

1. Introduction

Microbial physiology: problems and prospects

2. Metabolic diversity. Metabolic processes of energy transduction

Diversity and adaptability: the energetic basis of microbial life

3. Overview of central pathways of heterotrophic metabolism

Glycolysis, pentose phosphate pathway, Entner-Doudoroff pathway, glyoxylate cycle, the citric acid cycle and its modifications

4. Physiology of growth in extreme environments

Adaptations to extreme conditions. Temperature, pH and osmotic homeostasis

5. Membranes and solutes transport

Diversity of transport mechanisms

A functional-phylogenetic classification system for transmembrane solute transporters

6. Metabolic regulation

Transcription in Prokaryotes

Enzyme induction

Repression by catabolite

Repression and attenuation by final metabolites

Termination and anti-termination

7. Physiological adaptation and homeostasis

General introduction to the systems of signal transduction

Redox control and oxidative stress

Hierarchical regulation of transport and utilization of carbon sources

Regulation of gene expression associated with transition aerobic / anaerobic

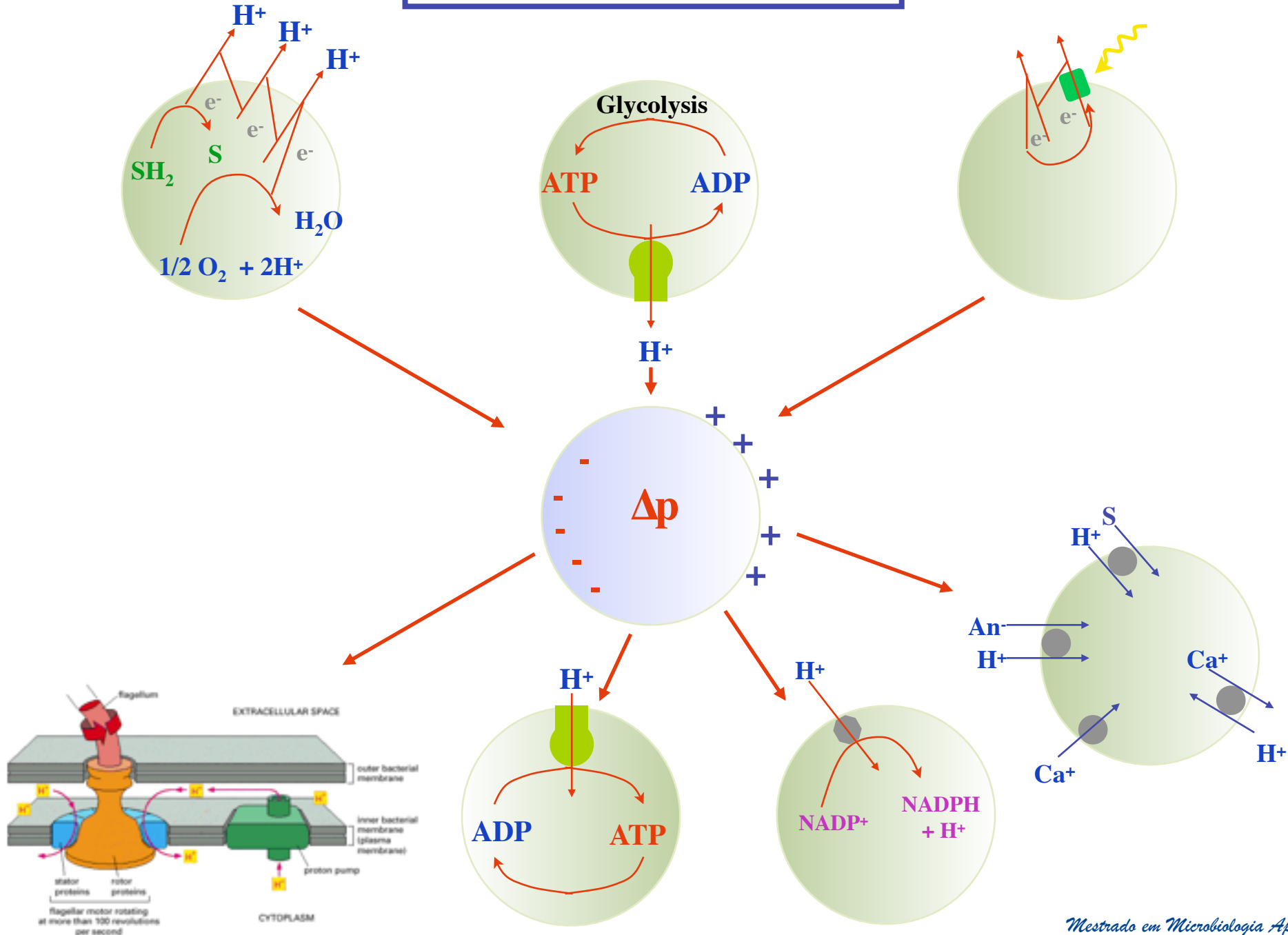
Photo-regulation in prokaryotes

8. Nitrate reduction and nitrogen cycle in archaea

9. Methanogenic archaea, methane and carbon cycle

10. "Omics" technology and Systems Microbiology

Nutrients and energy production



Energy Transduction

Gibbs-Helmholtz equation

$$\Delta G = \Delta H - T\Delta S$$

ΔG : Gibbs energy changes (free energy changes)

ΔH : enthalpy change

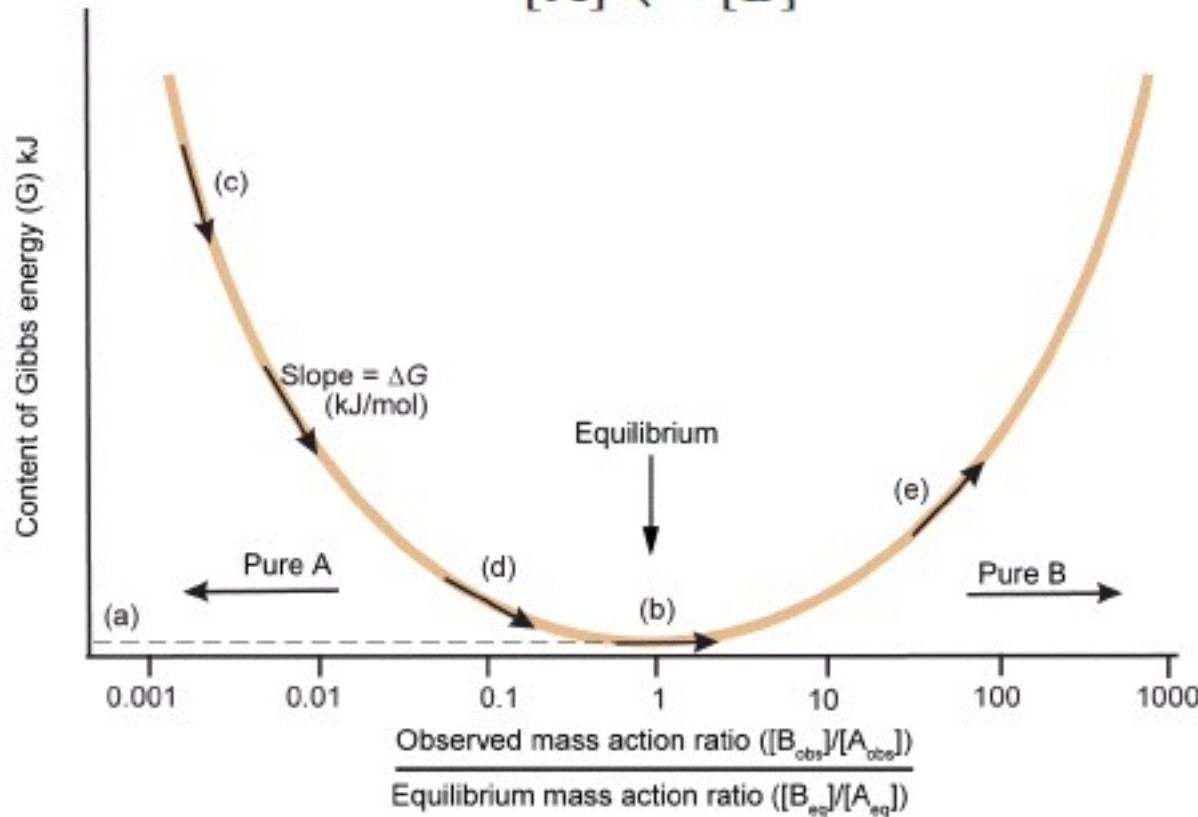
ΔS : entropy change



Manifestations of Gibbs energy are interconverted

- ✓ ΔG : used in the description of metabolic reactions and its displacement from equilibrium
- ✓ ΔE : redox potential changes, in the oxidation-reduction reactions occurring in the electron transfer pathways in respiration and photosynthesis
- ✓ $\tilde{\mu}_{\text{H}^+}$: the available energy in the ion electrochemical gradient
- ✓ $Nh\nu$: the energy available from the absorption of quanta of light in photosynthetic systems

ΔG : used in the description of metabolic reactions and its displacement from equilibrium



$$K = \frac{[C]_{eq}^c [D]_{eq}^d}{[A]_{eq}^a [B]_{eq}^b} \text{Molar}^{(c+d-a-b)}$$

$$\Gamma = \frac{[C]_{obs}^c [D]_{obs}^d}{[A]_{obs}^a [B]_{obs}^b} \text{Molar}^{(c+d-a-b)}$$

$$\Delta G = -2.3RT \log_{10} \left\{ \frac{K}{\Gamma} \right\}$$

$$\Delta G^\circ = -2.3RT \log_{10} K$$

observed mass action ratio $\Gamma = \frac{[B]_{obs}}{[A]_{obs}}$

equilibrium constant $K = \frac{[B]_{eq}}{[A]_{eq}}$

$$\Delta G = \Delta G^\circ + 2.3RT \log_{10} \left\{ \frac{[C]_{obs}^c [D]_{obs}^d}{[A]_{obs}^a [B]_{obs}^b} \right\}$$

Energy Transduction

* ΔG and the redox potential

Biological oxidation-reduction reactions

1. Direct electron transfer



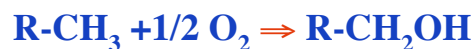
2. Hydrogen transfer (2e⁻ reduction followed by de addition of 2H⁺)



3. Transfer of hydride ion (:H⁻ 2e⁻ reduction followed by de addition of 1H⁺)

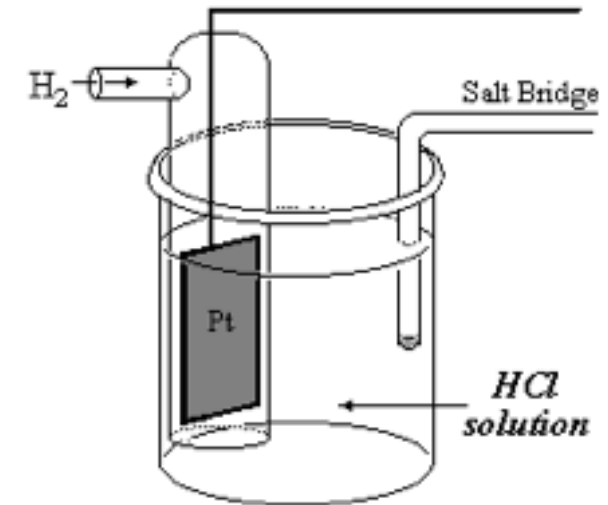


4. Direct incorporation of oxygen



Reducing equivalent (redox equivalent): generic term refers to any number of chemical species which transfer the equivalent of one electron in redox reactions

E_h redox potential

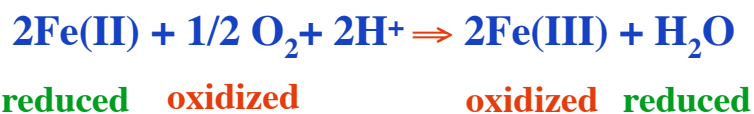


standard hydrogen electrode : H_2
 $E = 0 \text{ V}$ at 25°C

E_h redox potential is a measure of the tendency of a chemical species to acquire electrons and be reduced. Reduction potential is measured in volts (V), or millivolts (mV).

Energy Transduction

➡ **E: a measure of affinity for e⁻**



$$E = E^\circ + 2,3 \frac{RT}{nF} \left[\frac{[\text{e}^- \text{ acceptor} - \text{oxidized}]}{[\text{e}^- \text{ donor} - \text{reduced}]} \right]$$

n: number of electrons transferred
F: Faraday constant (9,6485 x 10⁴ C)

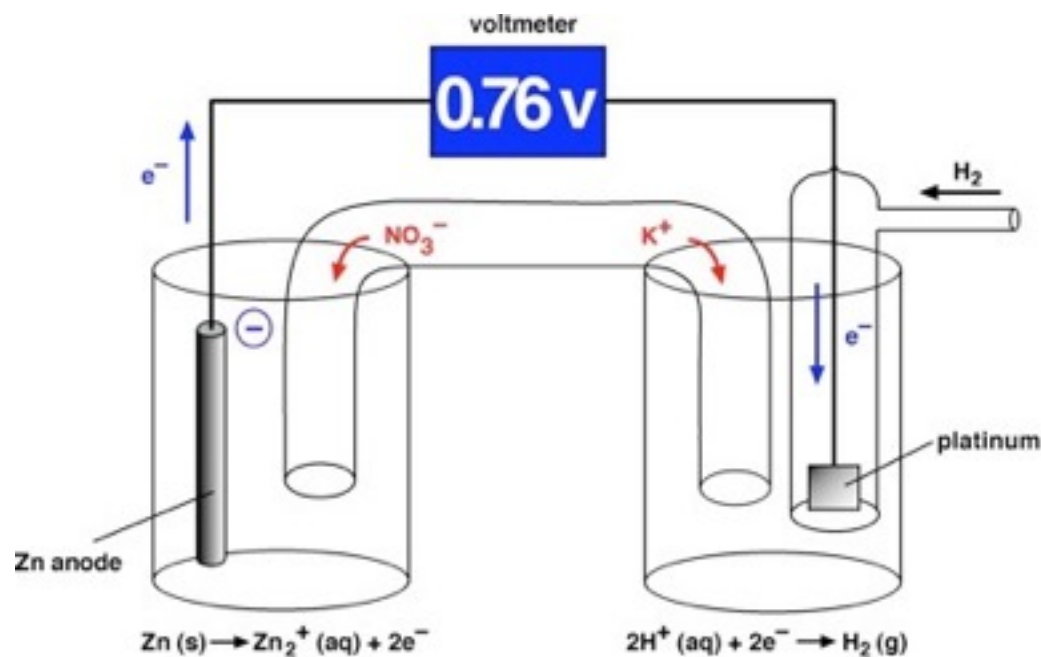
$$\Delta G = -nF \Delta E_h$$

E is a measure of Δ*G* in electrochemical units

$$\Delta E_h = E (\text{acceptor couple}) - E (\text{donor couple})$$

O₂ - oxidizing agent - relatively + redox potential *E*

2Fe(II) - reducing agent - relatively - redox potential *E*



Energy Transduction

* $\Delta\tilde{\mu}_X^{m+}$ Electrochemical potential

Ion electrochemical potential across a membrane

✓ Ion concentration gradient

ΔG associated to the transfer of 1 mol of solute across a membrane from a concentration $[X]_A$ to a concentration $[X]_B$.

In the absence of a membrane potential

$$\Delta G \text{ (Jmol}^{-1}\text{)} = 2,3RT \log_{10} \frac{[X]_B}{[X]_A}$$

✓ Electric potential difference - $\Delta\psi$ membrane potential

ΔG associated to the transfer of 1 mol of cation X^{m+} , transported down an electrical potential of $\Delta\psi$ (mV)

$$\Delta G \text{ (Jmol}^{-1}\text{)} = -mF\Delta\psi$$

m : valence of the ion

F : Faraday constant $9,6485 \times 10^4$ C

✓ $\Delta\tilde{\mu}_H^+$ Electrochemical gradient

ΔG associated to the transfer of 1 mole do catião X^{m+} , of cation X^{m+} , transported down an electrical potential of $\Delta\psi$ (mV), from a concentration $[X]_A$ to a concentration $[X]_B$

$$\Delta G \text{ (Jmol}^{-1}\text{)} = -mF\Delta\psi + 2,3RT \log_{10} \frac{[X^{m+}]_B}{[X^{m+}]_A}$$

Energy Transduction

* $\Delta\tilde{\mu}_{\text{H}^+}$ Proton electrochemical gradient

$$\Delta\tilde{\mu}_{\text{H}^+} (\text{Jmol}^{-1}) = -F\Delta\psi + 2,3 RT \log_{10} \frac{[\text{H}^+]_e}{[\text{H}^+]_i}$$

$$\text{Log} ([\text{H}^+]_e / [\text{H}^+]_i) = \log[\text{H}^+]_e - \log[\text{H}^+]_i \Leftrightarrow -\log[\text{H}^+]_i - (-\log[\text{H}^+]_e) = \text{pH}_i - \text{pH}_e = \Delta\text{pH}$$

$$\Delta\tilde{\mu}_{\text{H}^+} (\text{Jmol}^{-1}) = -F\Delta\psi + 2,3RT\Delta\text{pH}$$

The electrochemical gradient that is established when a proton cross the membrane is function of the electrical potential (membrane potential - $\Delta\psi$) and the difference of $[\text{H}^+]$ between two phases separated by the membrane (ΔpH)

* Protonmotive force or protonic potential (Δp) in units of voltage

$$\Delta p (\text{mV}) = -(\Delta\tilde{\mu}_{\text{H}^+}/F) \qquad \Delta p (\text{mV}) = \Delta\psi - \frac{2,3RT}{F} \Delta\text{pH}$$

By convention $\Delta\psi$ is negative when the inner surface of the membrane is negative

$$\Delta\tilde{\mu}_{\text{H}^+} = 1 \text{ kJmol}^{-1} \Leftrightarrow \Delta p = -10.4 \text{ mV} \qquad \Delta p = -200 \text{ mV} \Leftrightarrow \Delta\mu_{\text{H}^+} = 19 \text{ kJmol}^{-1}$$

$$\text{a } 25^\circ\text{C } \Delta p (\text{mV}) = \Delta\psi - 59\Delta\text{pH}$$

