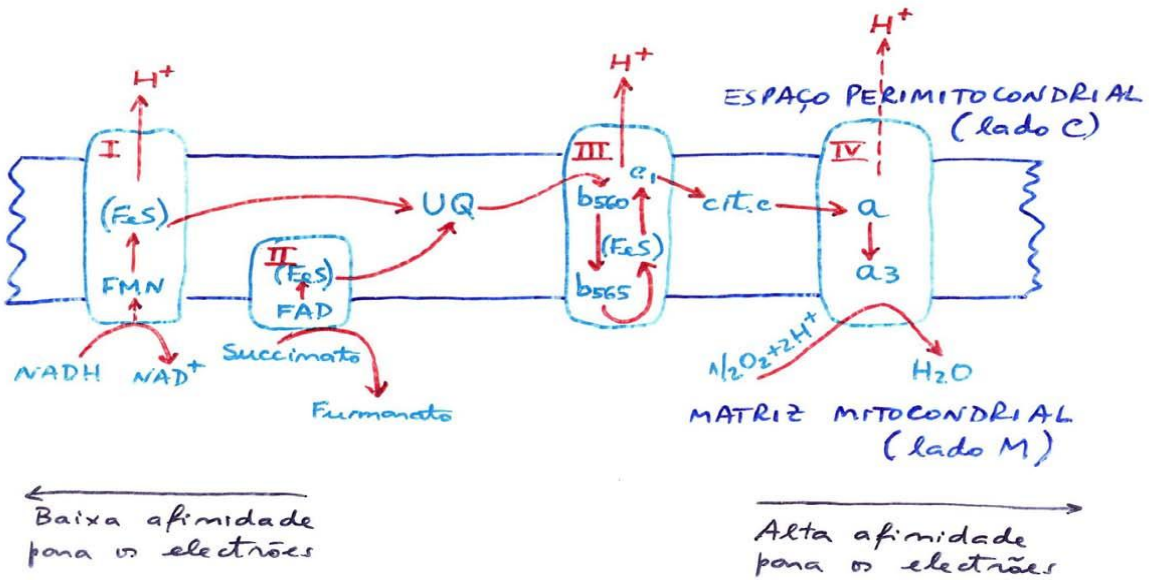
In the mETC, electrons move from a high energy state to a low energy state, i.e. from NADH to O_2.

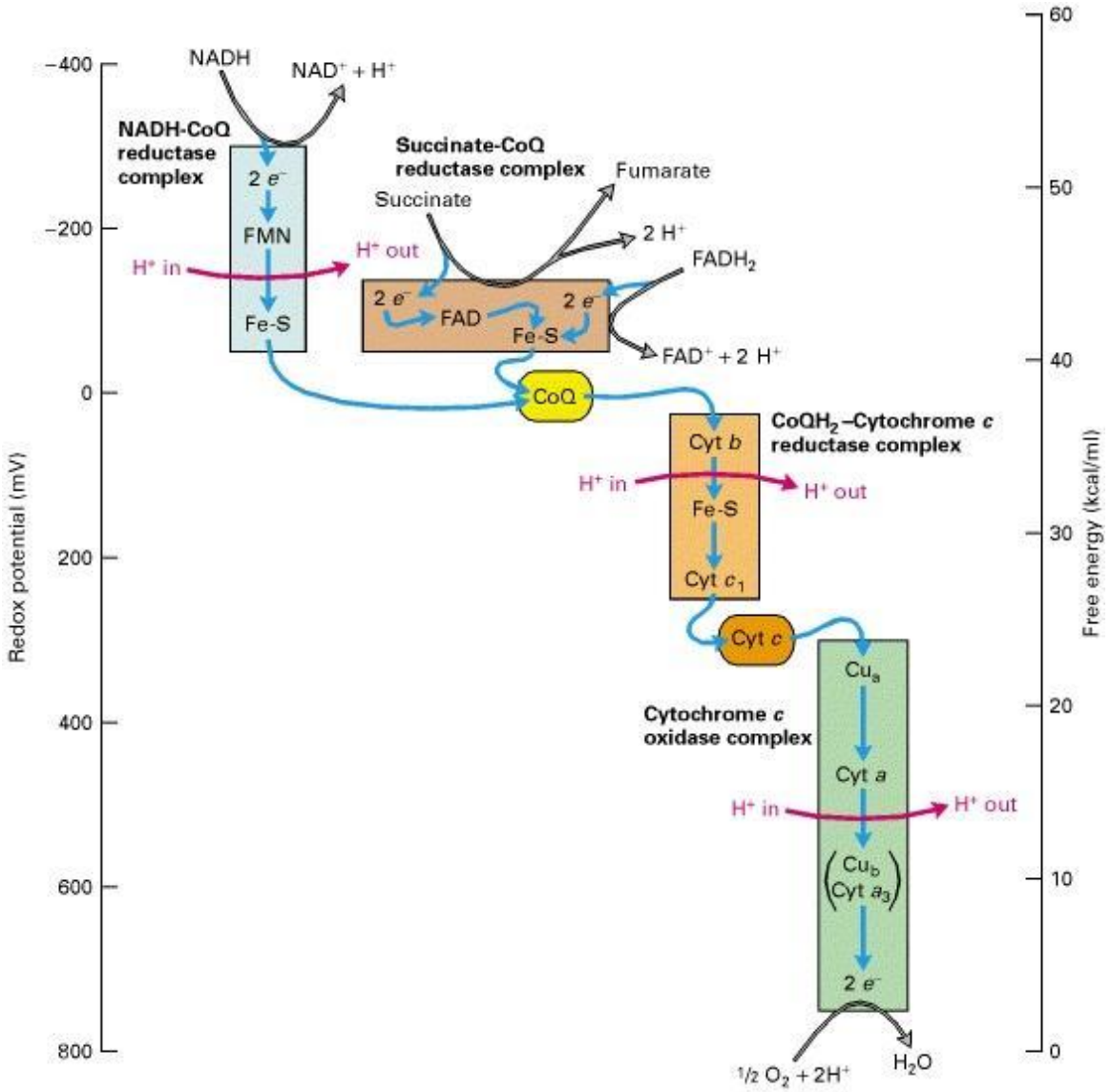
NADH, with a highly negative E'_o value exibits
- Very low affinity towards electrons
- A high electron transfer potential
- A high level of energy
- It is a reduced compound

O_2, with a highly positive E'_o value exibits
- Very high affinity towards electrons
- A low electron transfer potential
- A low level of energy
- It is an oxidised compound

The electrons then move spontaneously from NADH to O_2, releasing a large quantity of energy (-220 kJ/mol).

VIA PRINCIPAL DE TRANSPORTE DE ELECTRÕES DOS MITOCÔNDRIOS VEGETAIS (DO NADH AO O_2), MOSTRANDO OS COMPLEXOS SUPRAMOLECULARES I, II, III e IV.




NADH
NAD⁺ + H⁺
NADH-CoQ reductase complex
2 e⁻
FMN
Fe-S
H⁺ in
H⁺ out
Succinate-CoQ reductase complex
Succinate
Fumarate
2 H⁺
FADH₂
2 e⁻
FAD
Fe-S
2 e⁻
FAD⁺ + 2 H⁺
CoQ
CoQH₂–Cytochrome c reductase complex
Cyt b
H⁺ in
H⁺ out
Fe-S
Cyt c₁
Cyt c
Cytochrome c oxidase complex
Cuₐ
Cyt a
H⁺ in
H⁺ out
Cu_b
Cyt a₃
2 e⁻
½ O₂ + 2H⁺
H₂O
Redox potential (mV)
−400
−200
0
200
400
600
800
Free energy (kcal/ml)
60
50
40
30
20
10
0

Organization of mitochondrial electron transport chain

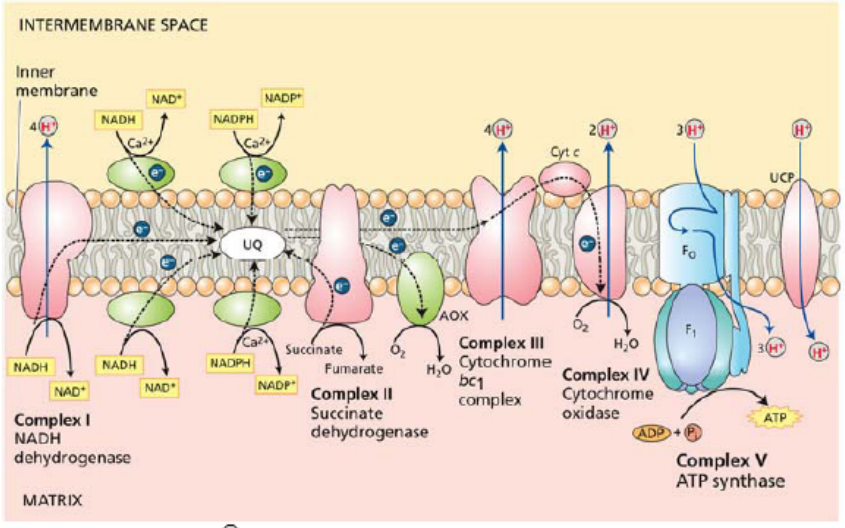
NADH Dehydrogenase (complex I)
- oxidizes NADH
- transfers e^- to Ubiquinone (UQ)
- pumps $1H^+$ per e^-

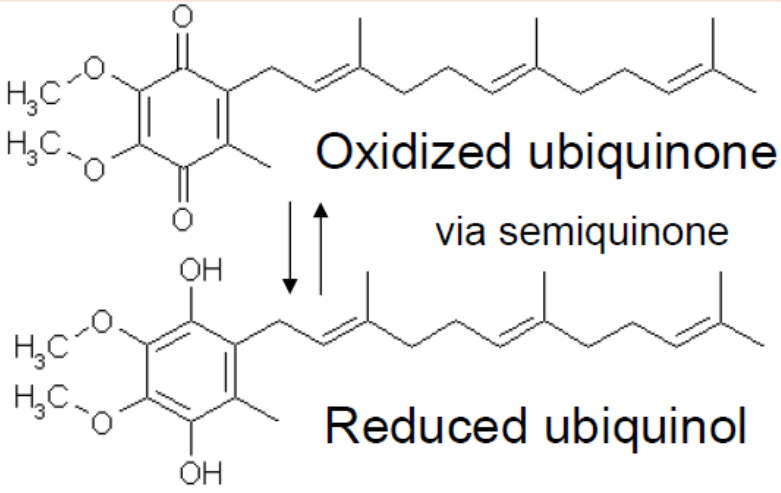
Succinate Dehydroganase (complex II)
- oxidation of succinate (from citric acid cycle)
- e^- are transferred via $FADH_2$
- does not pump protons

Cytochrome bc_1 complex (complex III)
- oxidizes reduced UQ (= ubiquinol)
- pumps $1H^+$ per e^-

Cytochrome c oxidase (complex IV)
- reduces O_2 to H_2O
- pumps $1H^+$ per e^-

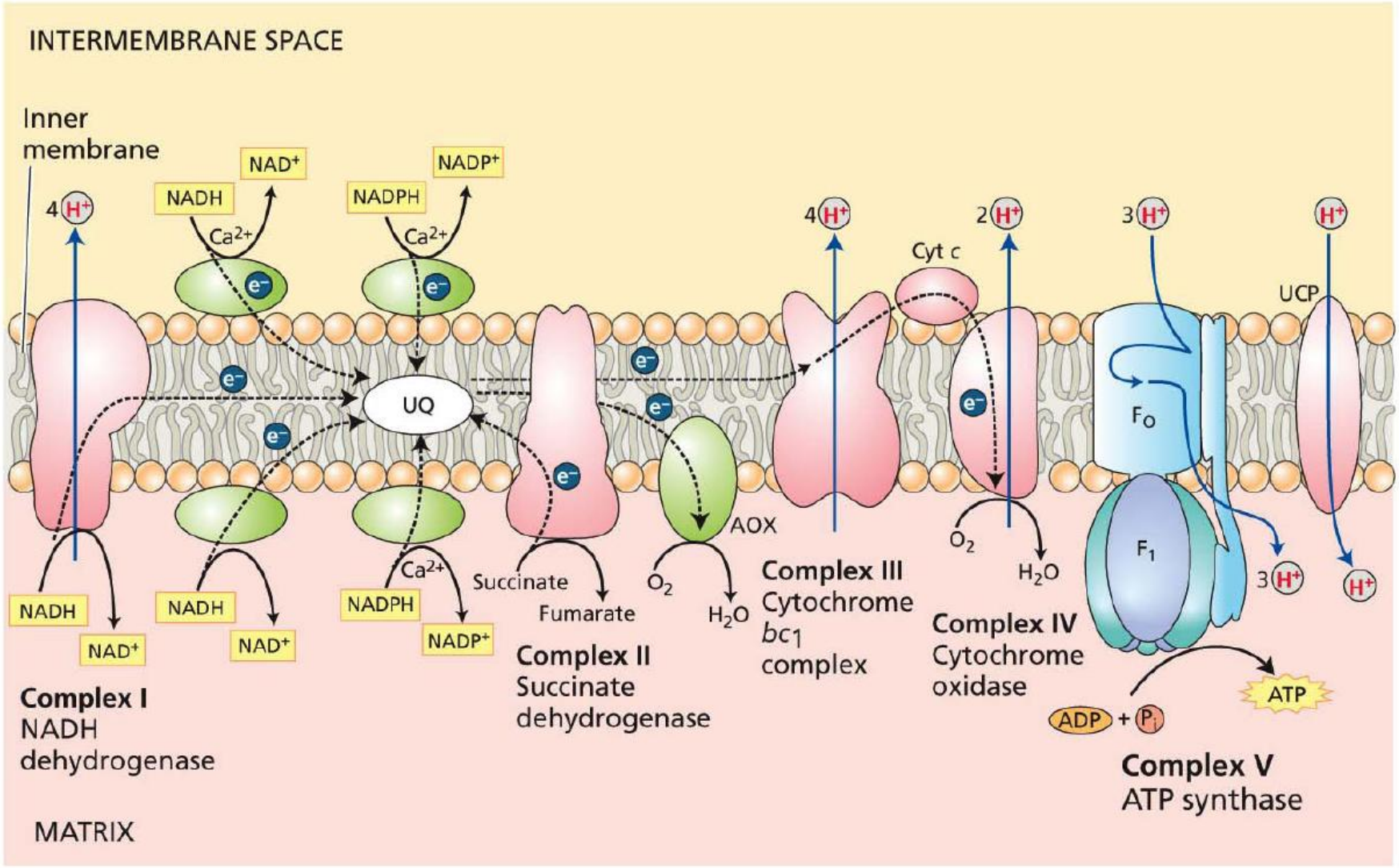
ATP synthase (complex V)
- uses electrochemical proton gradient to synthesize ATP





As vias alternativas de transporte de electrões dos mitocôndrios vegetais

Organization of mitochondrial electron transport chain

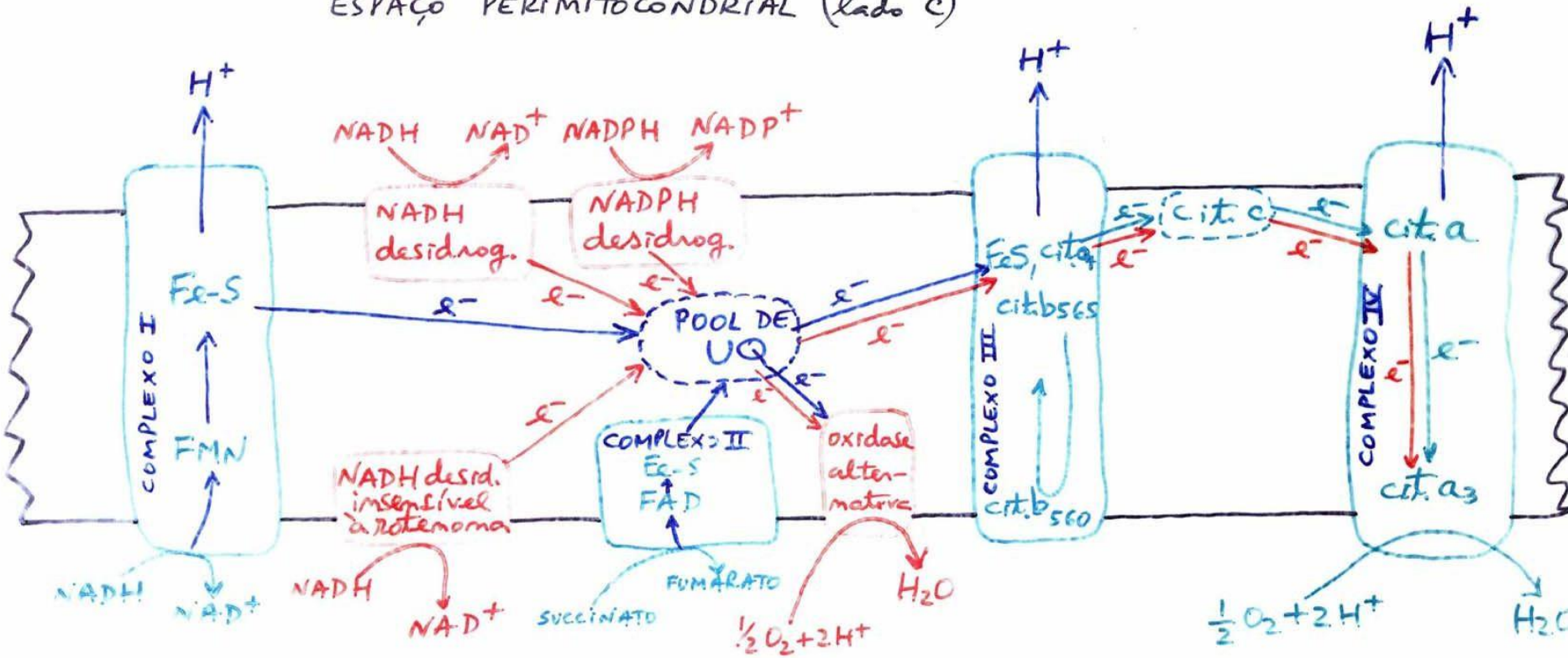


Plant mitochondria contain additional enzymes (in green), which do not pump protons

VIAS DE TRANSPORTE DE ELECTRÕES DOS MITOCÔNDRIOS VEGETAIS:

- Complexos I a IV da cadeia normal
- 2 complexos de NADH desidrogenases
- 1 complexo NADPH desidrogenase
- A oxidase alternativa insensível ao cianeto

Os 3 centros de conservação de energia estão representados por setas verticais, referentes à extrusão de protões

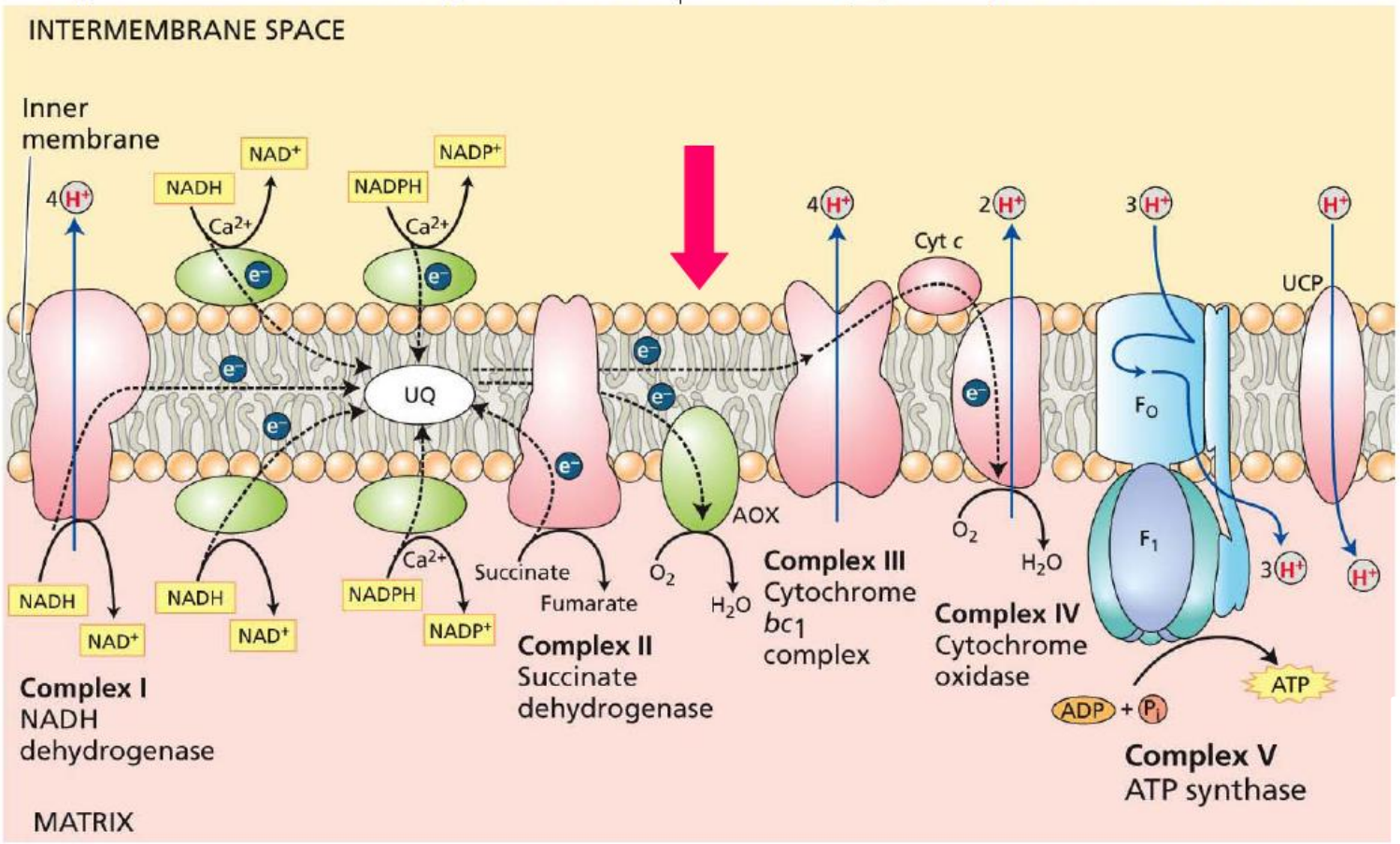


Mechanisms of plants to lower ATP yield –

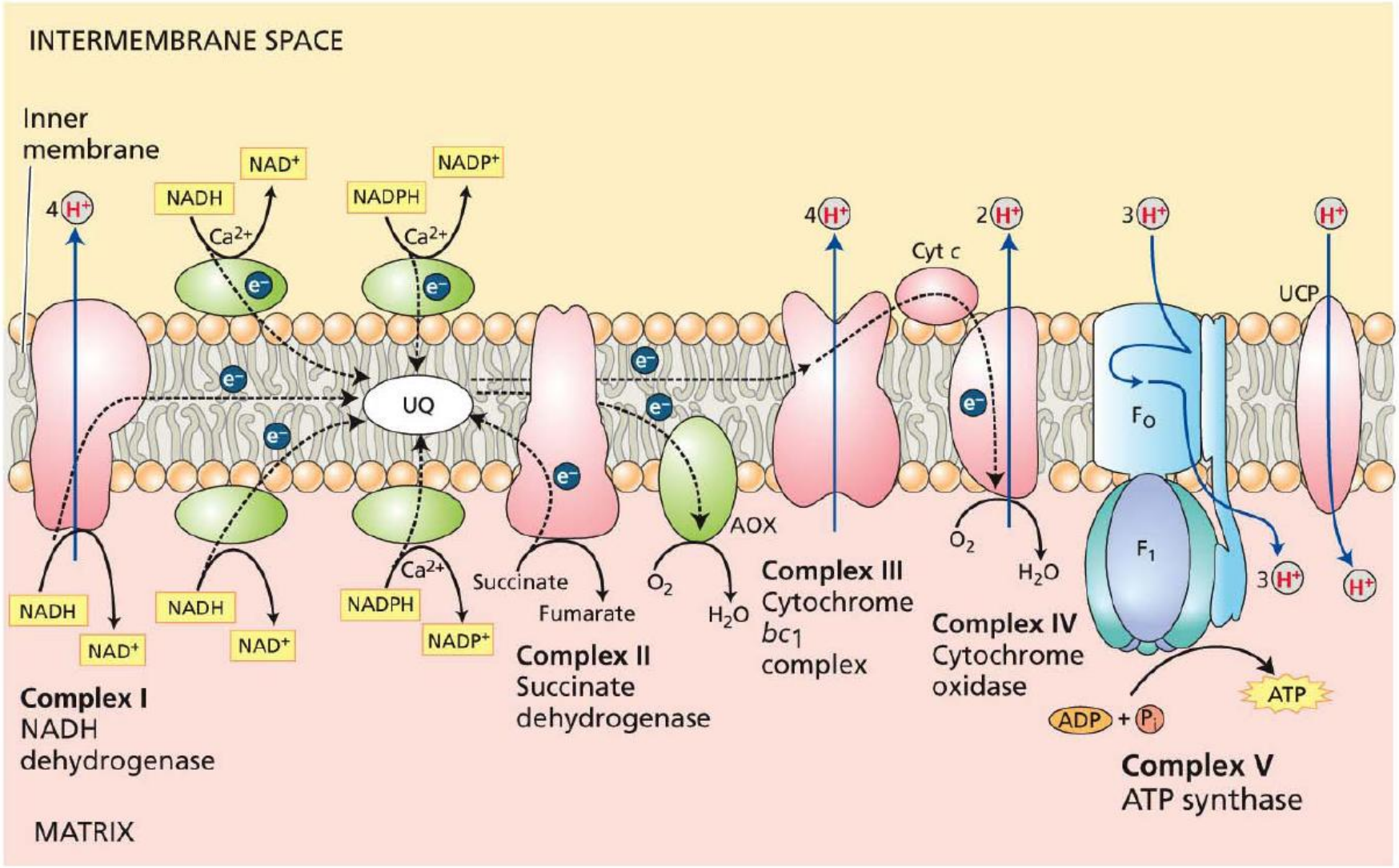
The role of the Alternative Oxidase and the Uncoupling Protein

Alternative oxidase

- some plants have cyanide-resistant respiration; can be 10-25%, even up to 100% of uninhibited control rate
- enzyme responsible for this cyanide-resistant oxygen uptake → Alternative oxidase

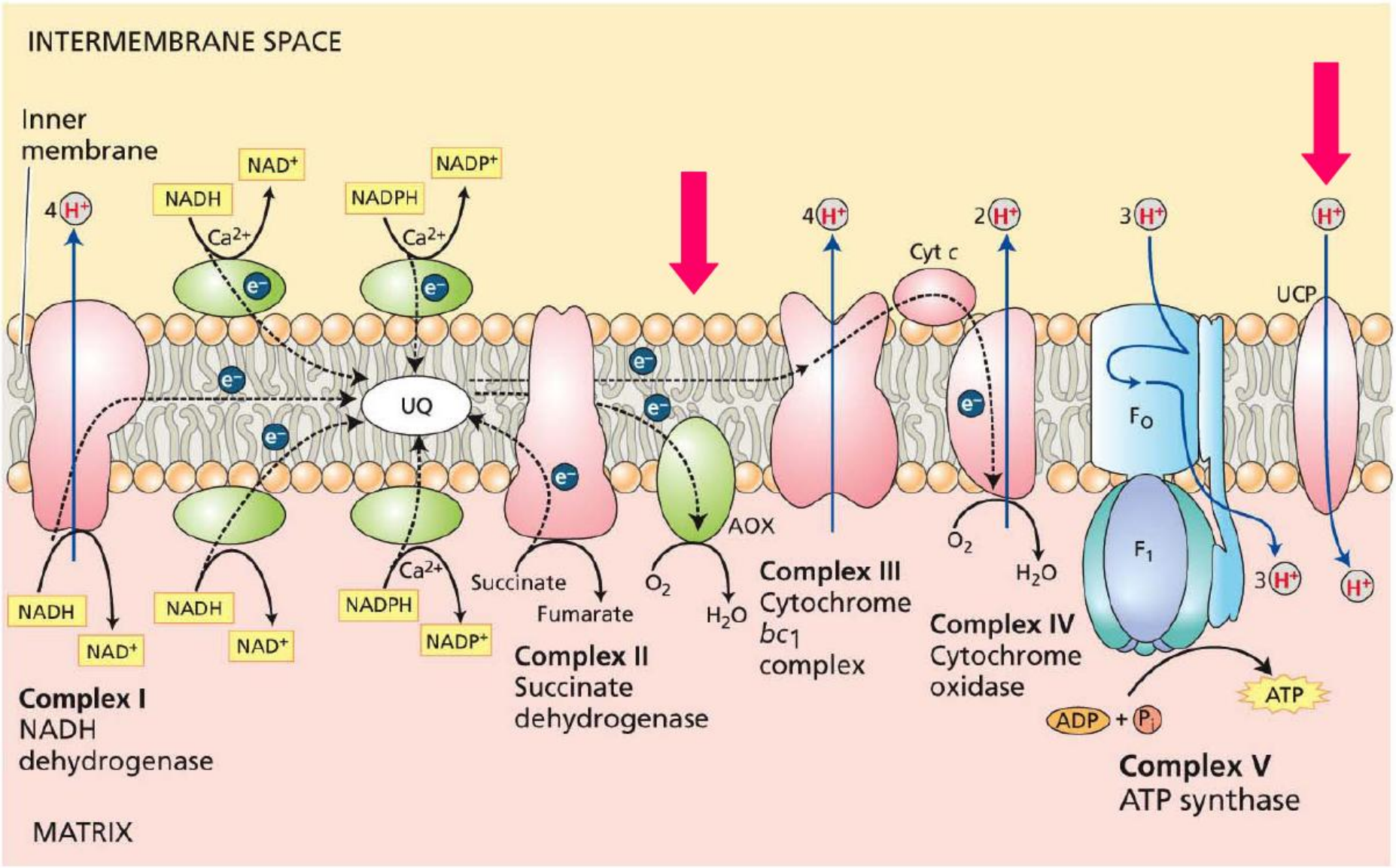


Organization of mitochondrial electron transport chain



Plant mitochondria contain additional enzymes (in green), which do not pump protons

Organization of mitochondrial electron transport chain



Plant mitochondria contain additional enzymes (in green), which do not pump protons

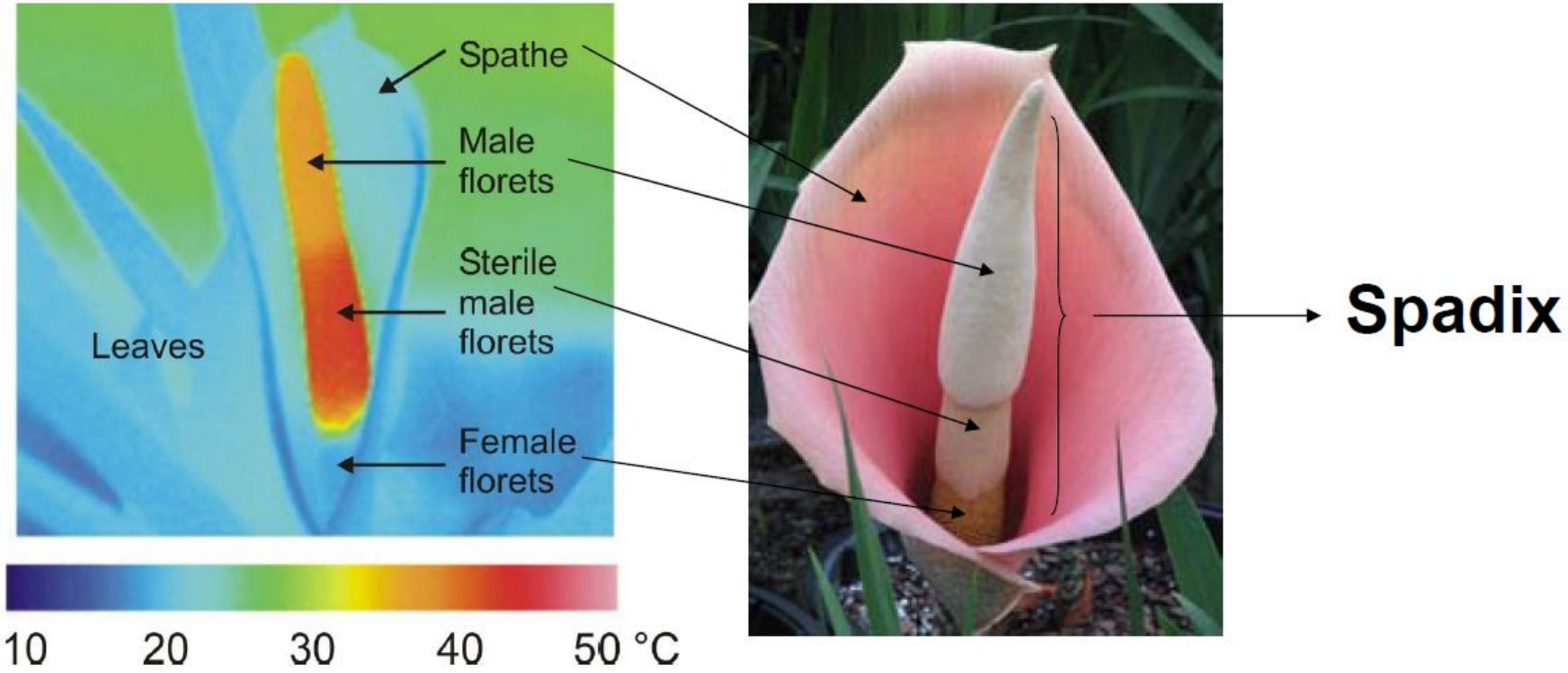
Mechanisms of plants to lower ATP yield –

The role of the Alternative Oxidase and the Uncoupling Protein

Alternative oxidase

How can this energetically wasteful process be of importance for plant metabolism?

