Microbes as Energy Transducers

- The Metabolic Menu
- Metabolic Strategies
- Respiration & Fermentation
- Chemolithotrophy
- Photoautotrophy
- Biogeochemical Cycles
- Metabolism in Primitive Organisms

All major types of nutrition and metabolism evolved among prokaryotes: they are the ultimate biochemists

The prokaryotes exhibit some unique modes of nutrition as well as **every type** of nutrition found in eukaryotes.

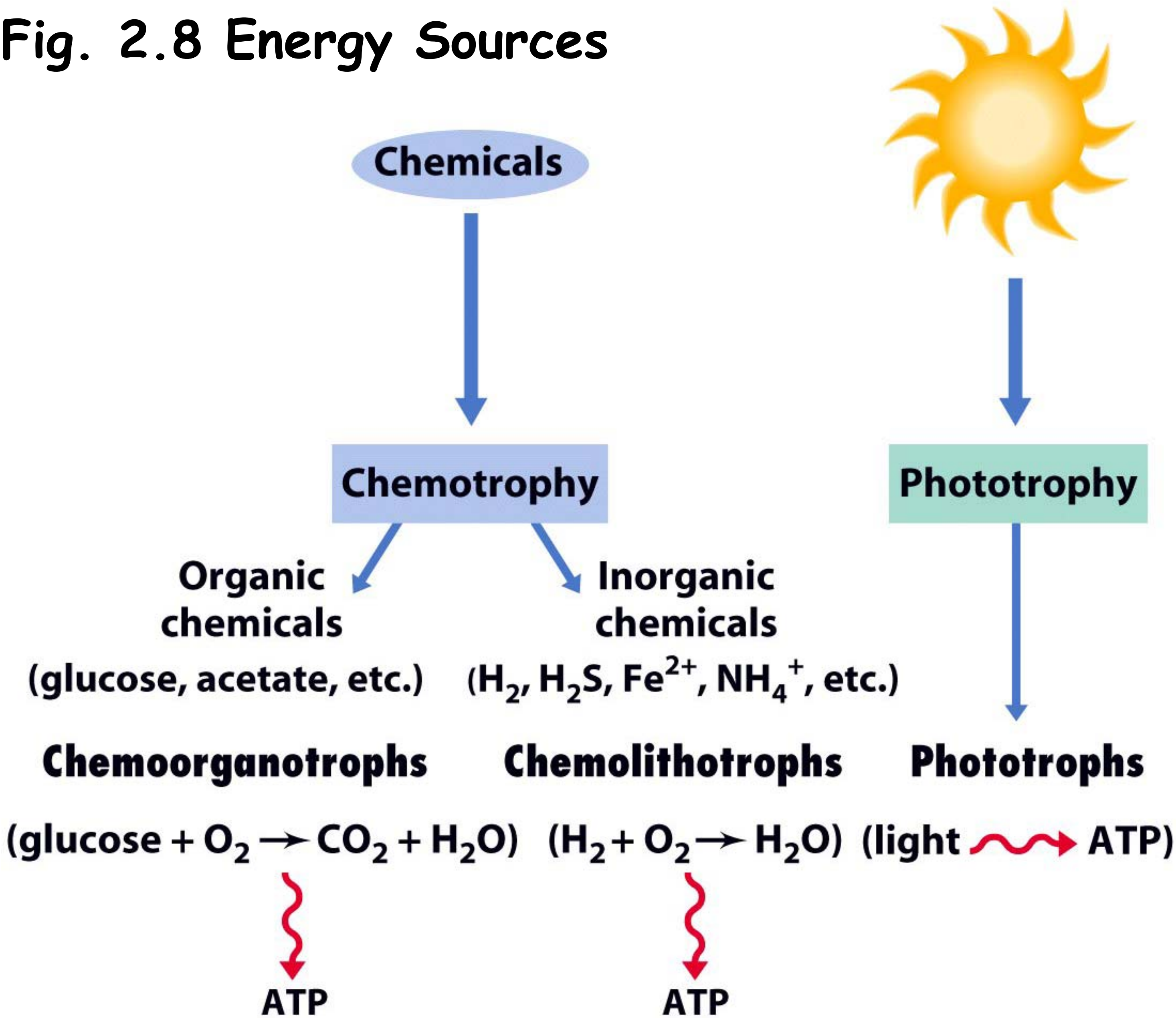
Major Modes of Nutrition:

Prokaryotes exhibit a great diversity in how they obtain the necessary resources **(energy and carbon)** to synthesize organic compounds.