Energy Transduction

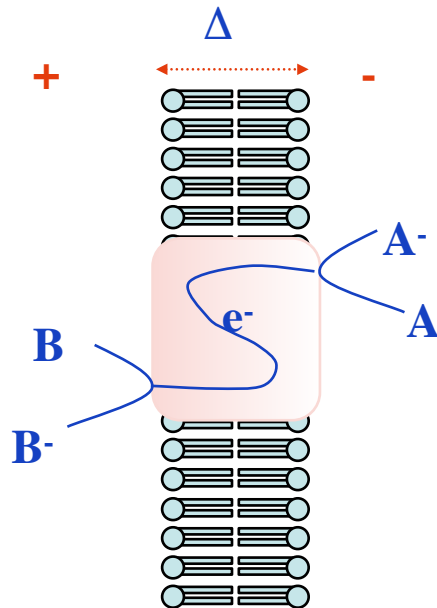
- * ΔE_h and ΔG for an electron transfer between redox couples located on opposite sites of a membrane sustaining a membrane potential

$$\Delta G = -nF \Delta E_h$$

$$\Delta E_h = E (\text{acceptor couple}) - E (\text{donor couple})$$

$$\Delta E = E_B - E_A$$

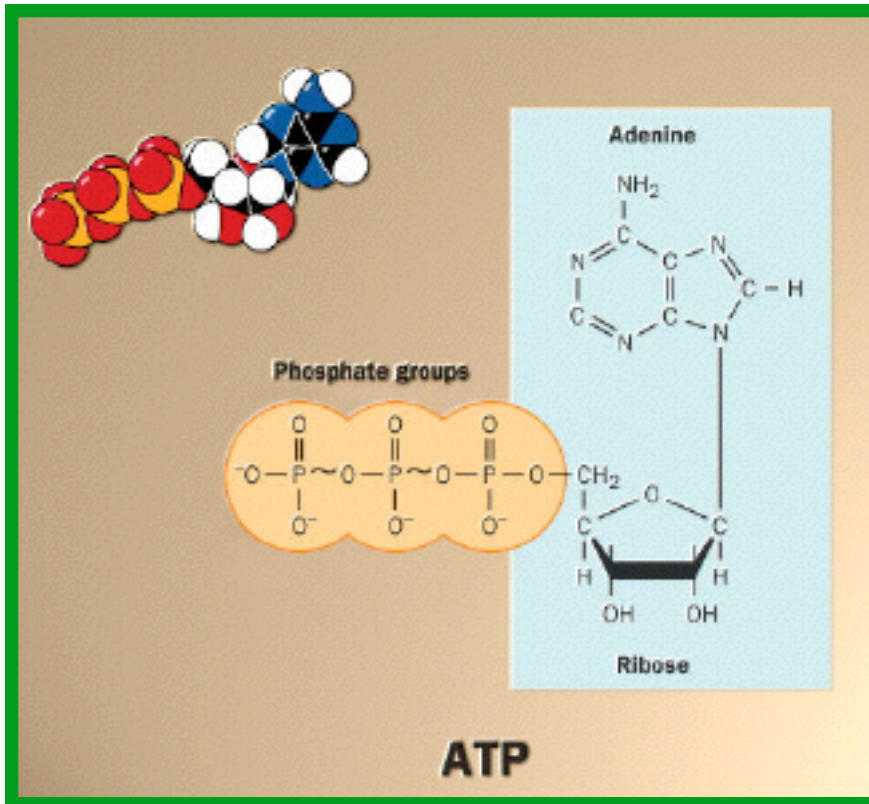
$$\Delta G = -nF (\Delta E + \Delta\psi)$$



The value of the membrane potential must be added to the redox potential difference to calculate the effective Gibbs energy change ΔG

Energy Transduction

* Phosphorylation potential



* In the cytoplasm the chemical energy available for endergonic reactions: ATP, PEP, nucleotide derivatives, acyl-phosphates and acyl-CoA

* ~ bond with a high free energy of hydrolysis

* The energy input required to break a bond is known as bond energy.

$$\text{P - O : bond energy} = + 413 \text{ kJ} \\ \Delta G = -35 \text{ kJ}$$

* Bonds with high energy hydrolysis \Leftrightarrow
high potential for group transfer

✓ Under physiological conditions (physiological []s of ATP, ADP and Pi), the energy hydrolysis or ΔG for ATP synthesis is designated as **phosphorylation potential** (ΔG_p).

$$\Delta G_p = \Delta G^\circ + 2,3RT \log_{10} \left[\frac{[\text{ATP}]}{[\text{ADP}] [\text{Pi}]} \right]$$

near the equilibrium: $\Delta G_p / F = y \Delta p$

$$\Delta G_p = -50 \text{ kJ (518 mV)} \quad e \quad y = 3$$

Under these conditions the hydrolysis of 1 ATP $\Leftrightarrow \Delta p = -173 \text{ mV}$

The chemiosmotic hypothesis

* Energy coupling - energy-transducing membranes

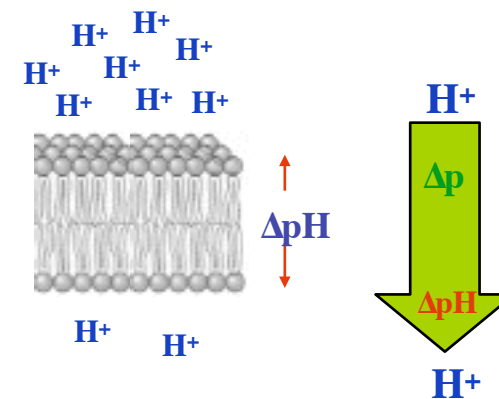
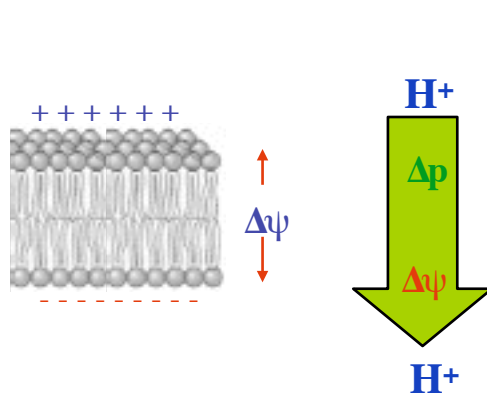
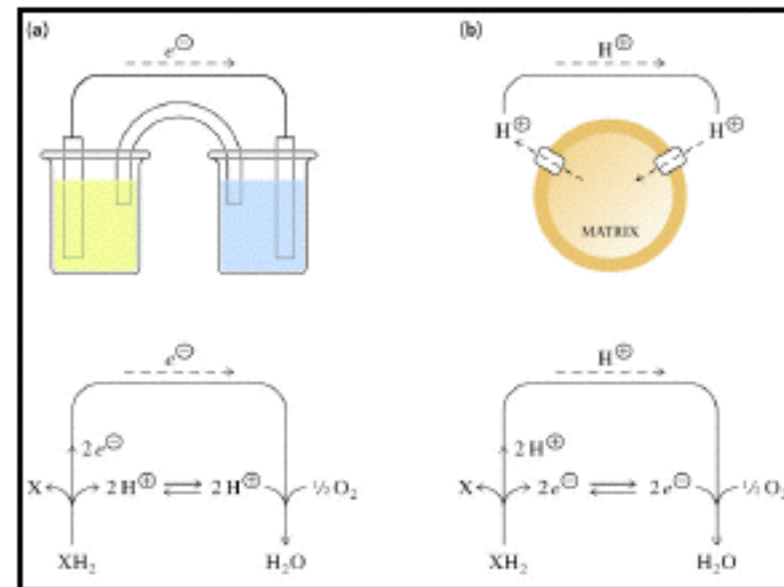
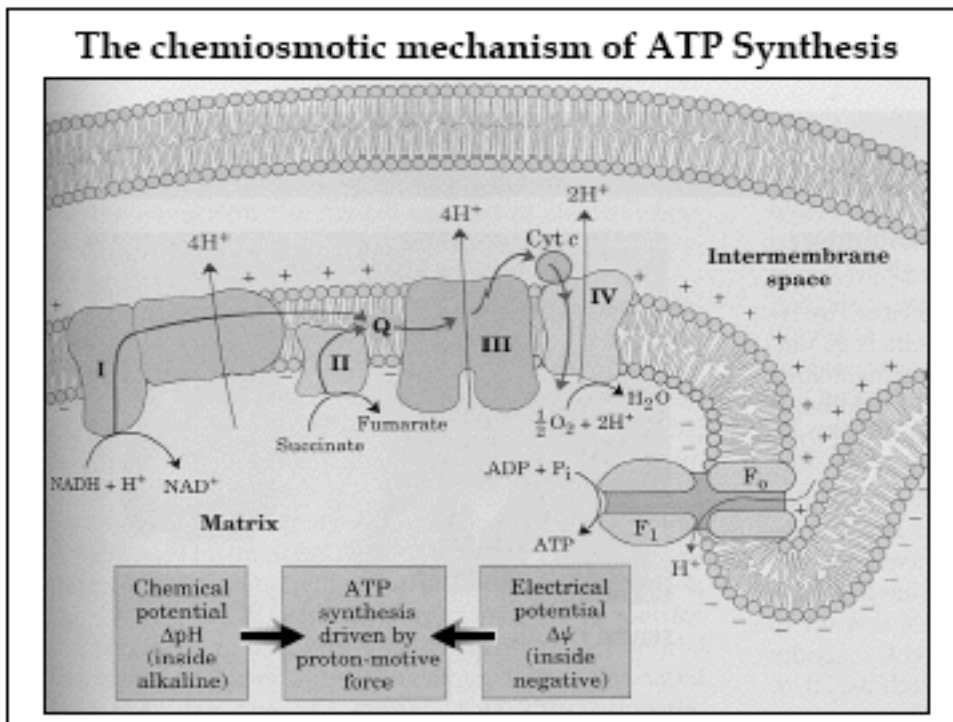
The ATP synthetase complex is associated with energy-transducing membranes:

- ✓ Plasma membrane of prokaryotic cells
- ✓ Inner membrane of mitochondria
- ✓ Plasma membrane of photosynthetic bacteria
- ✓ Thylakoid membrane of chloroplasts

* Energy-transducing membranes features

- A low proton permeability (a low effective proton conductance)
- Contains two proton pumps: primary and secondary pumps
- The nature of the primary proton pump depends on the energy source
- Secondary pump = **ATP synthase**
- The primary and secondary pumps are coupled through translocation of protons ($\Delta\tilde{\mu}_{H^+}$)

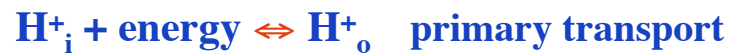
The chemiosmotic hypothesis



The chemiosmotic hypothesis

* Dissipation of Δp

✓ Solute transport



✓ ADP phosphorylation

■ F_0 and F_1 complexes

■ F_0 : united integrated in the membrane
(ab_2c_{12})

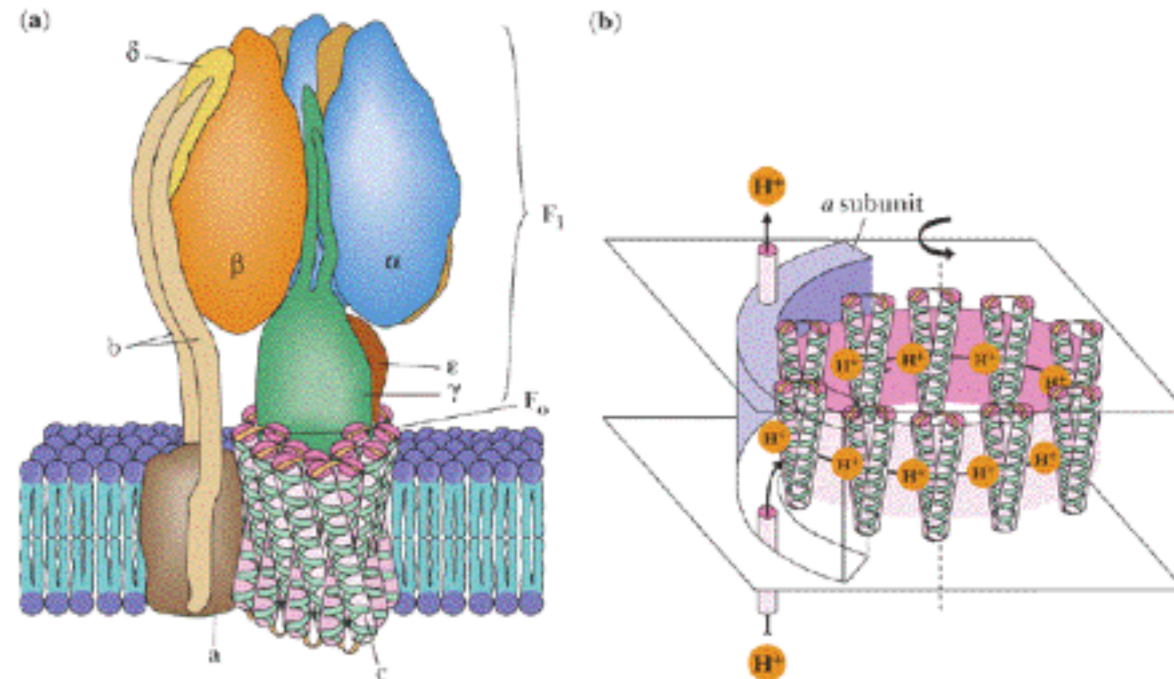
F_1 : catalytic unit ($\alpha_3\beta_3\gamma\delta\epsilon$)

Proton flux \Rightarrow rotation of unit c \Rightarrow rotation of unit γ \Rightarrow conformational change

✓ Reversed electron transfer

○ Electron transfer to more negative redox potentials

○ ATP hydrolysis



The laws of cell energetics

The first law

The living cell avoids direct utilization of external energy sources in the performance of useful work. It transforms energy of these sources to a convertible energy currency, i.e. ATP, $\Delta\tilde{\mu}_{H^+}$ or $\Delta\tilde{\mu}_{Na^+}$, which is then spent to support various types of energy-consuming processes

The cell prefers to deal with energy in a money-type circulation rather than with barter

A célula prefere negociar com a "energia em circulação" (dinheiro) em vez de negociar por "câmbio"

The second law

Any living cell always possesses at least two energy currencies, one water-soluble (ATP) and the other membrane-linked ($\Delta\tilde{\mu}_{H^+}$ and/or $\Delta\tilde{\mu}_{Na^+}$)

The cell always has some currency in cash and some in cheques

A célula tem sempre alguma energia "em caixa" e alguma em "cheques"

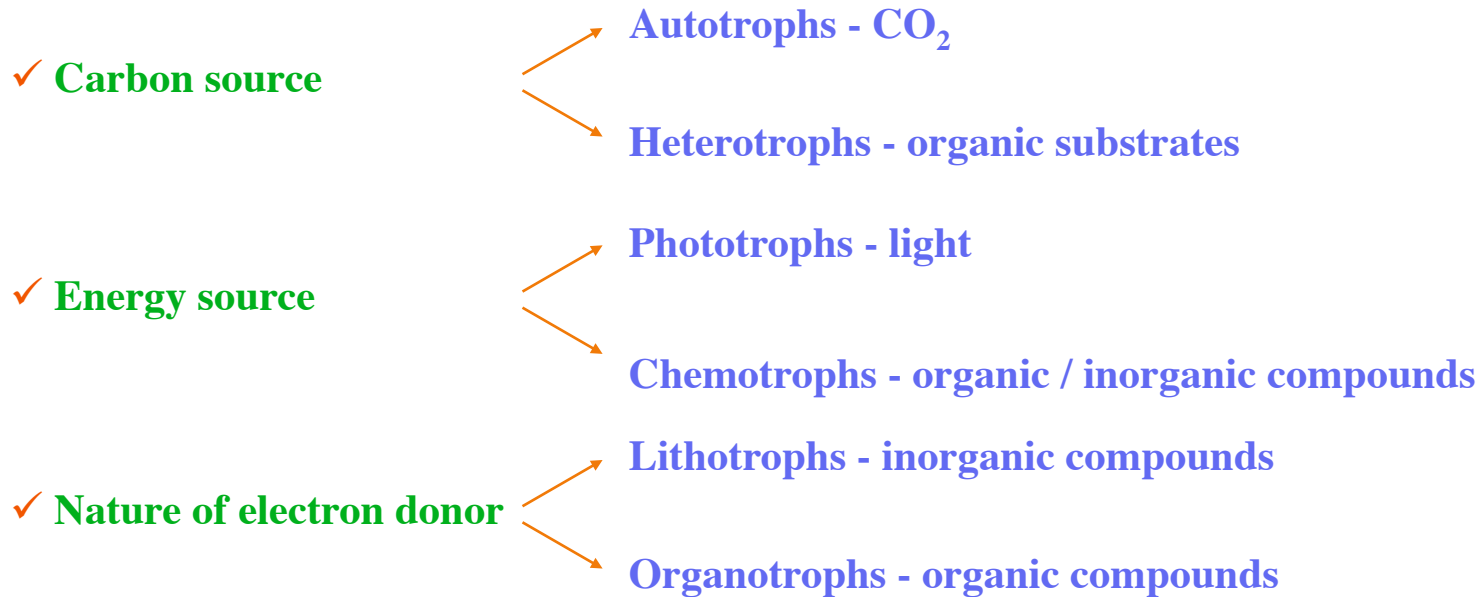
The third law

All the energy requirements of the living cell can be satisfied if at least one of three convertible energy currencies is produced at the expense of external energy sources. The cell is always competent in the $ATP \rightarrow \Delta\tilde{\mu}_{H^+}$ and/or $ATP \rightarrow \Delta\tilde{\mu}_{Na^+}$ interconversions due to reversibility of H^+ -ATP e Na^+ -ATP synthases

It does not matter how an income is received, in cash or in cheques, as long as they are interconvertible

Não interessa como é recebido o salário, em dinheiro ou em cheque, visto que eles são convertíveis

Energy classes of microorganisms



○ **Photolithotrophy**

- ✓ Light - energy source
- ✓ CO₂ - carbon source
- ✓ inorganic compound - e⁻ donor

○ **Photoorganotrophy**

- ✓ Light - energy source
- ✓ organic substrates - C source
- ✓ organic substrates - e⁻ donor

○ **Chemolithotrophs**

- ✓ inorganic compound - energy
- ✓ CO₂ - carbon source
- ✓ inorganic compound - e⁻ donor

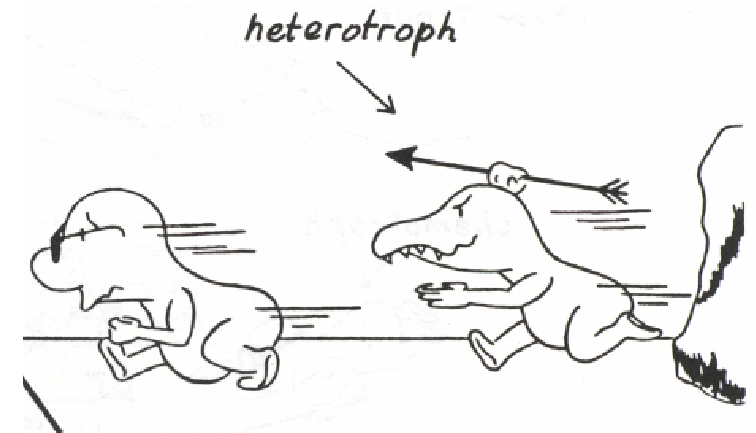
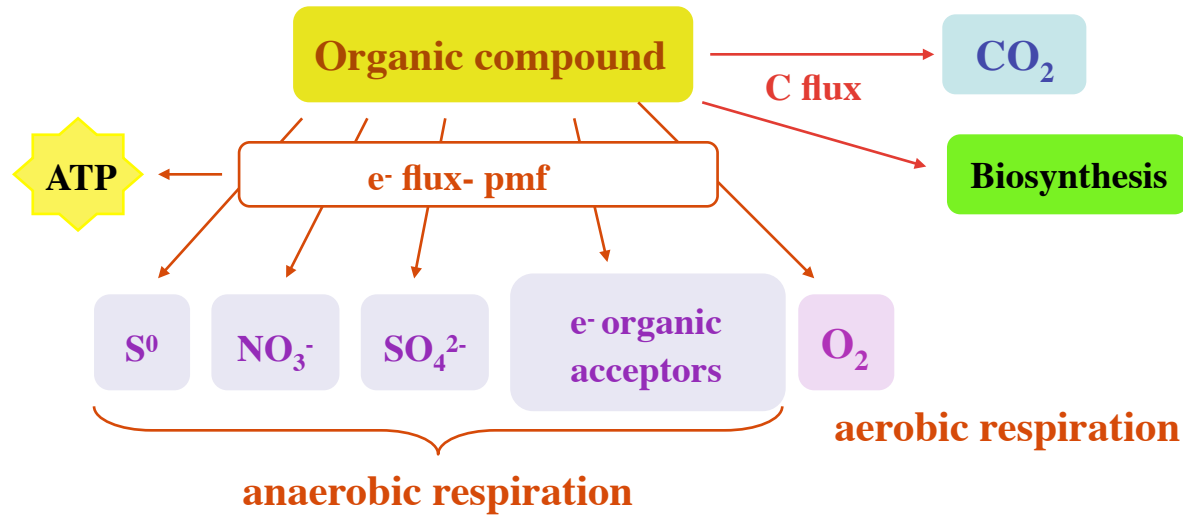
○ **Chemoorganotrophy**