Example: floral development in some members of the Araceae (arum family), e.g. voodoo lily (*Sauromatum guttatum*) → Thermogenesis

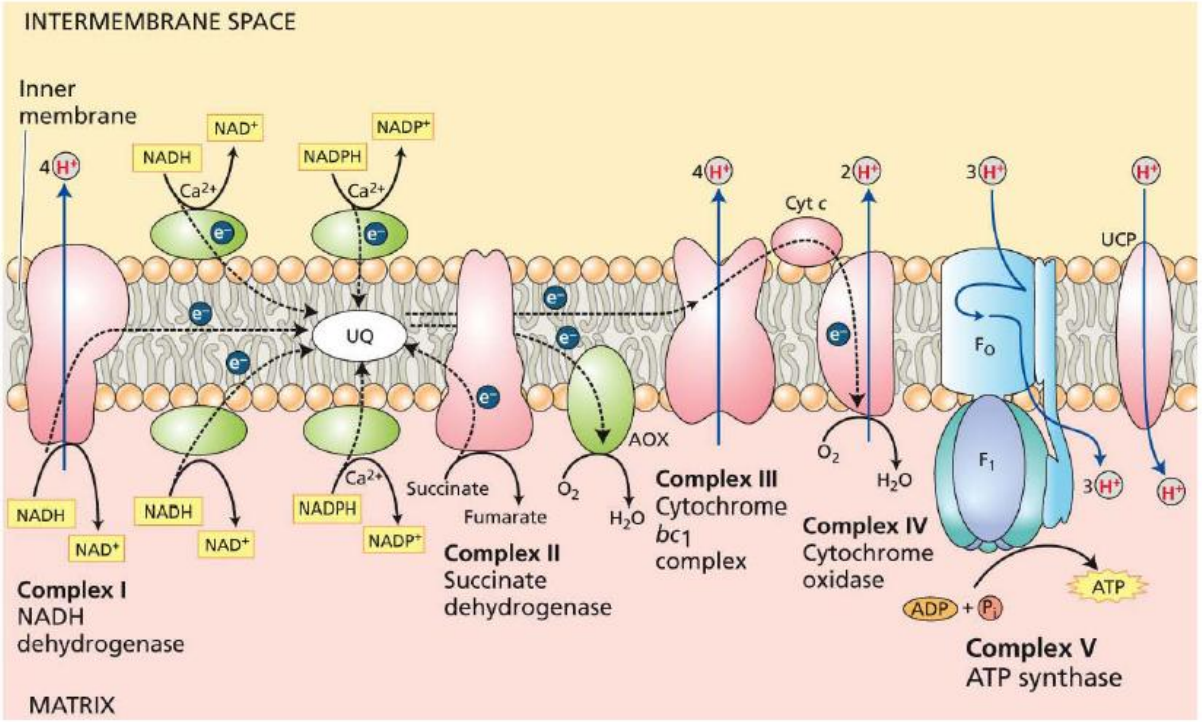


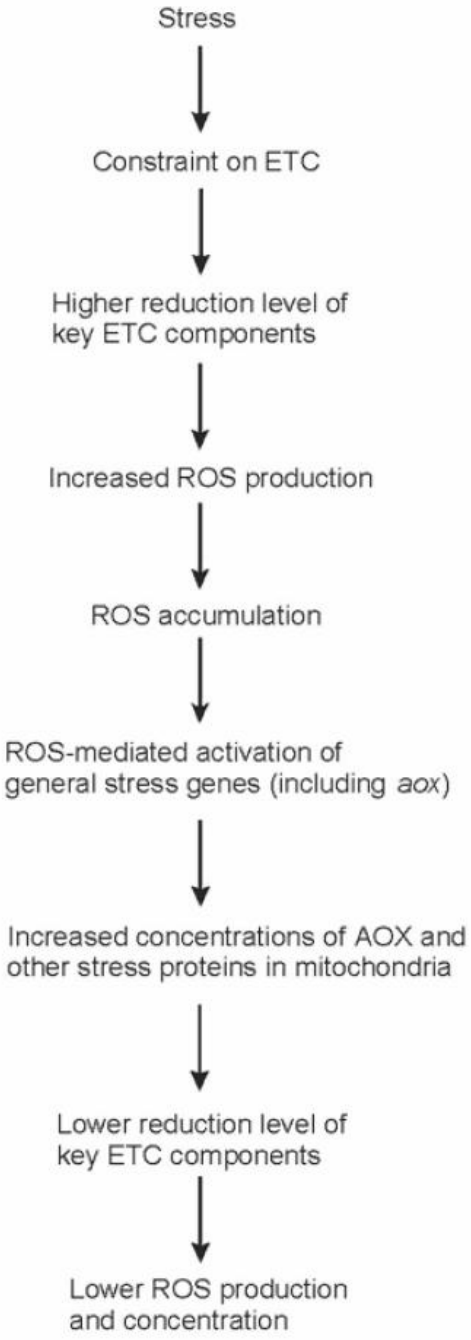
Alternative oxidase pathway

- Only in plant mitochondria
- No ATP synthesis, so heat is generated

Role of alternative oxidase pathway

1. Heat generation
2. Regulation of ATP synthesis
3. Regulation of metabolite synthesis
4. Helps overcome environmental stresses



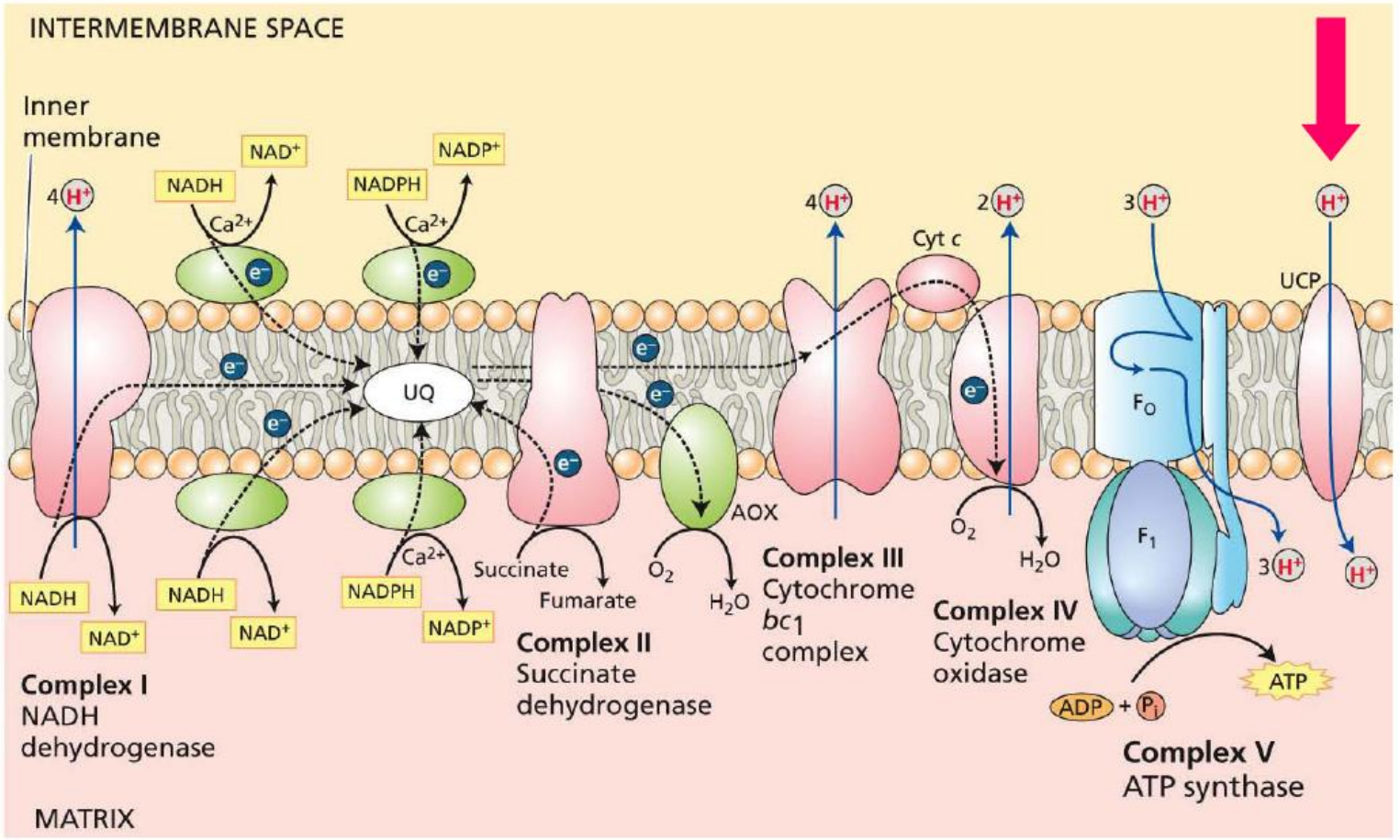


Mechanisms of plants to lower ATP yield –

The role of the Alternative Oxidase and the Uncoupling Protein

Uncoupling protein

- Increases proton permeability of inner mitochondrial membrane
- acts as an uncoupler → less ATP, more heat is produced
- main function in mammalian cells



Uncoupling proteins (UCP) belong to the mitochondrial anion carrier family of proteins, which are localized in the inner membrane; they partially uncouple respiration from ATP synthesis by catalysing proton leakage. All of these carriers have a molecular mass close to 33 kDa and consist of three tandemly repeated homologous domains, each with two hydrophobic stretches.

In cold-adapted brown adipose tissue (BAT), UCP1 levels can reach up to about 5% of total mitochondrial proteins and it plays a key role in non-shivering thermogenesis. However, the recent findings that **plant uncoupling proteins** are expressed in nonthermogenic tissues bring into question their involvement in thermogenesis. These results suggest that plant UCPs are involved in the regulation of energy metabolism or in the reduction of reactive oxygen species in mitochondria. Recently it was also shown, by using a knockout mutant, that UCP1 in *Arabidopsis* leaves is related to photosynthetic metabolism.

Natural Uncouplers Convert the Mitochondria in Brown Fat into Heat-generating Machines

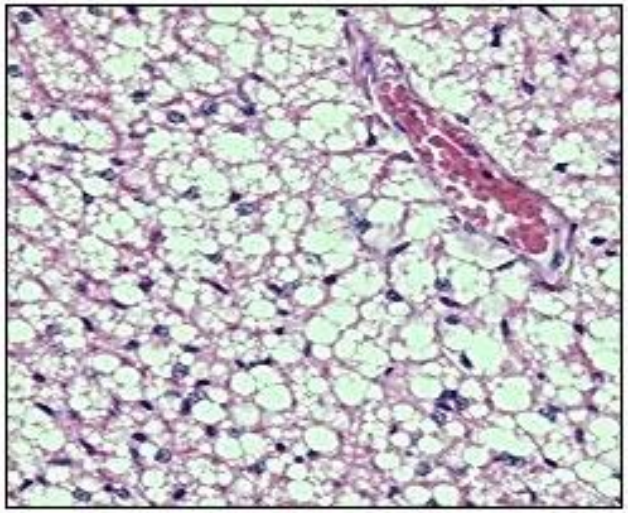
In some specialized fat cells, mitochondrial respiration is normally uncoupled from ATP synthesis. In these cells, known as brown fat cells, most of the energy of oxidation is dissipated as heat rather than being converted into ATP. The inner membranes of the large mitochondria in these cells contain a special transport protein that allows protons to move down their electrochemical gradient, by-passing ATP synthase. As a result, the cells oxidize their fat stores at a rapid rate and produce more heat than ATP. Tissues containing brown fat serve as “heating pads,” helping to revive hibernating animals and to protect sensitive areas of newborn human babies from the cold.

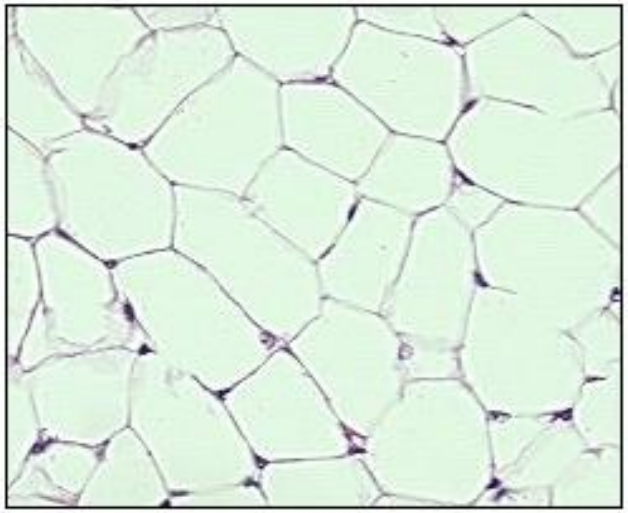
Brown fat tissue: Cell Biology and Function

Brown fat is of particular importance in neonates, small mammals in cold environments, and animals that hibernate, because it has the ability to dissipate stored energy as heat.

In contrast to other cells, including white adipocytes,brown adipocytes express **mitochondrial uncoupling protein 1** (UCP-1), which gives the cell's mitochondria an ability to uncouple oxidative phosphorylation and utilize substrates to generate heat rather than ATP.

Exposure to cold leads to sympathetic stimulation of brown adipocyte via norepinephrine binding to β–adrenergic receptors. As in white fat, sympathetic stimulation promotes hydrolysis of triglyceride, with release of fatty acids and glycerol. However, within brown adipocytes, most fatty acids are immediately oxidized in mitochondria and, because of the uncoupling protein, a large amount of heat is produced. This process is part of what is called non-shivering thermogenesis.





Examination of sections of white and brown fat at low magnification reveal dramatic differences in structure, as seen on the left images of mouse tissues.

White adipocytes (*right panel*) have a scant ring of cytoplasm surrounding a single large lipid droplet. Their nuclei are flattened and eccentric within the cell.

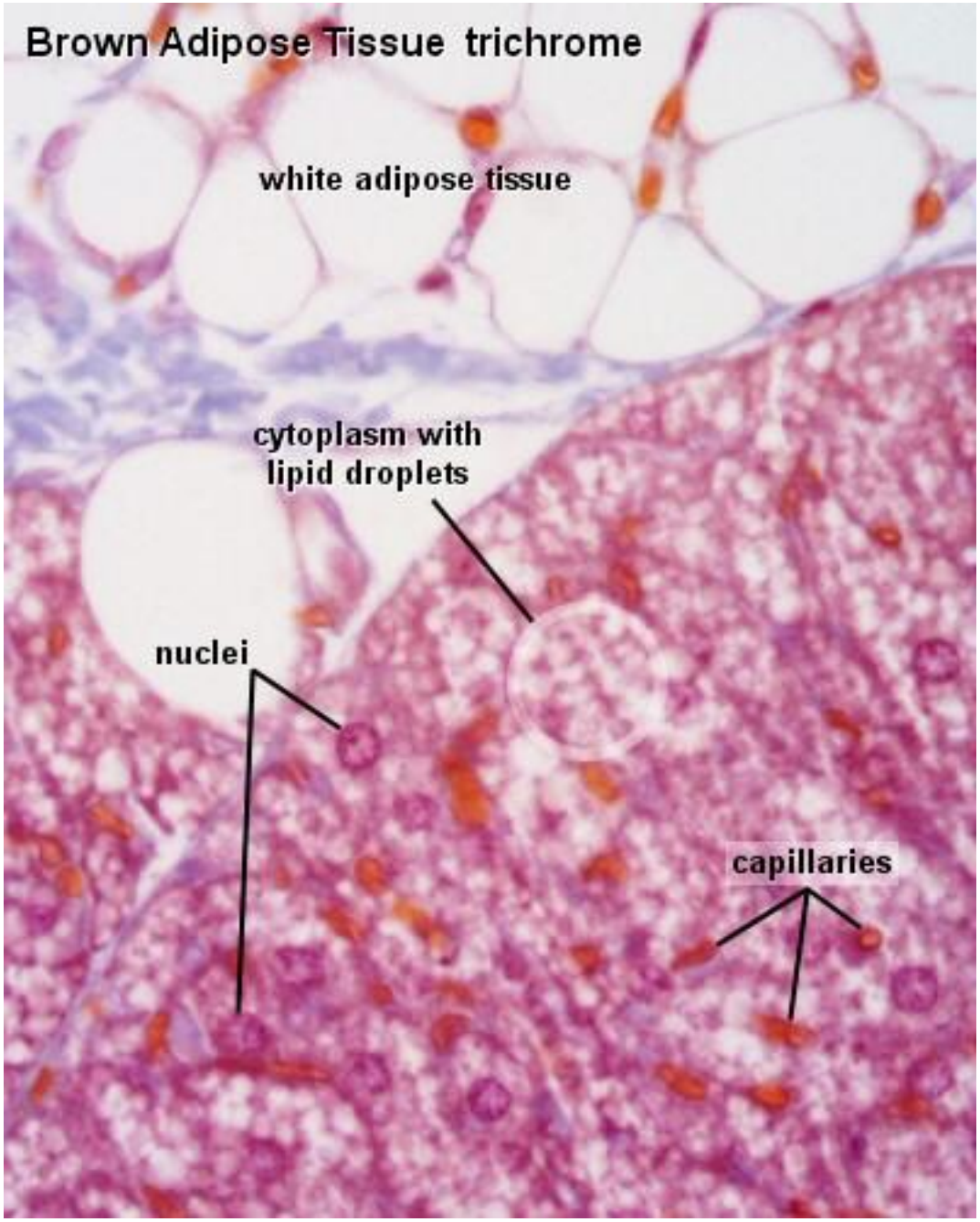
Brown adipocytes (*left panel*) are polygonal in shape, have a considerable volume of cytoplasm and contain multiple lipid droplets of varying size. Their nuclei are round and almost centrally located.

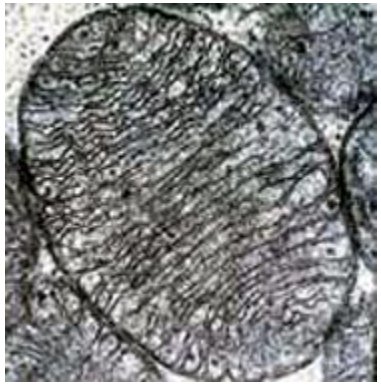
Electron micrographs of brown fat cells reveal one of their hallmarks: an extraordinary number of mitochondria, which, as described below, are involved in heat generation. The mitochonria are typically round, with cristae across their entire width.

Kidney – trichrome

In the renal sinus, islands of brown adipose tissue are often surrounded by white adipose tissue, which emphasises the different appearances of the two tissue types. In brown adipose tissue, the nuclei of adipocytes are round and located more or less centrally in a cytoplasm which, after the extraction of lipids during tissue preparation, looks very frothy. Cell borders can be difficult to identify. Capillaries are very frequent.

Note the characteristic features of white and brown adipose if both types are present side by side.





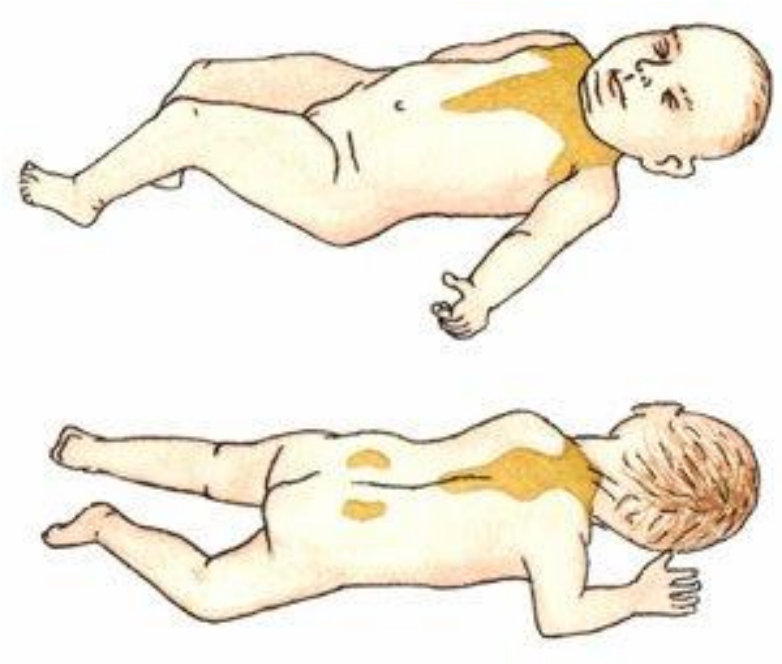
What Keeps us Warm?

The body's generation of heat is called **thermogenesis**. There are 2 kinds of thermogenesis.

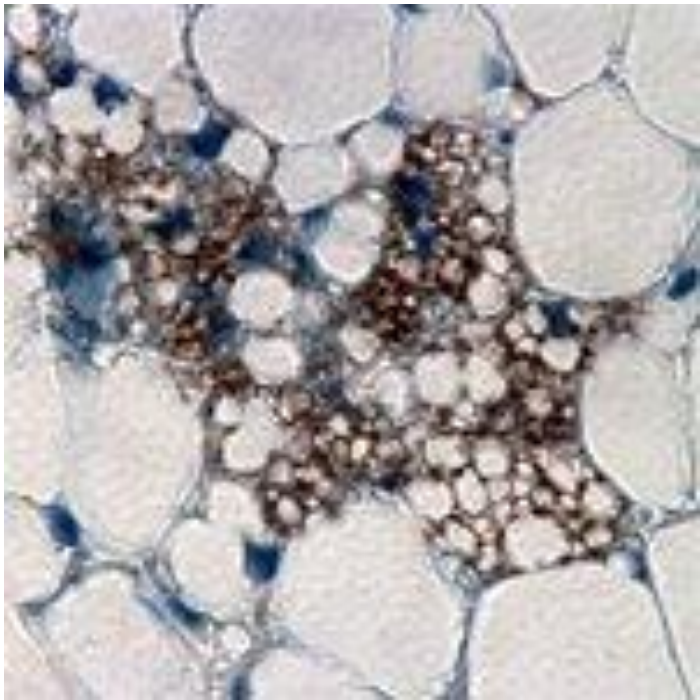
Shivering thermogenesis (or just "shivering" in layman's terms) works, producing between 10 and 15 kJ/min, but it has the significant downside of impairing coordination, or "skilled performance". It's really only functional as a "bridge" to non-shivering thermogenesis. And if it doesn't bridge you to non-shivering thermogenesis in about 30 min or so, it's more of "plank-walk" to hypothermia… Shivering is controlled by the hypothalamus, that part of the brain that also regulates sub-conscious or half-conscious bodily functions such as heartrate and breathing.

Non-shivering thermogenesis is the real ticket; that's what will keep you warm outside for the long-haul. And the part of your body that plays the most important role in this process is the rather gross-sounding brown fat, or brown adipose tissue. Brown fat isn't the fat on your gut or your ass (that's white fat); it generally surrounds blood vessels and internal organs and comprises less than 2% of your total body mass. In infants, brown fat comprises more like 5% of total body mass, due to the challenges of keeping a smaller body (with its higher surface area-to-volume ratio) warm. Brown fat is packed with fat cells and capillaries, and its primary function is keeping you warm.

The heat produced in brown fat can actually be imaged using a thermal (infrared) camera. If one takes such a picture of an unswaddled infant sleeping at room temperature, "hot spots" can be seen in the skin overlying brown fat deposits in the neck and interscapular area. Brown fat thermogenesis also seems to be of considerable importance to animals coming out of hybernation, allowing them to rewarm.



Finally, it seems that brown fat plays a non-trivial role in control of body weight, and that mitochondrial uncoupling proteins may be one of many factors involved in development of obesity. An interesting demonstration of this is found in a report in which transgenic mice with genetic ablation of brown fat developed obesity in the absense of overeating.

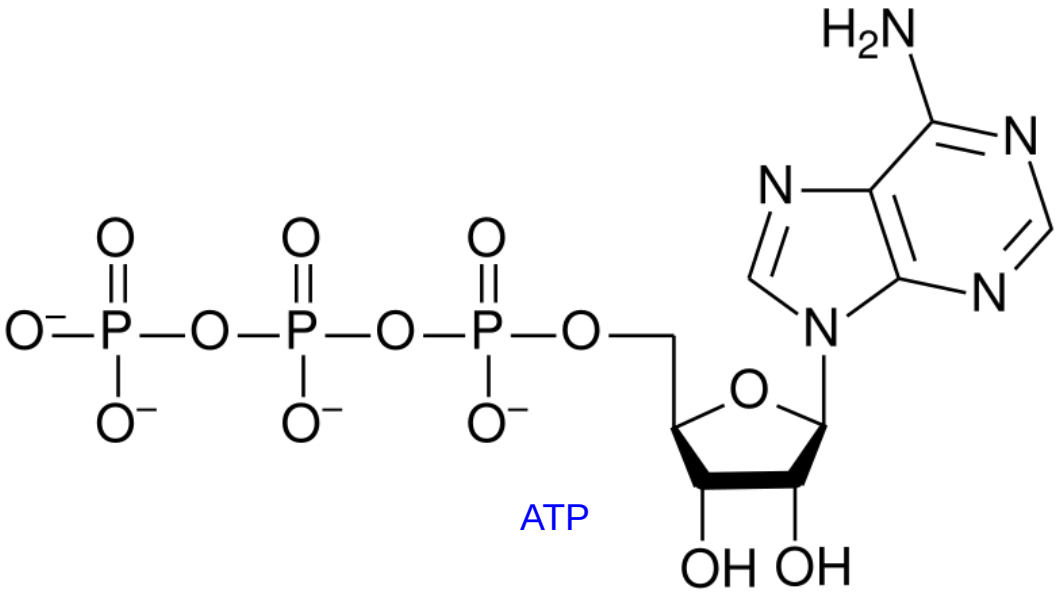


A Wee Bit O' Chemistry

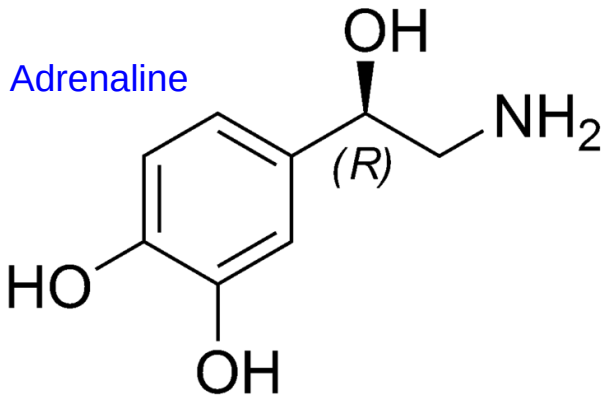
The fat cells in brown bat are packed with an unusually high number of mitochondria.

Mitochondria primary job is to generate energy for the cell, in the form of ATP. ATP is the biochemical fuel that powers every cell in our bodies.

In brown fat the ATP-producing reaction is altered by a special kind of protein in the mitochondrial membrane wall called an **uncoupling protein**, which causes the reaction to produce heat instead of ATP.



ATP



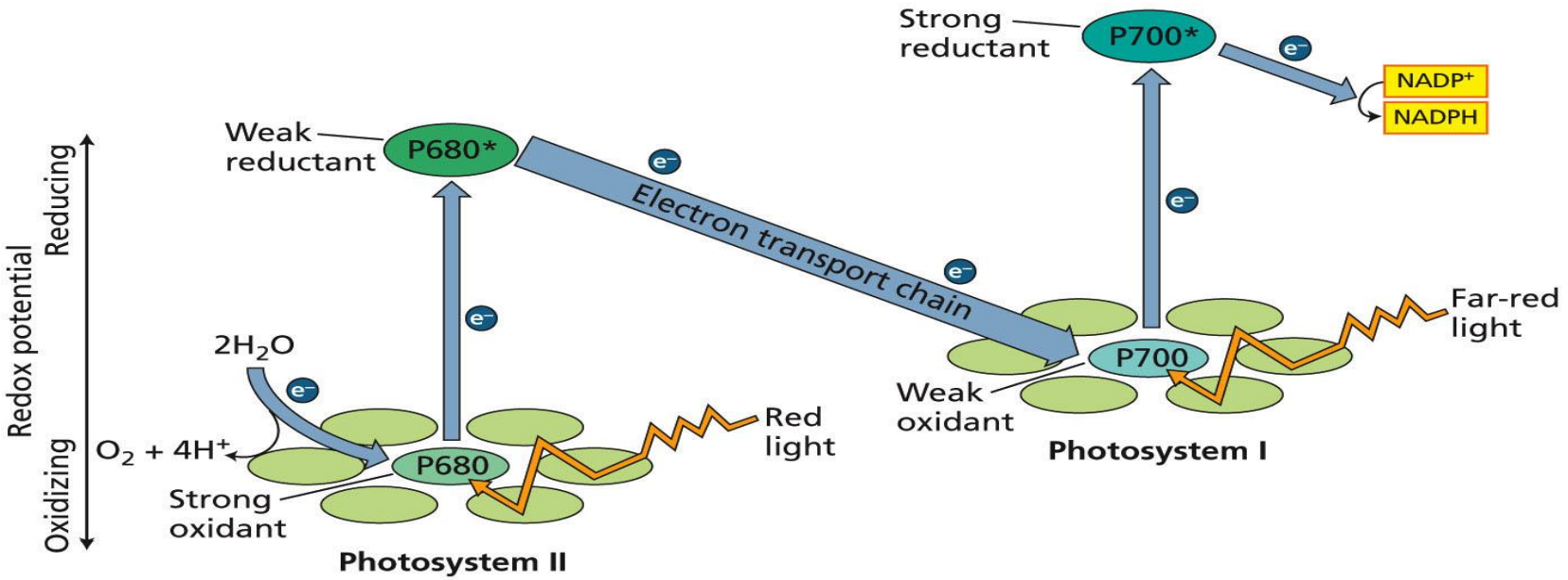
Adrenaline

The primary regulator of this heat-producing reaction is a hormone called noradrenaline, which acts to depolarize, or reduce the voltage across the cell membranes, and thereby accelerate the uncoupled-protein-modified, would-be ATP-producing reaction.

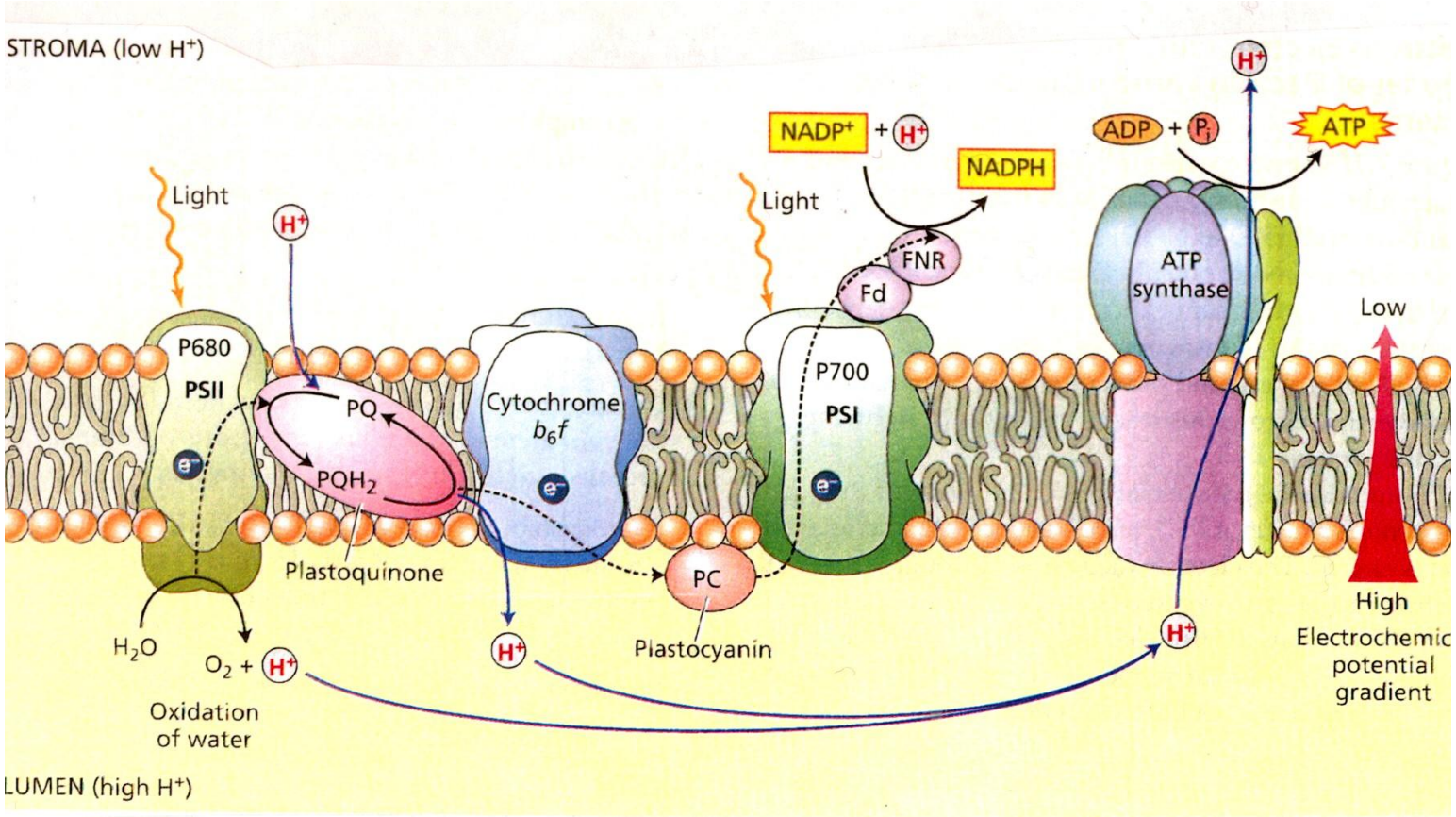
The chloroplast electron transport chain (cETC)

Esquema em Z

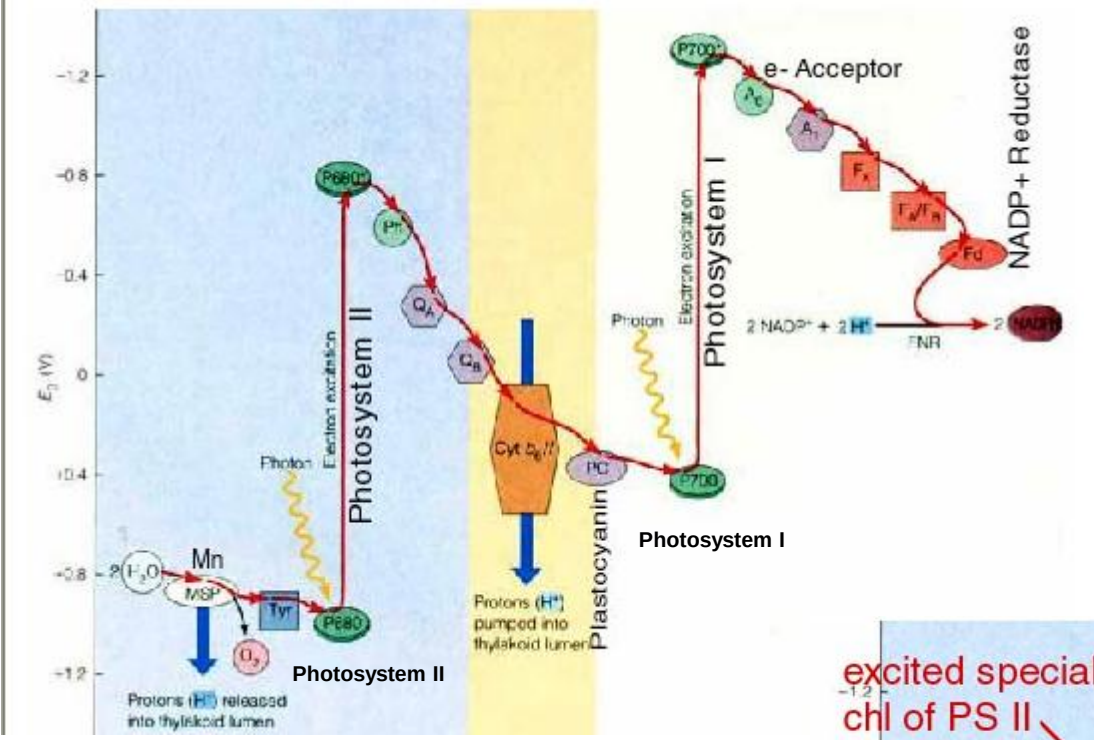
A luz vermelha (680 nm) absorvida pelo Fotossistema II (**PSII**) produz um composto **oxidante forte** que oxida **a água**. A luz vermelha (700 nm) absorvida pelo Fotossistema I (**PSI**) produz um composto **redutor forte** que reduz o **$NADP^+$ a NADPH**



Durante o **transporte de electrões** entre a água e o $NADP^+$ gera-se um gradiente de protões entre o lúmen do tilacóide (pH mais baixo) e o estroma (pH mais elevado)

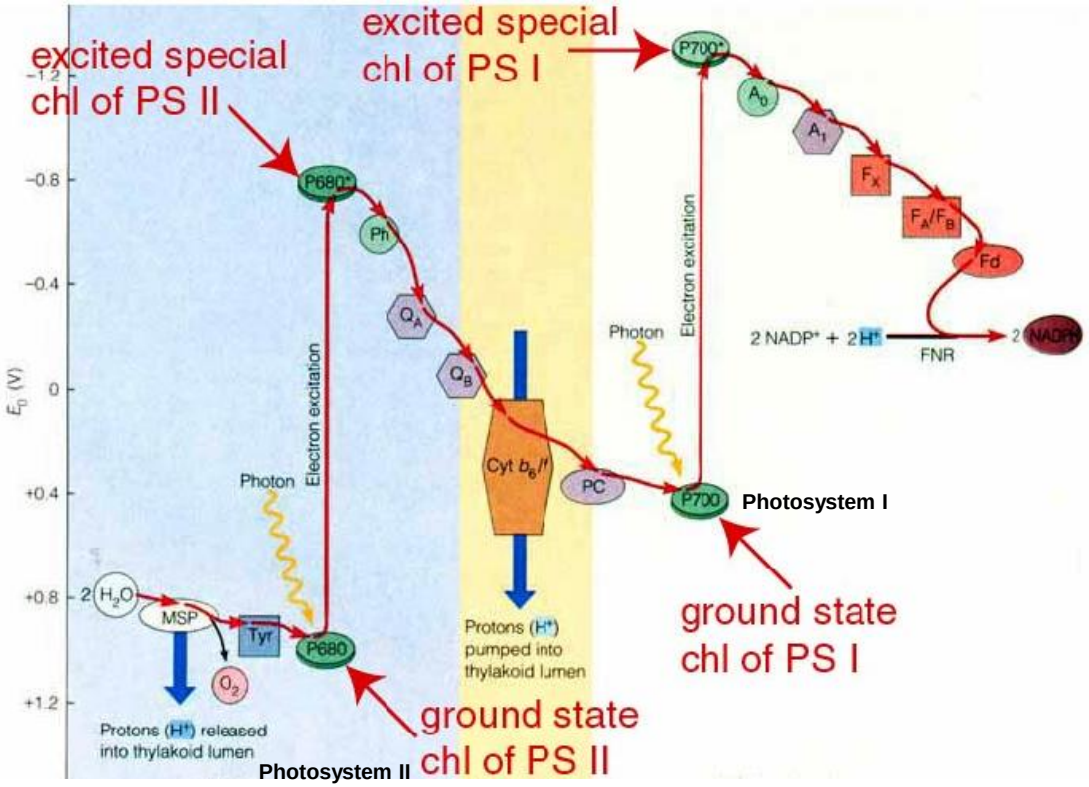


Os prováveis transportadores de electrões que se movem entre o PSII e O PSI ao longo dos tilacóides são a Plastoquinona e a Plastocianina

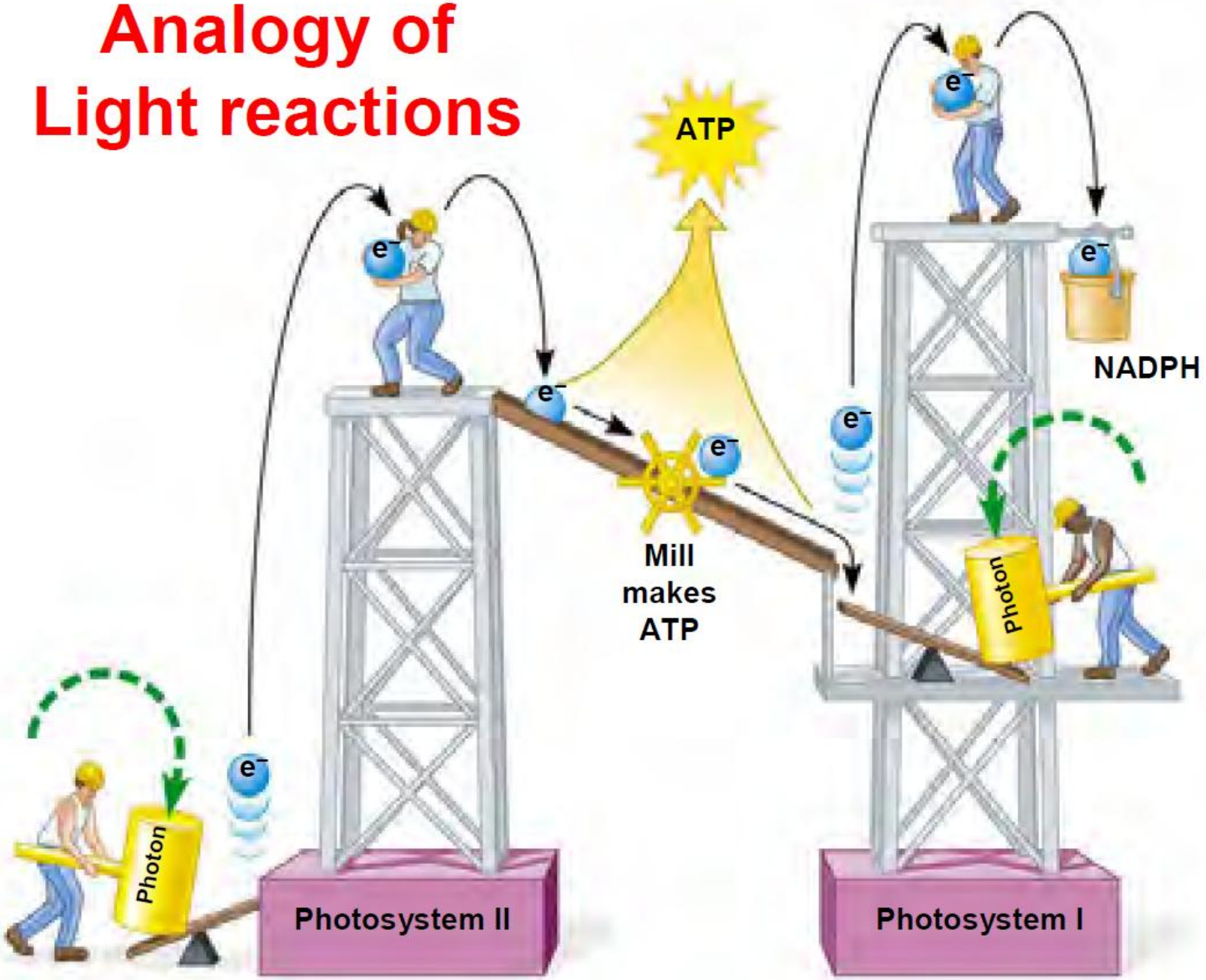


Photosystem I

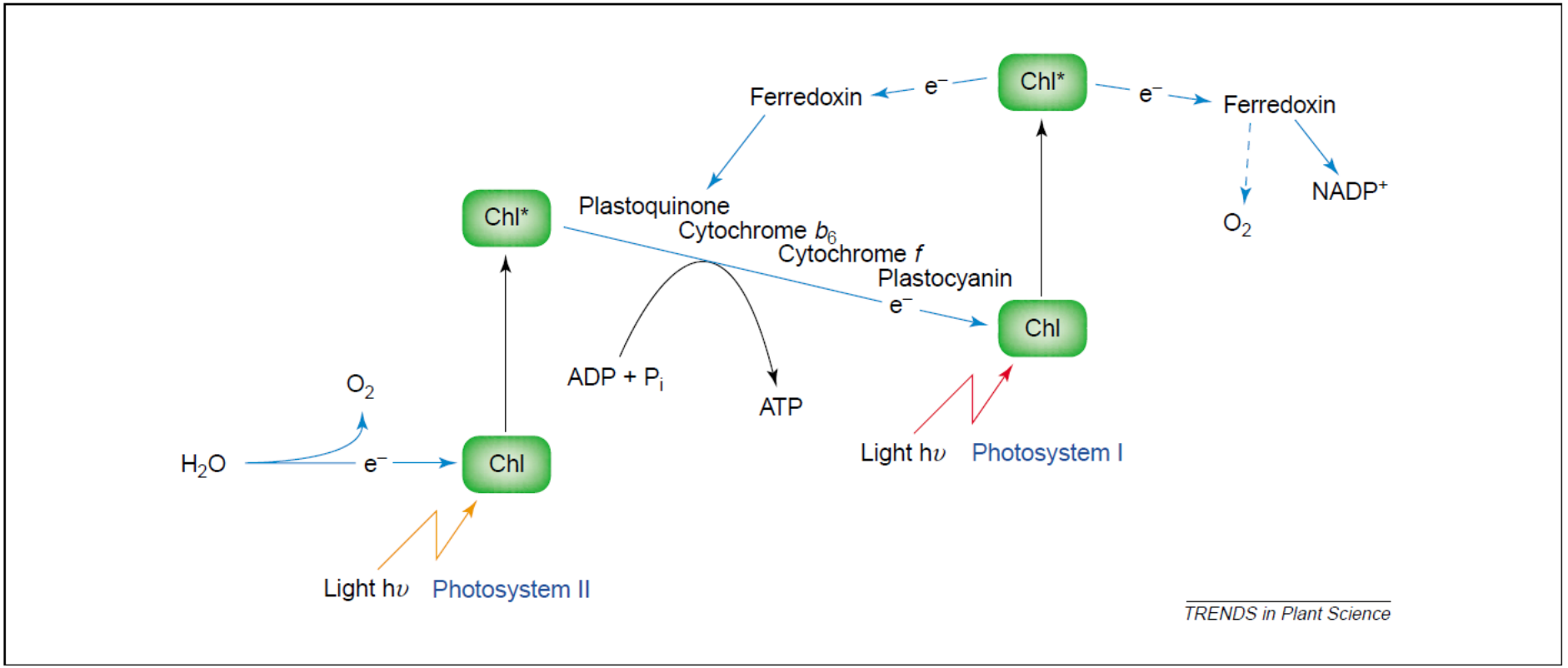
Photosystem II



Analogy of Light reactions



Cyclic, pseudocyclic and noncyclic photophosphorylation



Abbreviations: Chl, chlorophyll; Chl*, the excited state of Chl; e^-, electron; P_i, inorganic phosphate.

In pseudocyclic photophosphorylation, the terminal electron acceptor is O_2 instead of $NADP^+$.

Energy Conservation from the Proton Motive Force

- When electrons are transported through an electron transport chain, protons are extruded to the outside of the membrane, forming the **proton motive force**.

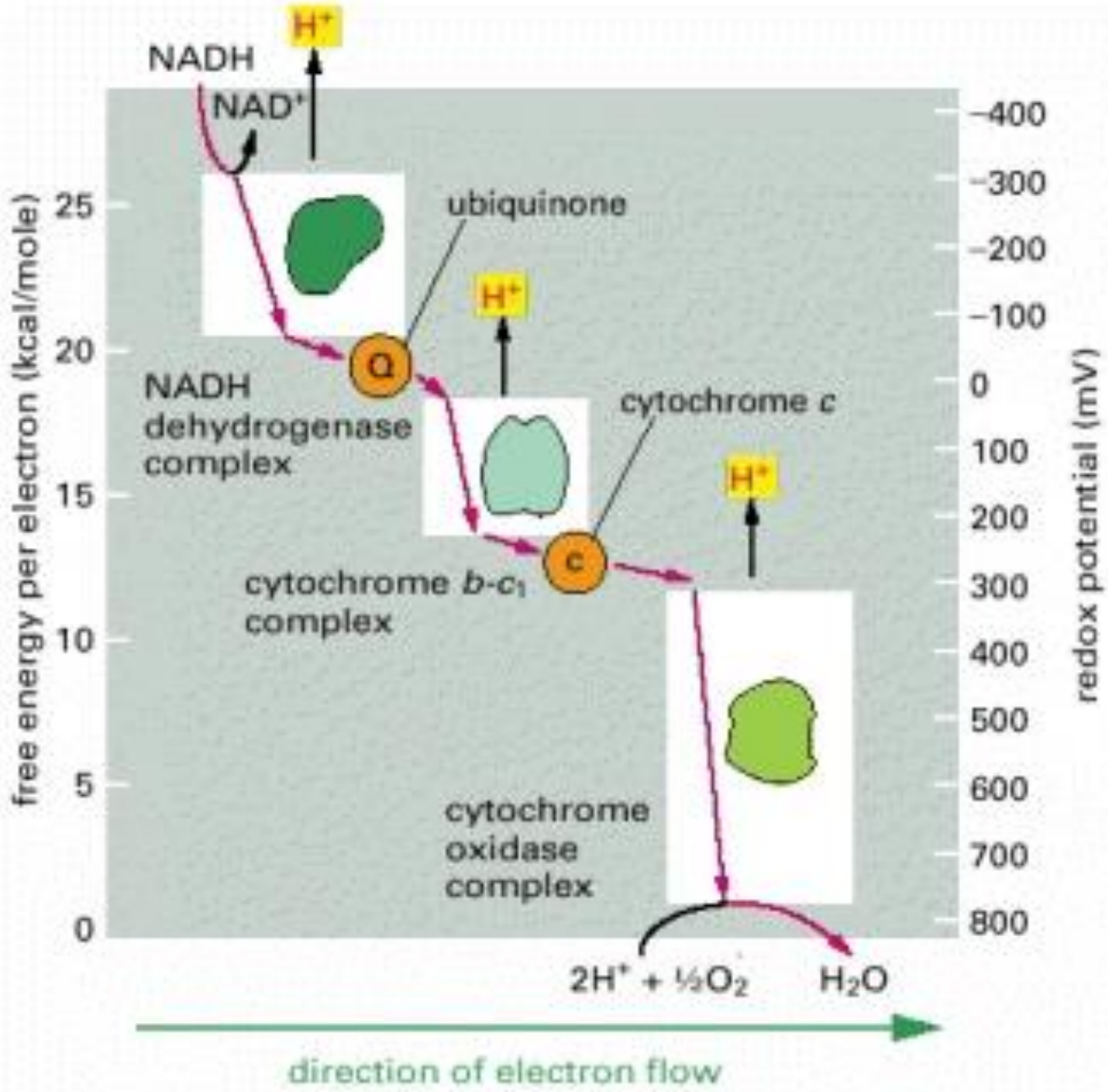
Why do the electrons move spontaneously along the ETC ????

How do they move spontaneously from NADH to O_2 in the mETC ????

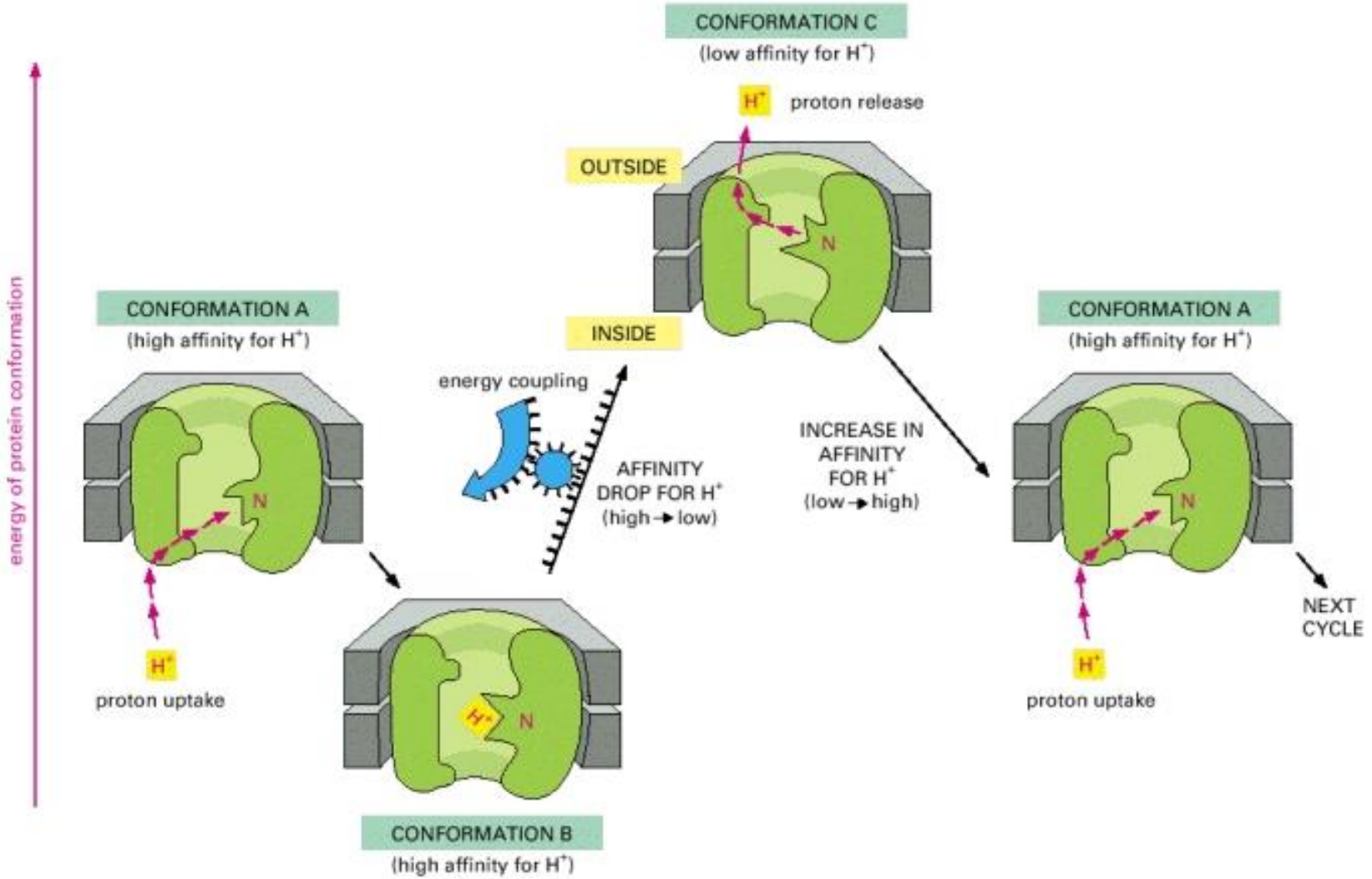
How do they move spontaneously from H_2O to NADPH in the cETC ????

Why are protons extruded across the membranes, spontaneously and against an electrochemical gradient ????

Bioenergética da cadeia mitocondrial de transporte de electrões



Modelo proposto para o funcionamento acoplado da translocação de protões / cadeia de transporte de electrões



As alterações de conformação A→B e C→A ocorrem com ΔG < 0 e, por isso, espontaneamente.

As mudanças de conformação B→C são endergónicas (ΔG > 0), requerendo, por isso, o fornecimento de energia para funcionarem de modo espontâneo. Essa energia é libertada e fornecida pelo transporte de electrões ao longo da CTE.

O funcionamento do ciclo global A→B→C→A ... ocorre com ΔG < 0, o que faz com que os protões sejam translocados contra um gradiente electroquímico, da matriz mitocondrial para o espaço perimitocondrial.

The electron cycle of aerobic organisms

Table of standard reduction potentials for half-reactions important in biochemistry

The values below are standard reduction potentials for half-reactions measured at 25°C, 1 atmosphere and a pH of 7 in aqueous solution.

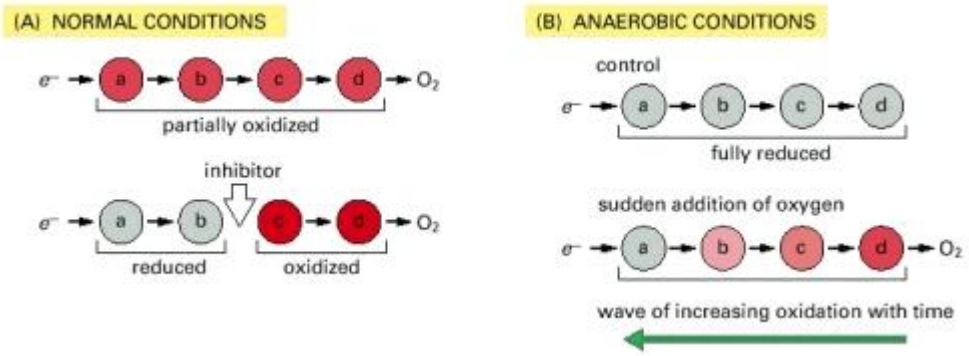
Half-reaction	Δξ°'(V)
$CH_3COOH + 2\ H^+ + 2\ e^- \rightarrow CH_3CHO + H_2O$	-0.581
$2\ H^+ + 2\ e^- \rightarrow H_2$	-0.421
$NAD^+ + H^+ + 2\ e^- \rightarrow NADH$	-0.320
$NADP^+ + H^+ + 2\ e^- \rightarrow NADPH$	-0.320
$FAD + 2\ H^+ + 2\ e^- \rightarrow FADH_2$ (coenzyme bonded to flavoproteins)	-0.22
$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O_2$	+0.7
$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$	+1.64
$P680^+ + e^- \rightarrow P680$	~ +1.0

Table 18–2 Standard reduction potentials for respiratory chain and related electron carriers

Redox reaction (half-reaction)	E_0' (V)
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.55
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816

Half-reaction	Δξ°'(V)
$CH_3COOH + 2\ H^+ + 2\ e^- \rightarrow CH_3CHO + H_2O$	-0.581
$2\ H^+ + 2\ e^- \rightarrow H_2$	-0.421
$NADP^+ + H^+ + 2\ e^- \rightarrow NADPH$	-0.320
$FAD + 2\ H^+ + 2\ e^- \rightarrow FADH_2$ (coenzyme bonded to flavoproteins)	~0
$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O_2$	+0.295
$1/2\ O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O$	+0.815
$P680^+ + e^- \rightarrow P680$	~ +1.0

REDUCTION POTENTIALS	
Half Reaction	$\mathcal{E}^{o'}$ (Volts)
$O_2 + 2\ H^+ + 2\ e^- \rightarrow H_2O$	0.816 V
$SO_4^{2-} + 2\ H^+ + 2\ e^- \rightarrow SO_3^{2-} + H_2O$	0.480 V
fumarate + $2H^+ + 2\ e^-$ → succinate	0.030 V
acetaldehyde + $2\ H^+ + 2\ e^-$ → ethanol	− 0.163 V
oxaloacetate + $2\ H^+ + 2\ e^-$ → malate	− 0.175 V
$FAD + 2H^+ + 2\ e^- \rightarrow FADH_2$	− 0.180 V
$NAD^+ + 2H^+ + 2\ e^- \rightarrow NADH + H^+$	− 0.180 V
pyruvate + $CO_2 + 2H^+ + 2\ e^-$ → malate	− 0.330 V

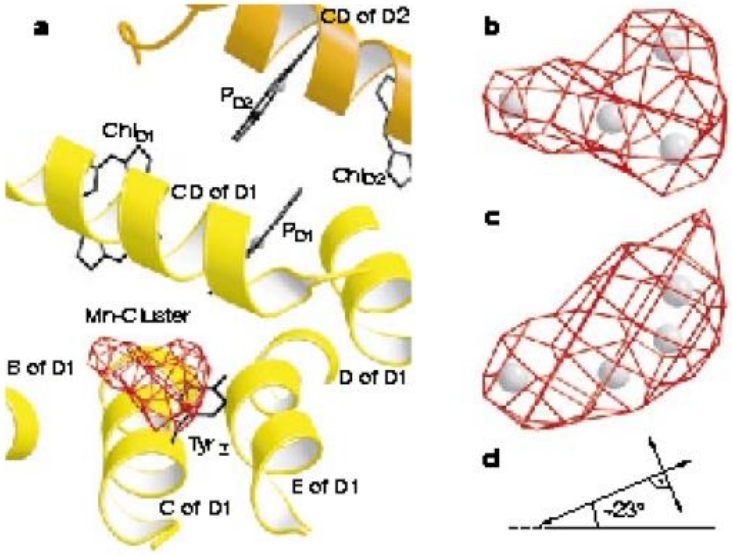


The general methods used to determine the path of electrons along an electron-transport chain

The extent of oxidation of **electron carriers** a, b, c, and d is continuously monitored by following their distinct spectra, which differ in their oxidized and reduced states. In this diagram an increased degree of oxidation is indicated by a *darker red.* (A) Under normal conditions, where oxygen is abundant, all carriers are in a partly oxidized state. The addition of a specific inhibitor causes the downstream carriers to become more oxidized *(red)* and the upstream carriers to become more reduced. (B) In the absence of oxygen, all carriers are in their fully reduced state *(gray).* The sudden addition of oxygen converts each carrier to its partly oxidized form with a delay that is greatest for the most upstream carriers.

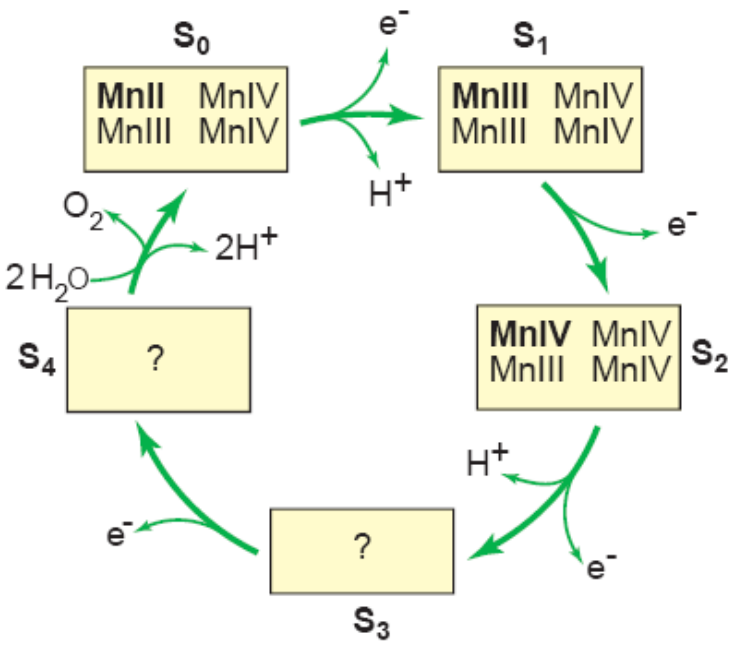
Water splitting reaction

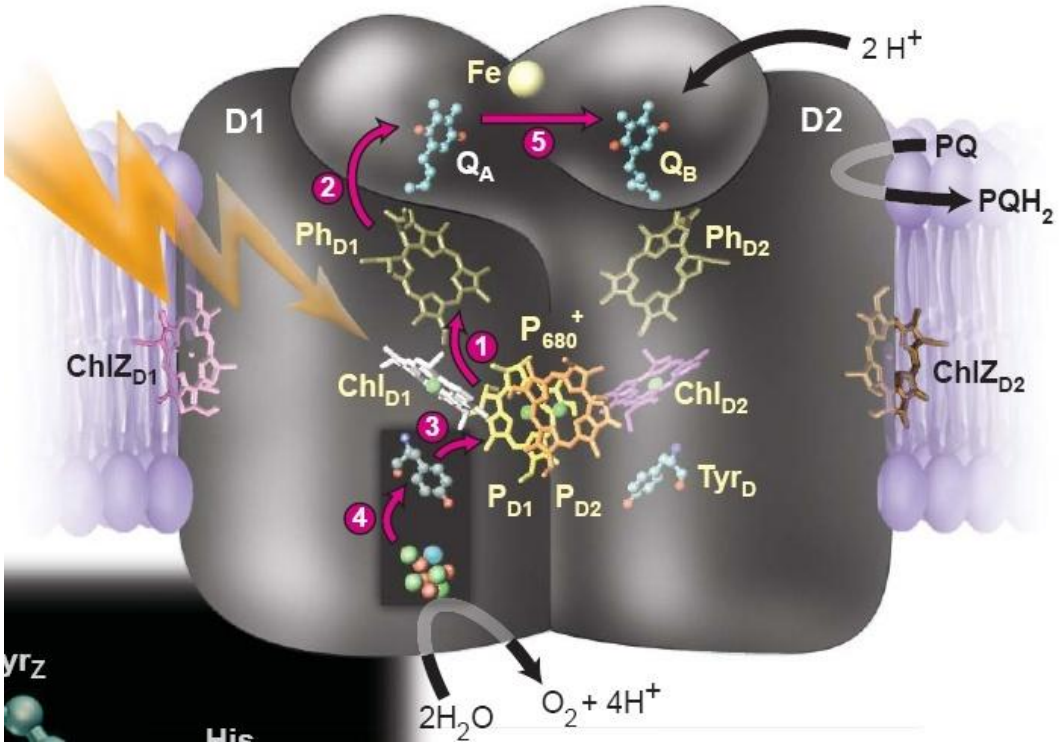
- The photo-excited P_{680}^{+} is reduced by a tyrosine residue, Tyr_z.
- Tyr_z^{+} in turn abstracts an electron from the Mn cluster.
  - Located 7 Å from the Mn cluster.
- Four photon absorption steps lead to 4Mn being oxidised to $4Mn^{+}$.
  - Highly electropositive.
  - Spontaneously accepts 4 electrons from H_2O ($E_{m,7}$ of the $O_2/2H_2O$ couple is 810 mV).
  - **Most electropositive reaction in nature**.
- Centre-to-centre distance from the Mn cluster to the P_{680} chlorophylls is 18.5 Å to P_{D1} & 25.1 Å to P_{D2}.