- ✓ organic substrates - energy
- ✓ organic substrates - C source
- ✓ organic substrates - e⁻ donor

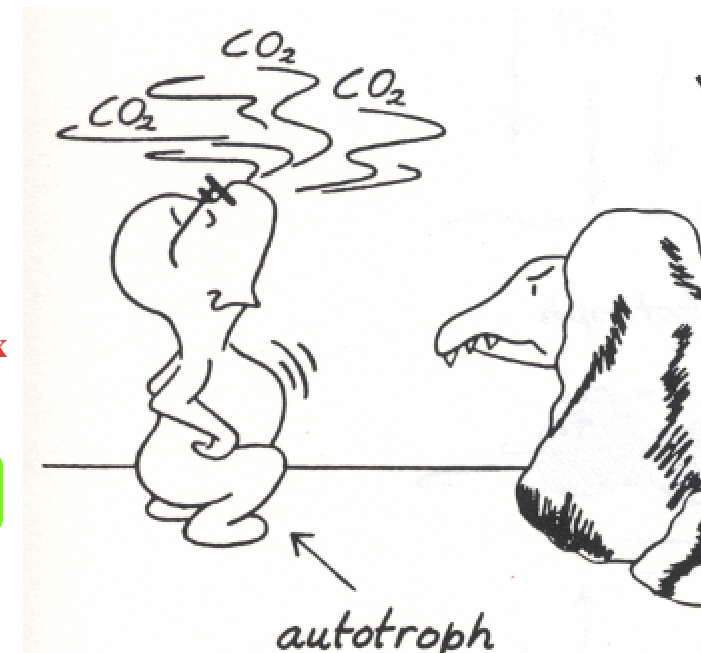
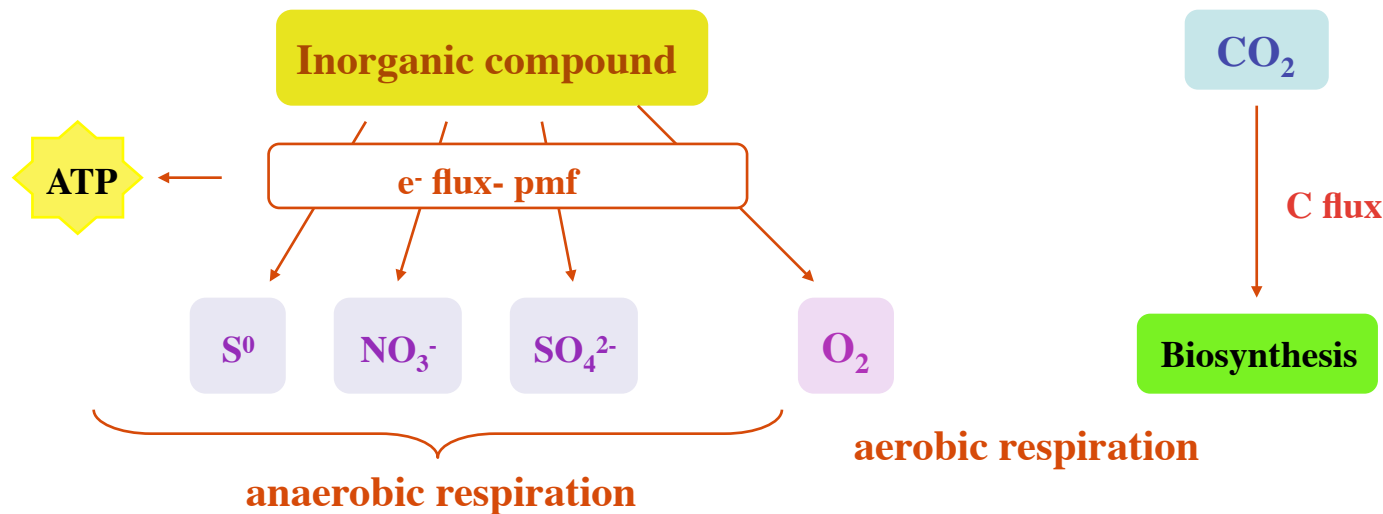
○ **Mixotrophy** - inorganic compound - energy source; organic substrates - C source

Energy classes of microorganisms

✓ **Chemoorganotrophic metabolism**

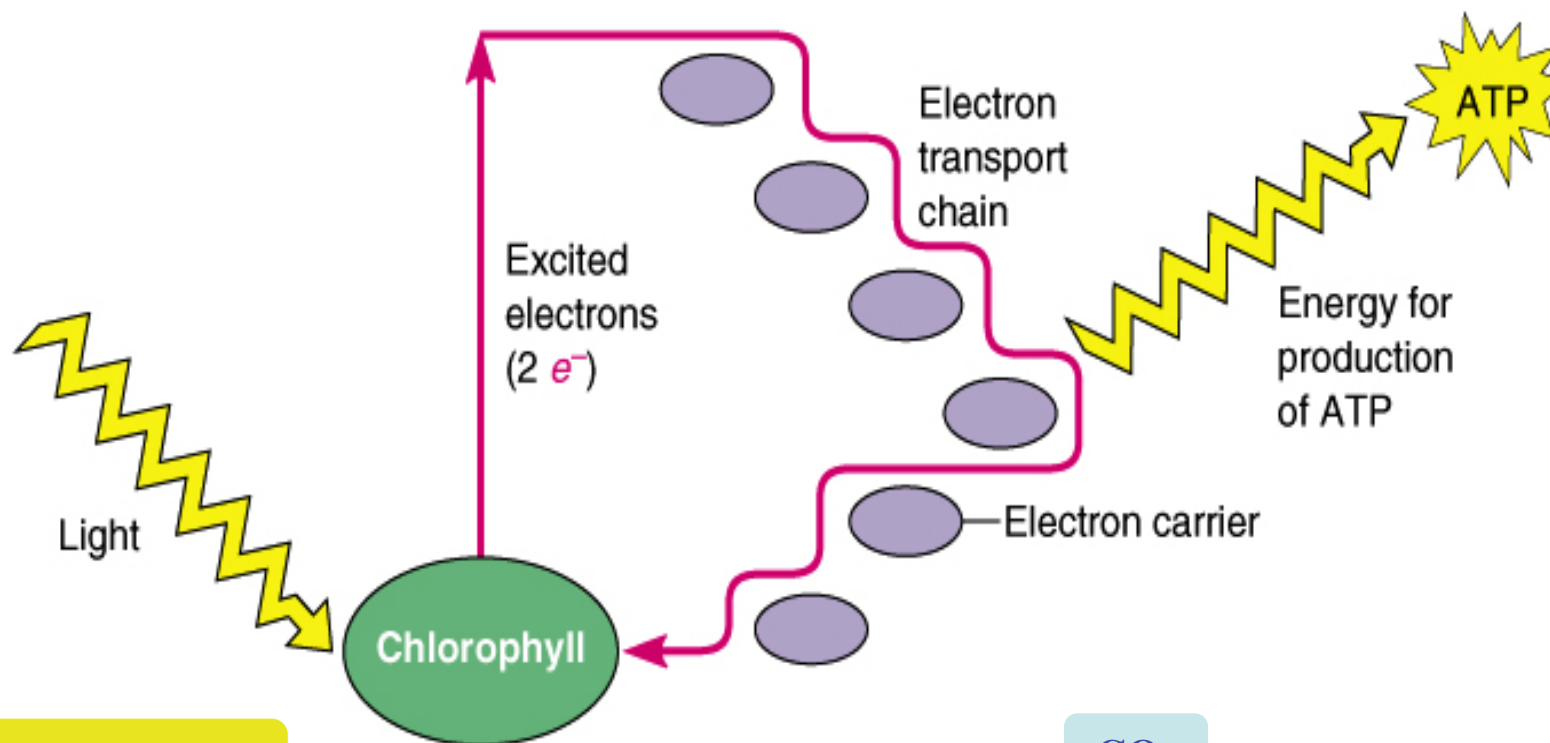


✓ **Chemolithotrophic metabolism**



Energy classes of microorganisms

✓ **Phototrophic metabolism**



organic compound

C flux

Biosynthesis

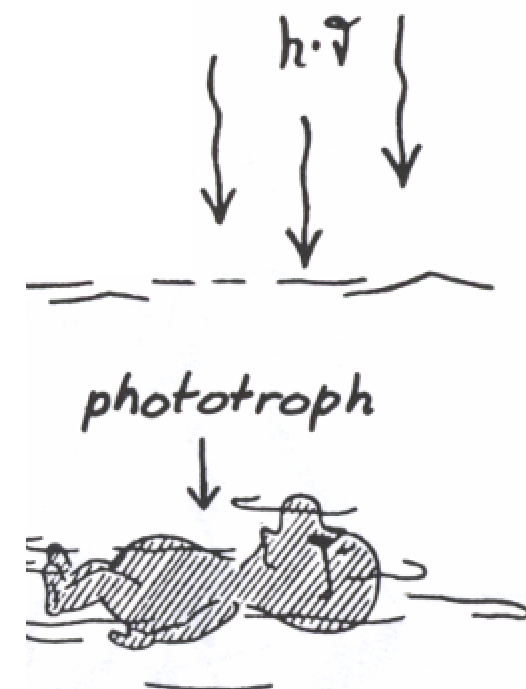
Photoheterotrophy

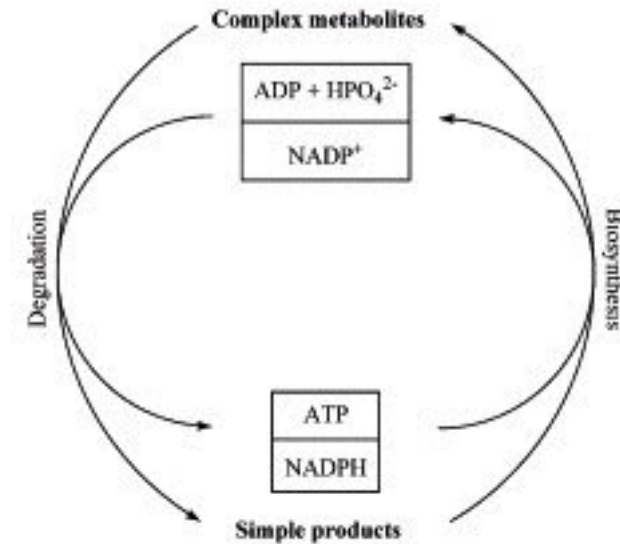
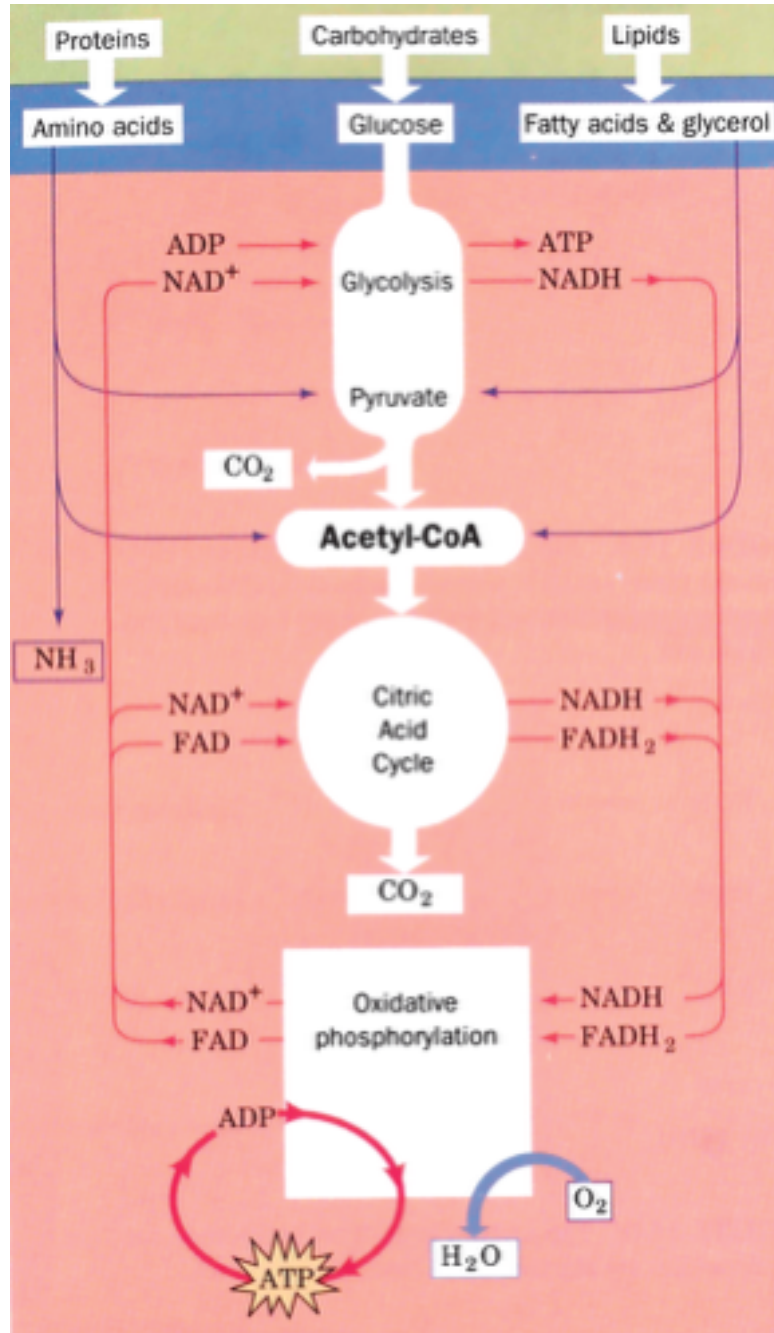
CO₂

C flux

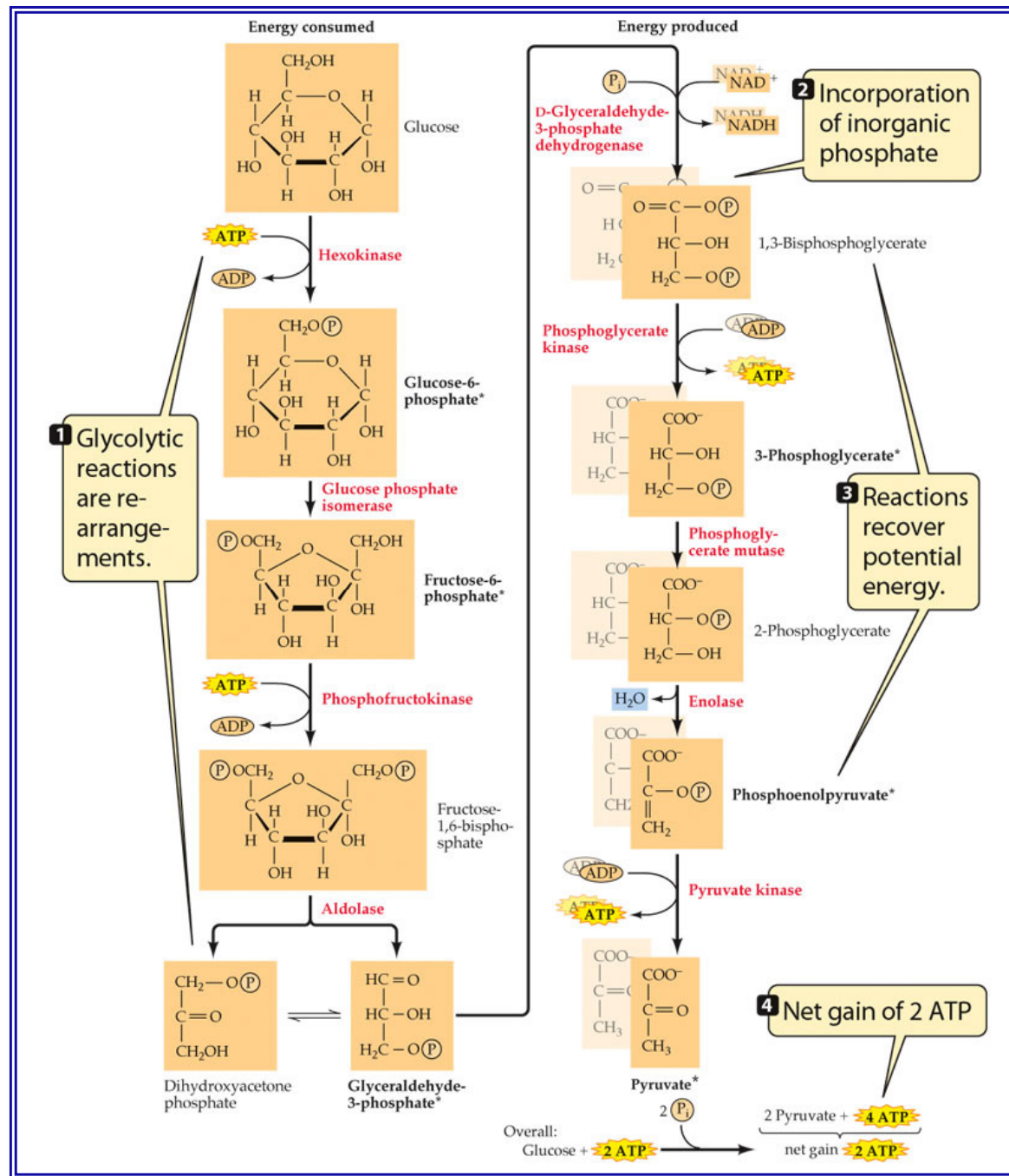
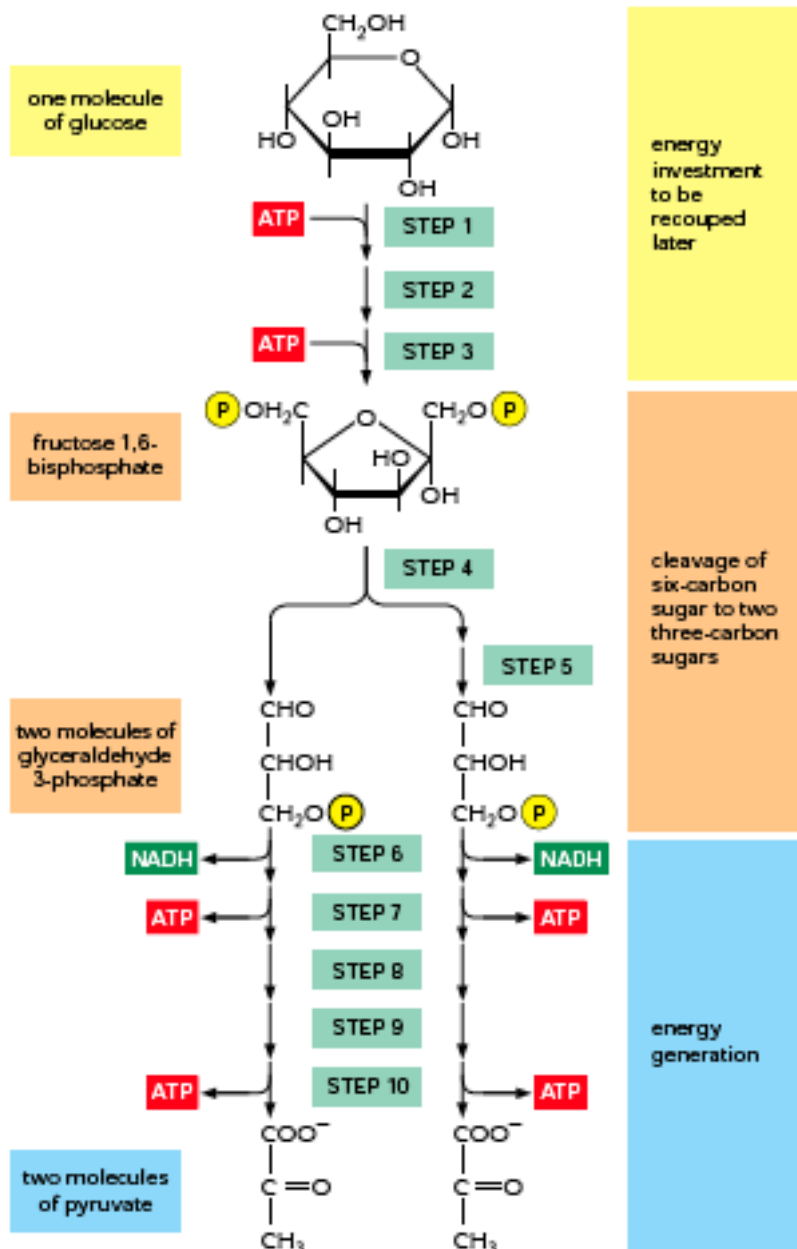
Biosynthesis