Water splitting reaction

- The enzyme accumulates four positive charge-equivalents
- Deprotonation occurs to compensate the charge accumulation on some steps, before oxidizing $2H_2O$ and releasing O_2.
- The valence of the Mn ions increases on the S0 to S1 to S2 steps;
- Less certain for the S3 & S4 steps.





Se o O_2 exibe uma afinidade extremamente elevada para os electrões, sendo ele, por isso, o aceitador terminal de electrões da mETC, como é possível retirar electrões da H_2O para circularem na cETC, com libertação de oxigénio molecular?
A molécula, ou melhor, o complexo de moléculas capaz de arrancar os electrões da H_2O é *The water-splitting complex*, com um valor do potencial redox de oxidação-redução de

It is the most electropositive reaction in nature.

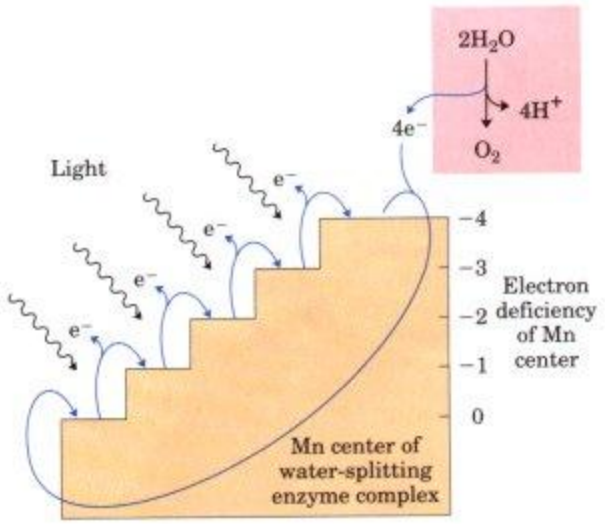
About 3 billion years ago, evolution of primitive photosynthetic bacteria (the progenitors of the modern cyanobacteria) produced a photosystem capable of taking electrons from a donor that is always available-water. In this process two water molecules are split, yielding four electrons, four protons, and molecular oxygen: $2H_2O\ 4H^+ + 4e^- + O_2$. A single photon of visible light does not possess enough energy to break the bonds in water; four photons are required in this photolytic cleavage reaction.

The four electrons abstracted from water do not pass directly to $P680^+$, which can only accept one electron at a time. Instead, a remarkable molecular device, the **water-splitting complex**, passes four electrons one at a time to $P680^+$. The immediate electron donor to $P680^+$ is a Tyr residue (often represented by the symbol Z) in protein D_1 of the photosystem II reaction center:
This Tyr residue regains its missing electron by oxidizing a cluster of four manganese ions in the water-splitting complex. With each single electron transfer, this Mn cluster becomes more oxidized; four single electron transfers, each corresponding to the absorption of one photon, produce a charge of +4 on the Mn complex.

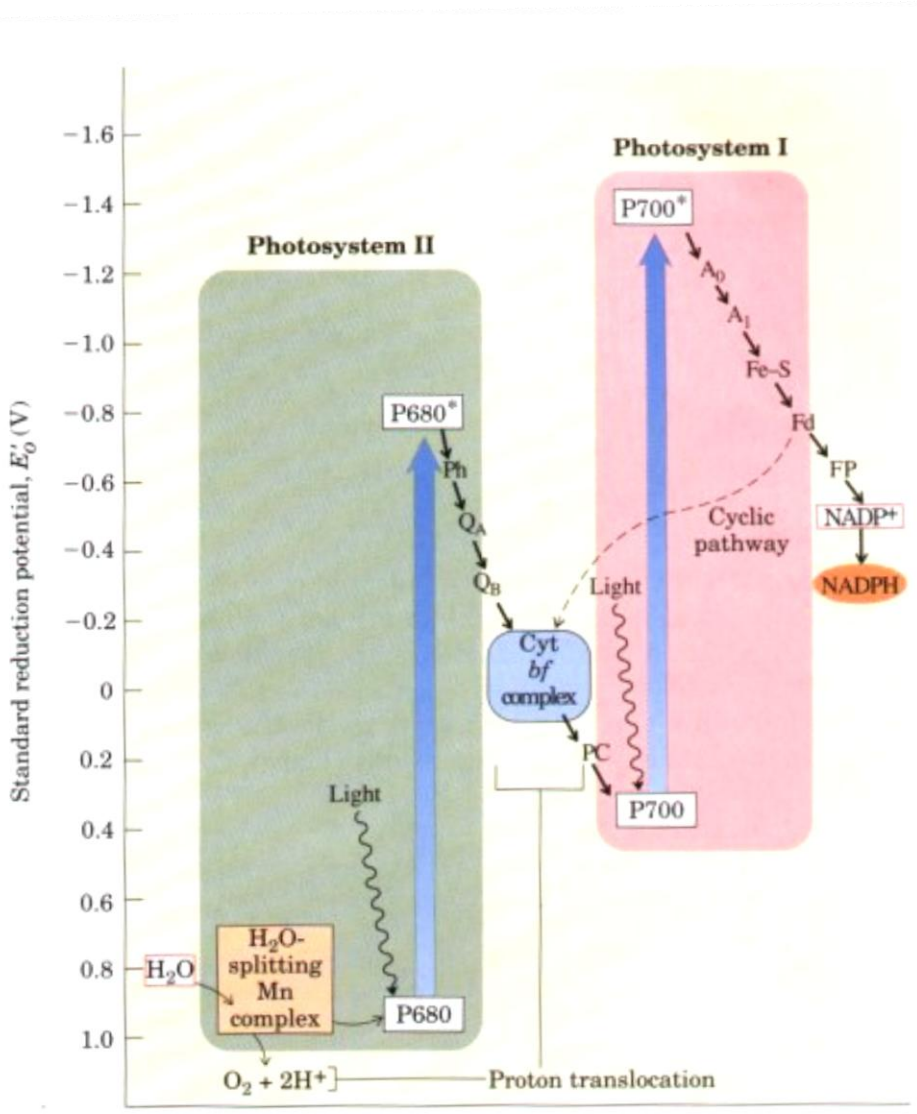
In this state, the Mn complex can take four electrons from a pair of water molecules, releasing $4H^+$ and 0_2:

$$[\text{Mn complex}]^{4+} + 2\ H_2O \rightarrow [\text{Mn complex}]^0 + 4H^+ + O_2$$

The water-splitting activity is an integral part of the photosystem II reaction center, and it has proved exceptionally difficult to purify. The detailed structure of the Mn cluster is not yet known. Manganese can exist in stable oxidation states from +2 to +7, so a cluster of four Mn ions can certainly donate or accept four electrons; the chemical details of this process, however, remain to be clarified.



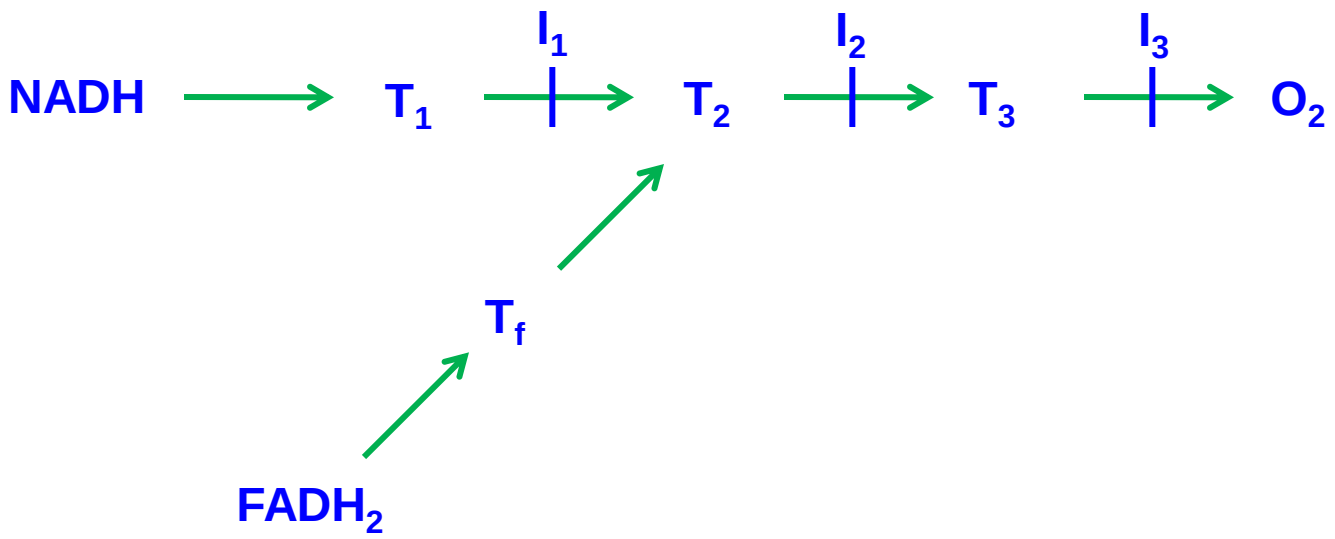
The four-step process that produces a four-electron oxidizing agent, believed to be a complex of several Mn ions, in the water-splitting complex of photosystem II. The sequential absorption of four photons, each causing the loss of one electron from the Mn center, produces an oxidizing agent that can take four electrons from two molecules of water, producing O_2. The electrons lost from the Mn center pass one at a time to a Tyr residue (Z^+) in a reaction-center protein.



The integration of photosystems I and II. This "Z scheme" shows the pathway of electron transfer from H_2O (lower left) to $NADP^+$ (upper right) in noncyclic photosynthesis. The position on the vertical scale of each electron carrier reflects its standard reduction potential. To raise the energy of electrons derived from H_2O to the energy level required to reduce $NADP^+$ to NADPH, each electron must be "lifted" twice (heavy arrows) by photons absorbed in photosystems I and II. One photon is required per electron boosted in each photosystem. After each excitation, the high-energy electrons flow "downhill" via the carrier chains shown. Protons move across the thylakoid membrane during the water-splitting reaction and during electron transfer through the cytochrome bf complex, producing the proton gradient that is central to ATP formation. The dashed arrow is the path of cyclic electron transfer, in which only photosystem I is involved; electrons return via the cyclic pathway to photosystem I, instead of reducing $NADP^+$ to NADPH. Ph, pheophytin; Q_A, plastoquinone; Q_R, a second quinone; PC, plastocyanin; A_0 electron acceptor chlorophyll; A_1, phylloquinone; Fd, ferredoxin; FP, ferredoxin-$NADP^+$ oxidoreductase.

Problema - 1

Considere uma cadeia de transporte de electrões hipotética, constituída pelos transportadores de electrões T_1, T_2, T_3 e T_f, que se encontra acoplada à síntese de ATP.



O emprego dos inibidores I_1, I_2 e I_3 bloqueia o fluxo de electrões nos pontos indicados. As razões P / $2e^-$ (i.e., o número de moléculas de ATP produzidas por par de electrões que passa ao longo da cadeia) obtidas na presença e na ausência dos inibidores foram as seguintes:

a) Na presença de NADH e na ausência de inidores	P / $2e^-$ = 3
b) Na presença de NADH e de I_1	P / $2e^-$ = 1
c) Na presença de NADH e de I_2	P / $2e^-$ = 1
d) Na presença de NADH e de I_3	P / $2e^-$ = 2
e) Na presença de $FADH_2$ e na ausência de inidores	P / $2e^-$ = 3

A partir dos dados localize, o mais precisamente possível, as transferências de electrões que libertam energia suficiente para a fosforilação do ADP quando o substrato da cadeia de transporte de electrões é o NADH e quando é o $FADH_2$.

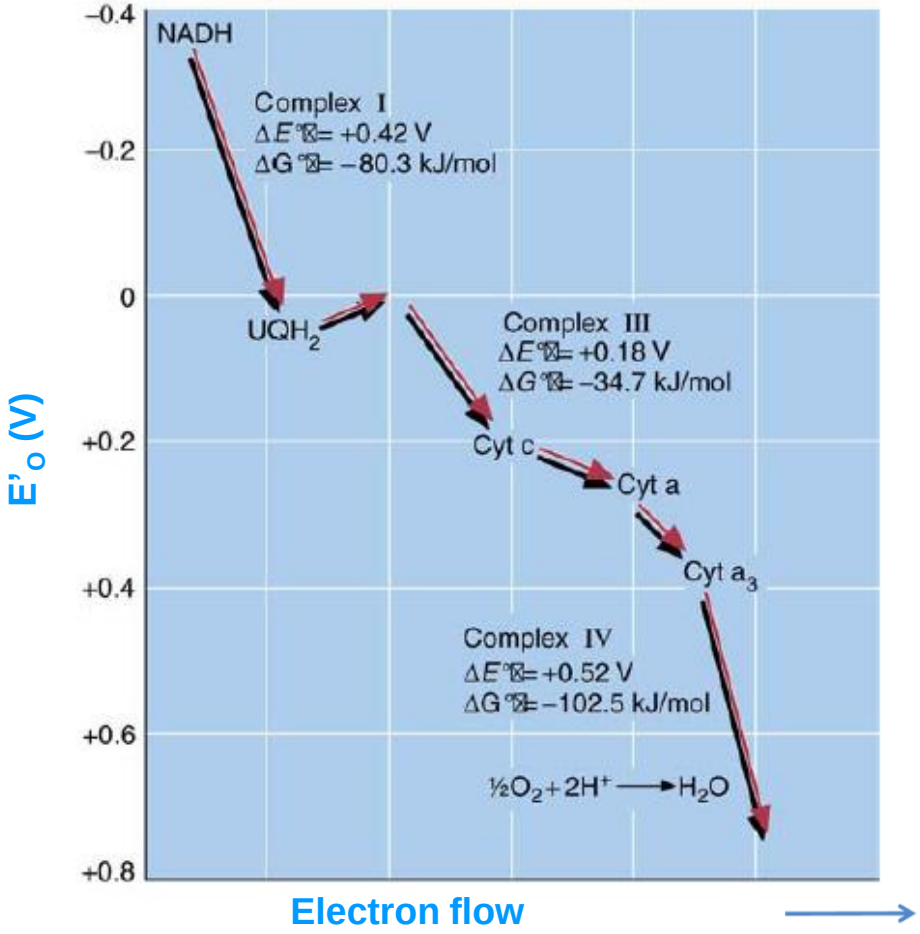
Problema - 2

Considere uma cadeia de transporte de electrões hipotética constituída pelos seguintes transportadores de electrões, a que correspondem os potenciais–padrão de oxidação-redução:

A : -0,12 V
B : -0,32 V
C : -0,02 V
D : -0,23 V
E : -0,52 V
F : -0,18 V

Coloque-os pela ordem em que circulam os electrões. Justifique.

Problema - 3



De acordo com a figura apresentada, explique como é possível para os electrões passarem espontaneamente (i.e., com ΔG < O) da UBQ_2 para o complexo III, com uma pequena descida no valor de E'_o.

Pista: Relembre a diferença entre ΔG e $\Delta G^{o'}$.

The Paradox of Aerobiosis

- **Oxygen is essential, but toxic.**
- **Aerobic cells face constant danger from reactive oxygen species (ROS).**
- **ROS can act as mutagens, they can cause lipid peroxidation and denature proteins.**

Reactive oxygen species (ROS) - Definition

ROS are reactive molecules that contain the oxygen atom. They are very small molecules that include oxygen ions and peroxides and can be either inorganic or organic. They are highly reactive due to the presence of unpaired valence shell electrons. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress (e.g. UV or heat exposure) ROS levels can increase dramatically, which can result in significant damage to cell structures. This cumulates into a situation known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation.

- The Earth was originally anoxic
- Metabolism was anaerobic
- O_2 started appearing ~2.5 x 10^9 years ago

Anaerobic metabolism-glycolysis

$$\text{Glucose} + 2\text{ADP} + 2P_I \longrightarrow \text{Lactate} + 2\text{ATP} + 2H_2O$$

O_2 an electron acceptor in aerobic metabolism

$$\text{Glucose} + 6O_2 + 36\text{ADP} + 36P_I \longrightarrow 6CO_2 + 36\text{ATP} + 6H_2O$$

Reactive Oxygen Species (ROS)

Radicals:	
$O_2^{\cdot-}$	Superoxide
$^{\cdot}OH$	Hydroxyl
$RO_2^{\cdot}$	Peroxyl
$RO^{\cdot}$	Alkoxyl
$HO_2^{\cdot}$	Hydroperoxyl

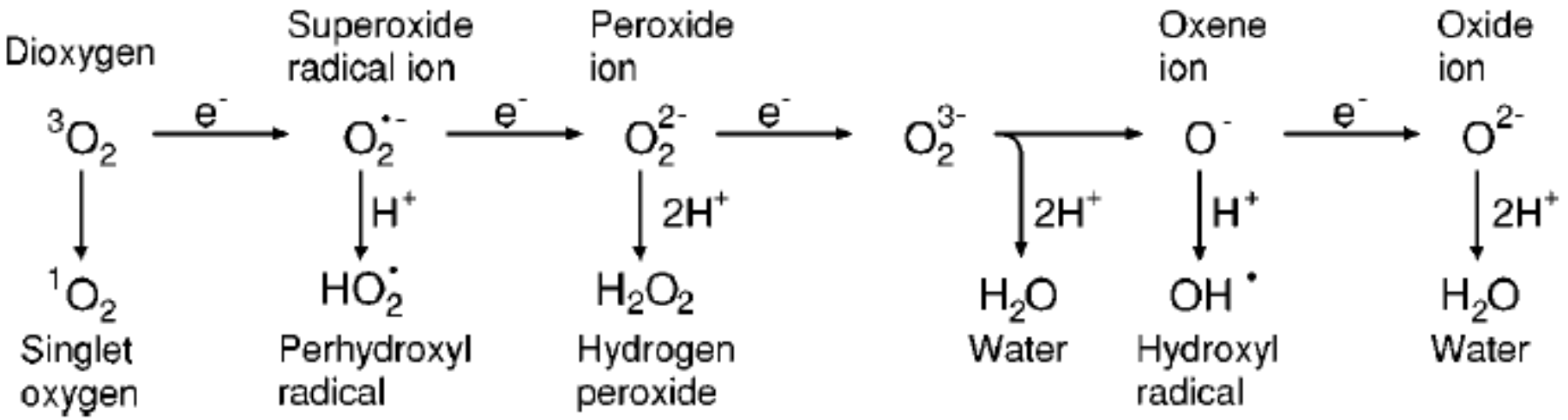
Non-Radicals:	
H_2O_2	Hydrogen peroxide
$HOCl^{-}$	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^{-}$	Peroxynitrite

"Longevity" of reactive species

Reactive Species	Half-life
Hydrogen peroxide Organic hydroperoxides Hypohalous acids	~ minutes
Peroxyl radicals Nitric oxide	~ seconds
Peroxynitrite	~ milliseconds
Superoxide anion Singlet oxygen Alcoxyl radicals	~ microsecond
Hydroxyl radical	~ nanosecond

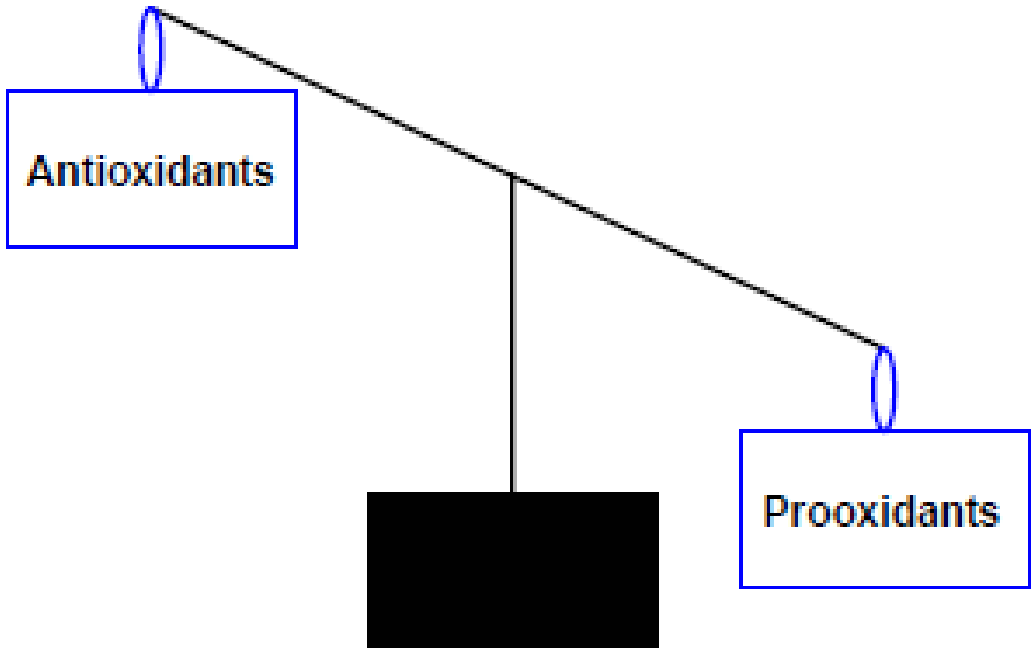
Oxidative Stress

1. Classic definition: The production of reactive oxygen in excess of antioxidant mechanisms
2. Modern definition: Altered homeostatic balance resulting from oxidant insult.



Generation of different ROS by energy transfer or sequential univalent reduction of ground state triplet oxygen.

Oxidative Stress



"An imbalance favoring prooxidants and/or disfavoring antioxidants, potentially leading to damage" -H. Sies

Major sources of ROS:

The ETCs – both the mETC and the cETC,

under normal metabolic conditions

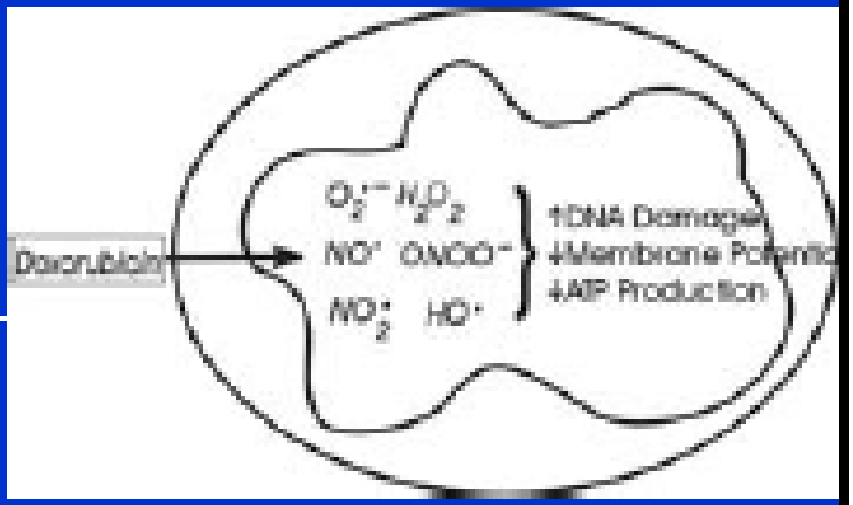
under stress conditions:

In plants – hot, dry, summer afternoon

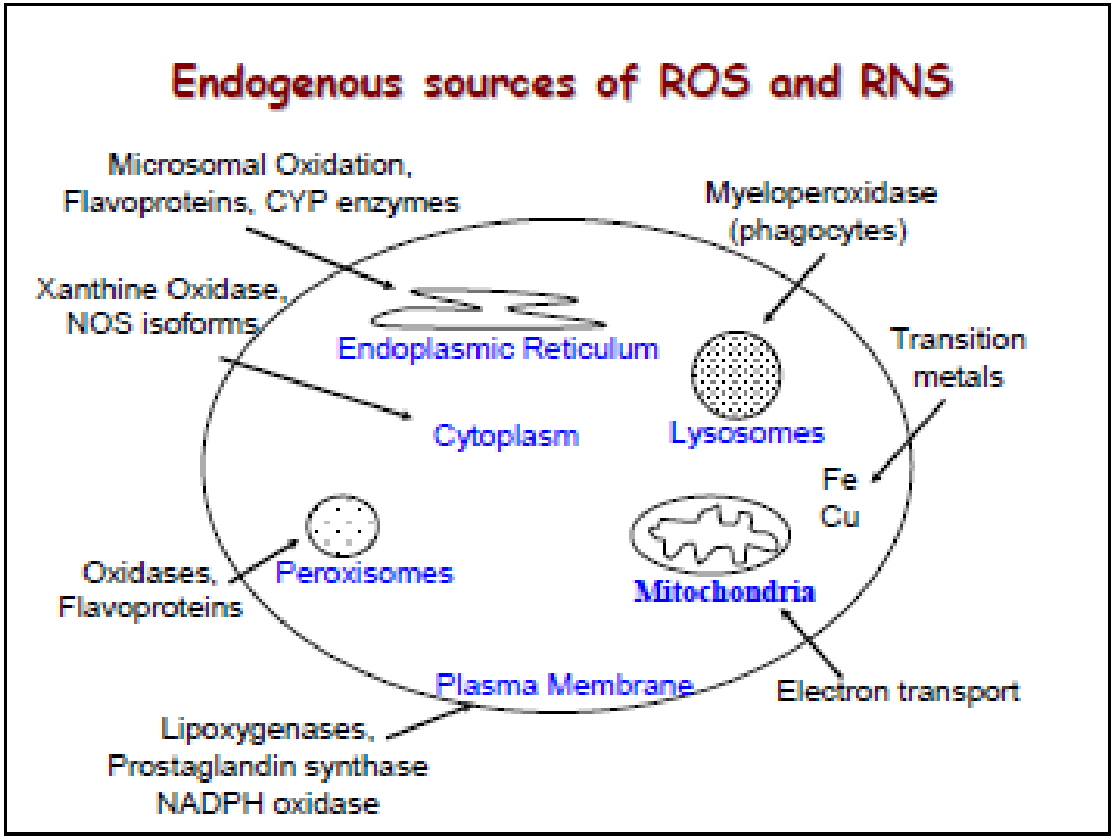
In man – exposure to certain herbicides

Cytochrome oxidase is estimated to account for 90-95% of the total oxygen uptake in most cells

- What happens to other 1-5%
- $O_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$, $HO_2^{\bullet}$ (hydroperoxyl radical)
- Respiring cells avoid $O_2^{\bullet-}$ formation 99-95% of the time



Endogenous sources of ROS and RNS



Sources of ROS

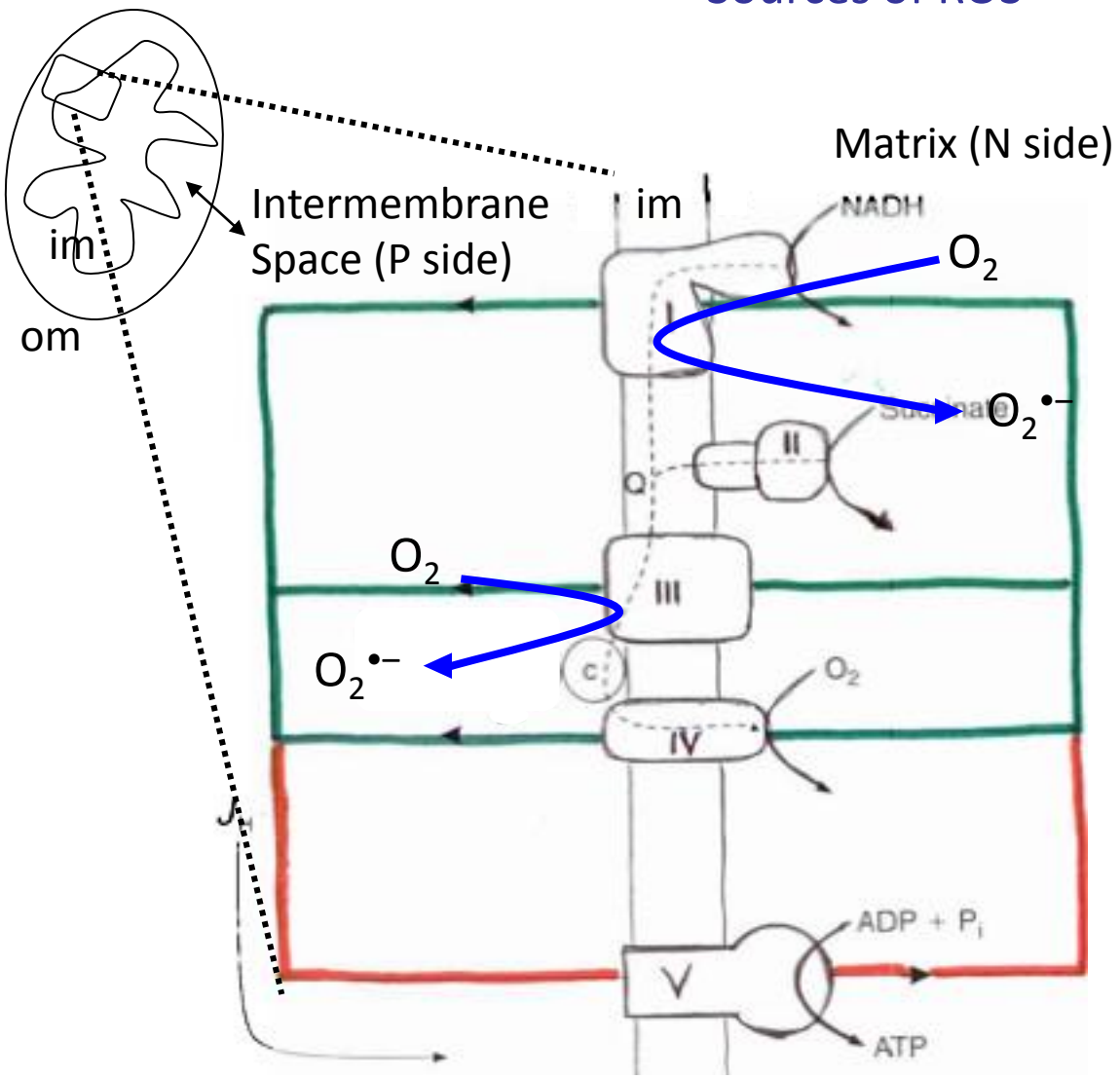
Enzymes located in microsomes, peroxisomes, and cytosol can contribute $O_2^{\bullet -}$ and H_2O_2, especially in response to certain drugs or in response to ischemia/reperfusion

Main ROS generator is mitochondria

Electron "fumbling" or "leakage" by respiratory chain gives rise to $O_2^{\bullet -}$ and hence H_2O_2, in isolated mitochondria, submitochondrial particles, and isolated respiratory complexes.

Main generators are Complexes I and III

Sources of ROS



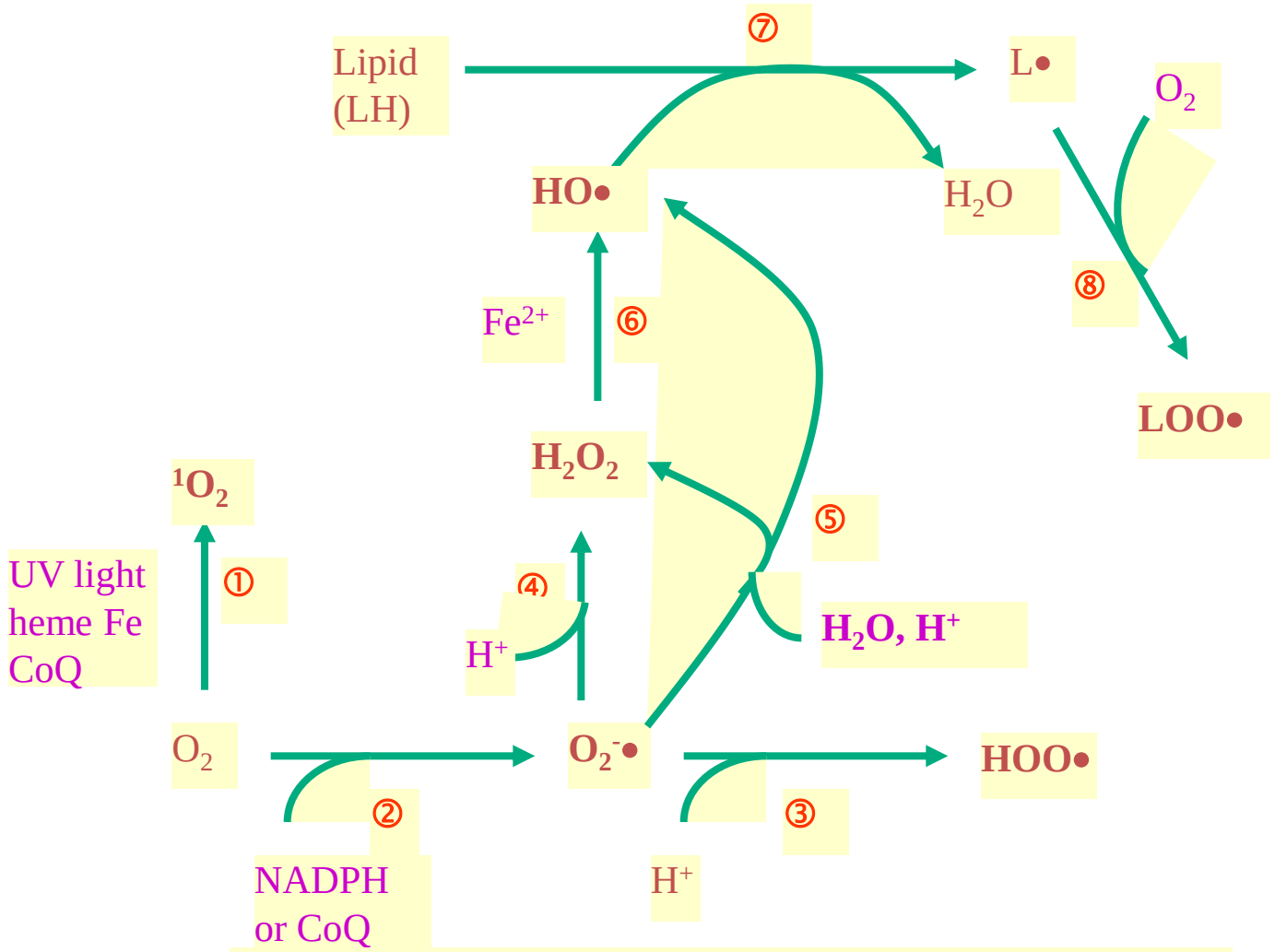
$O_2^{\bullet-} + H^+ \rightarrow HO_2^{\bullet}$

$pK_a = 4.7$.

To measure superoxide in intact mitos, need to measure H_2O_2, because H_2O_2 can cross membrane.

Assays for H_2O_2 should be specific, and the H_2O_2 generated should not be scavenged by other antioxidants, and should avoid other agents that could affect or interfere with the assay.

There is potential for fumbling e- at many sites: cytochromes, Fe/S clusters, flavins And $Q^{\bullet-}$. Many studies implicate $Q^{\bullet-}$ and flavins the major culprits.



Pathways for the formation of reactive oxygen species

① Singlet oxygen

② Superoxide radical anion

③ Peroxyl radical

④ Superoxide dismutase

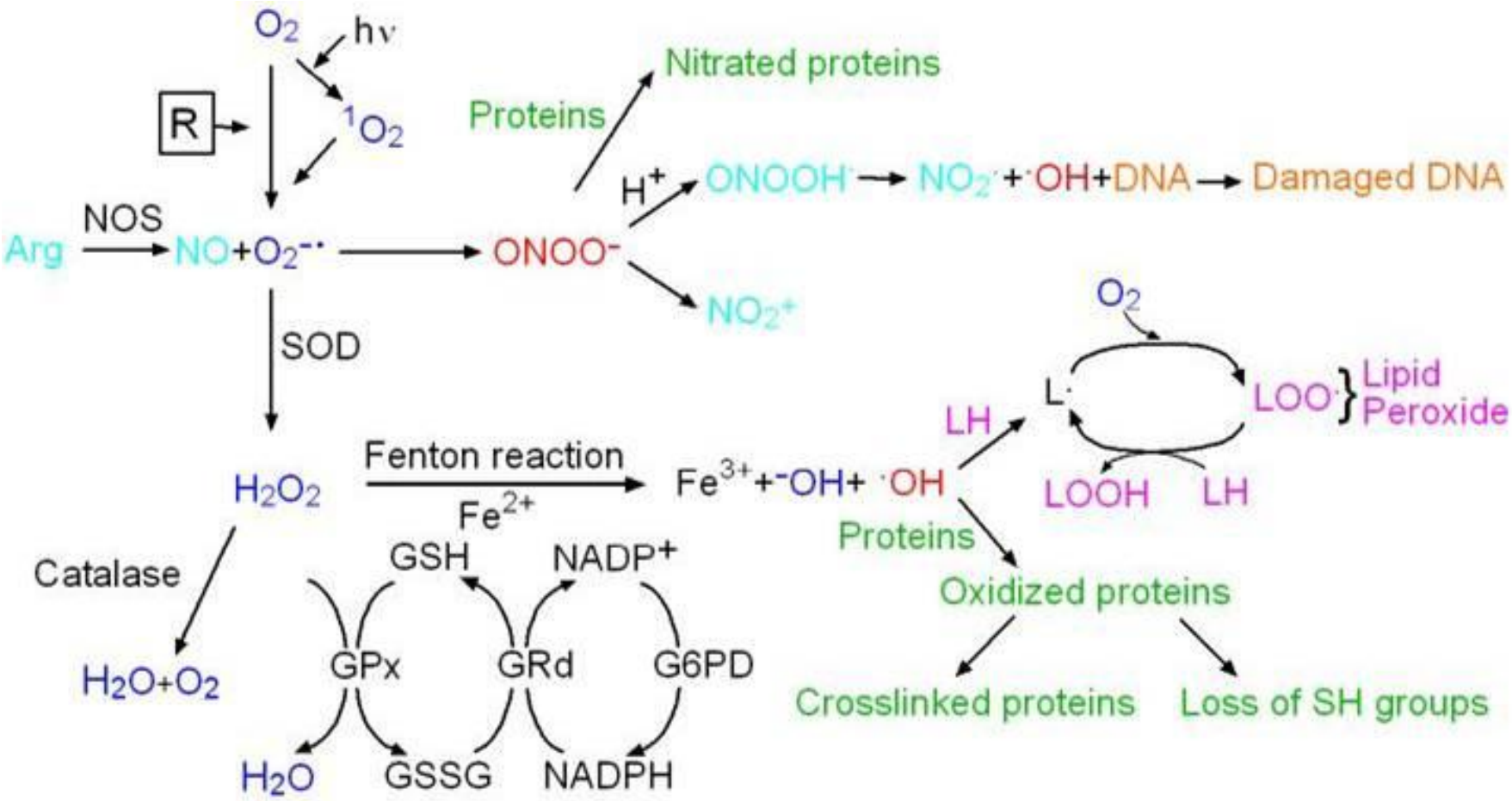
⑤ Haber-Weiss reaction;

⑥ Fenton reaction

⑦ lipid radical

⑧ lipid peroxyl radical

Partial reduction of oxygen generates ROS



Exogenous sources of free radicals

- **Radiation**
  UV light, x-rays, gamma rays
- **Chemicals that react to form peroxides**
  Ozone and singlet oxygen
- **Chemicals that promote superoxide formation**
  Quinones, nitroaromatics, bipyrimidiulium herbicides
- **Chemicals that are metabolized to radicals**

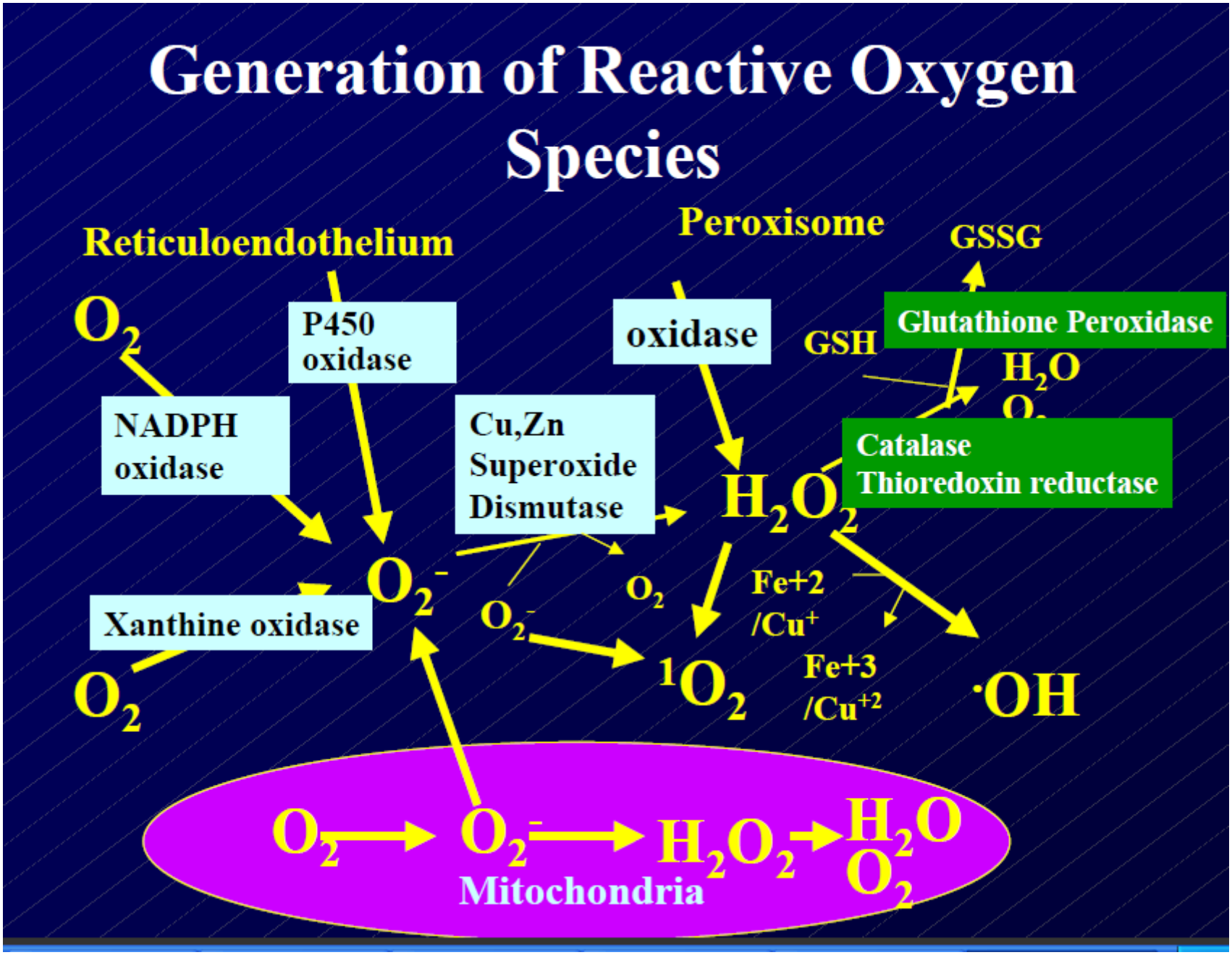
  e.g., polyhalogenated alkanes, phenols, aminophenols
- **Chemicals that release iron**
  ferritin

ROS Arise as a Result of Exposure to:

- Ozone
- Sulfur dioxide
- High light
- Herbicides
- Extremes of temperature
- Salinity
- Drought

Pathological conditions that involve oxidative stress

- Inflammation
- Atherosclerosis
- Ischemia/reperfusion injury
- Cancer
- Aging


Generation of Reactive Oxygen Species
Reticuloendothelium
Peroxisome
GSSG
O_2
P450 oxidase
oxidase
GSH
Glutathione Peroxidase
H_2O
O_2
NADPH oxidase
Cu,Zn Superoxide Dismutase
Catalase
Thioredoxin reductase
H_2O_2
O_2^-
O_2
Fe+2 /Cu^+
Xanthine oxidase
O_2^-
$^1O_2$
Fe+3 /Cu^{+2}
$\cdot OH$
O_2
$O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow H_2O$ O_2
Mitochondria

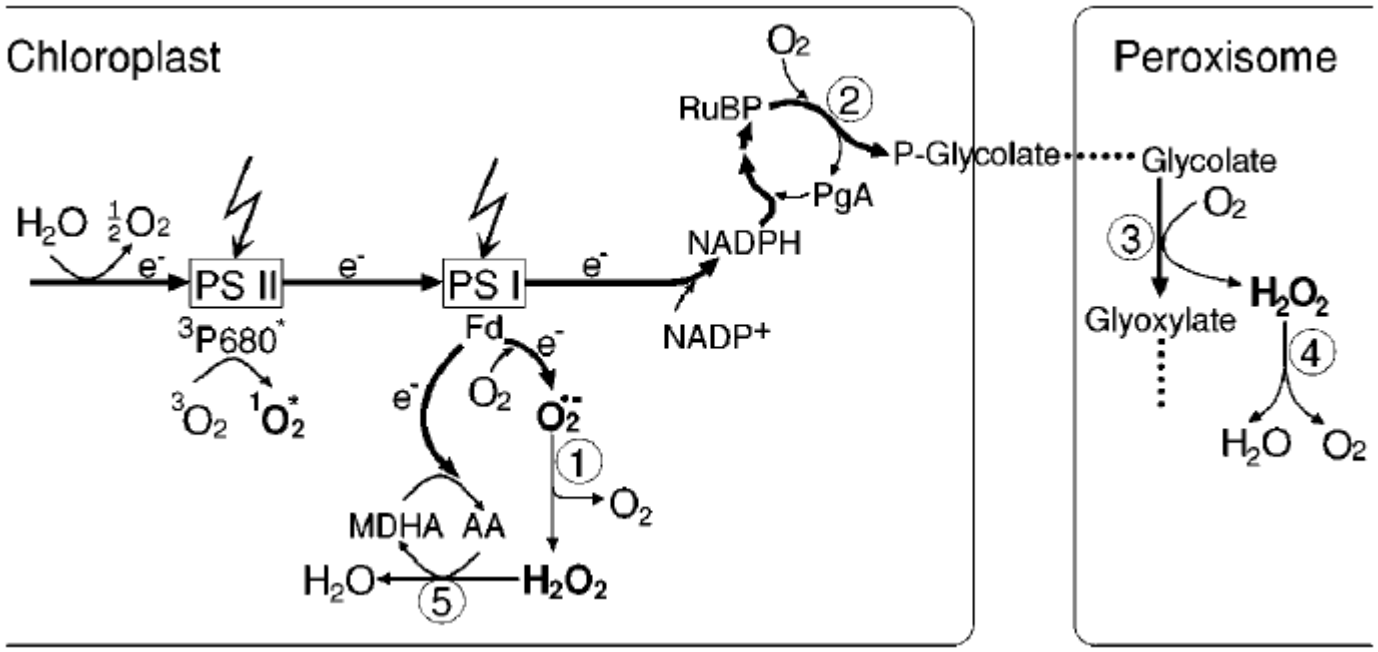
Reactive Oxygen Species

Endoplasmic Reticulum

O_2 → H_2O_2 → •OH

Mitochondrion

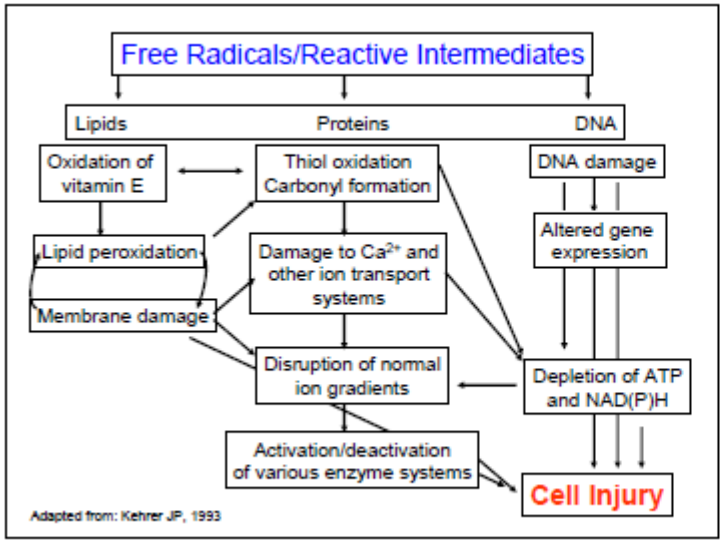
- •Damage DNA, RNA
- •Oxidize Proteins (enzymes, histones)
- •Oxidize Lipids
- •Activate Cell Suicide



The principal features of photosynthetic electron transport under high light stress that lead to the production of ROS in chloroplasts and peroxisomes. Two electron sinks can be used to alleviate the negative consequences of overreduction of the photosynthetic electron chain: (*a*) the reduction of oxygen by PSI that generates superoxide and H_2O_2, and (*b*) the Rubisco oxygenase reaction and the photorespiratory pathway that lead to H_2O_2 generation within the peroxisome. Under light stress, increasing amounts of singlet oxygen are produced within PSII. Bold arrows show the main routes of electron transport.Key enzymes discussed in the text are shown in encircled numbers: 1) superoxide dismutase, 2) Rubisco, 3) glycolate oxidase, 4) catalase, and 5) ascorbate peroxidase.

ROS – consequences

- Unless ROS are removed from biological systems, they cause damage to:
  - Lipids (leakage of solutes);
  - Proteins (loss of function, aggregation);
  - Nucleic acids (mutation, nicks)
- The evolution of aerobic life forms has gone hand in hand with the evolution of highly conserved mechanisms for ROS removal and sensing of ROS.



Consequences of lipid peroxidation

- **Structural changes in membranes**
  - alter fluidity and channels
  - alter membrane-bound signaling proteins
  - increases ion permeability
- **Lipid peroxidation products form adducts/crosslinks with non lipids**
  - e.g., proteins and DNA
- **Cause direct toxicity of lipid peroxidation products**
  - e.g., 4-hydroxynonenal toxicity
- **Disruptions in membrane-dependent signaling**
- **DNA damage and mutagenesis**

Consequences of protein thiol oxidation

Oxidation of catalytic sites on proteins

- loss of function/abnormal function
- BUT(!): sometimes it is gain in function!

Formation of mixed sulfide bonds

- Protein-protein linkages (RS-SR)
- Protein-GSH linkages (RS-SG)
- Alteration in 2º and 3º structure

Increased susceptibility to proteolysis

Consequences of DNA oxidation

- **DNA adducts/AP sites/Strand breaks**
  - mutations
  - initiation of cancer
- **Stimulation of DNA repair**
  - can deplete energy reserves (PARP)
  - imbalanced induction of DNA repair enzymes
  - induction of error prone polymerases
  - activation of other signaling pathways

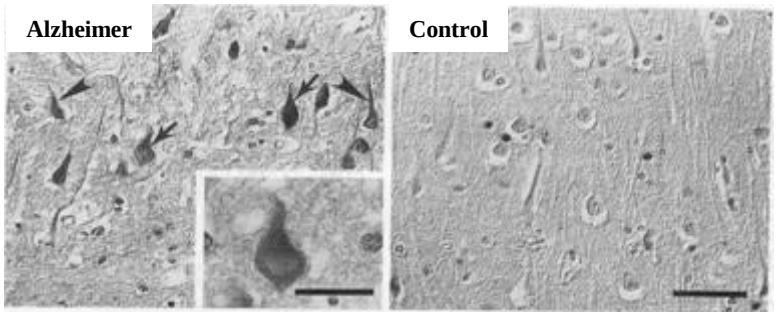
ROS pathology

• ROS are consequently implicated in a wide variety of disorders:

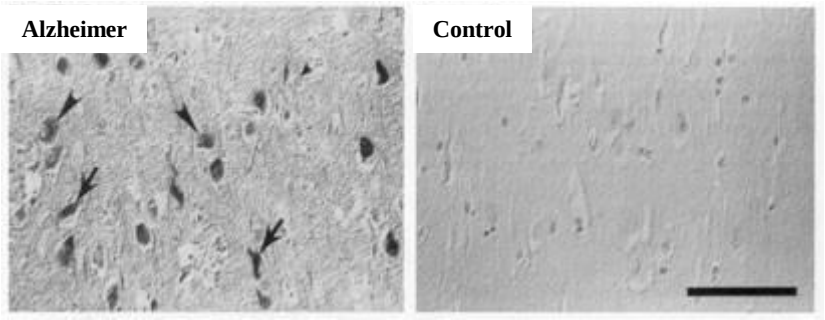
In plants:
– photorespiratory damage;
– pathogen attack;
– biotic and abiotic stresses
In animals:
– inflammatory disorders
– immune system
– cancer
– degenerative disorders such as Parkinson’s and Alzheimer's
– genetic disorders eg Lou Gehrig syndrome, Down’s syndrome

• The study of ROS is therefore one of the most vibrant areas of research in plant and animal science:

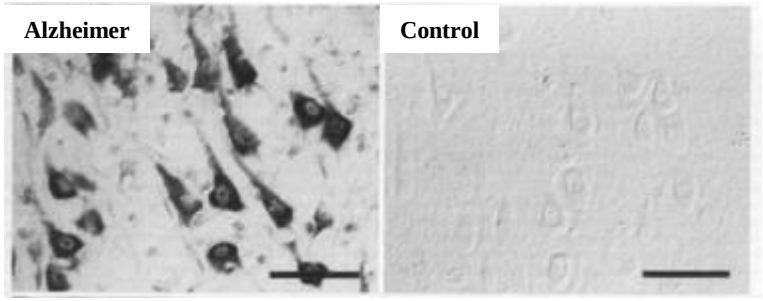
Oxidative Modifications Affect All Cellular Macromolecules



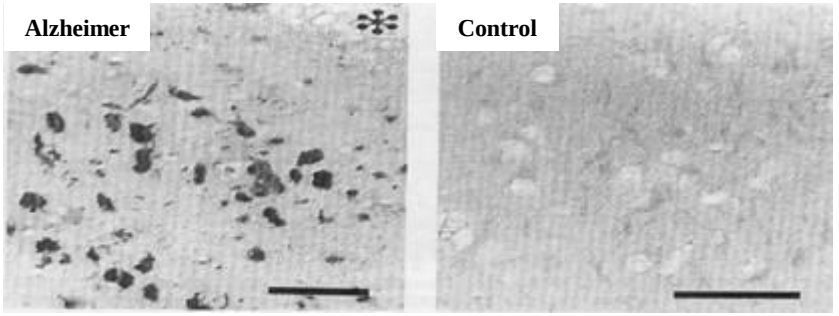
Lipid Peroxidation/Protein Adduction (4-HNE)



Protein Oxidation (Free Carbonyl Groups)

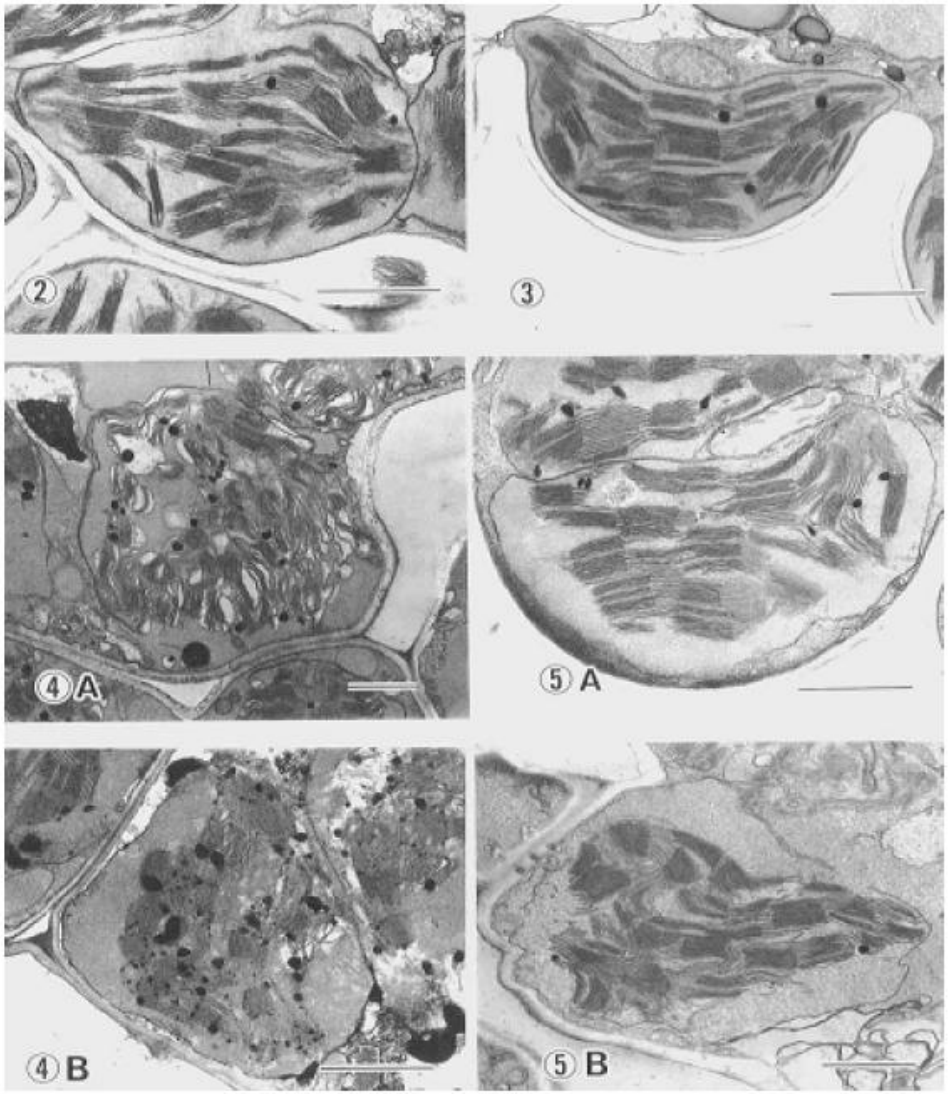


Nucleic Acids (8-OH-Guanosine)



Glycoxidation (Carboxymethyllysine)

ROS-mediated chloroplast damage (rice)

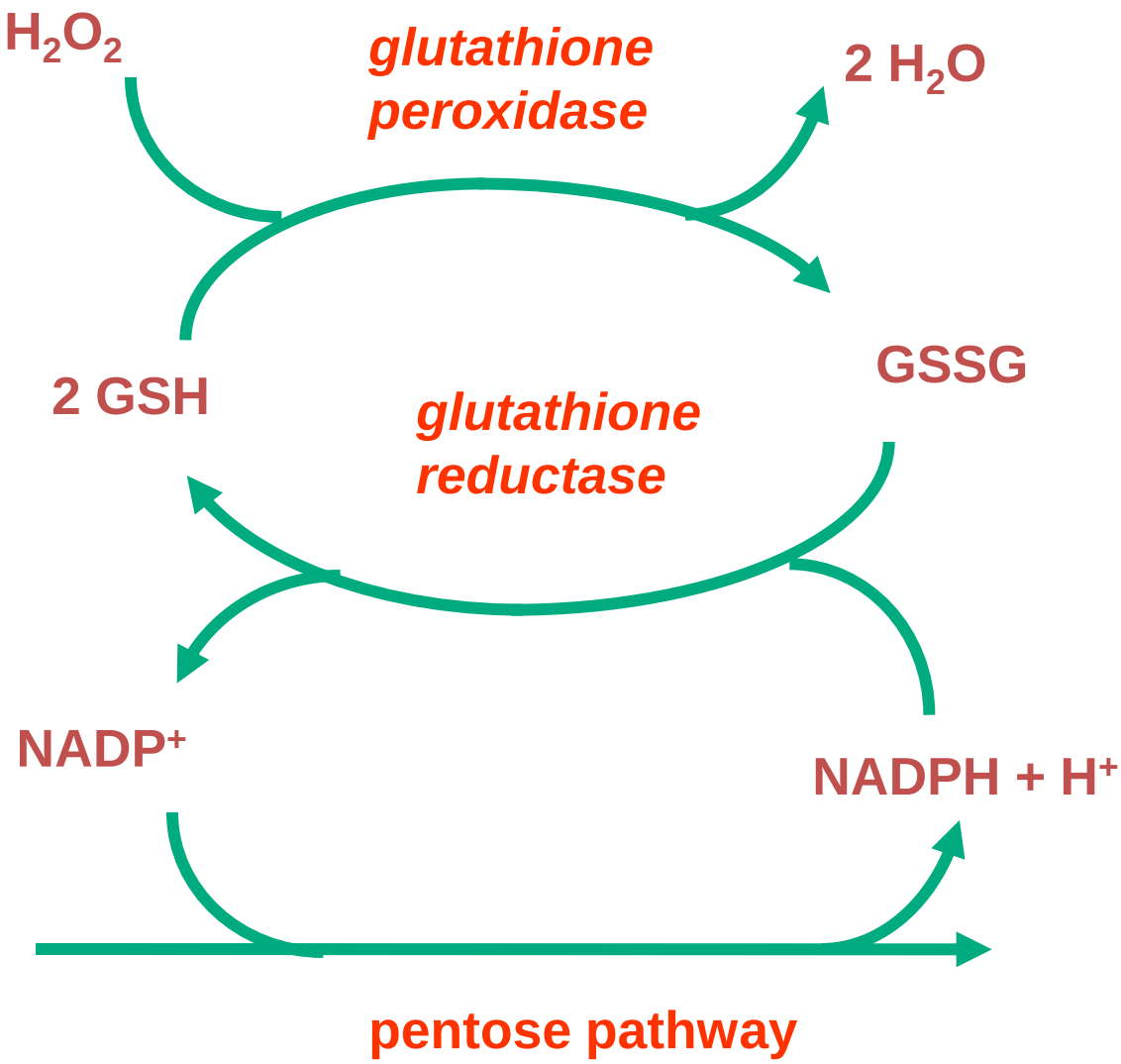


Methyl viologen

Salt

Reactive Oxygen Species and Antioxidants that Reduce them

Reactive Species	Antioxidant
Singlet oxygen $^1O_2$	Vitamin A, vitamin E
Superoxide radical ($O_2^{-}\bullet$)	superoxide dismutase, vitamin C
Hydrogen peroxide (H_2O_2)	Catalase; glutathione peroxidase
Peroxyl radical (ROO•)	Vitamin C, vitamin E
Lipid peroxyl radical (LOO•)	Vitamin E
Hydroxyl radical (OH•)	Vitamin C



Reactions of glutathione reduction and oxidation

ROS - Removal

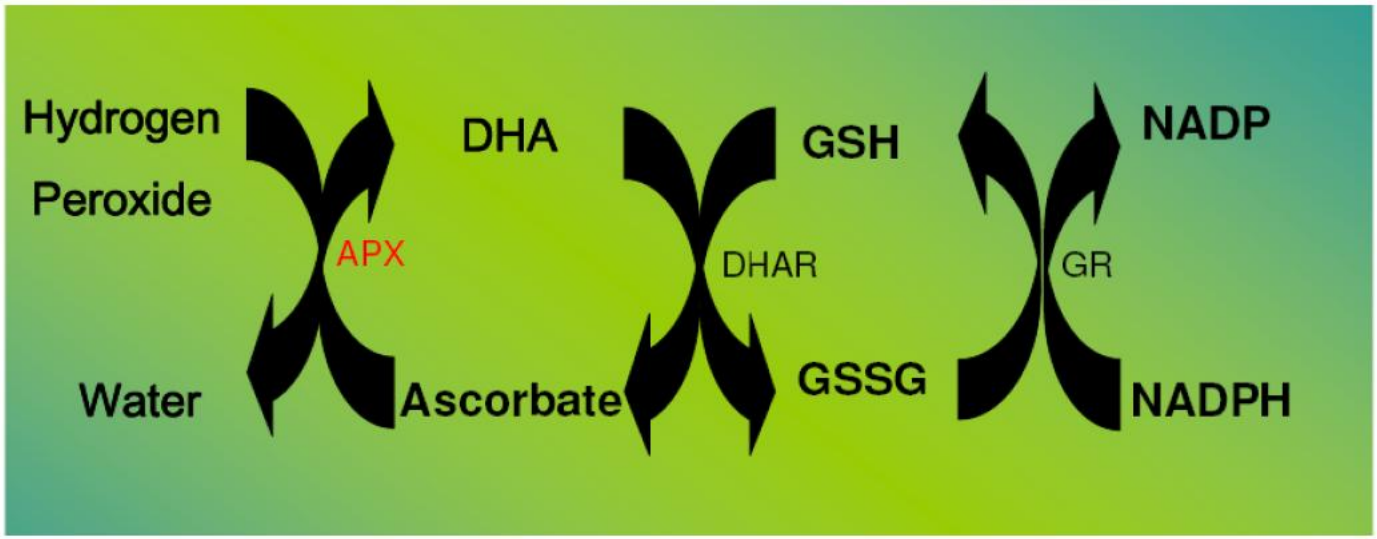
• Enzymatic processes:
– Superoxide dismutases
– peroxidases
– catalase
– thioredoxin/glutaredoxin