Photoautotrophy

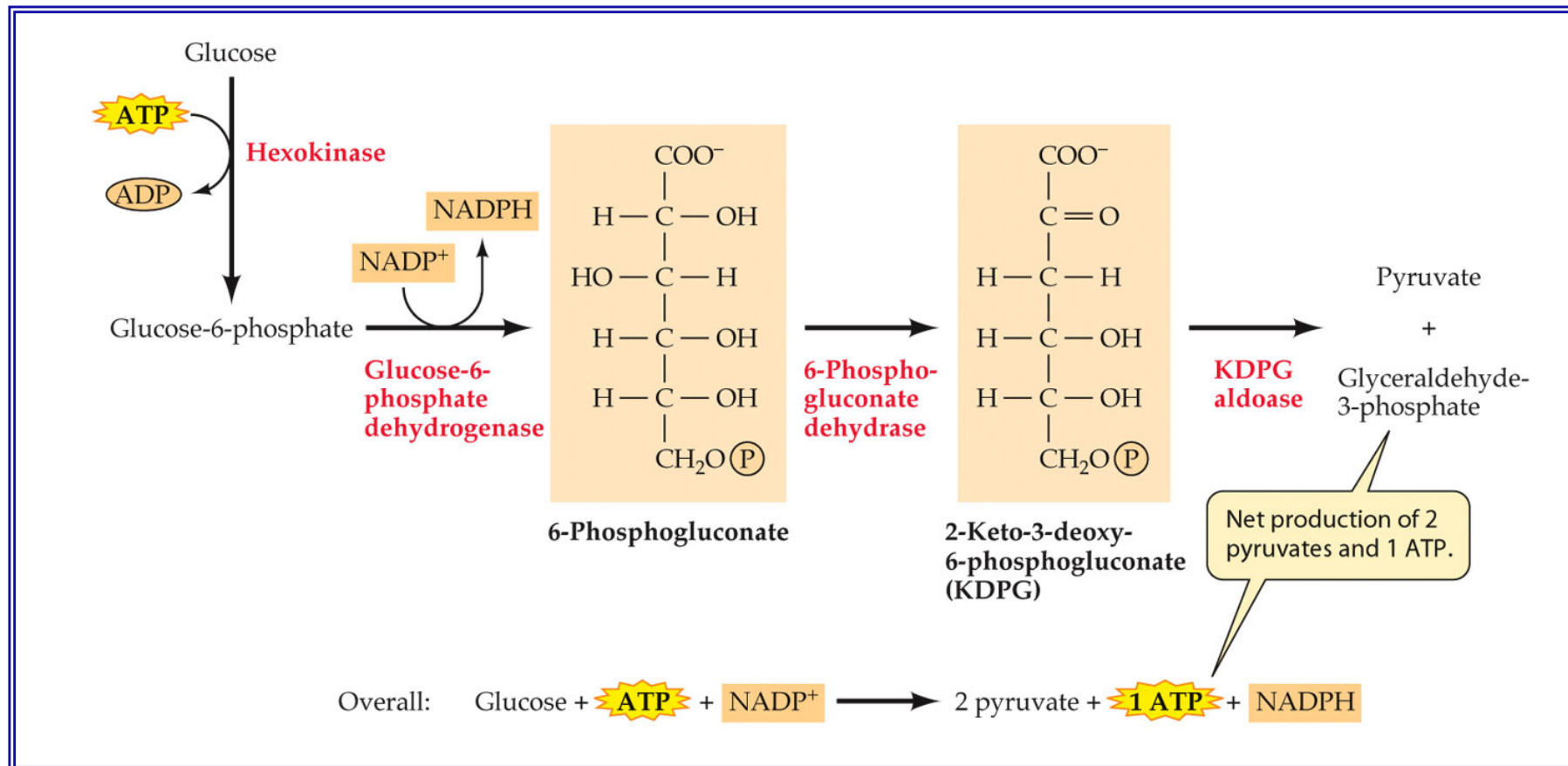


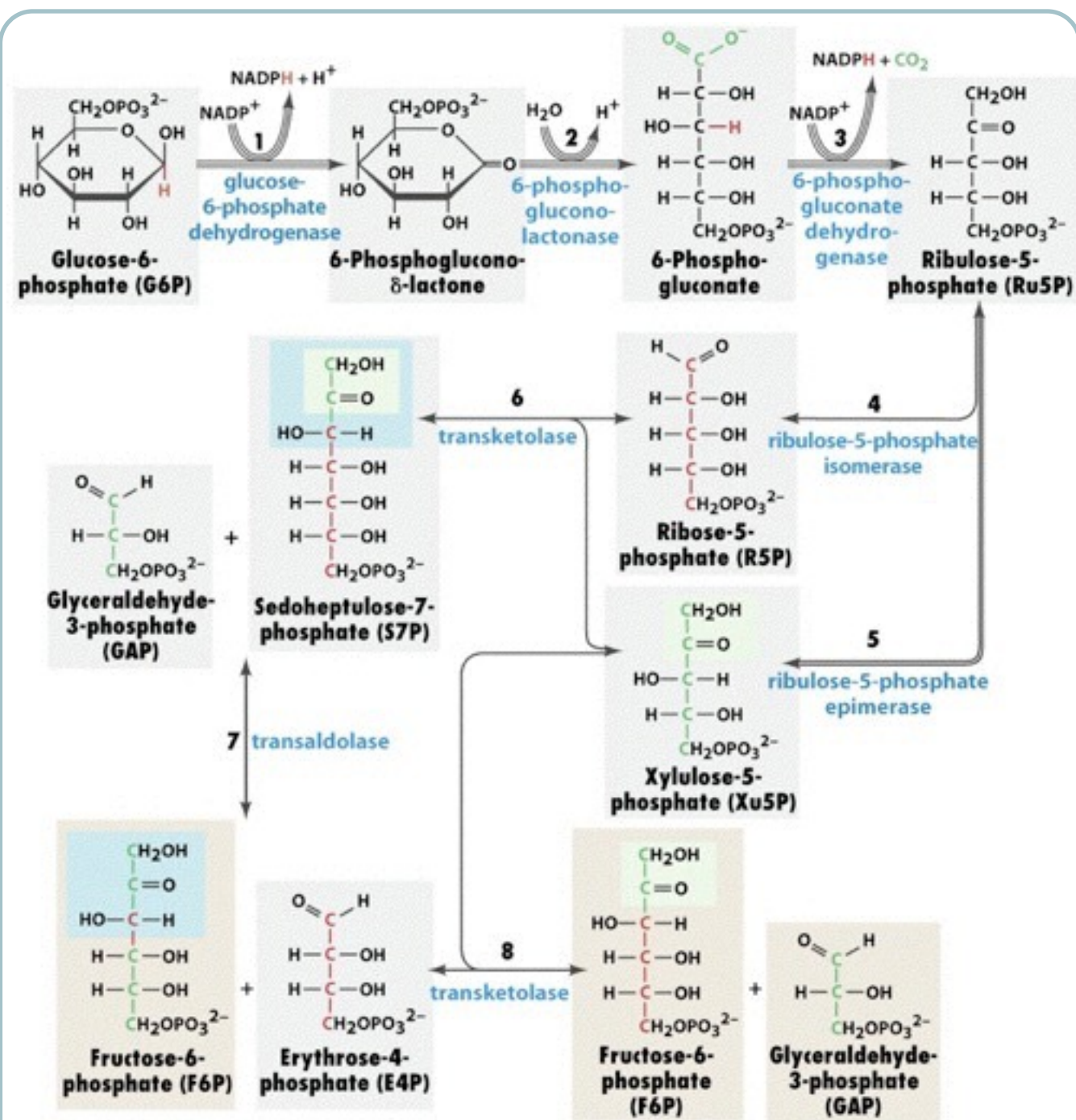


Glicólise



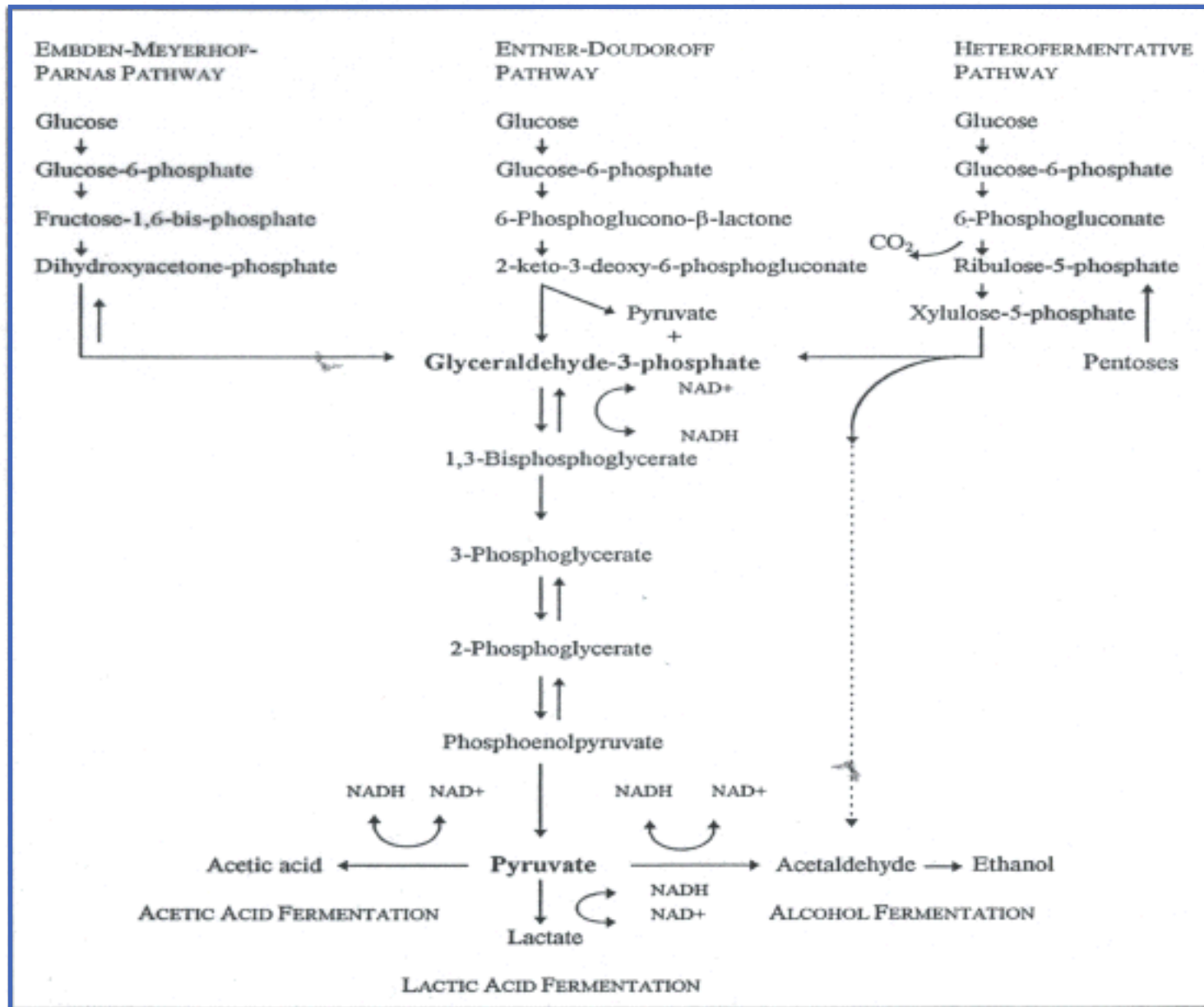
Via de Entner-Doudoroff



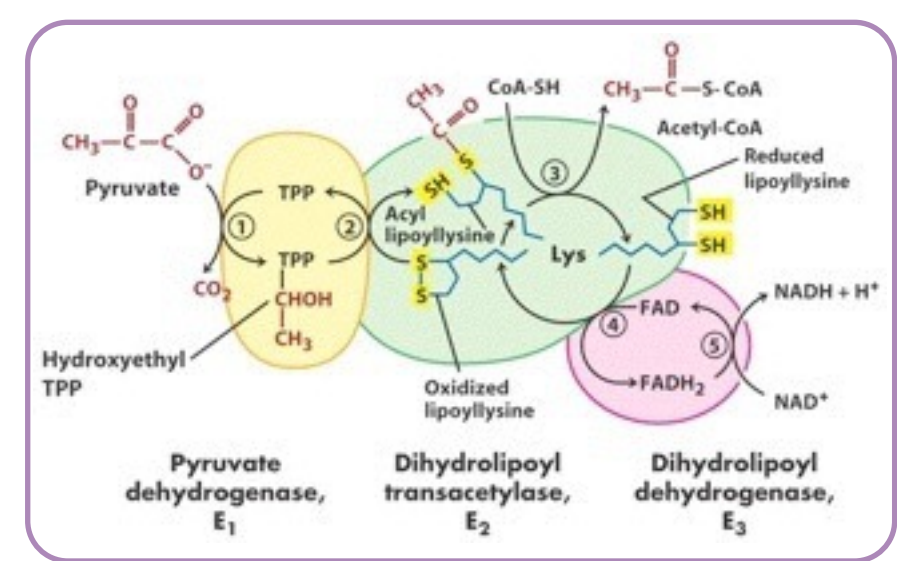
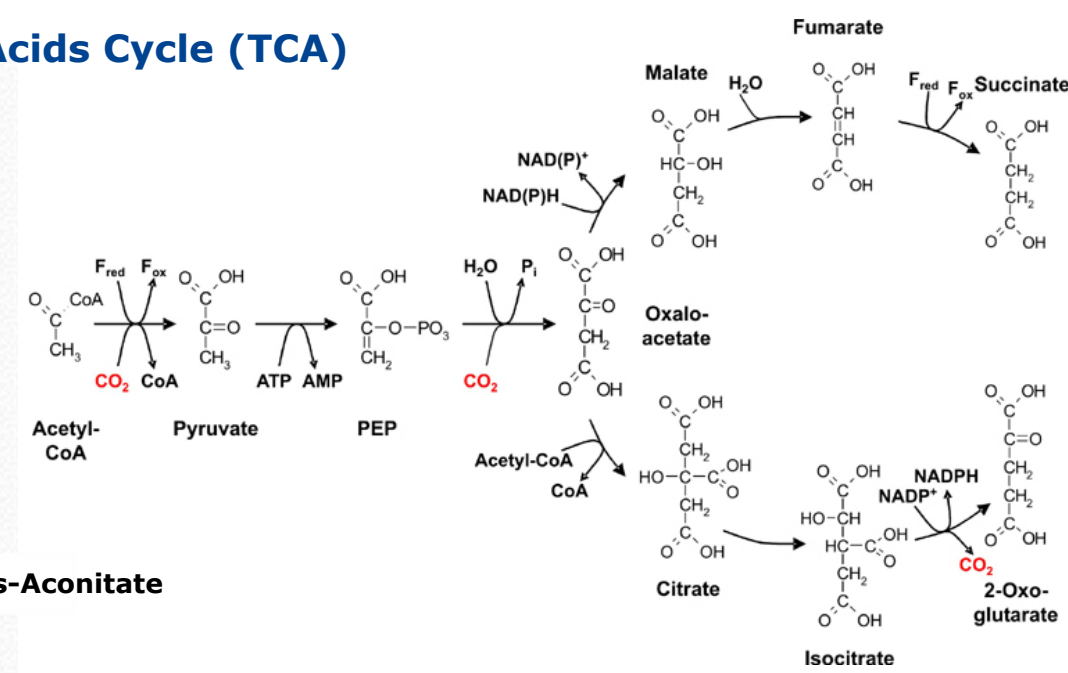
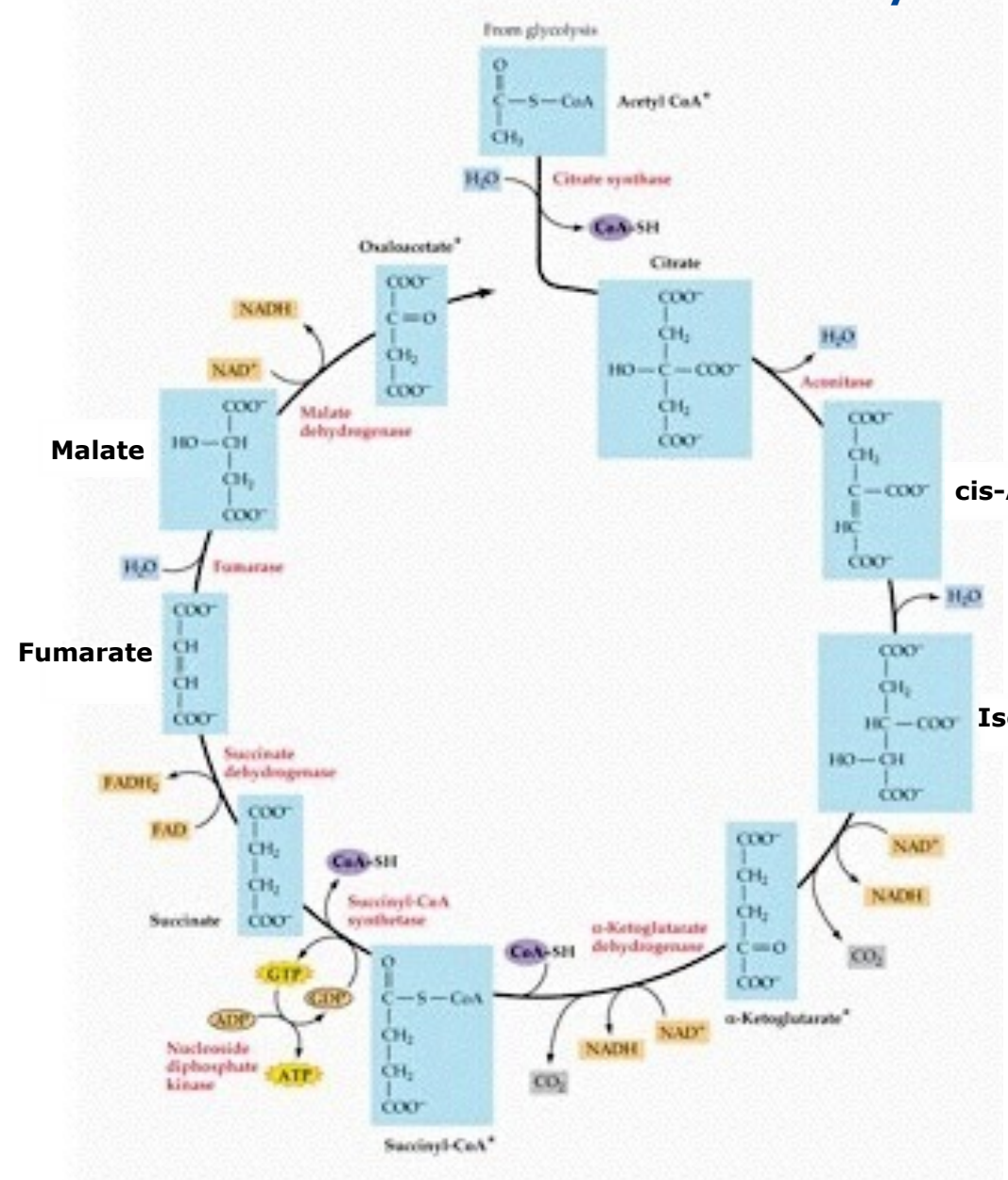