Some of these enzymes are selenoproteins

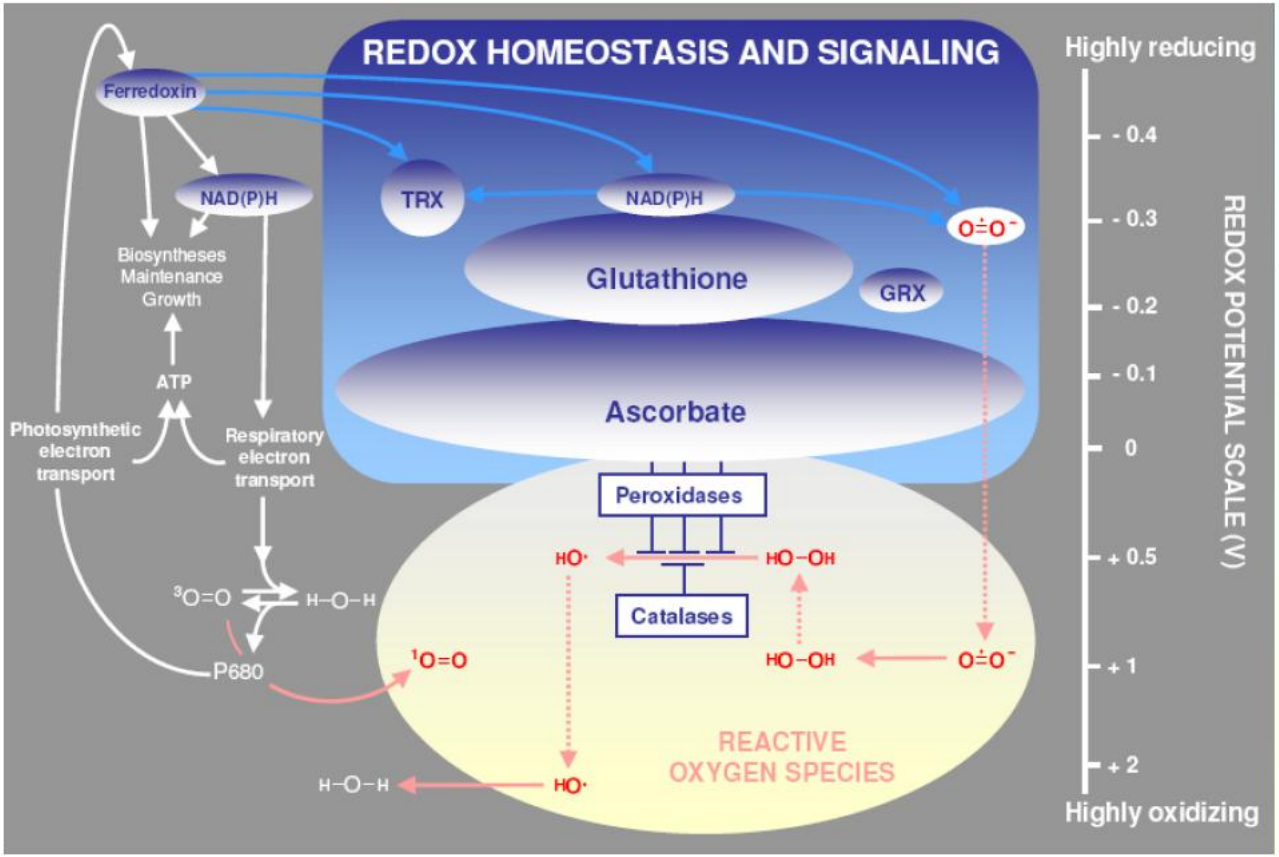
• Major antioxidant compounds/cofactors:
– NAD(P)H
– ascorbate
– tocopherols (vitamin E)
– glutathione
– pigments
– polysaccharides

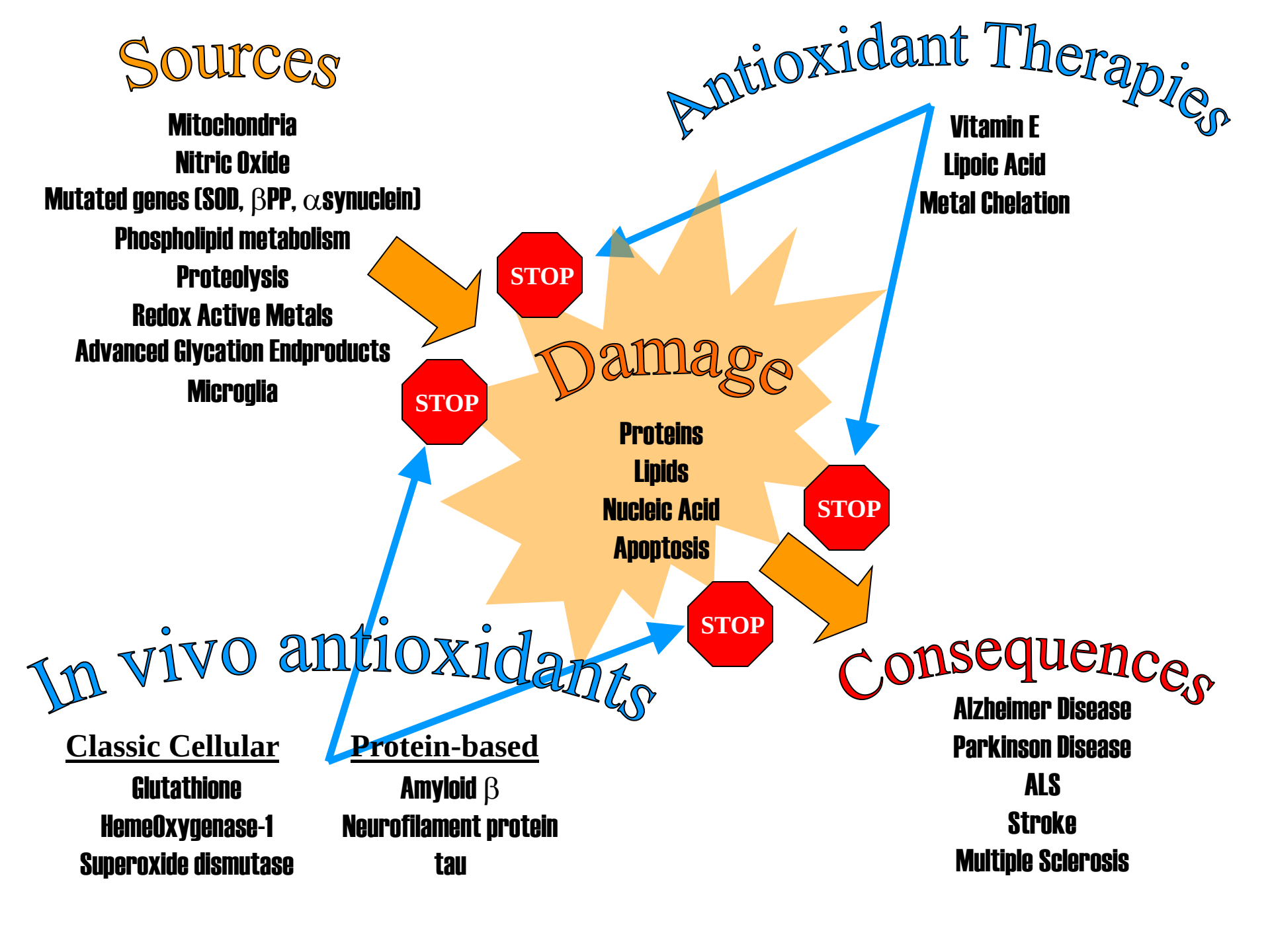
The overexagerated trend of antioxidants

The Ascorbate-Glutathione cycle



Redox homeostasis




Sources
Mitochondria
Nitric Oxide
Mutated genes (SOD, βPP, αsynuclein)
Phospholipid metabolism
Proteolysis
Redox Active Metals
Advanced Glycation Endproducts
Microglia
Antioxidant Therapies
Vitamin E
Lipoic Acid
Metal Chelation
STOP
STOP
STOP
STOP
Damage
Proteins
Lipids
Nucleic Acid
Apoptosis
In vivo antioxidants
Classic Cellular
Glutathione
HemeOxygenase-1
Superoxide dismutase
Protein-based
Amyloid β
Neurofilament protein
tau
Consequences
Alzheimer Disease
Parkinson Disease
ALS
Stroke
Multiple Sclerosis

Conclusions

- Metal catalyzed oxidative damage to all categories of macromolecules is increased.

- Antioxidant pathways and inflammatory responses are induced.

- “Pathological changes” may be compensations that are critical to maintaining oxidative homeostasis.

Free Radicals

Who's who on the wrecking crew: Some free radicals and their allies.

Sequence of events in the mitochondrial electron transport chain that lead to ROS formation

Animation at Site:

http://www.pathology.washington.edu/research/labs/rabinovitch/etc.ppt

Click the image for a larger version.

Movies

Mouse Movie

PowerPoint Animation

Use PowerPoint 2002 or better to view:

Free PowerPoint Viewer for Windows

Powerpoint animation of reactive oxygen generation from the mitochondrial electron transport chain (ETC)

Slide 1: Powerpoint animation of **reactive oxygen generation from the mitochondrial electron transport chain** (ETC).

1st mouse click: electron transport with generation of a proton gradient and production of ATP, with oxygen utilized as the final electron acceptor.

2nd mouse click: escape of free electrons from complex I and III of the electron transport chain, with production of superoxide.

3rd mouse click: Superoxide dismutase catalyzes the conversion of superoxide to hydrogen peroxide. The enzyme catalase removes hydrogen peroxide, with production of water.

4th mouse click: If hydrogen peroxide is not eliminated, it can generate the highly reactive hydroxyl radical (iron accelerates this process via the Fenton reaction). Hydroxyl radicals will damage cellular macromolecules, including components of the electron transport chain.

Slide 2: Mitochondrial function and **reactive oxygen (ROS) damage**.

1st mouse click: ***Mitochondrial ROS can damage mitochondrial DNA, mitochondrial proteins and the mitochondrial permeability transition pore (MPTP). Aconitase (within the Krebs cycle) and proteins with in ETC complex I, II and III have iron-sulfur centers which make them particularly sensitive to ROS damage. Opening of the MPTP secondary to ROS damage can induce cellular apoptosis.***

2nd mouse click: ***mitochondrial DNA codes for important components of the electron transport chain. ROS damage to mitochondrial DNA can result in mutated proteins, which may be less functional and result in greater leakage of electrons from the ETC, producing further DNA damage, etc. A vicious circle, or "error catastrophe" may result.***

The Rabinovitch Lab
Department of Pathology, University of Washington. USA

Catalase Mice

Our work on antioxidant overexpressing mice has been published in Science.

To determine the role of reactive oxygen species in mammalian longevity, we generated transgenic mice that overexpress human catalase localized to the peroxisome (PCAT), nucleus (NCAT), or mitochondrion (MCAT). **Median and maximum lifespans were maximally increased (average 5 months, and 5.5 months, respectively) in MCAT animals**. Cardiac pathology and cataract development were delayed, oxidative damage was reduced, H_2O_2 production and H_2O_2-induced aconitase inactivation were attenuated, and the development of mitochondrial deletions was reduced. **These results support the free radical theory of aging and reinforce the importance of mitochondria as a source of these radicals**.

View the Science paper:

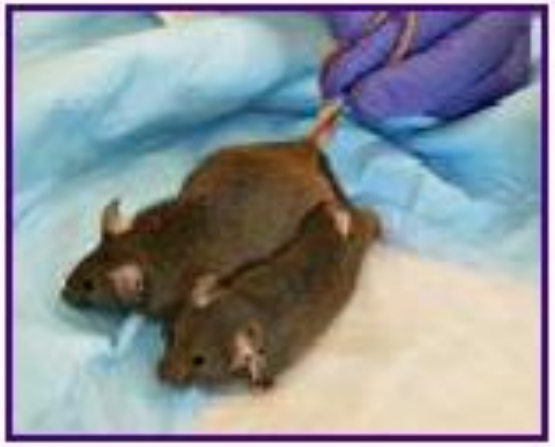
Extension of Murine Lifespan by Overexpression of Catalase Targeted to Mitochondria

Images

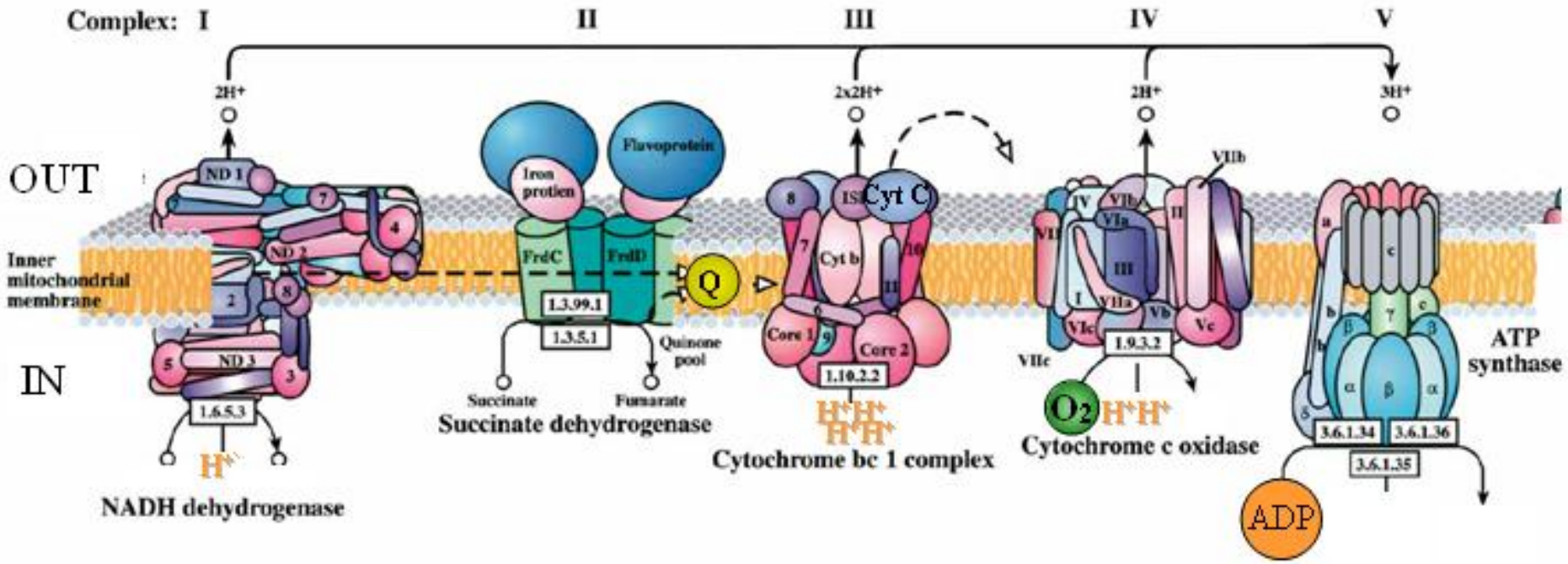
The MCAT mice and Dr. Nancy Linford

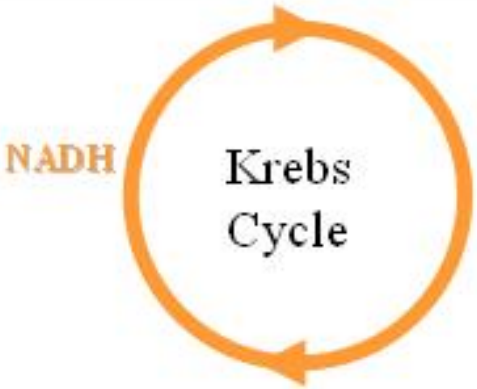




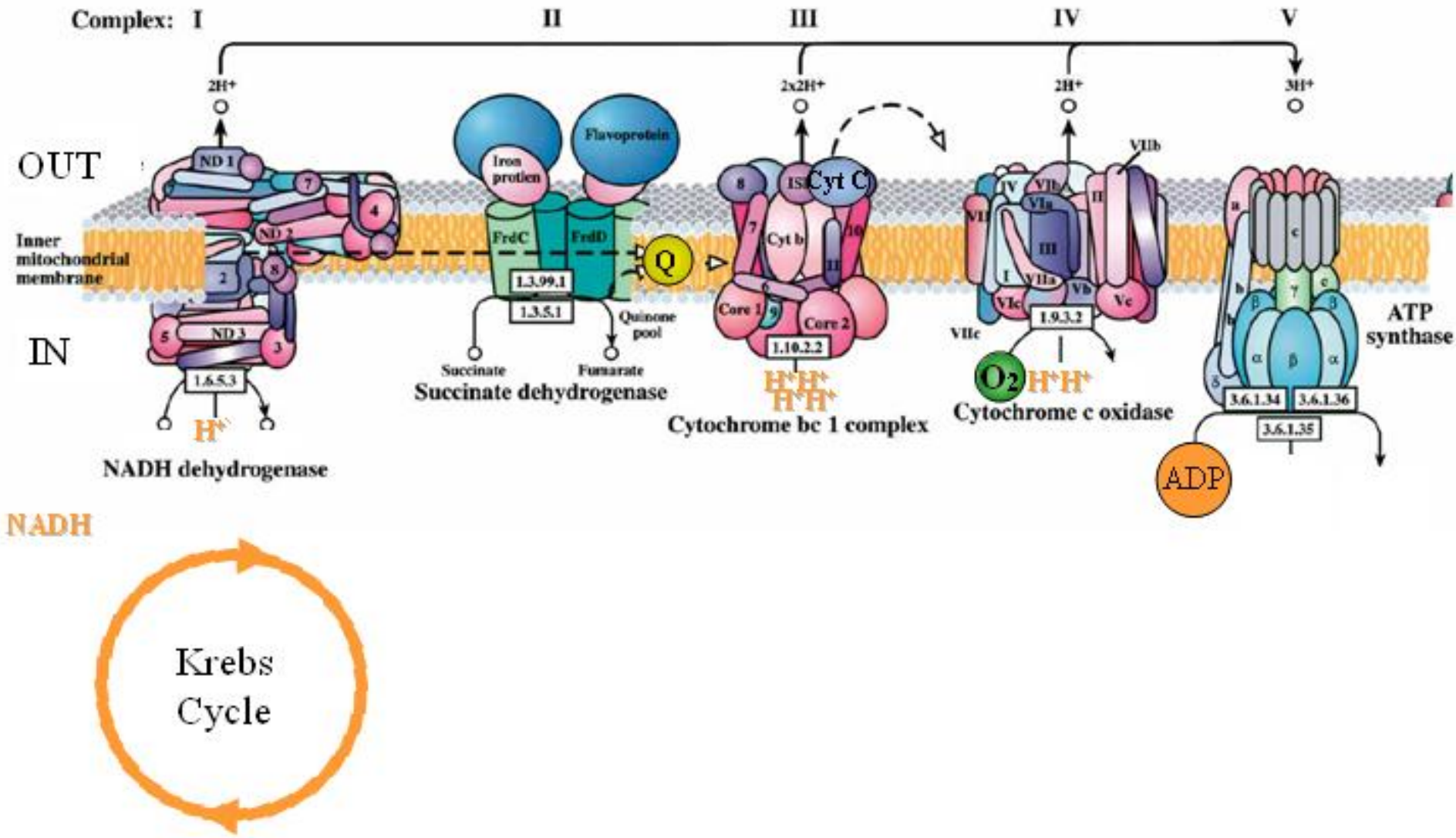



Mitochondrial Electron Transport Chain



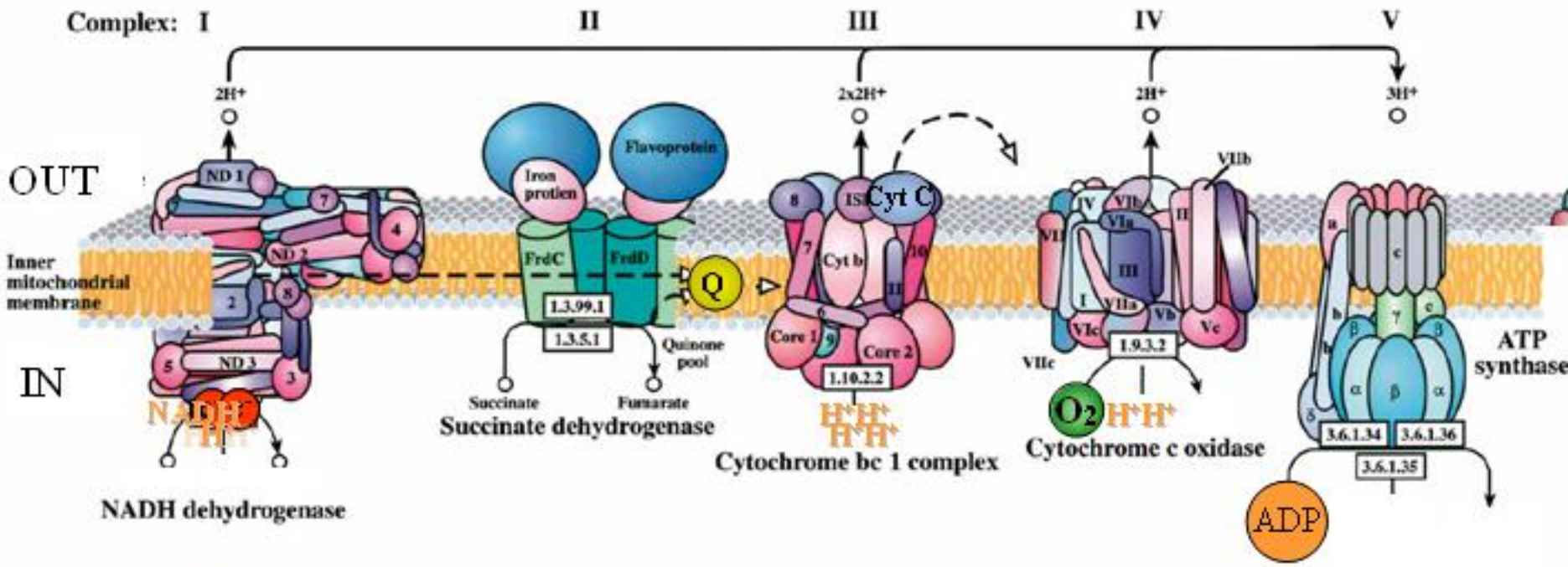


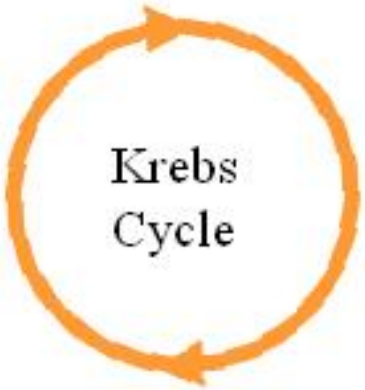
Mitochondrial Electron Transport Chain



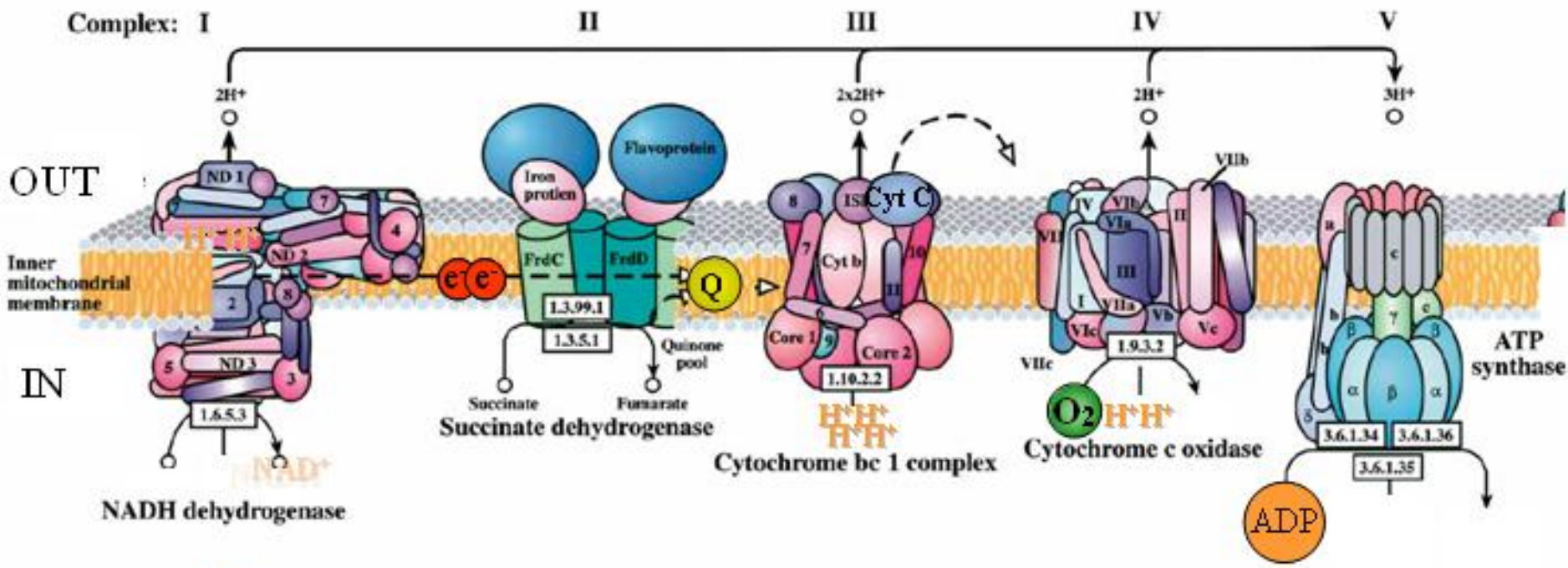
Krebs Cycle

Mitochondrial Electron Transport Chain



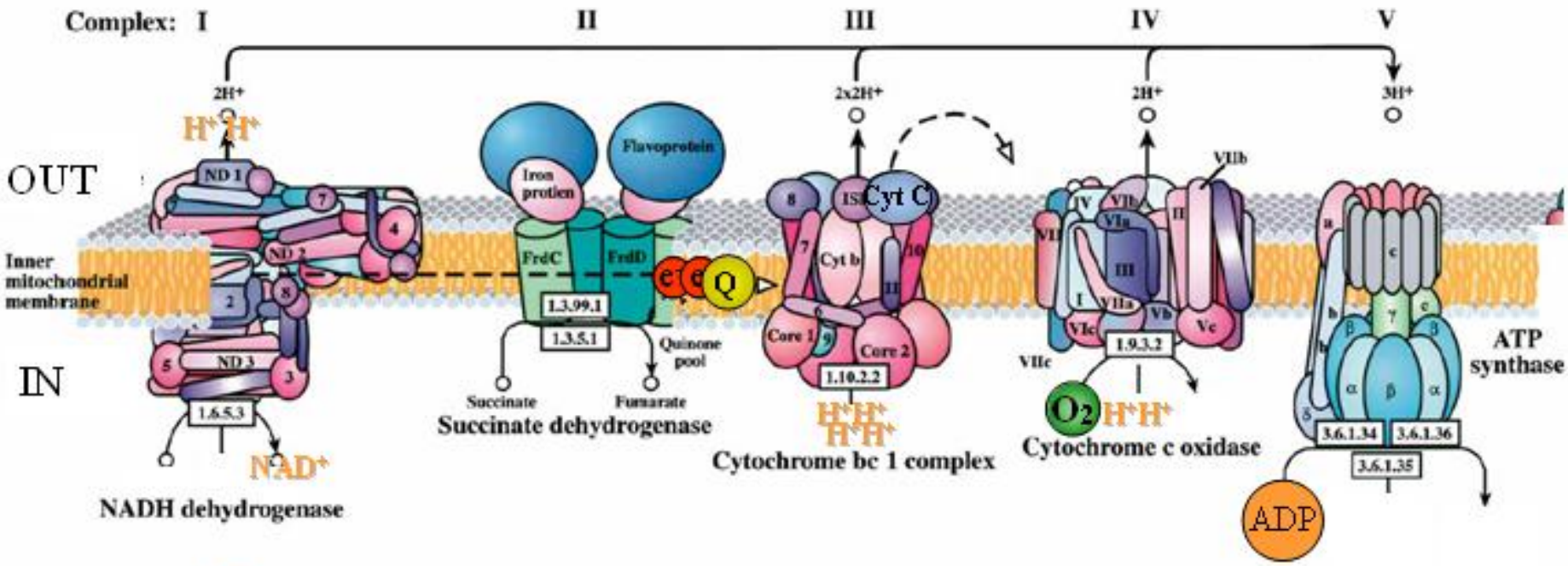


Mitochondrial Electron Transport Chain

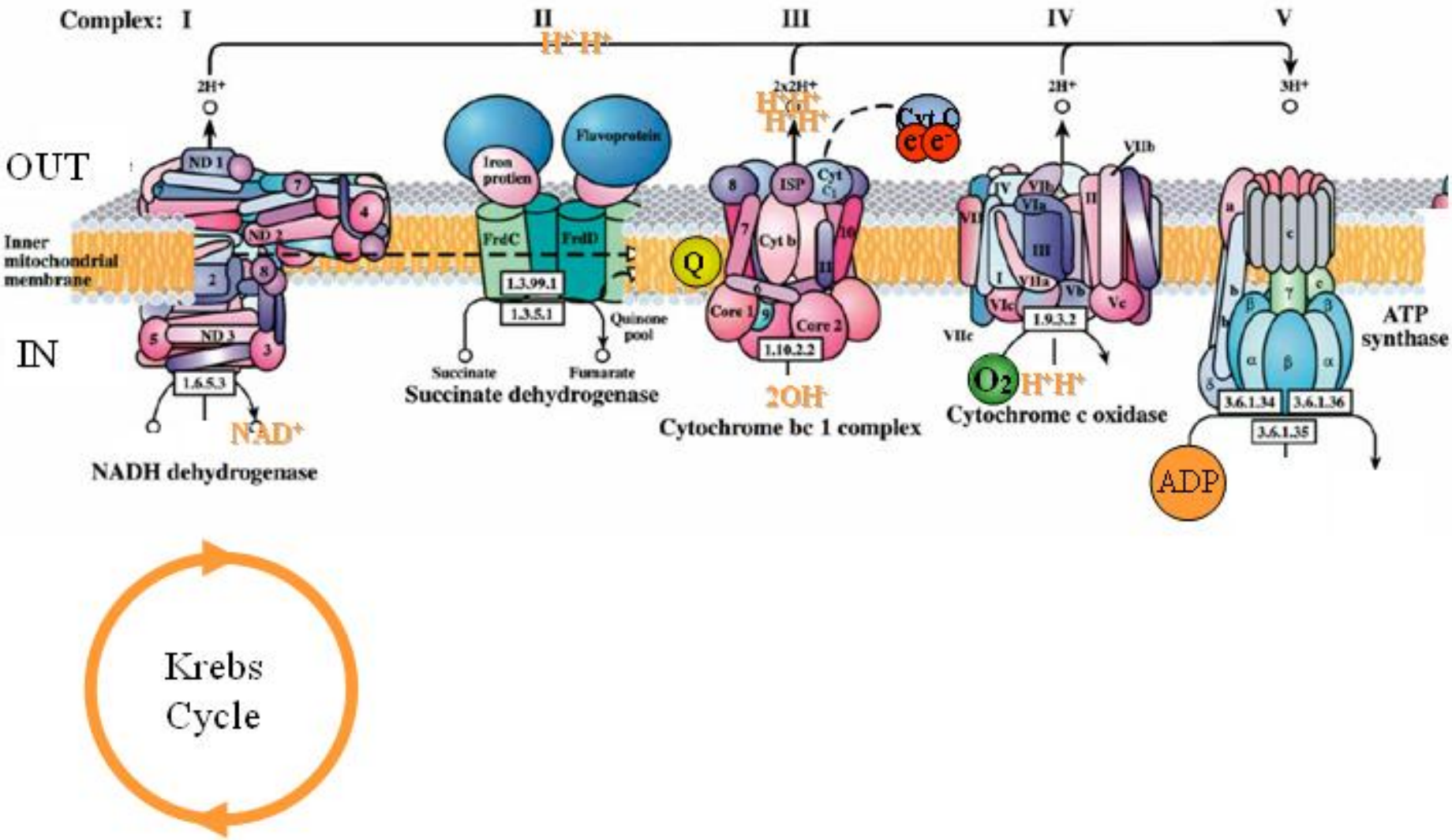


Krebs Cycle

Mitochondrial Electron Transport Chain

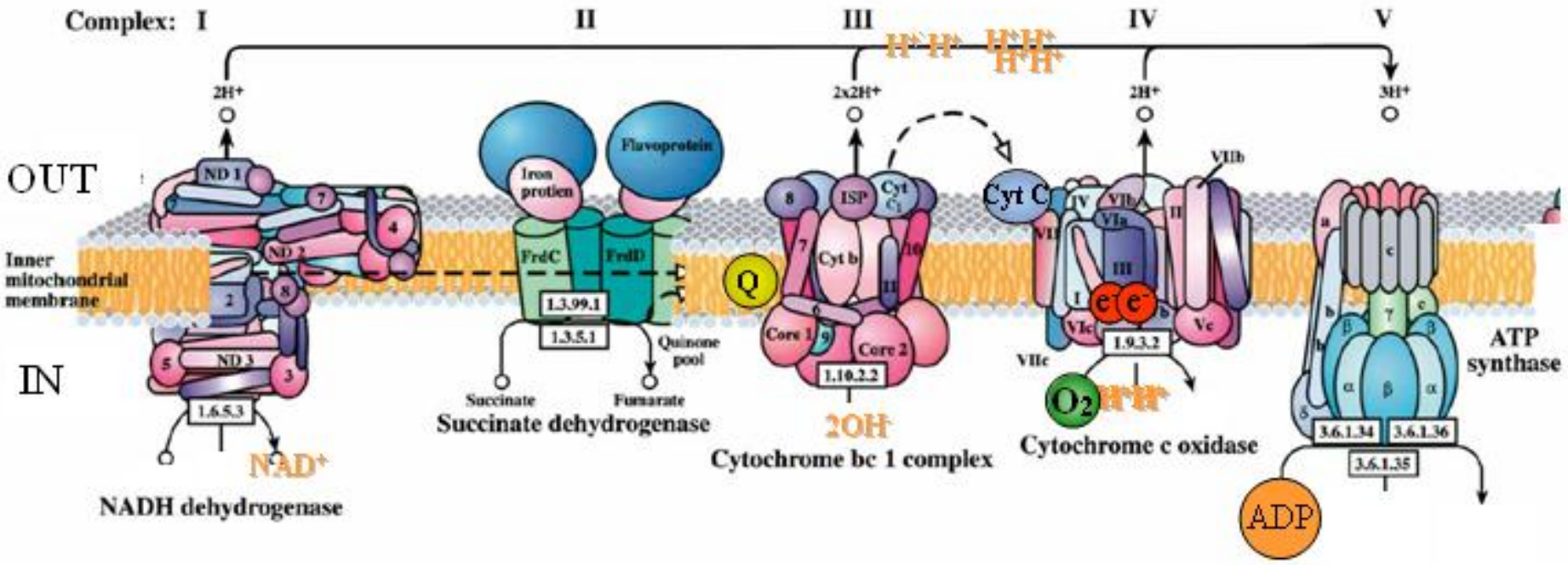


Mitochondrial Electron Transport Chain



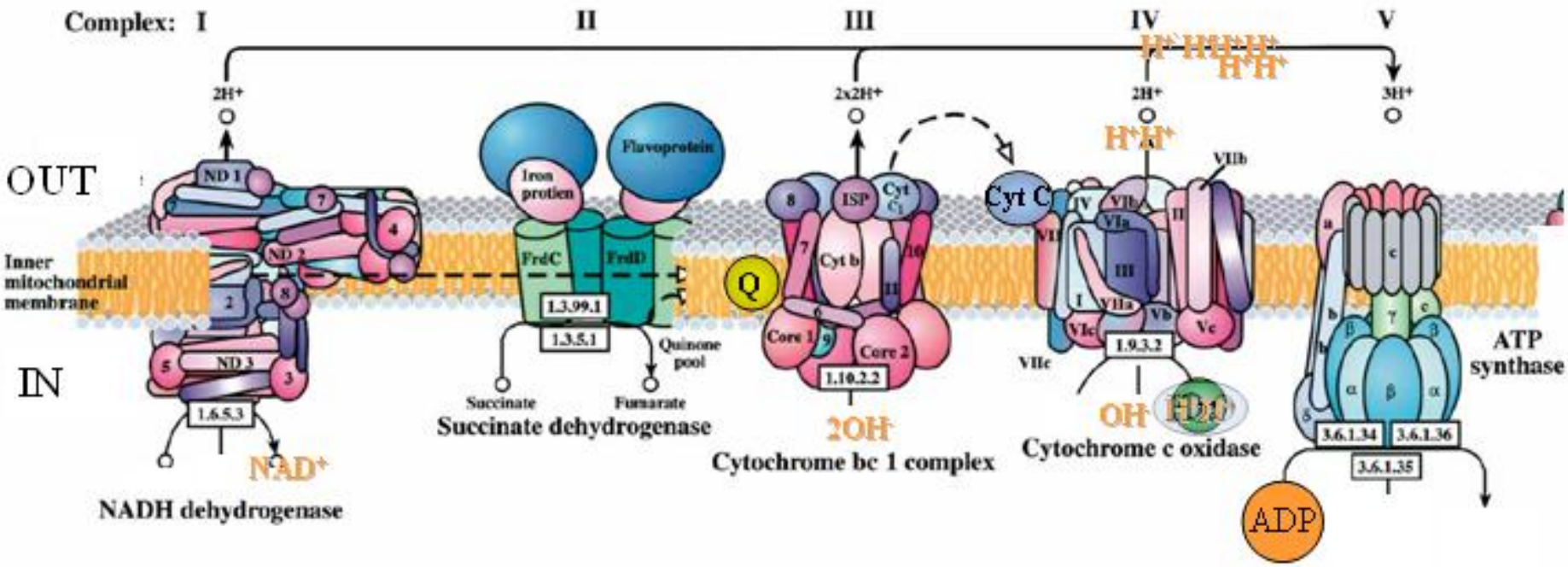
Krebs Cycle

Mitochondrial Electron Transport Chain



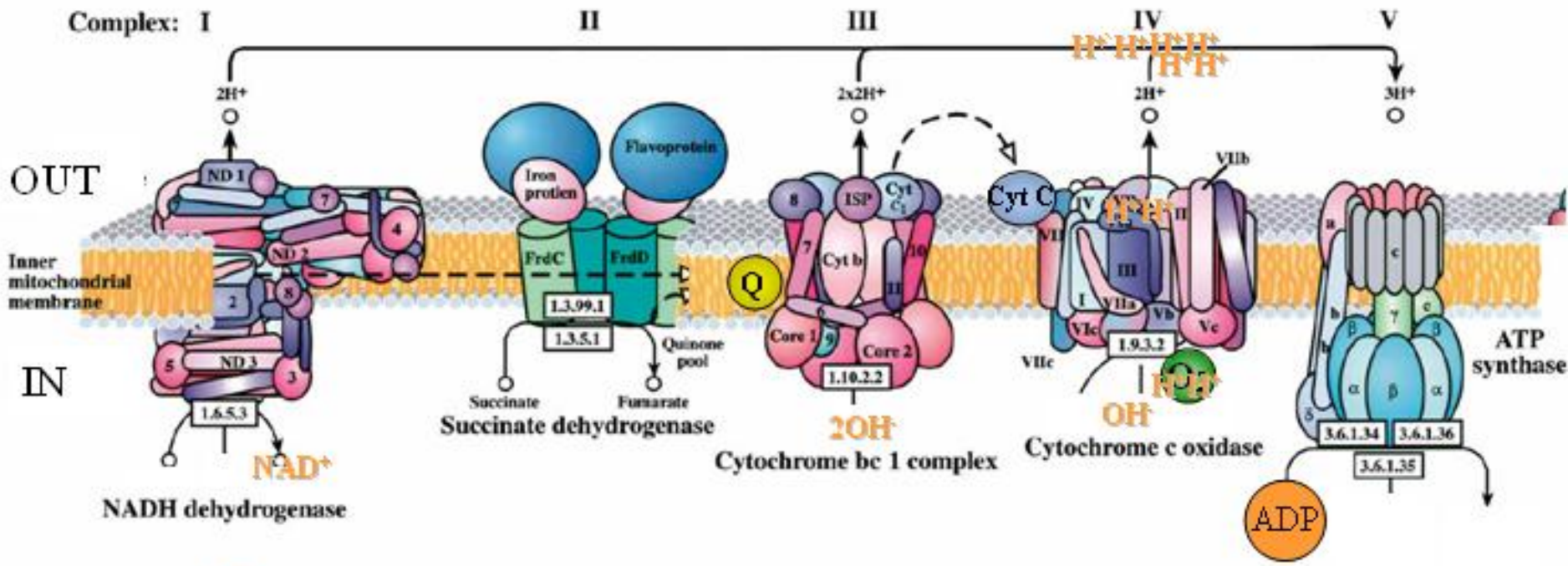
Krebs Cycle

Mitochondrial Electron Transport Chain

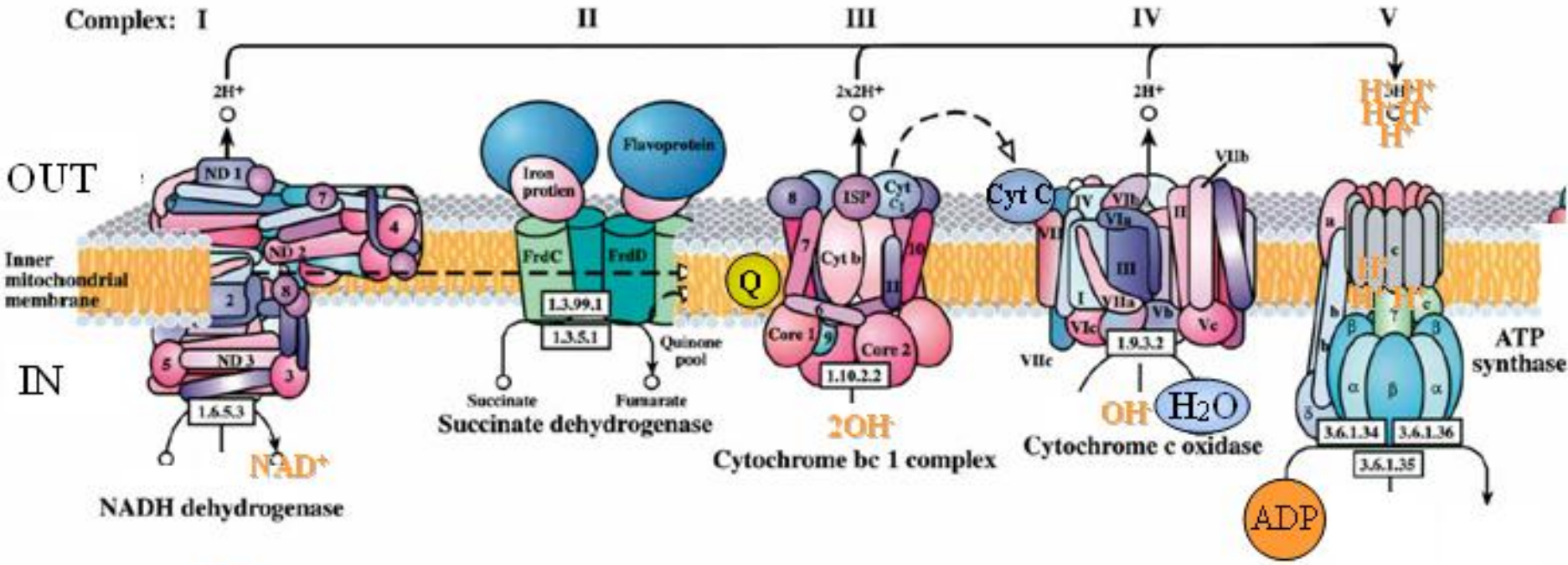


Krebs Cycle

Mitochondrial Electron Transport Chain

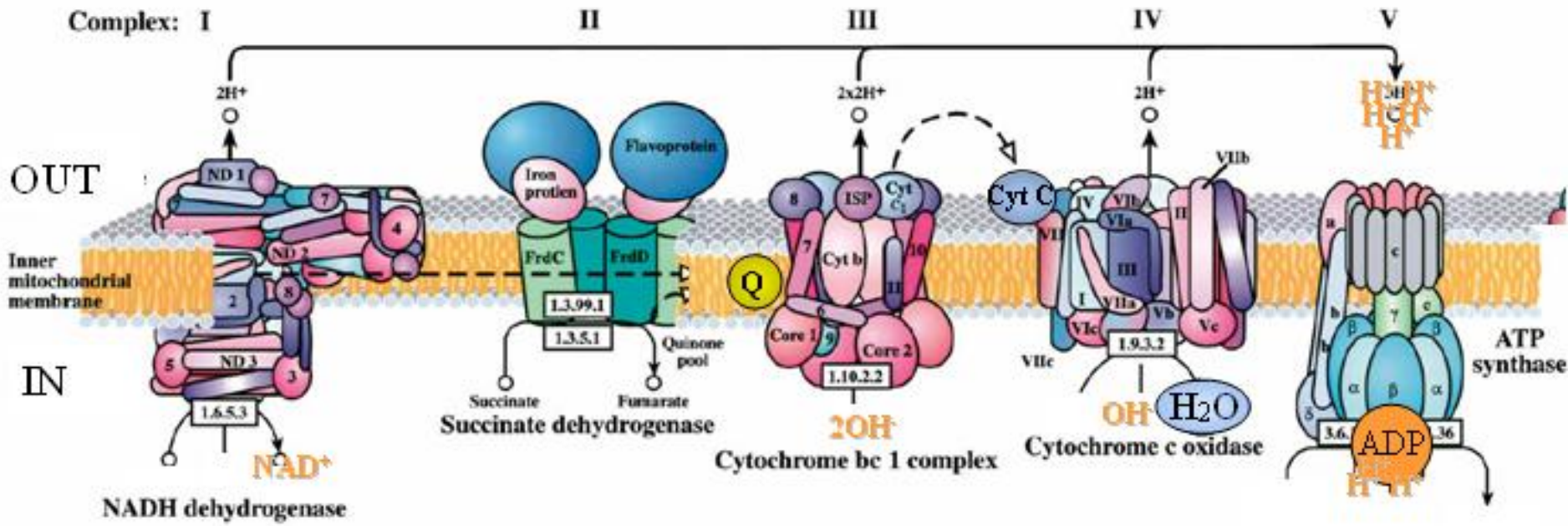


Mitochondrial Electron Transport Chain



Krebs Cycle

Mitochondrial Electron Transport Chain



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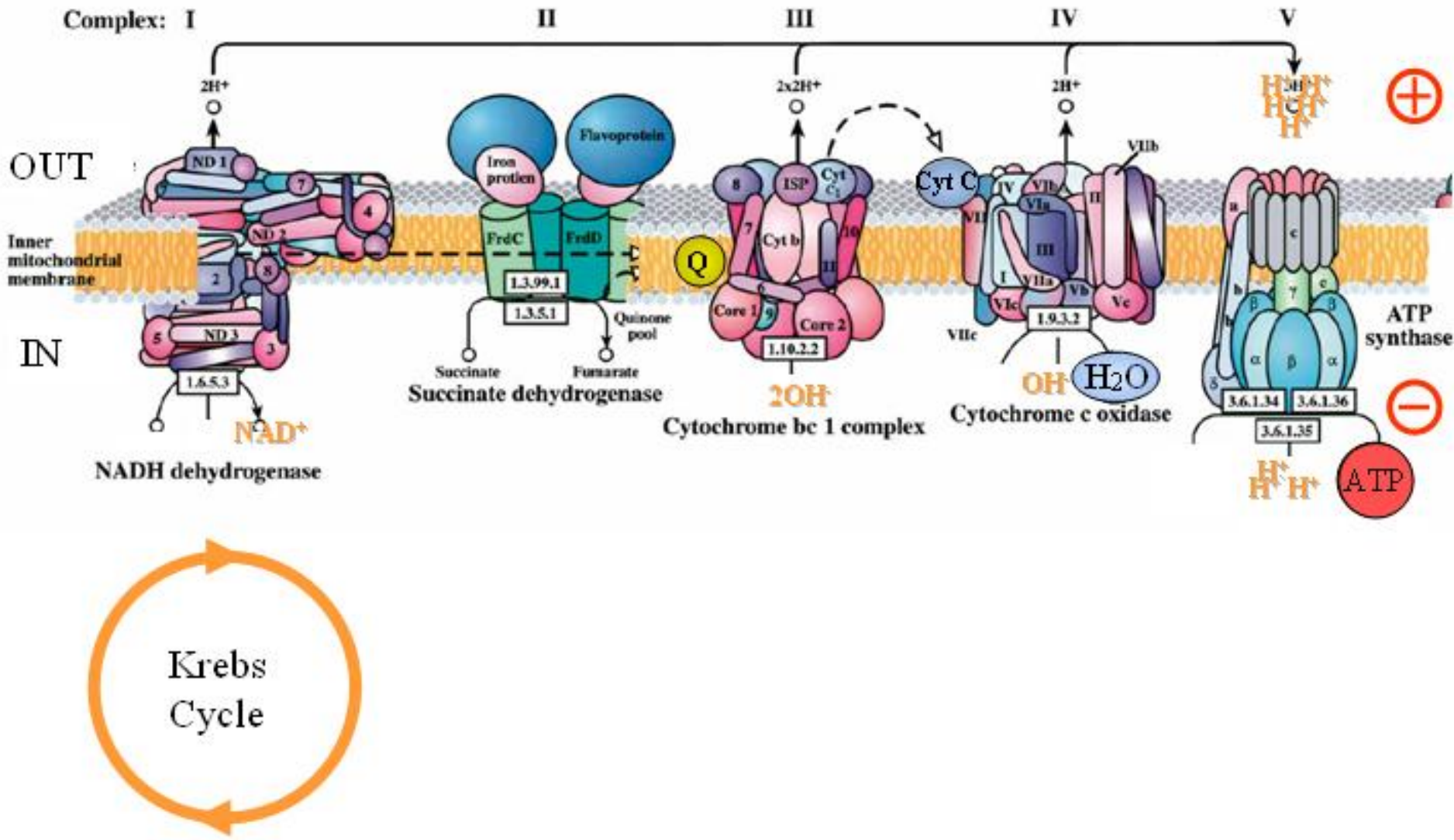

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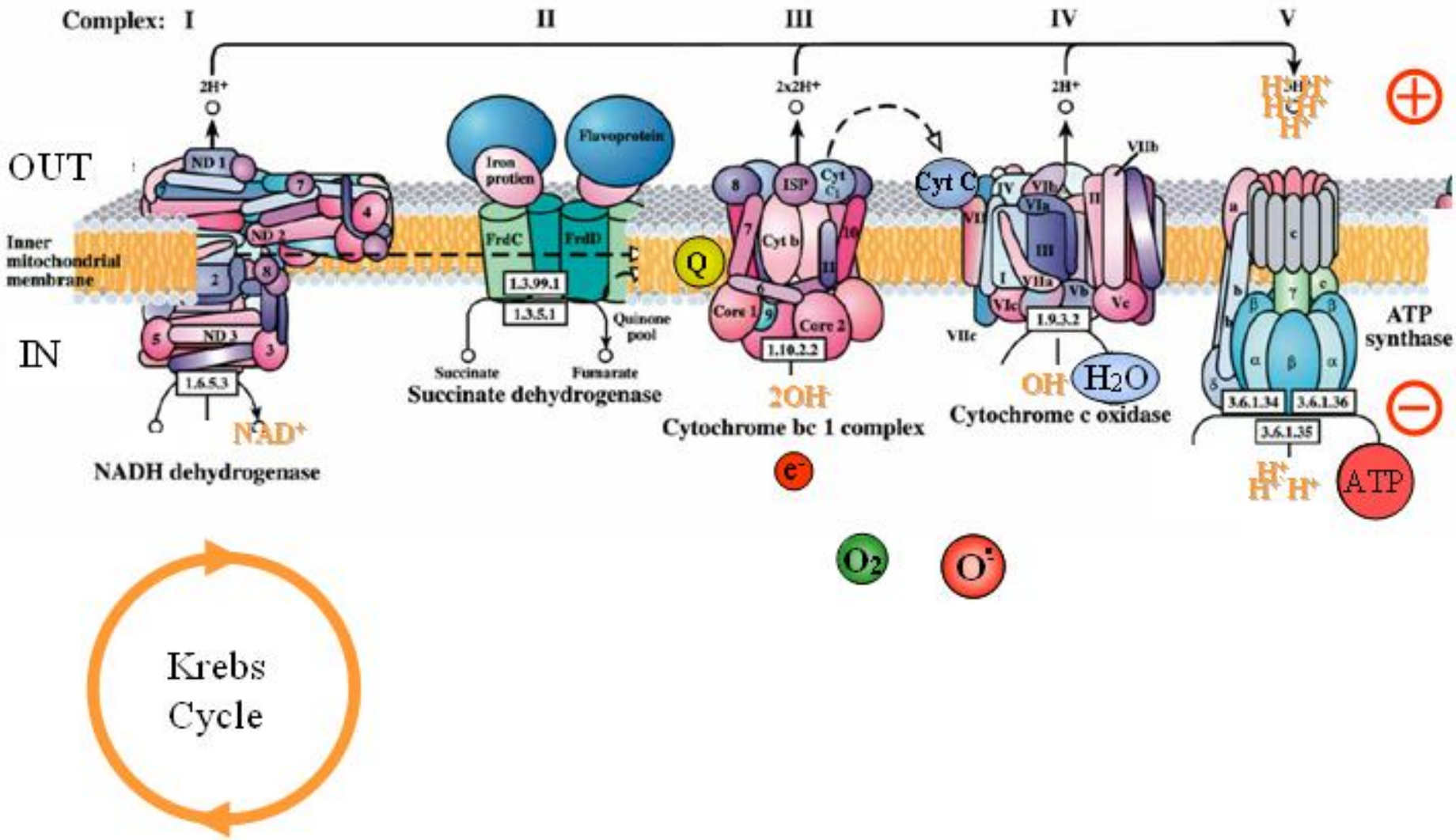
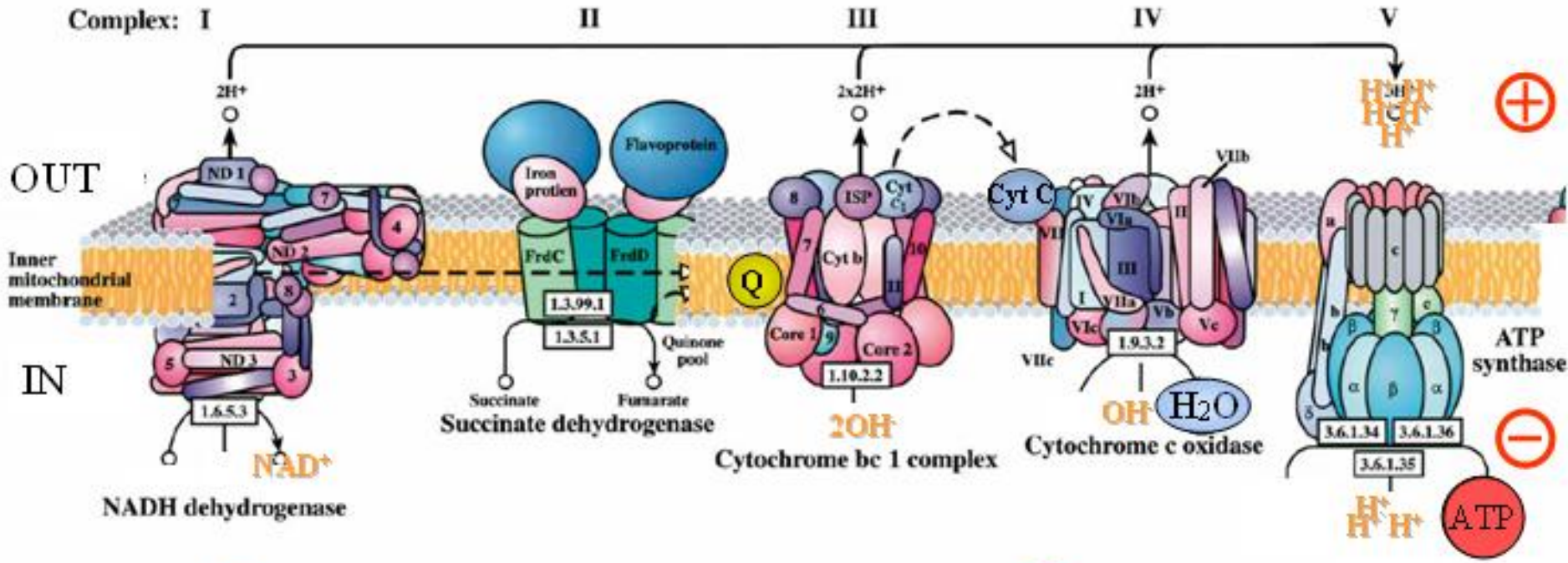
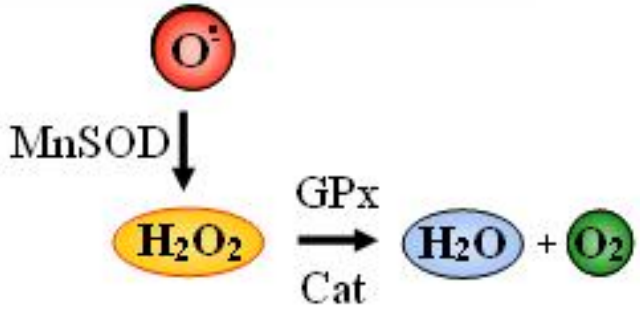