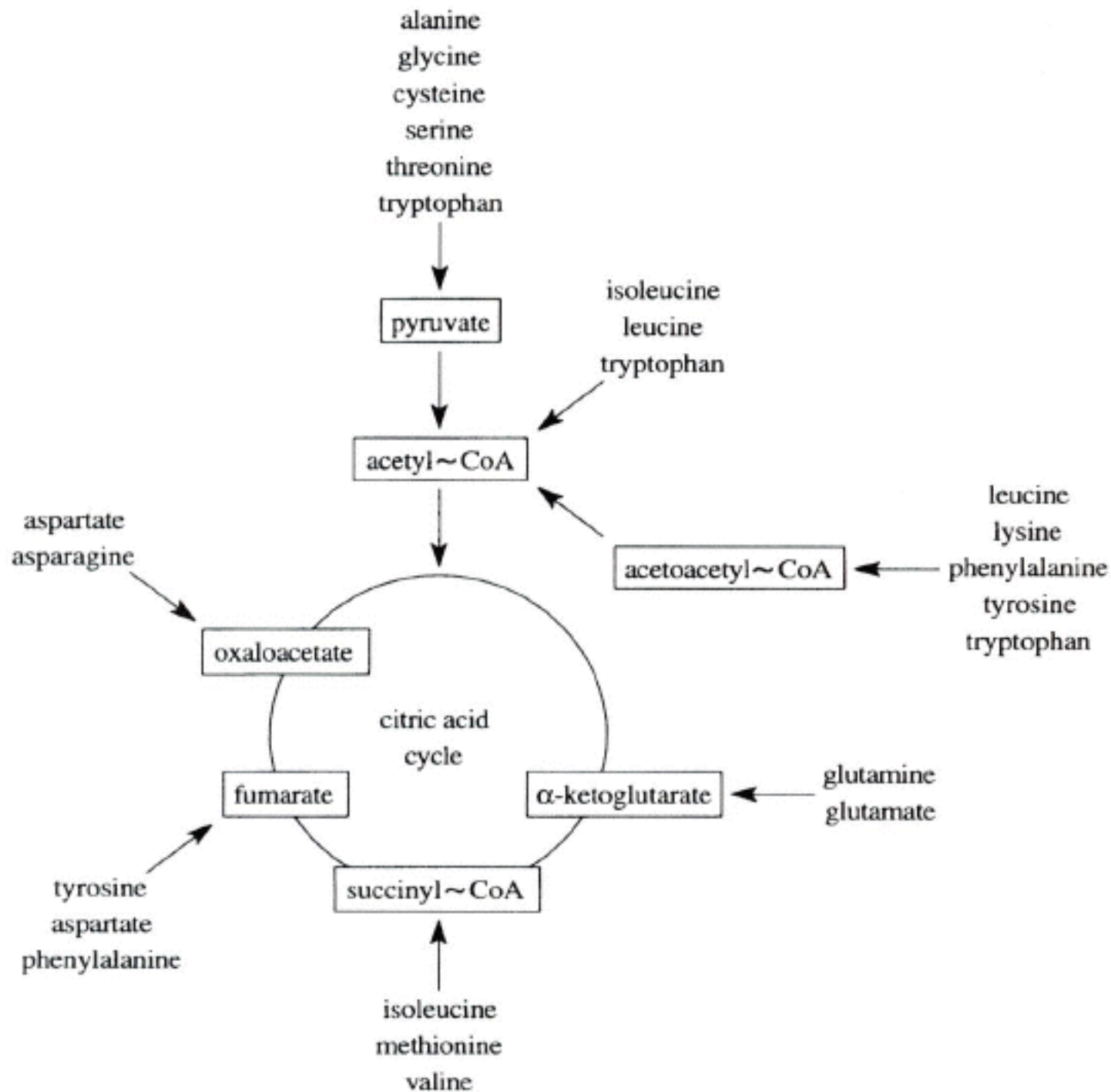
Via das pentoses fosfato

Figure 14-30 Fundamentals of Biochemistry, 2/e
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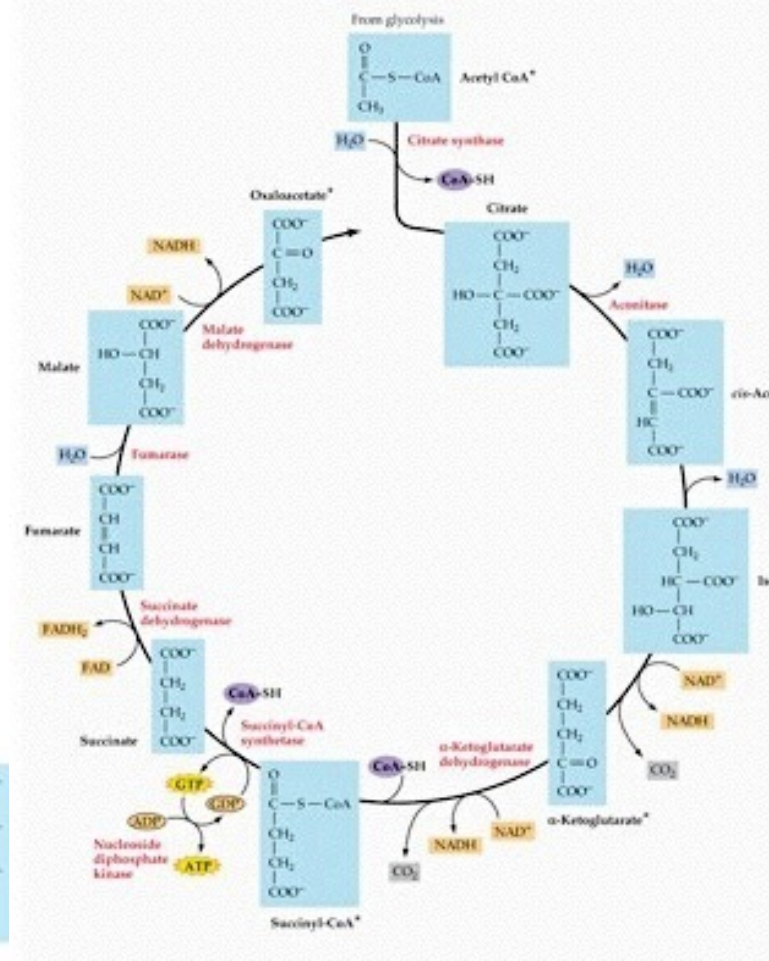
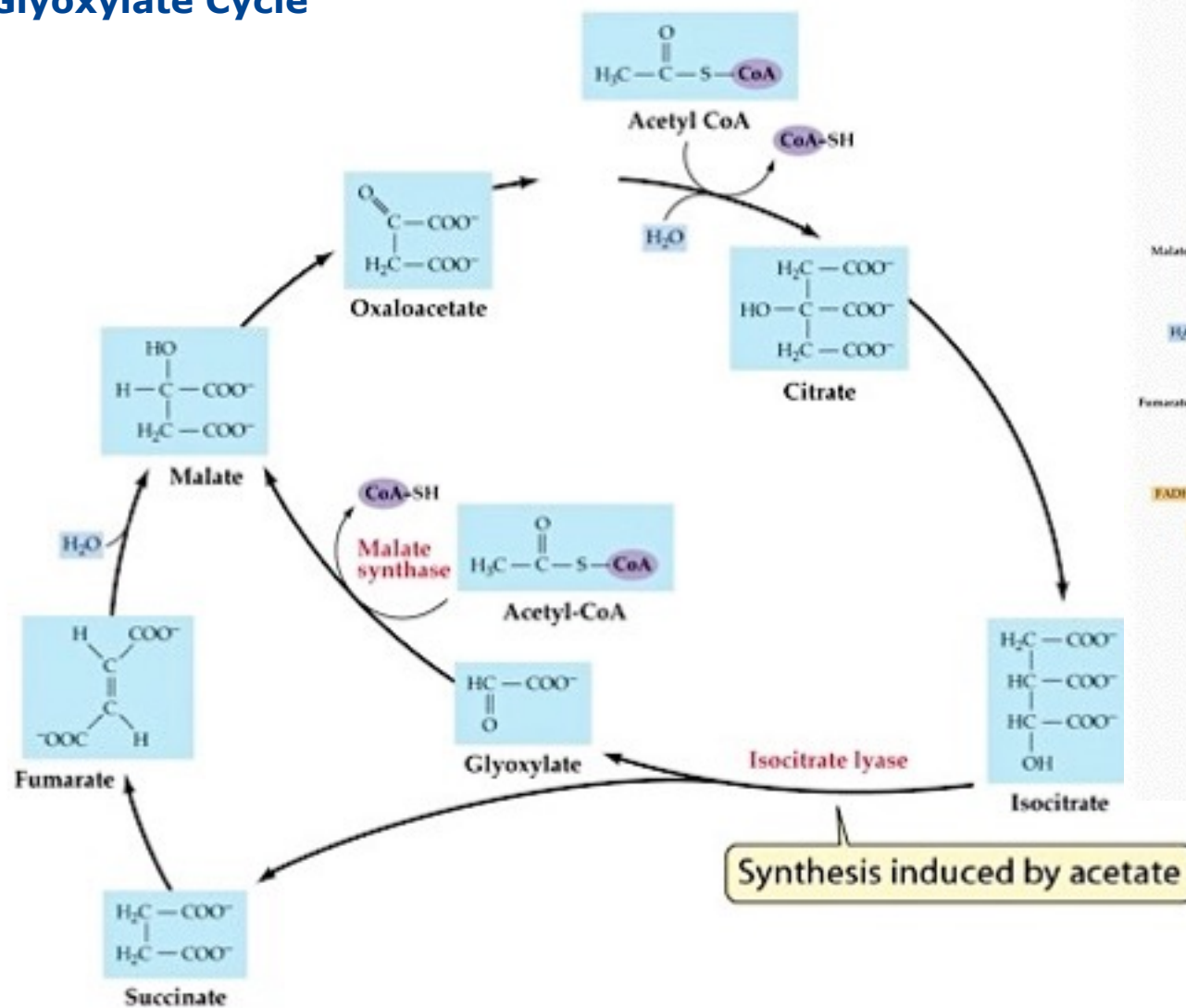


Tricarboxylic Acids Cycle (TCA)



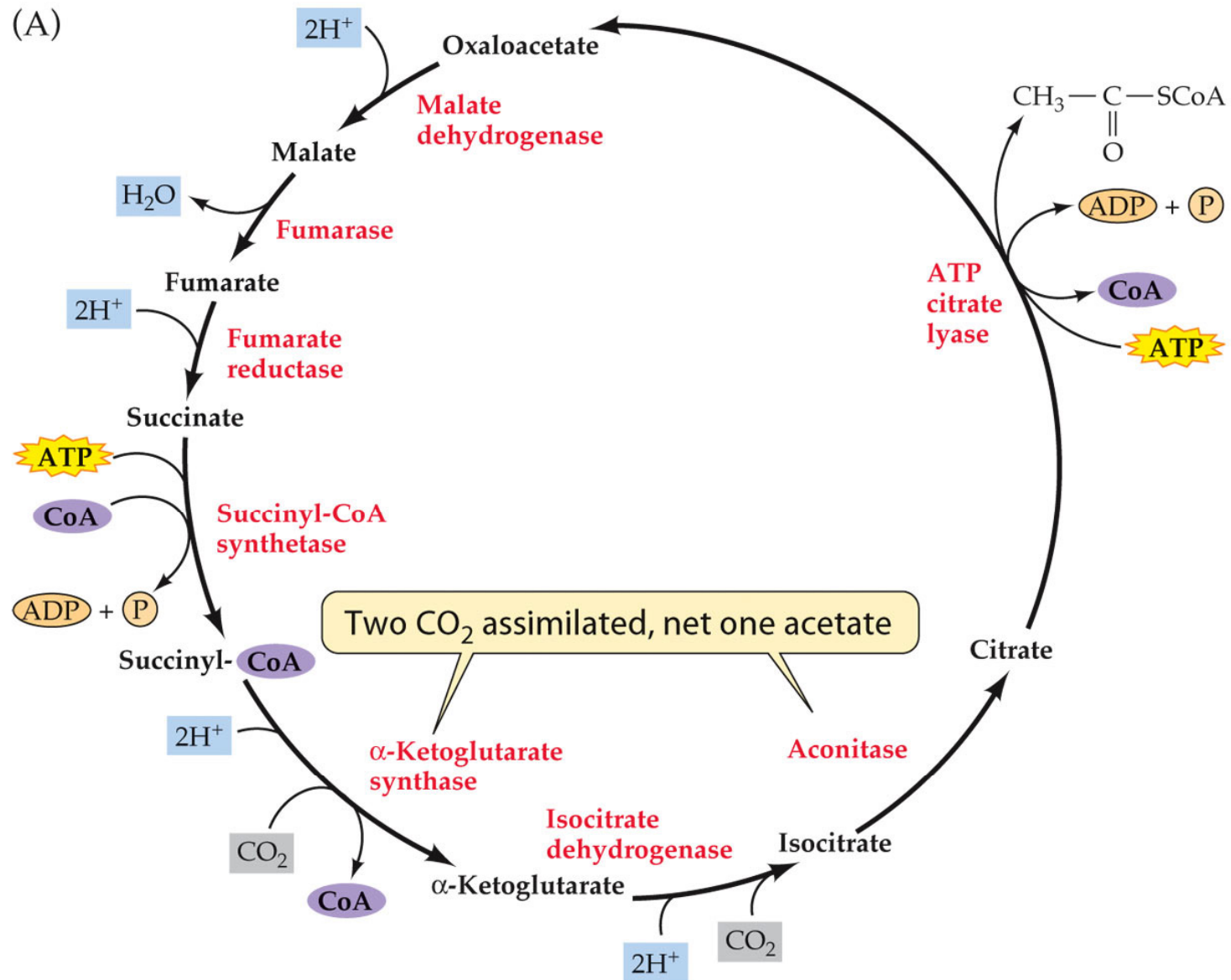


Glyoxylate Cycle



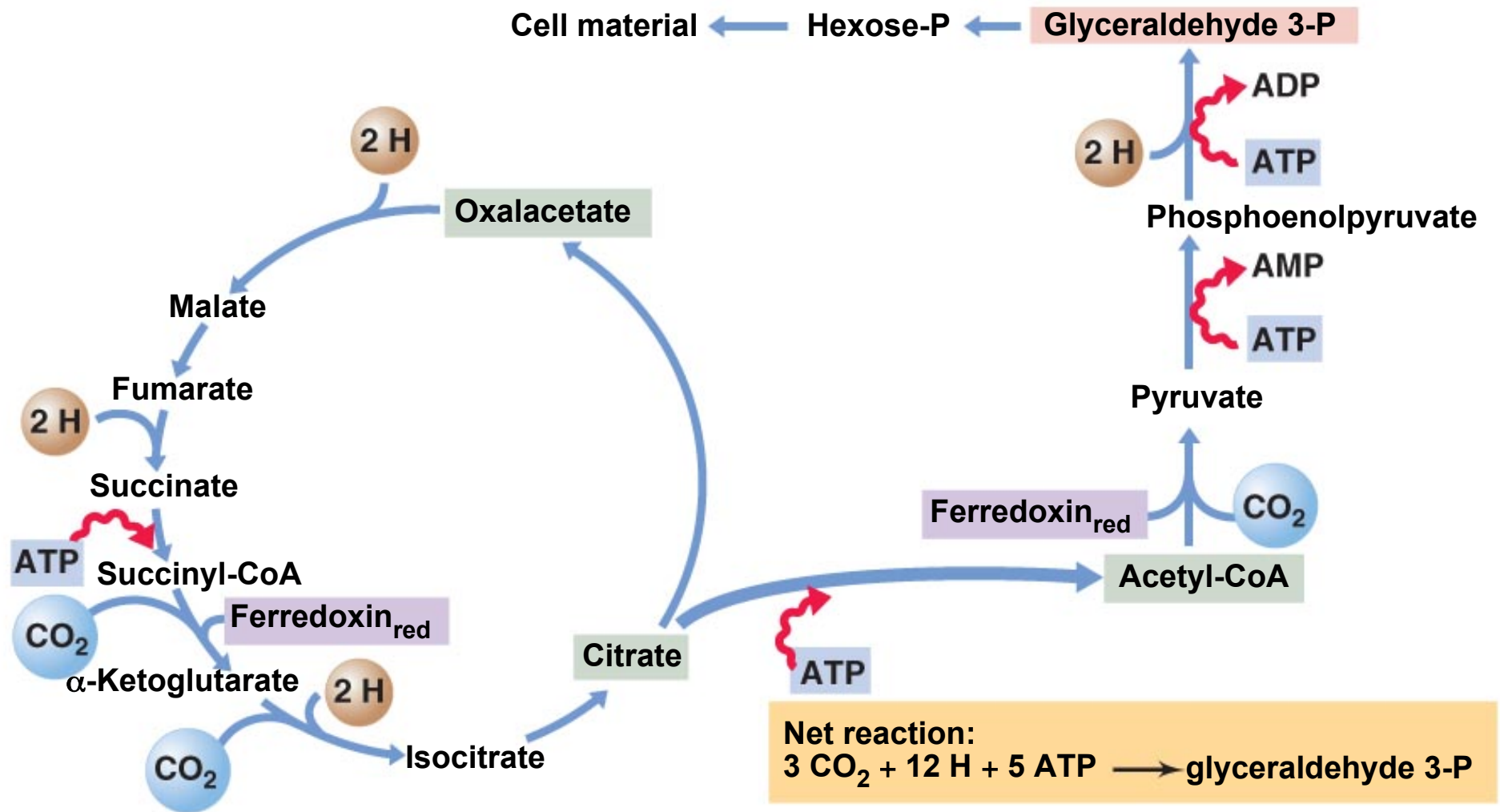
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Reverse TCA