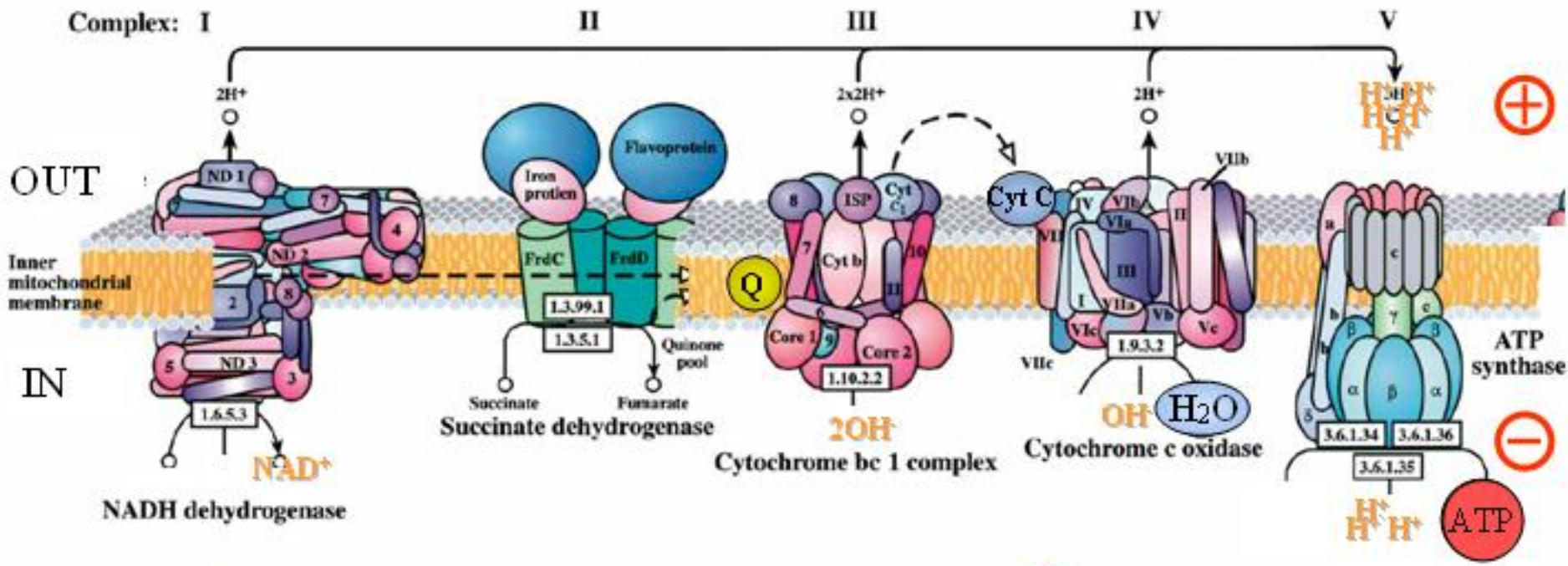
Krebs Cycle

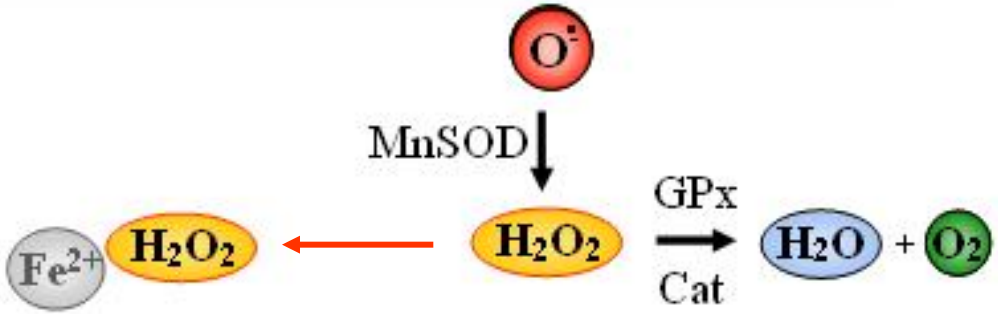
Mitochondrial Electron Transport Chain



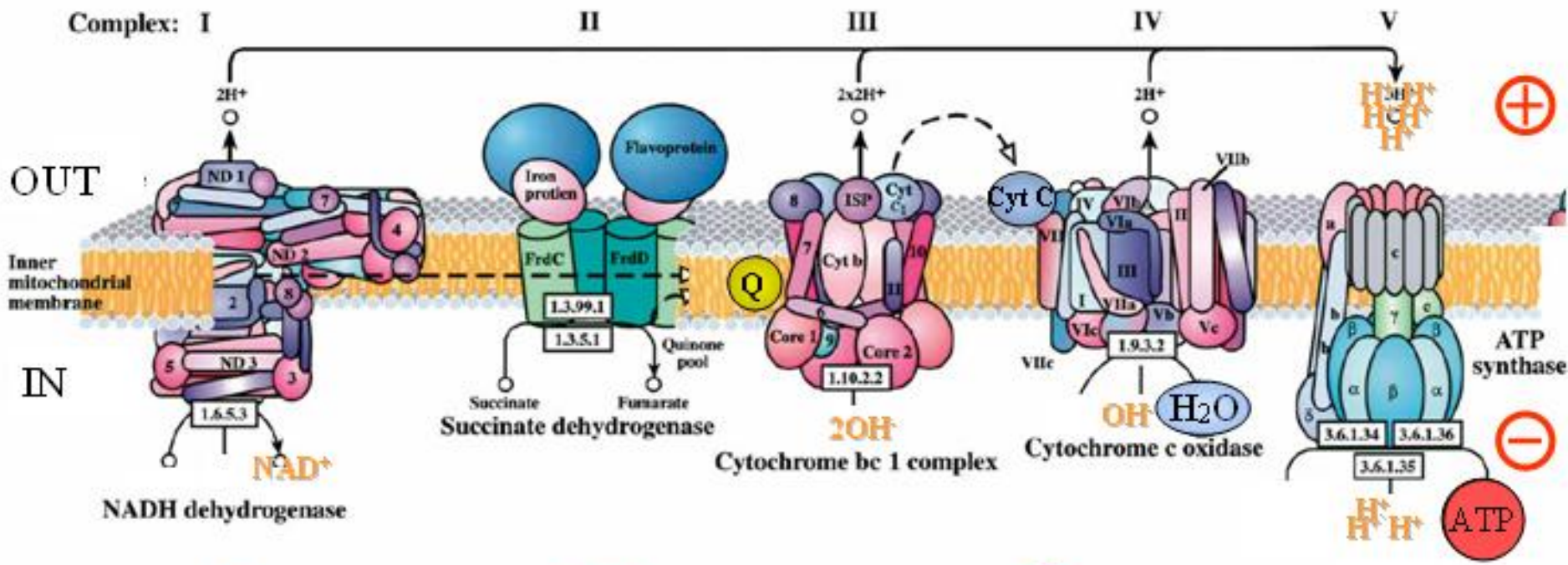


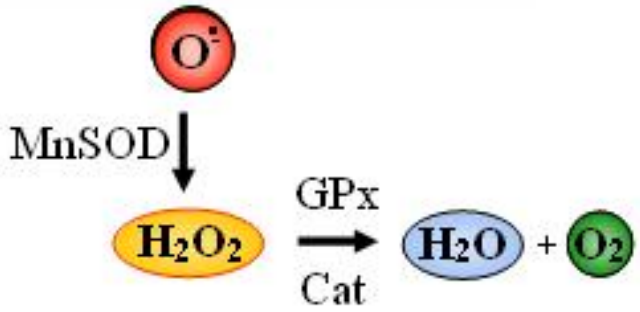
Mitochondrial Electron Transport Chain



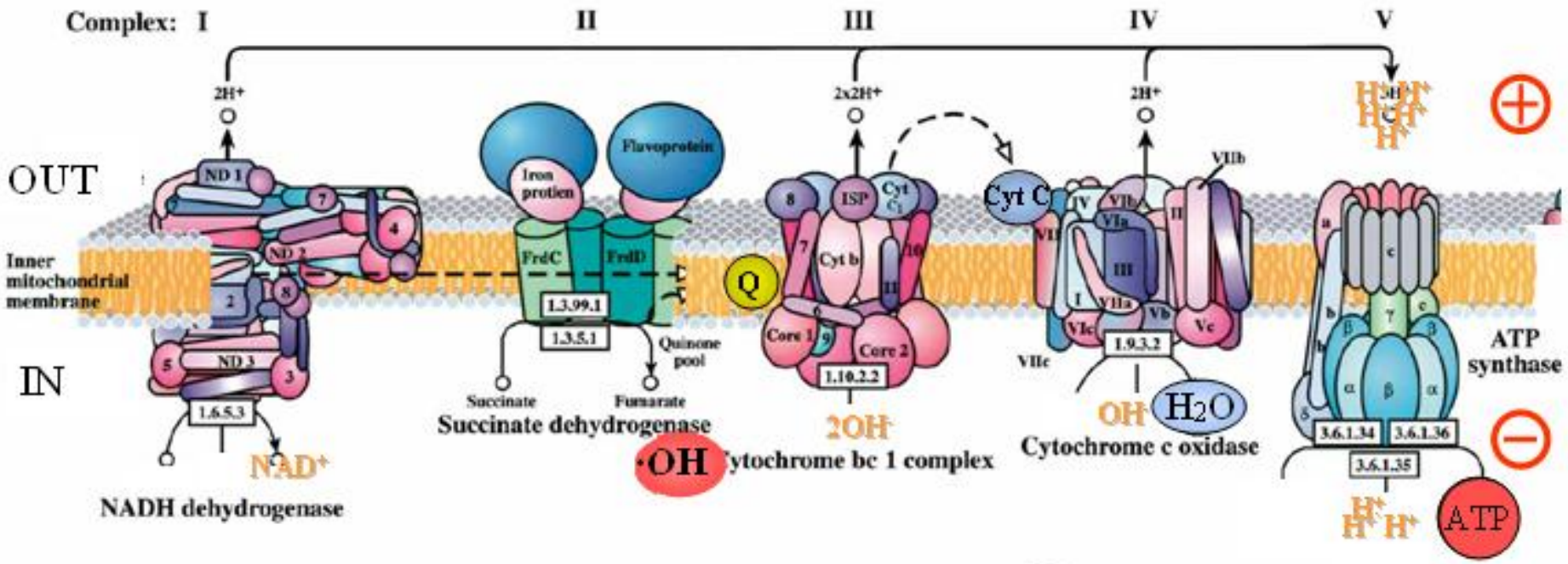


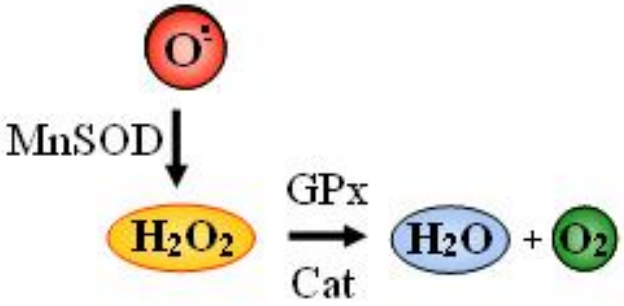
Mitochondrial Electron Transport Chain



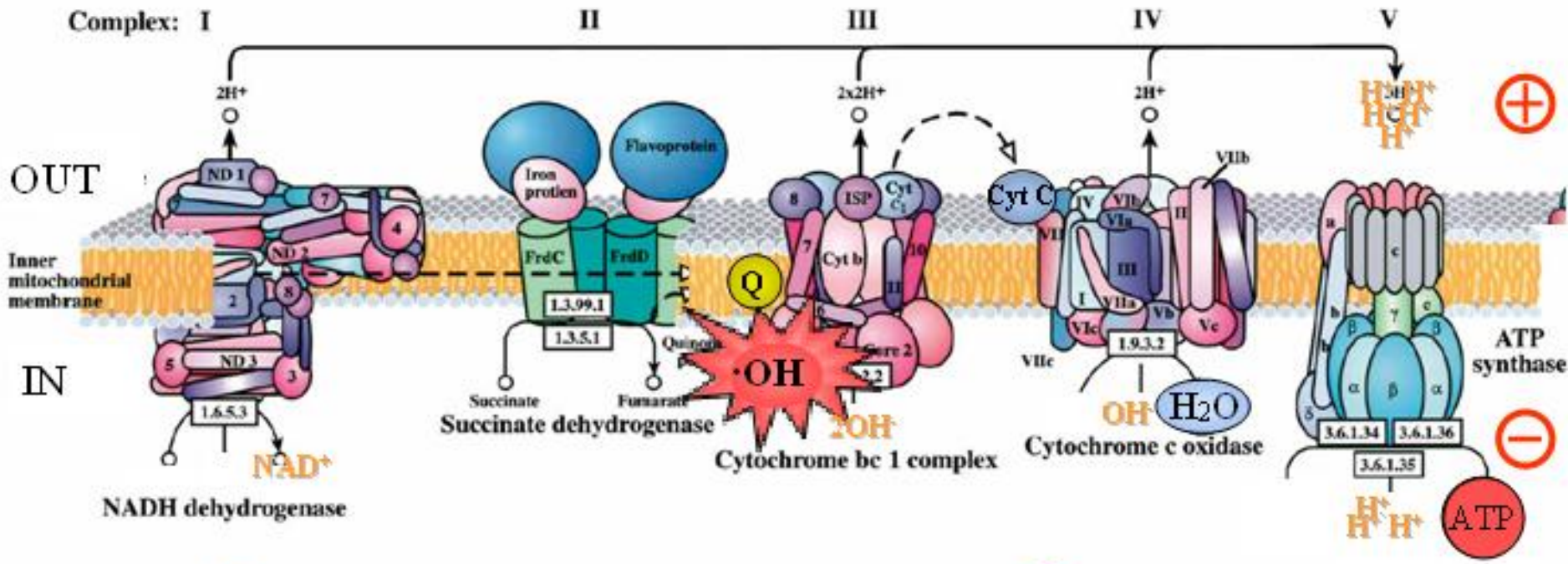


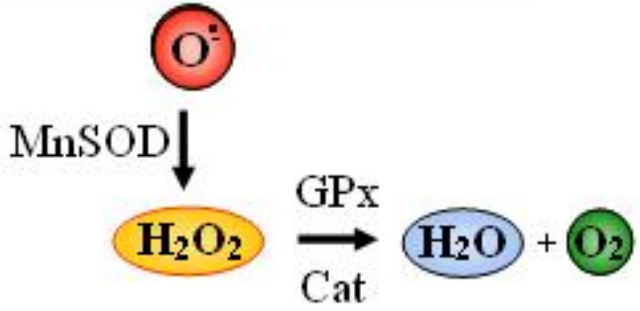
Mitochondrial Electron Transport Chain

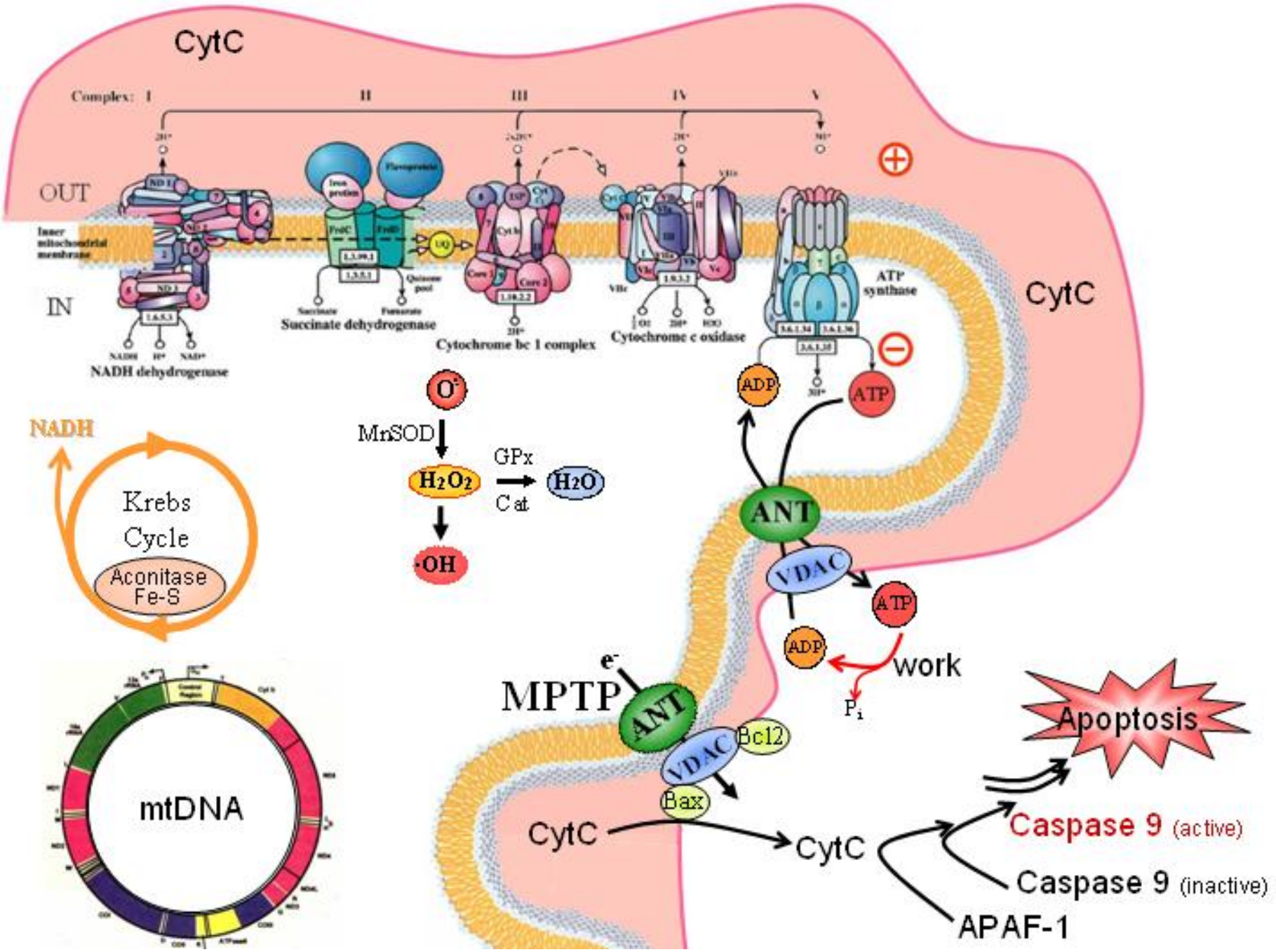


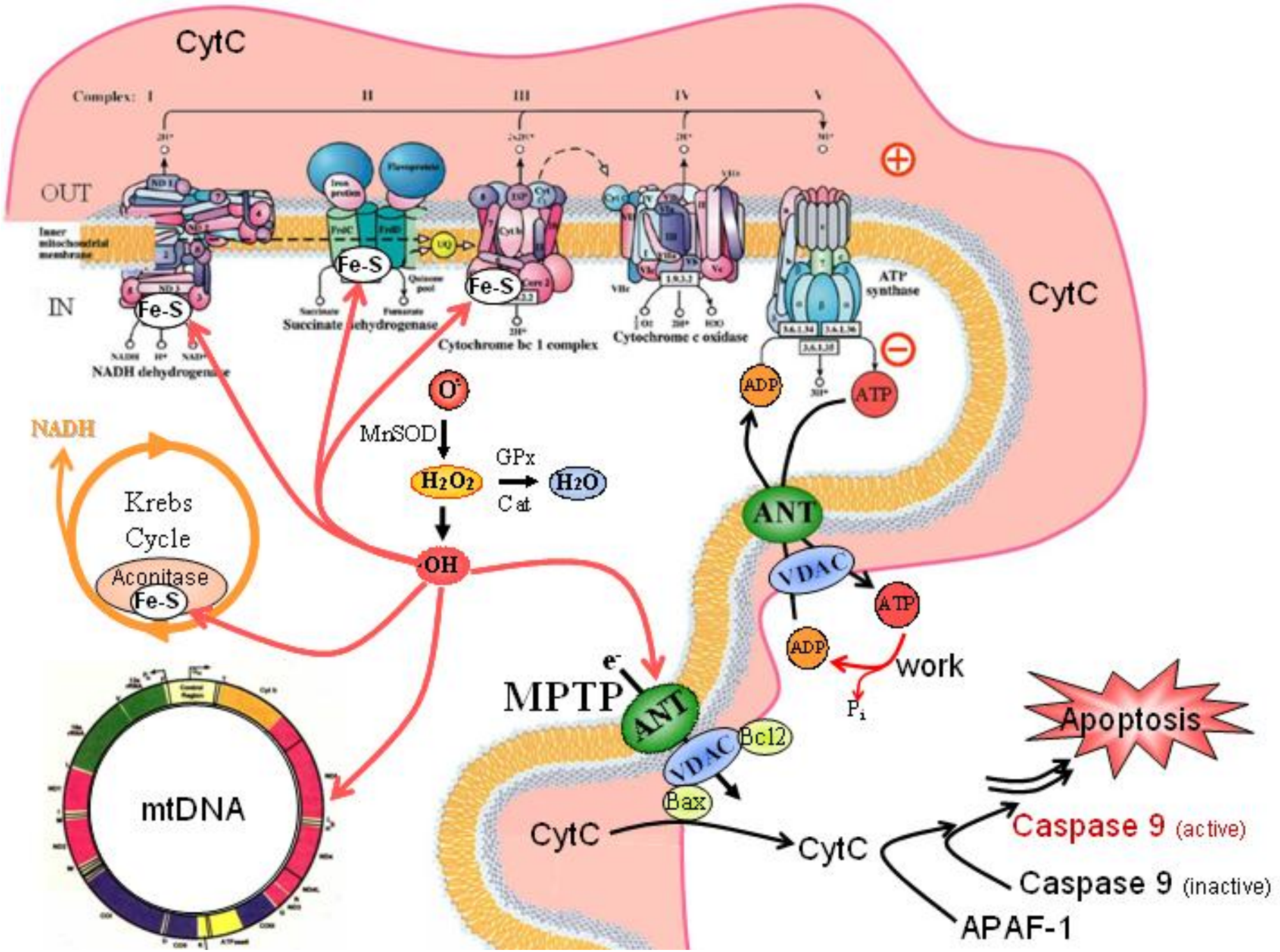


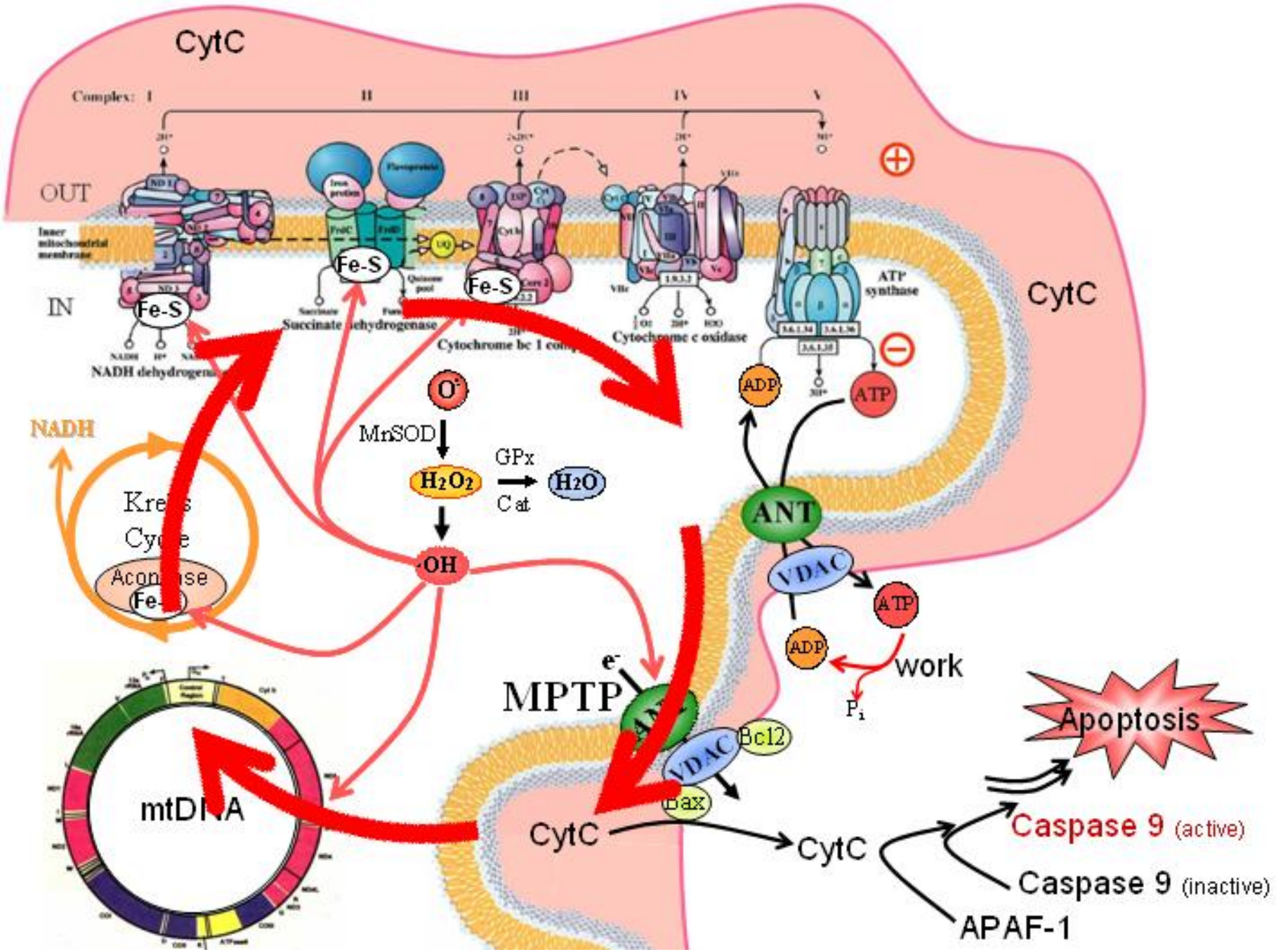
Mitochondrial Electron Transport Chain






CytC
Complex: I
II
III
IV
V
OUT
IN
Inner mitochondrial membrane
NADH dehydrogenase
Succinate dehydrogenase
Cytochrome bc 1 complex
Cytochrome c oxidase
ATP synthase
CytC
NADH
Krebs Cycle
Aconitase Fe-S
O-
MnSOD
H2O2
GPx
Cat
H2O
·OH
ADP
ATP
ANT
VDAC
ATP
ADP
work
Pi
MPTP
e-
ANT
VDAC
Bcl2
Bax
CytC
CytC
Apoptosis
Caspase 9 (active)
Caspase 9 (inactive)
APAF-1
mtDNA

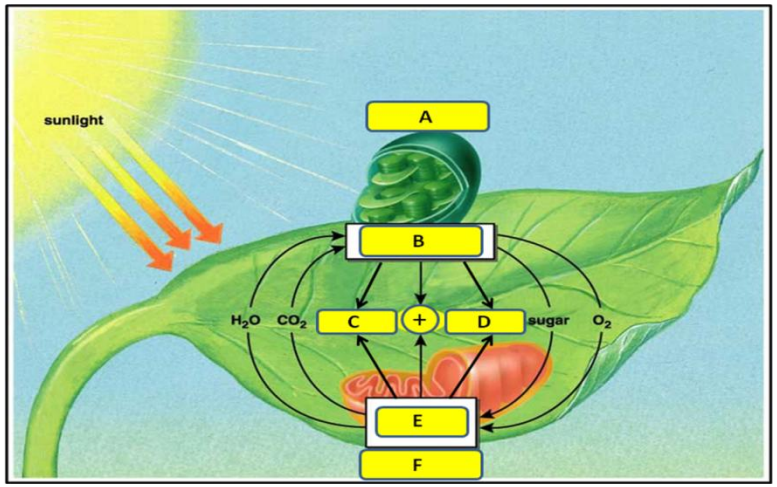

CytC
Complex: I II III IV V
OUT
IN
Inner mitochondrial membrane
Fe-S
Succinate dehydrogenase
Cytochrome bc 1 complex
Cytochrome c oxidase
ATP synthase
NADH dehydrogenase
CytC
ADP
ATP
NADH
Krebs Cycle
Aconitase
Fe-S
O2-
MnSOD
H2O2
GPx
Cat
H2O
·OH
ANT
VDAC
ATP
ADP
work
Pi
MPTP
e-
ANT
VDAC
Bcl2
Bax
mtDNA
CytC
CytC
Apoptosis
Caspase 9 (active)
Caspase 9 (inactive)
APAF-1


CytC
Complex: I II III IV V
OUT
IN
Fe-S
Succinate dehydrogenase
Cytochrome c oxidase
ATP synthase
CytC
NADH
Kreb's Cycle
Aconitase
Fe-S
O2-
MnSOD
H2O2
GPx
Cat
H2O
·OH
ANT
VDAC
ATP
ADP
work
Pi
MPTP
e-
Bc12
Bax
mtDNA
CytC
CytC
Apoptosis
Caspase 9 (active)
Caspase 9 (inactive)
APAF-1

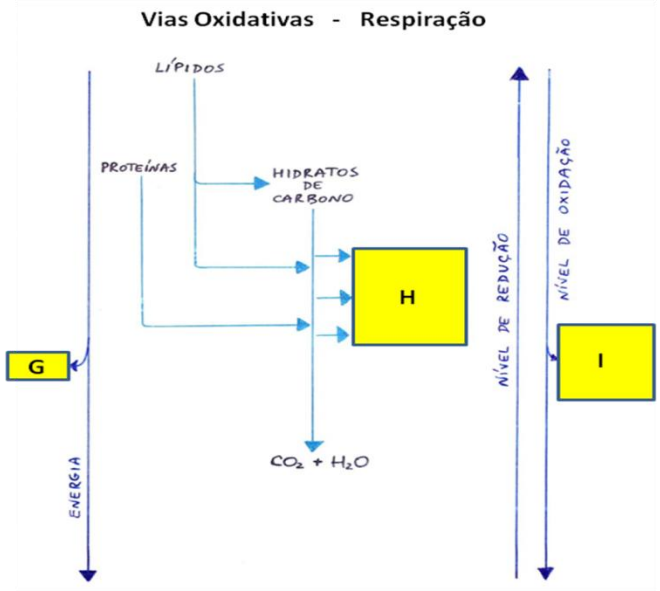
Questões

1. Considere a integração do metabolismo celular.

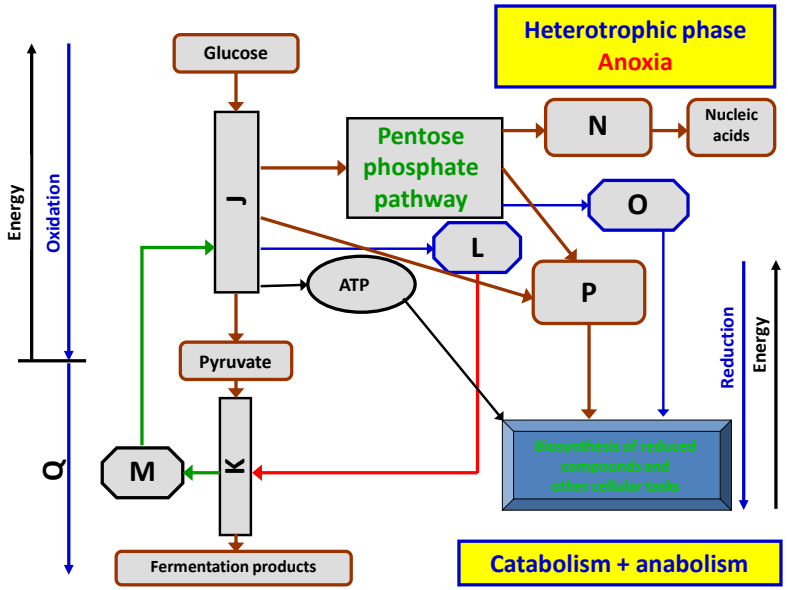
a) Identifique A, B, C, D, E e F na figura seguinte.



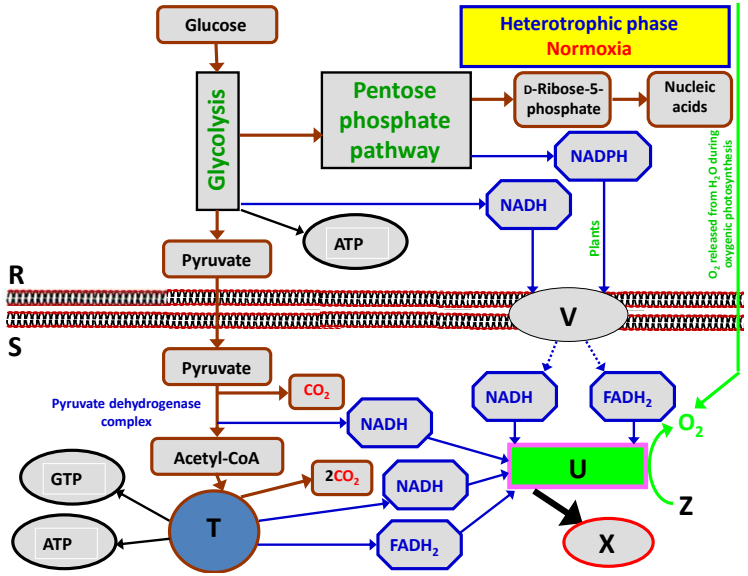
b) Identifique os três principais produtos da respiração, G, H e I.



c) Identifique os compostos/processos J, K, L, M, N, O, P e Q.



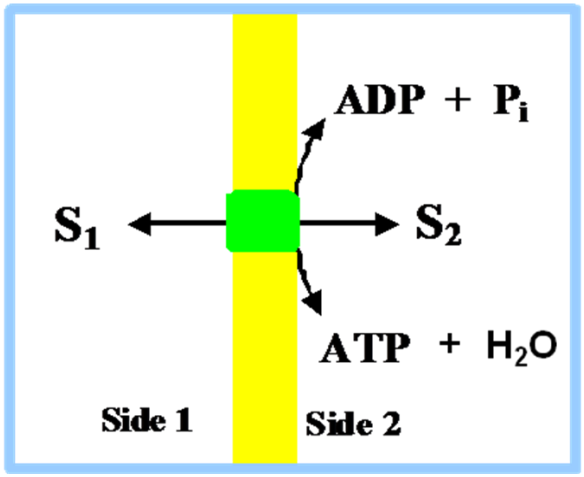
d)Identifique os compartimentos celulares/compostos/processos R, S, T, U, V, X e Z.



e)No esquema representado na alínea anterior (d)), há uma seta ao contrário. Identifique qual é.

f) No esquema representado na alínea anterior (d)), uma molécula de glucose (6C) é convertida pela glicólise em duas moléculas de piruvato (2 x 3C), cujos carbonos são seguidamente libertados sob a forma de CO**2**. Nestas condições, a célula não consegue crescer por falta de esqueletos carbonados para as reacções biossintéticas. Apresente duas razões que permitem às células ultrapassar esta situação.

2. Considerando a barra amarela como uma membrana celular, identifique as condições necessárias, em termos de concentrações, sentidos das setas e valores de ΔG**'**, para ocorrer:



a) Síntese de ATP, de acordo com a teoria quimiosmótica.

b) Transporte activo de um ião.

3. Considere uma cadeia de transporte de electrões hipotética constituída pelos seguintes transportadores de electrões, a que correspondem os potenciais–padrão de oxidação-redução:

A : -0,12 V
B : -0,32 V
C : -0,02 V
D : -0,23 V
E : -0,52 V
F : -0,18 V

Coloque-os pela ordem em que circulam os electrões. Justifique.

4. Considere o metabolismo global.

4.1 – Há três processos de formar ATP na Natureza. Indique quais são e onde ocorrem.

4.2 – O NADH é fundamentalmente produzido em quatro vias metabólicas e oxidado num processo, com um objectivo principal. Identifique as quatro vias, o processo e o objectivo.

4.3 – Há fundamentalmente dois processos de produzir NADPH na Natureza, com um objectivo principal. Identifique os dois processos e o objectivo.

4.4 – Três vias metabólicas principais fornecem os esqueletos carbonados necessários às reacções biossintéticas do metabolismo celular. Identifique quais são.

5. Responda sucintamente, mas objectivamente, às seguintes questões:

a) Quais as reacções e o nome das enzimas envolvidas na desaminação dos aminoácidos?

b) Qual a função biológica do ciclo da ureia?

c) Como classifica os aminoácidos proteicos de acordo com o fim metabólico dos seus esqueletos carbonados? Dê exemplos.

d) Como é que os organismo fixadores de azoto simbiontes das leguminosas resolveram o problema do seu complexo multienzimático nitrogenase ser inibido pelo oxigénio molecular?

e) O que são aminoácidos semi-essenciais para o homem? Dê exemplos.

f) Quais os três tipos principais de substratos respiratórios utilizados pelas células?

g) Identifique os três processos de produzir ATP na natureza e indique as vias metabólicas em que ocorrem.

6. Considere o desacoplamento das cadeias de transporte de electrões, o amónio, um bebé humano e uma flor do jarro.

Três mecanismos distintos permitem dissipar o gradiente electroquímico de hidrogeniões normalmente responsável pela síntese de ATP: um é prejudicial, mas dois são benéficos para os organismos em que ocorrem.

Descreva os mecanismos e os seus efeitos (prejudicial e benéficos).

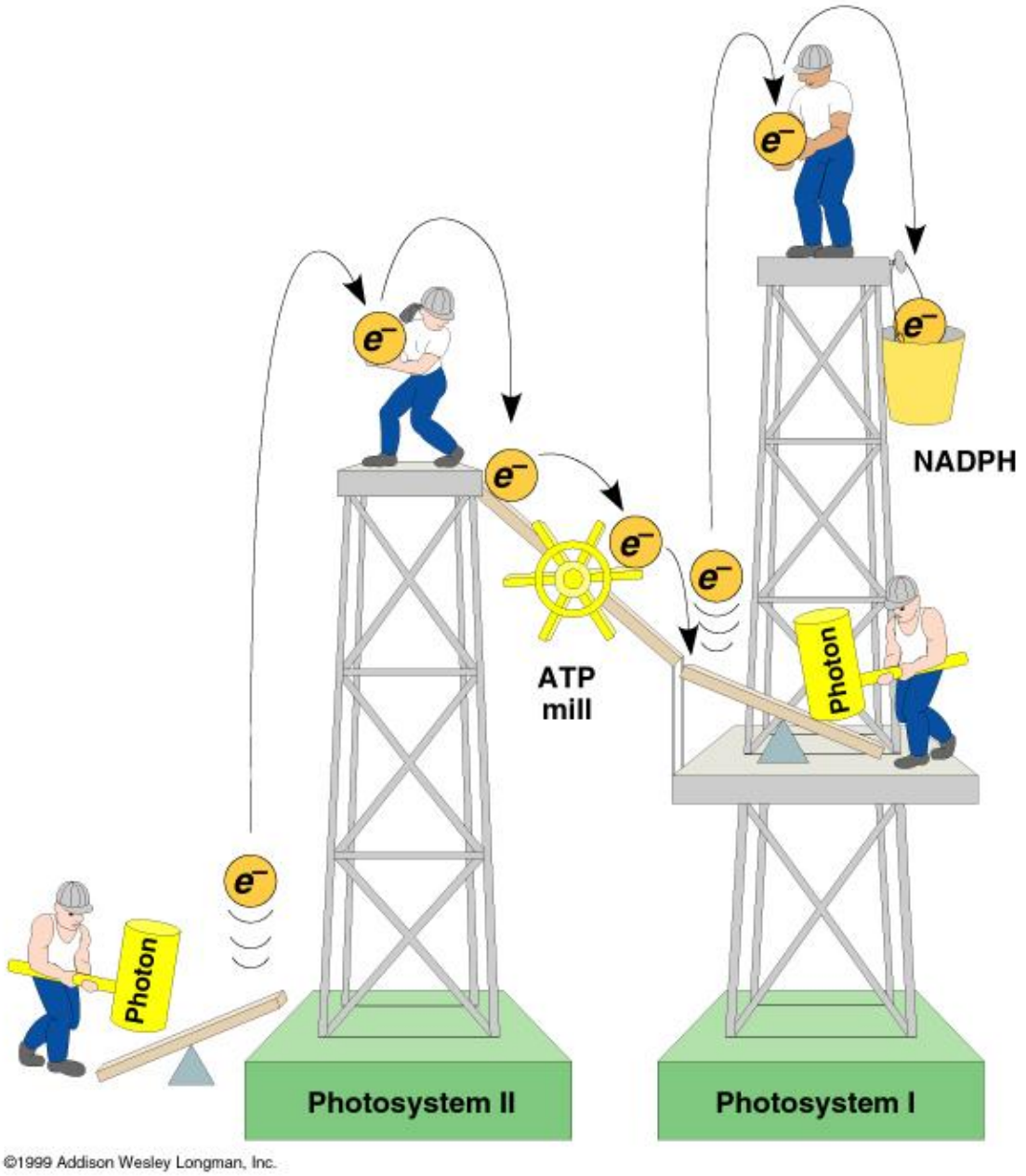
7 - a) O que representa a figura seguinte?

b) Ao construir um gráfico com esta figura, que unidades colocaria nos eixos das abcissas e das ordenadas?

c) Faça uma legenda sucinta, mas objectiva, da figura.

d) Explique o movimento espontâneo dos electrões na parte central da cadeia.

e) Explique a síntese espontânea do ATP associada à parte central da cadeia.



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