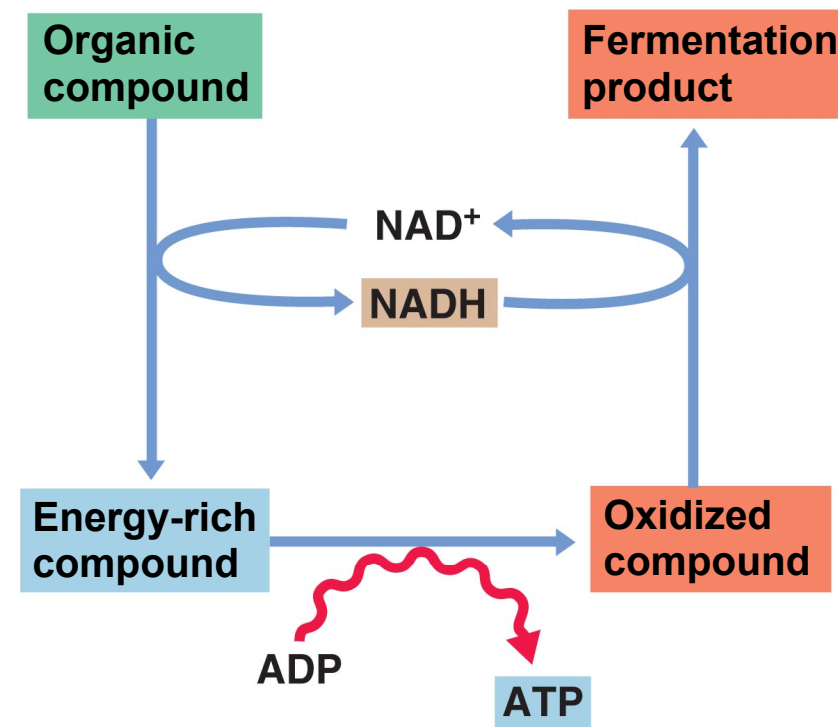
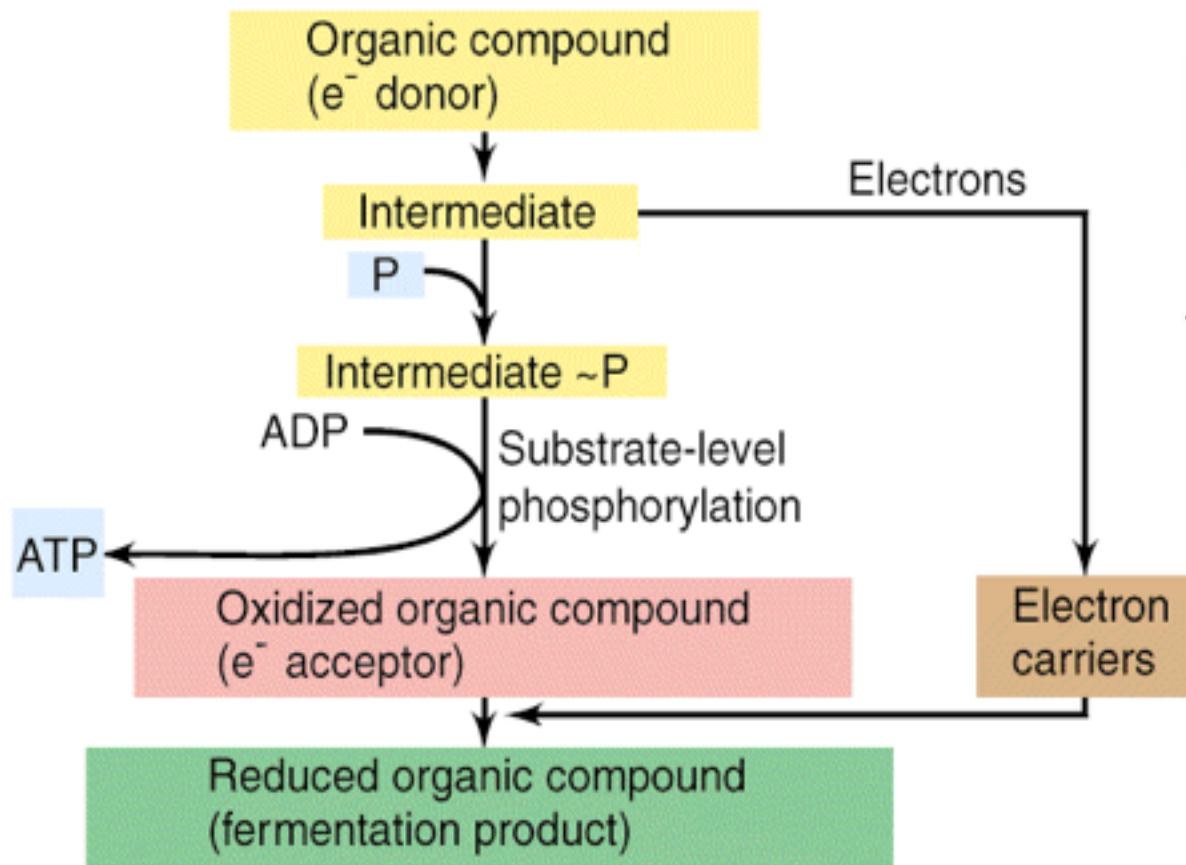


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Reverse TCA

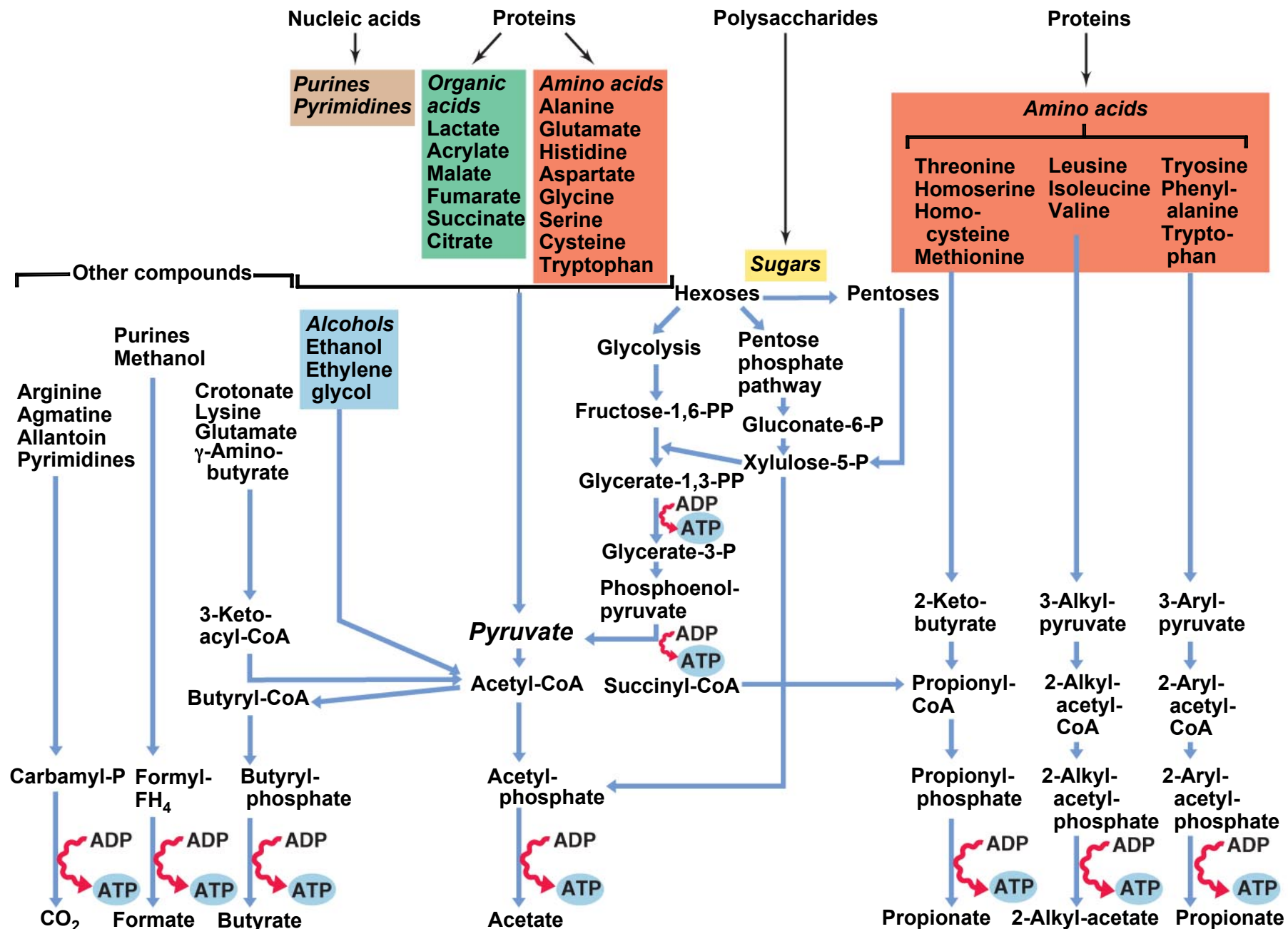


Fermentation and substrate level phosphorylation



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Fermentations

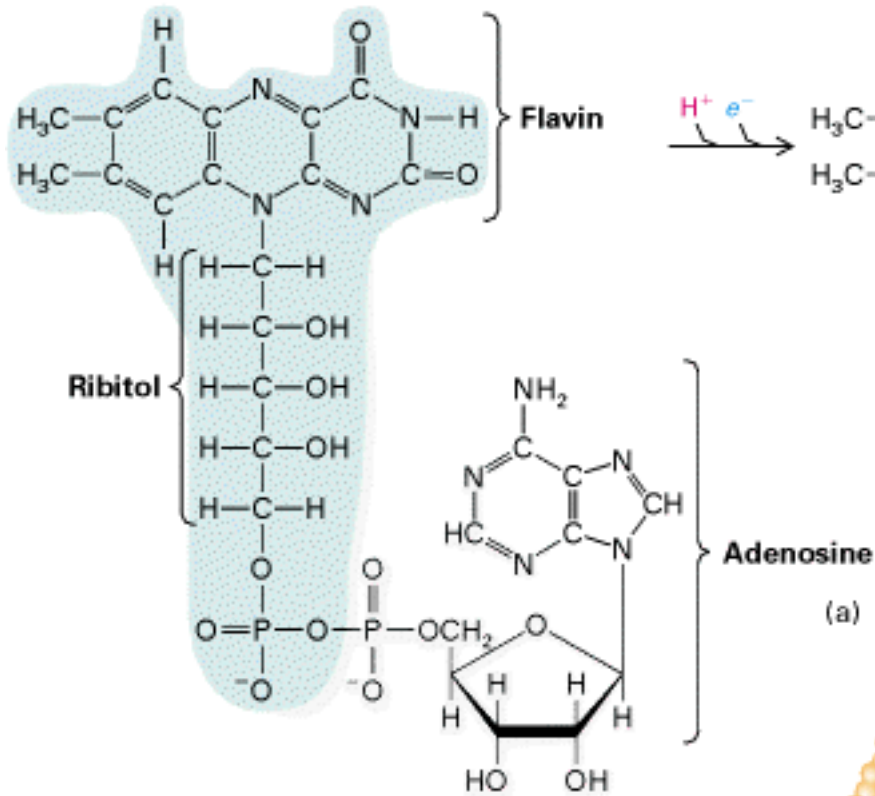


Electron Transport in Prokaryotes

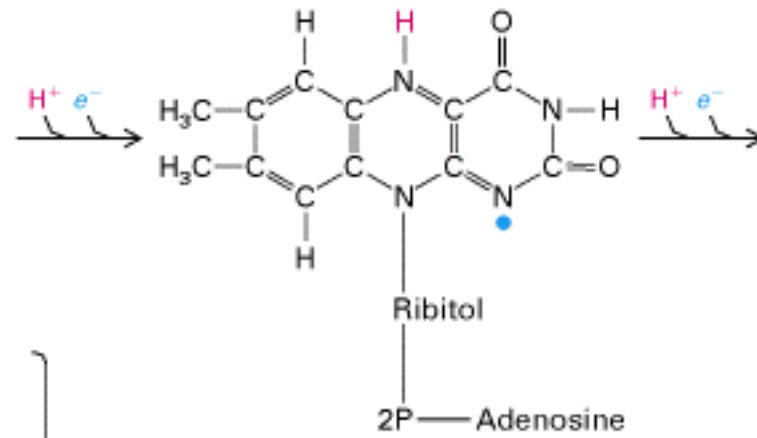
Electron Transporters

Flavoproteins

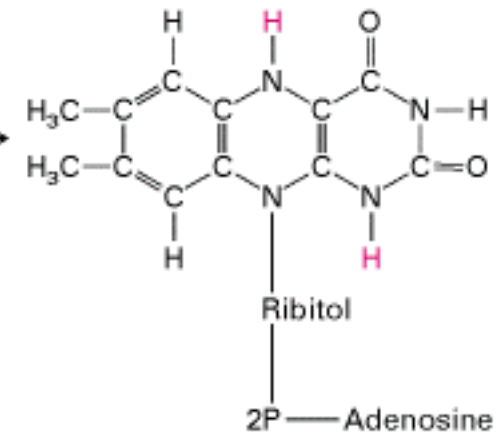
Oxidized: FAD



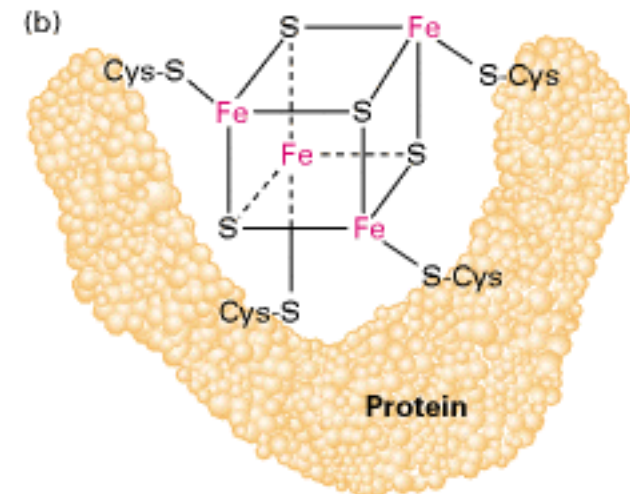
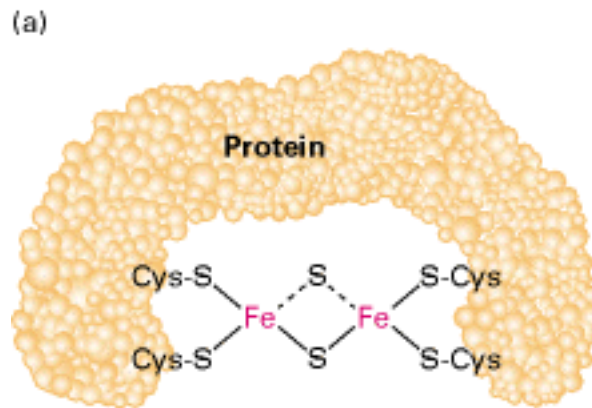
Semiquinone



Reduced: FADH₂



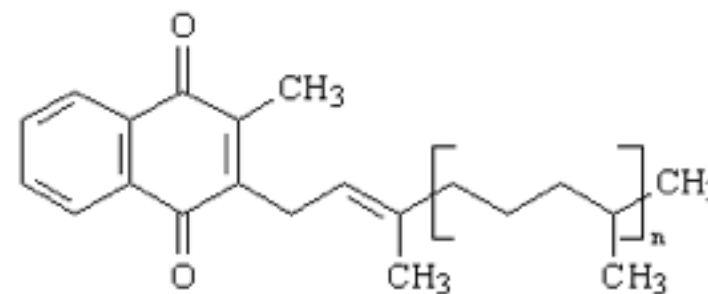
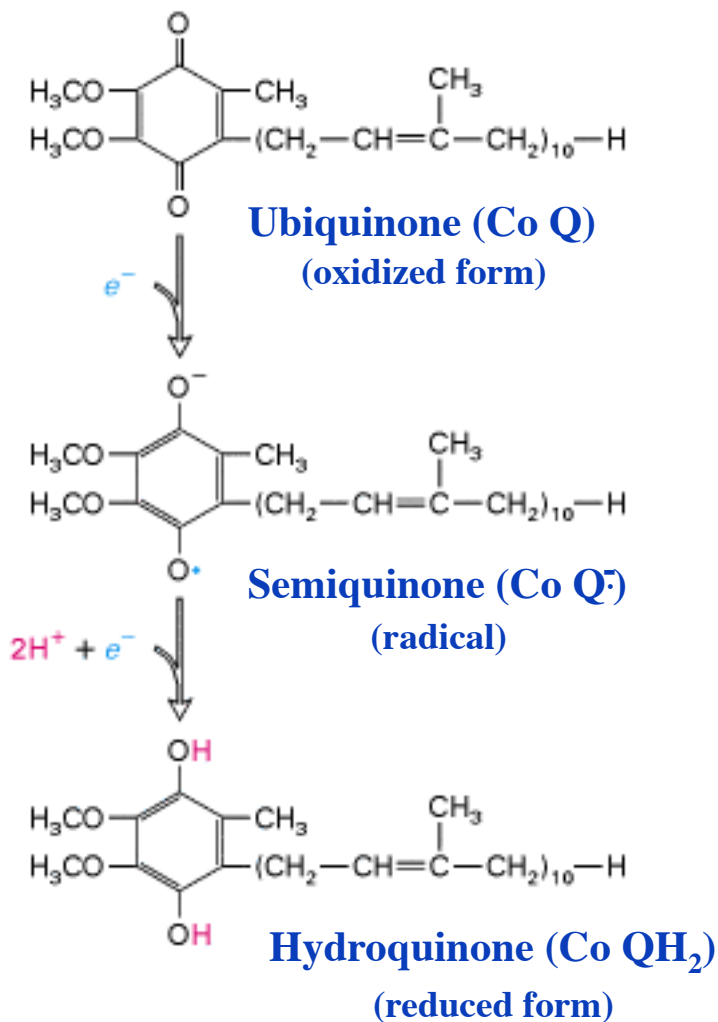
Fe-S Proteins



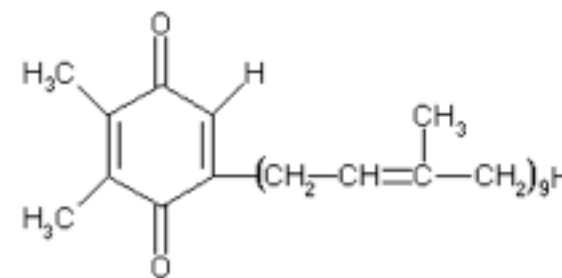
Electron Transport in Prokaryotes

Electron Transporters

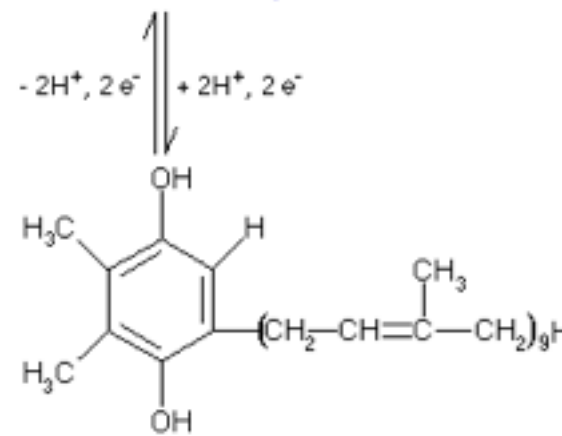
Quinones



Menaquinone (vitamine K1)



Plastoquinone A

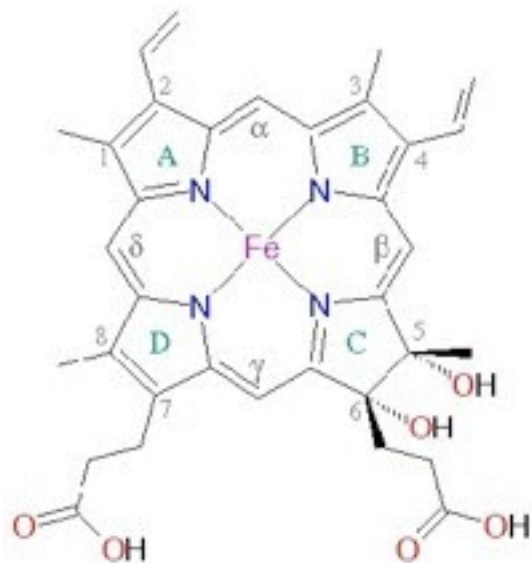


Plastohydroquinone A

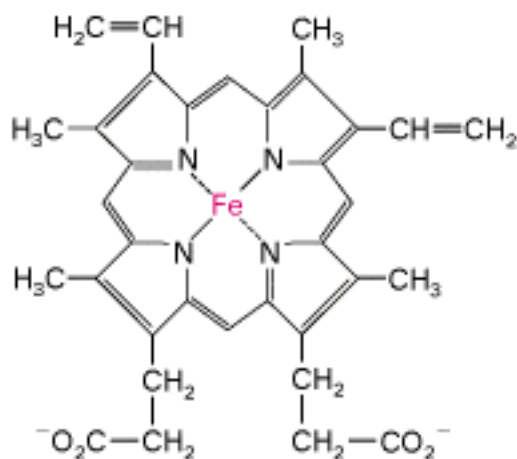
Electron Transport in Prokaryotes

Electron Transporters

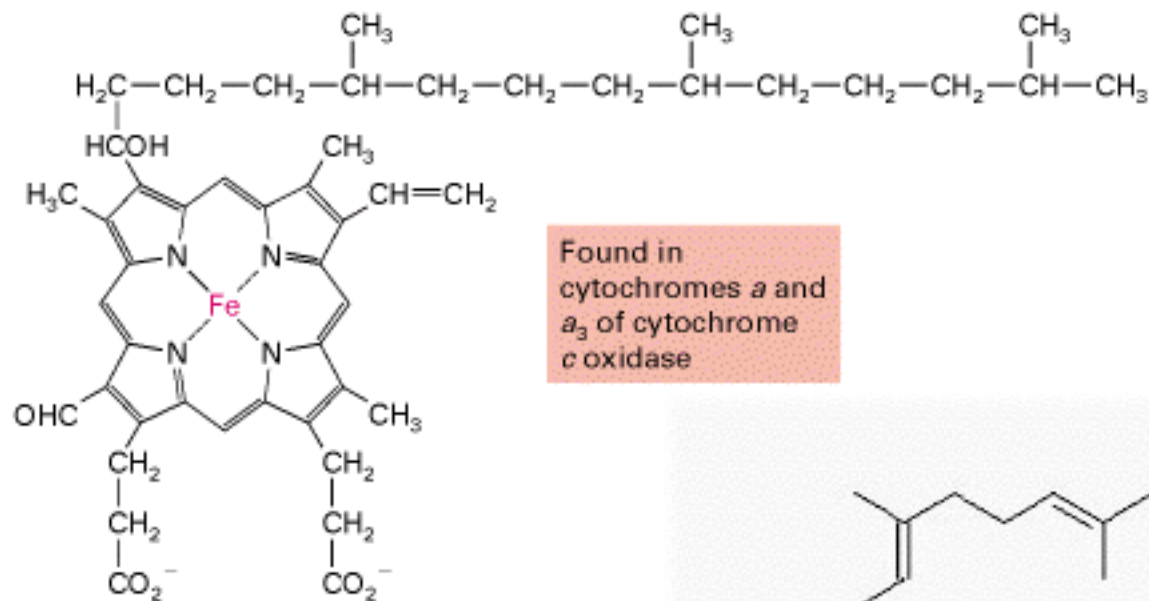
Cytochromes



d-type heme

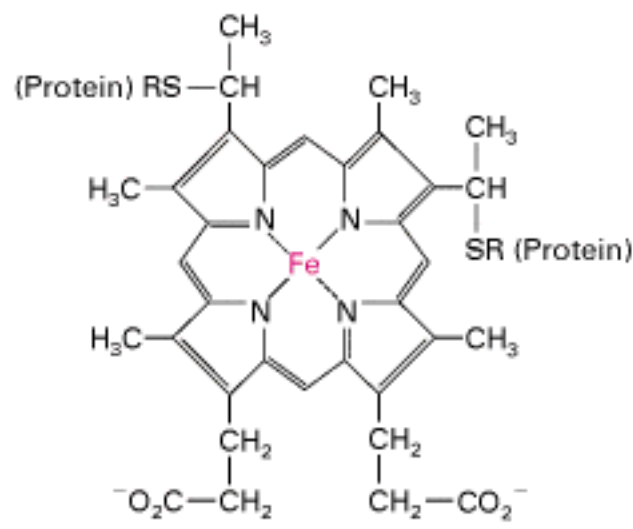


b-type heme

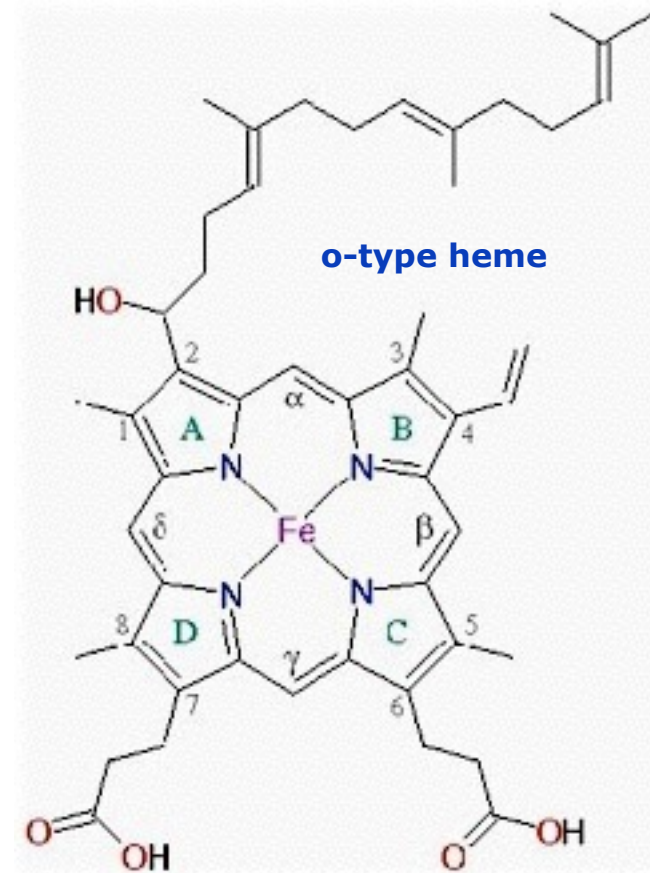


a-type heme

Found in cytochromes *a* and *a*₃ of cytochrome *c* oxidase



c-type heme

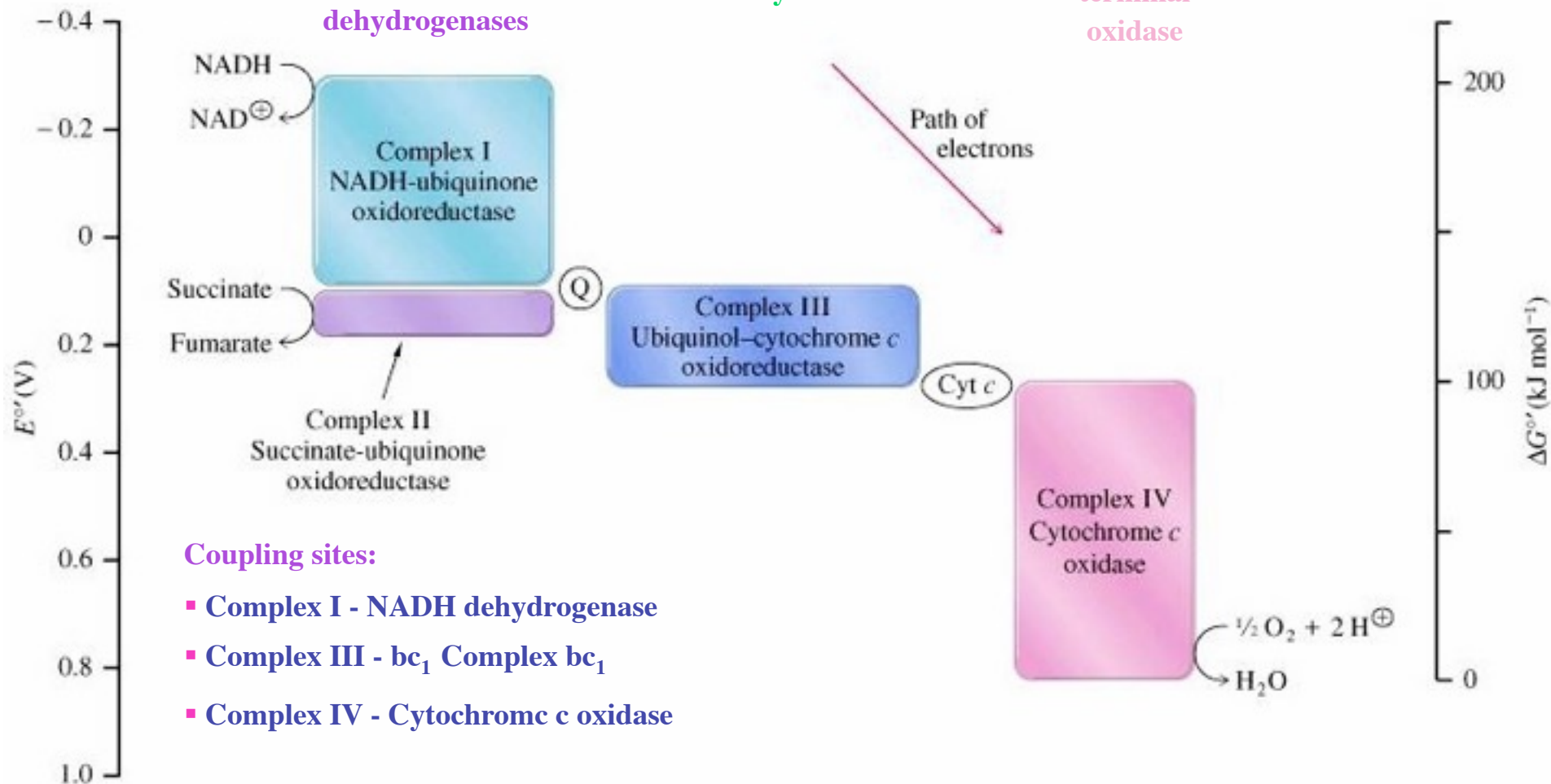
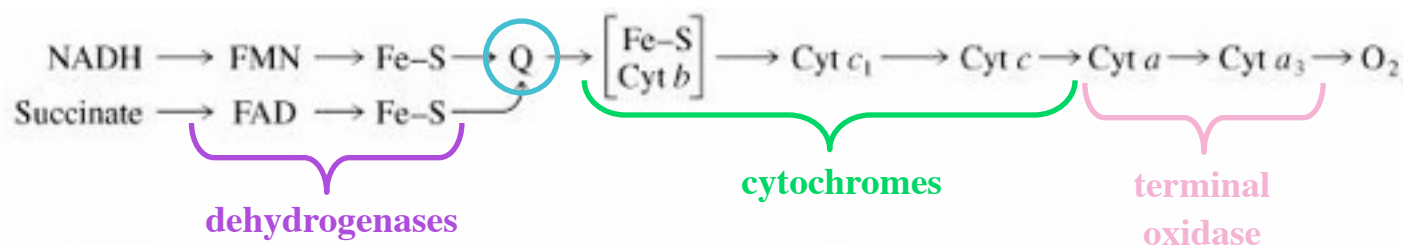


o-type heme

Redox Half-Reaction	Standard Reduction Potentials ($E^{0'}$) (V)
$2 \text{H}^+ + 2 e^- \rightarrow \text{H}_2$	-0.42
$\alpha\text{-Ketoglutarate} + \text{CO}_2 + 2 \text{H}^+ + 2 e^- \rightarrow \text{isocitrate}$	-0.38
$\text{NAD}^+ + \text{H}^+ + 2 e^- \rightarrow \text{NADH}$	-0.32
$\text{S} + 2 \text{H}^+ + 2 e^- \rightarrow \text{H}_2\text{S}$	-0.23
$\text{FAD} + 2 \text{H}^+ + 2 e^- \rightarrow \text{FADH}_2$	-0.22
$\text{Acetaldehyde} + 2 \text{H}^+ + 2 e^- \rightarrow \text{ethanol}$	-0.20
$\text{Pyruvate} + 2 \text{H}^+ + 2 e^- \rightarrow \text{lactate}$	-0.19
$\text{Oxaloacetate} + 2 \text{H}^+ + 2 e^- \rightarrow \text{malate}$	-0.166
$\text{Cu}^+ \rightarrow \text{Cu}^{2+} + e^-$	-0.16
$\text{Fumarate} + 2 \text{H}^+ + 2 e^- \rightarrow \text{succinate}$	-0.031
$\text{Cytochrome b (Fe}^{3+}) + e^- \rightarrow \text{cytochrome b (Fe}^{2+})$	+0.075
$\text{Cytochrome c}_1 (\text{Fe}^{3+}) + e^- \rightarrow \text{cytochrome c}_1 (\text{Fe}^{2+})$	+0.22
$\text{Cytochrome c (Fe}^{3+}) + e^- \rightarrow \text{cytochrome c (Fe}^{2+})$	+0.235
$\text{Cytochrome a (Fe}^{3+}) + e^- \rightarrow \text{cytochrome a (Fe}^{2+})$	+0.29
$\text{NO}_3^- + 2 \text{H}^+ + 2 e^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	+0.42
$\text{NO}_2^- + 8 \text{H}^+ + 6 e^- \rightarrow \text{NH}_4^+ + 2 \text{H}_2\text{O}$	+0.44
$\text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+}$	+0.77
$\frac{1}{2}\text{O}_2 + 2 \text{H}^+ + 2 e^- \rightarrow \text{H}_2\text{O}$	+0.82

Electron Transport Organization

Mitochondrial standard



Coupling sites:

- Complex I - NADH dehydrogenase
- Complex III - bc₁ Complex bc₁
- Complex IV - Cytochromc c oxidase

✿ Electron Transport Organization

➔ Energy transduction parameters

- ✓ **$H^+ / 2e^-$ (H^+ / O)** : number of translocated protons / 2 electrons transferred

NADH \rightarrow O₂ $H^+ / 2e^- = 10$

Complex I and III $H^+ / 2e^- = 4$

Complex IV $H^+ / 2e^- = 2$

- ✓ **P/O ($P / 2e^-$)** : number of ATP formed / 2 electrons transferred

NADH \rightarrow O₂ P/O = 3 \Leftrightarrow 3 coupling sites

succinato \rightarrow O₂ P/O = 2 \Leftrightarrow 2 coupling sites

citocromo c \rightarrow O₂ P/O = 1 \Leftrightarrow 1 coupling site

- ✓ **H^+ / ATP** : number of protons that enter the cell via ATP sintase

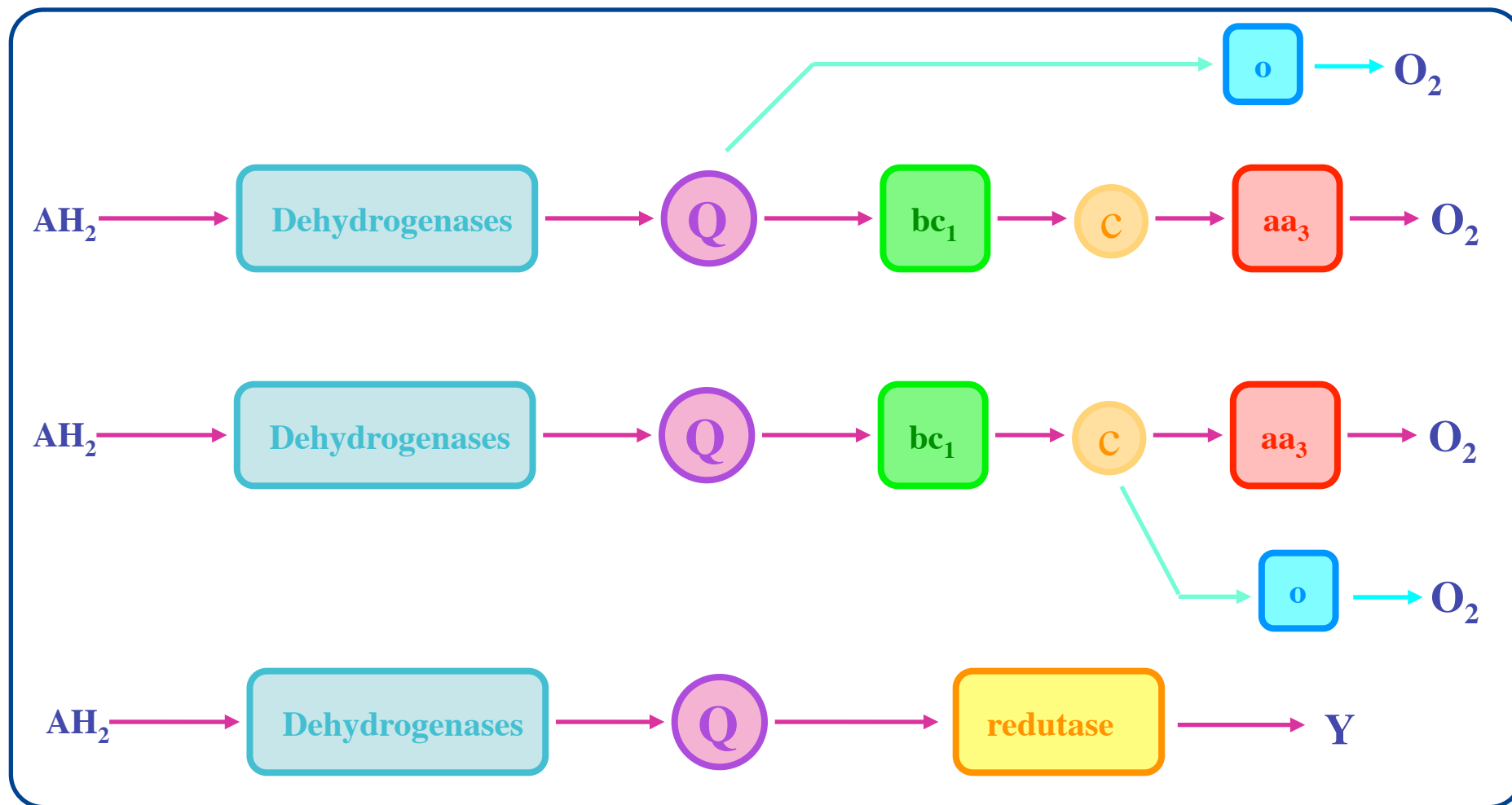
Mitochondria: $H^+ / ATP = 3 + 1$

Bacteria: $H^+ / ATP = 3$

- ✓ In the mitochondria if $H^+ / ATP = 4$ and $H^+ / 2e^- = 10 \Leftrightarrow$ maximum value of P/O = 2,5

Electron Transport Organization

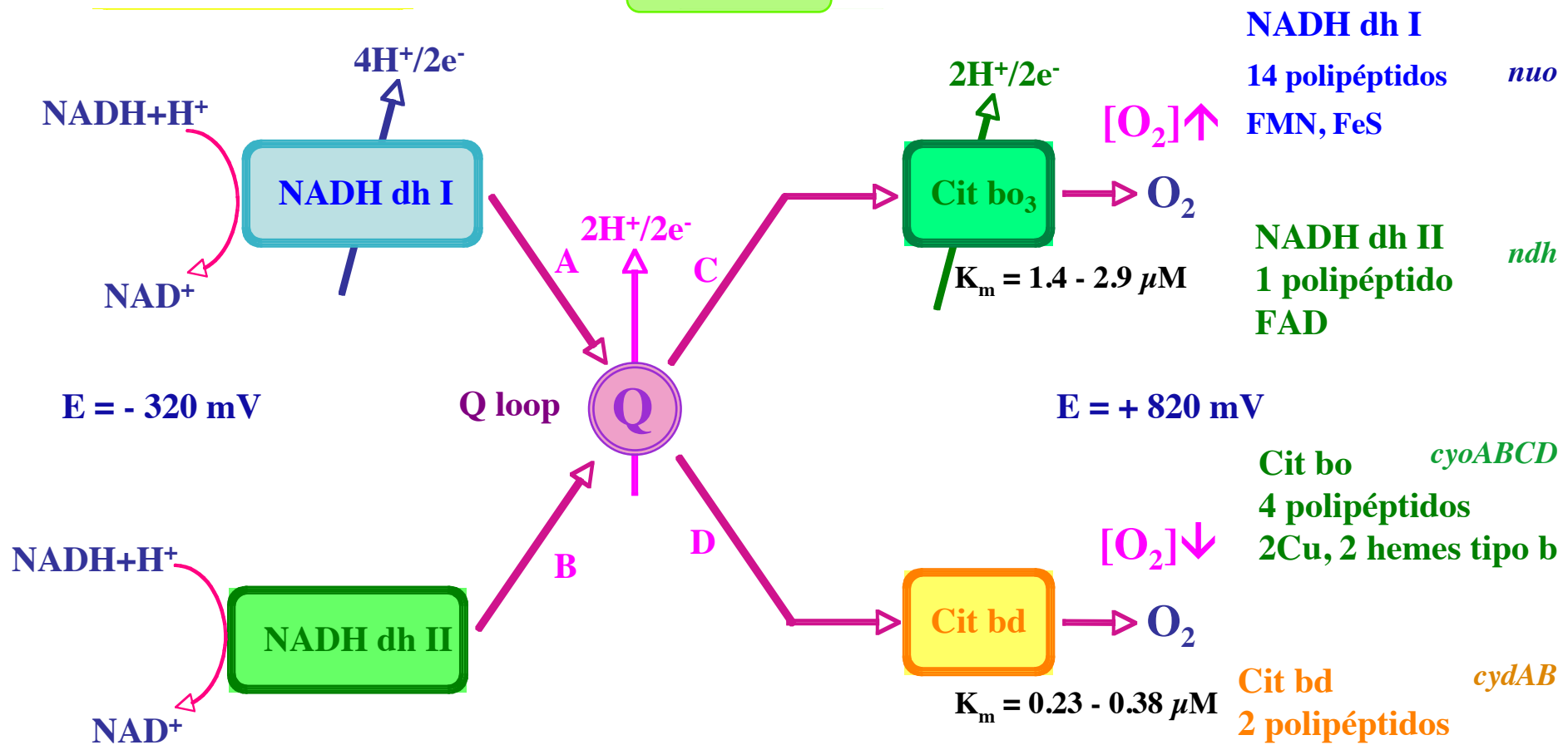
Bacterial standard



Electronic flow patterns in bacteria

Escherichia coli

Aerobiose

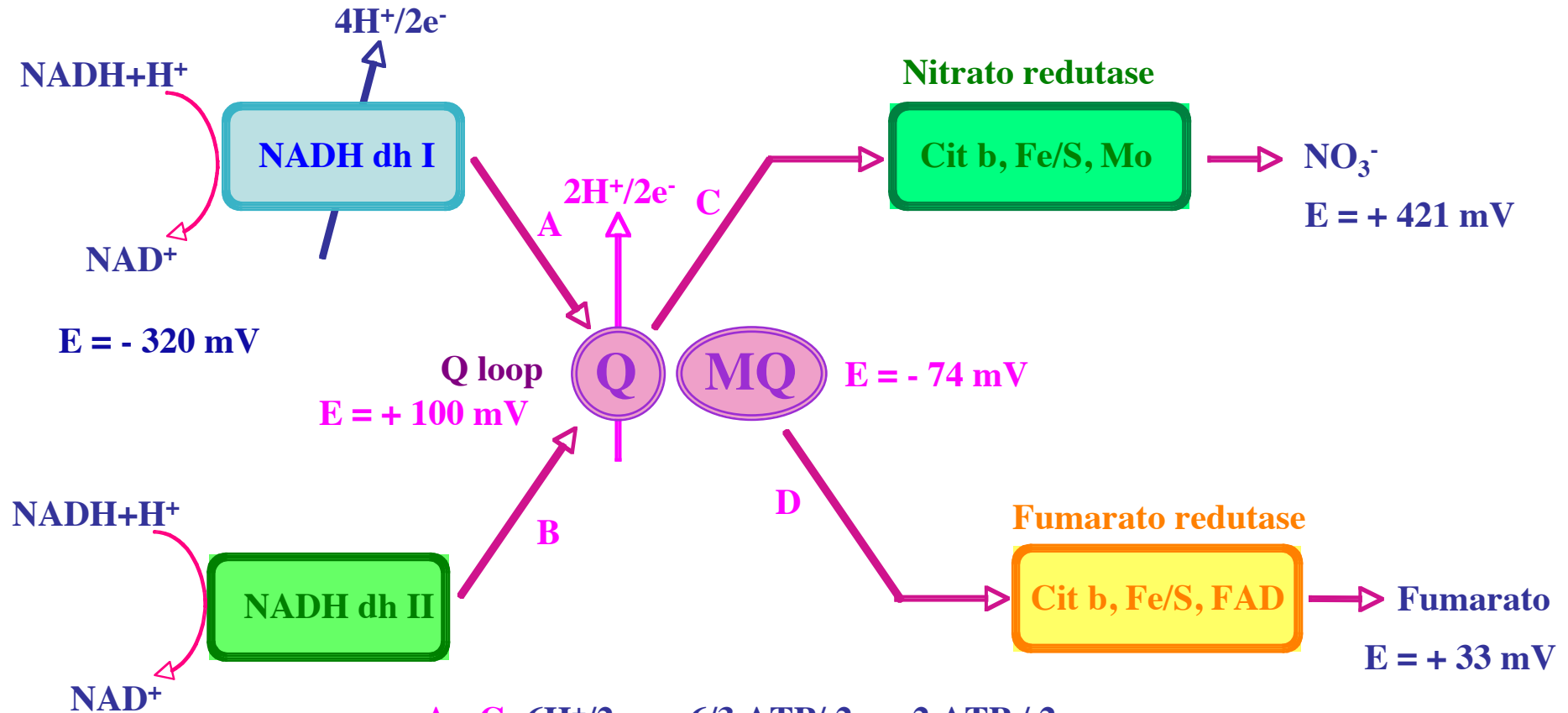


- A - C: $8\text{H}^+/2\text{e}^- \Rightarrow 8/3 \text{ ATP} / 2\text{e}^- \approx 2,7 \text{ ATP} / 2\text{e}^-$
- A - D: $6\text{H}^+/2\text{e}^- \Rightarrow 6/3 \text{ ATP} / 2\text{e}^- \approx 2 \text{ ATP} / 2\text{e}^-$
- B - C: $4\text{H}^+/2\text{e}^- \Rightarrow 4/3 \text{ ATP} / 2\text{e}^- \approx 1,3 \text{ ATP} / 2\text{e}^-$
- B - D: $2\text{H}^+/2\text{e}^- \Rightarrow 2/3 \text{ ATP} / 2\text{e}^- \approx 0,67 \text{ ATP} / 2\text{e}^-$

Electronic flow patterns in bacteria

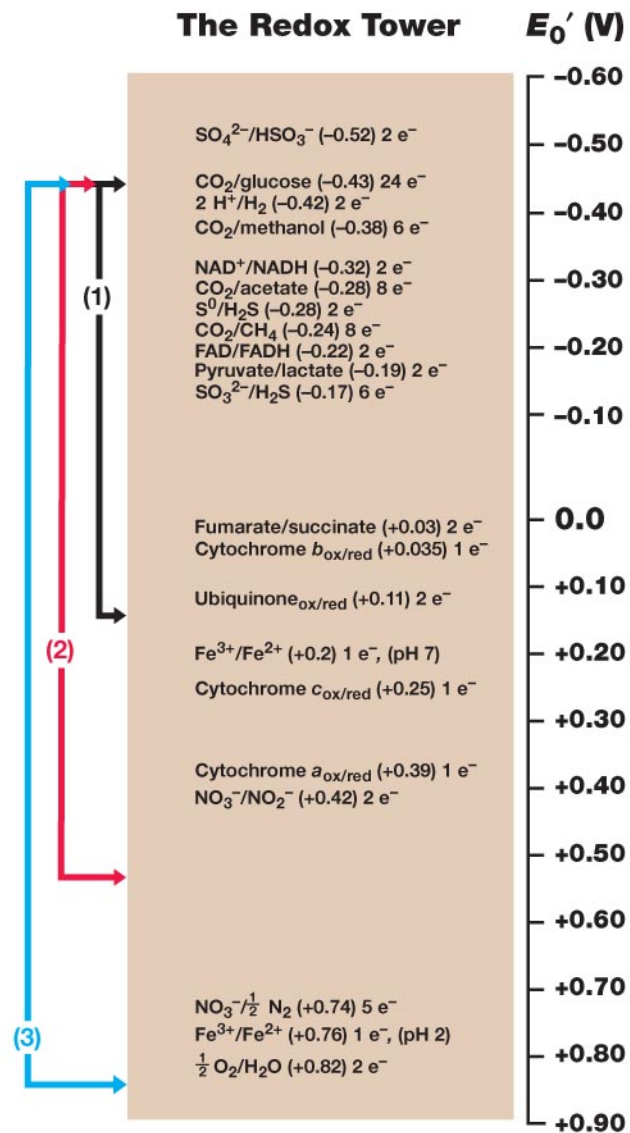
Escherichia coli

Anaerobiose



- A - C: $6\text{H}^+ / 2\text{e}^- \Rightarrow 6/3 \text{ ATP} / 2\text{e}^- \approx 2 \text{ ATP} / 2\text{e}^-$
- A - D: $6\text{H}^+ / 2\text{e}^- \Rightarrow 6/3 \text{ ATP} / 2\text{e}^- \approx 2 \text{ ATP} / 2\text{e}^-$
- B - C: $2\text{H}^+ / 2\text{e}^- \Rightarrow 2/3 \text{ ATP} / 2\text{e}^- \approx 0,67 \text{ ATP} / 2\text{e}^-$
- B - D: $2\text{H}^+ / 2\text{e}^- \Rightarrow 2/3 \text{ ATP} / 2\text{e}^- \approx 0,67 \text{ ATP} / 2\text{e}^-$

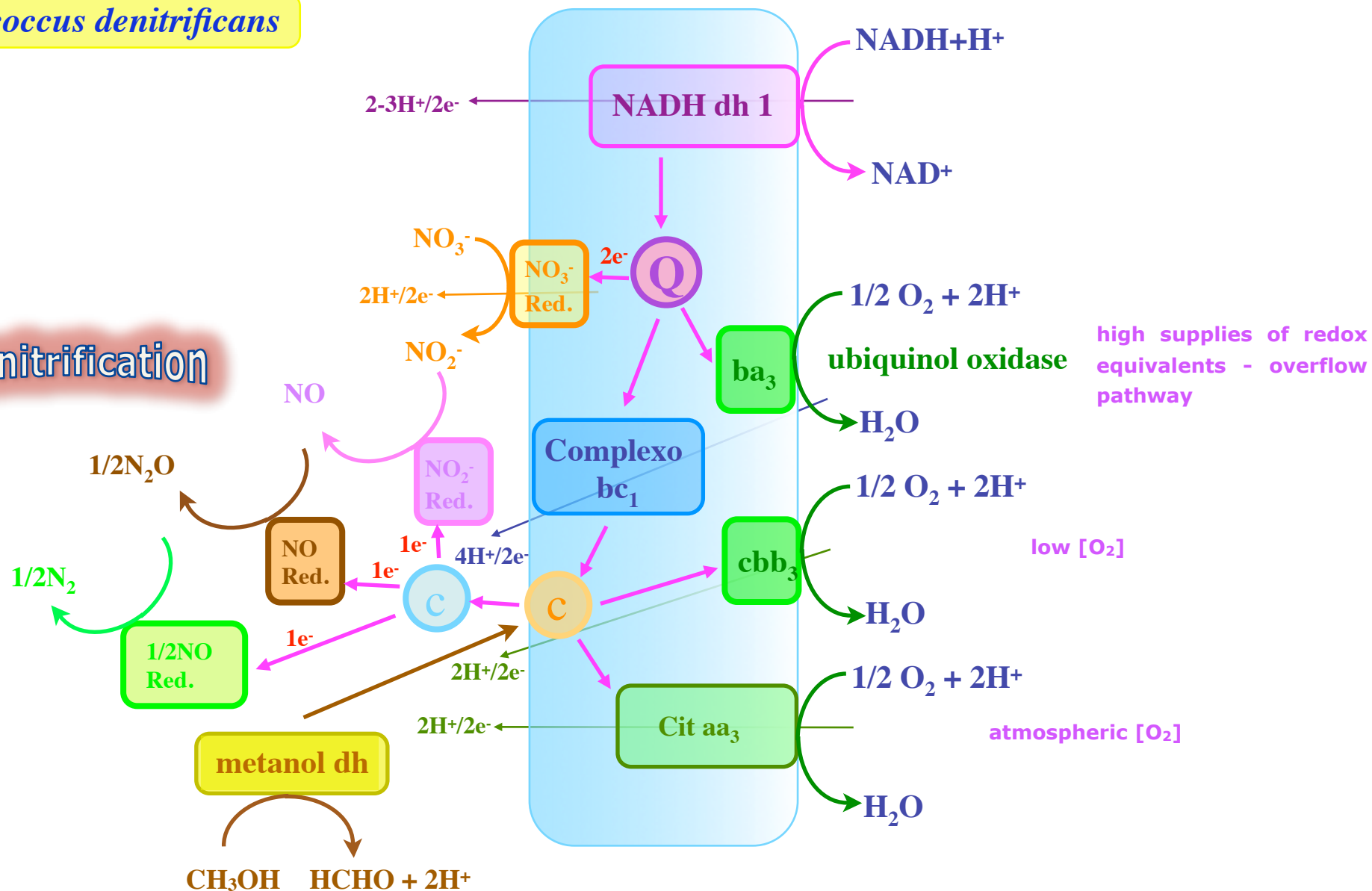
Electronic flow patterns in bacteria

 *Escherichia coli*


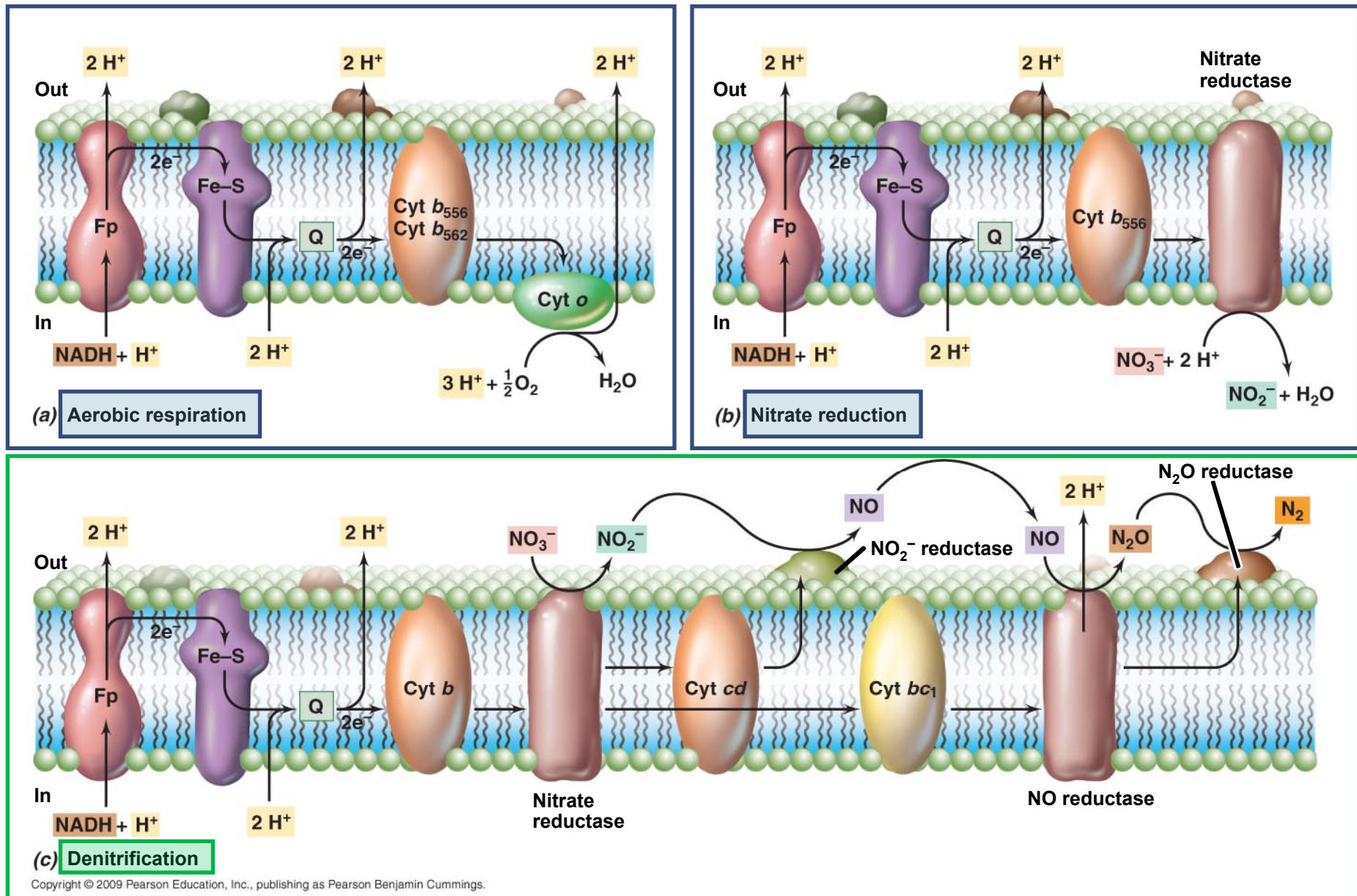
Electronic flow patterns in bacteria

Paracoccus denitrificans

Denitrification



Escherichia coli



Paracoccus denitrificans