- Some obtain energy from light **(phototrophs)**, while others use chemicals taken from the environment **(chemotrophs)**.

- Many can utilized CO_2 as a carbon source **(autotrophs)** and others require at least one organic nutrient as a carbon source **(heterotrophs)**.

Fig. 2.8 Energy Sources



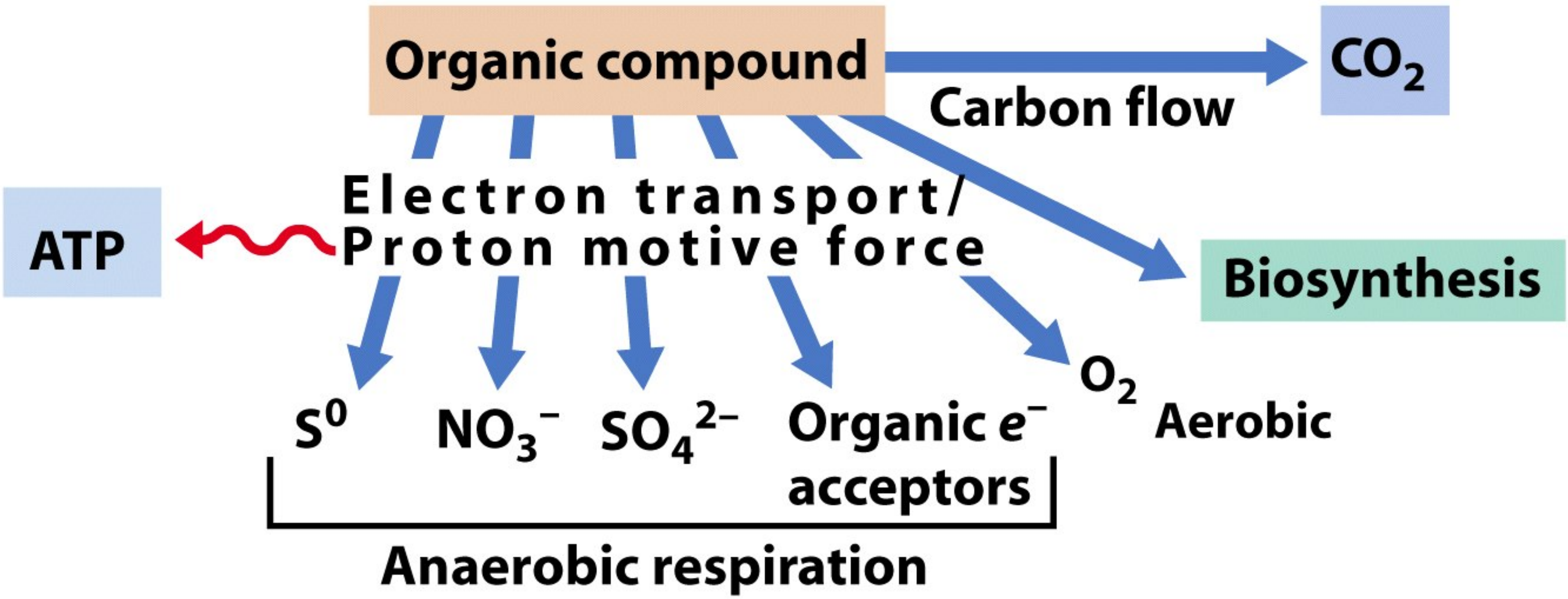
Depending upon the <u>energy source</u> **AND** the <u>carbon source</u>, prokaryotes have **four** possible nutritional modes:

1. **<u>Photoautotrophs</u>:** Use light energy to synthesize organic compounds from CO_2 – Includes the cyanobacteria. (Actually all photosynthetic eukaryotes fit in this category.)

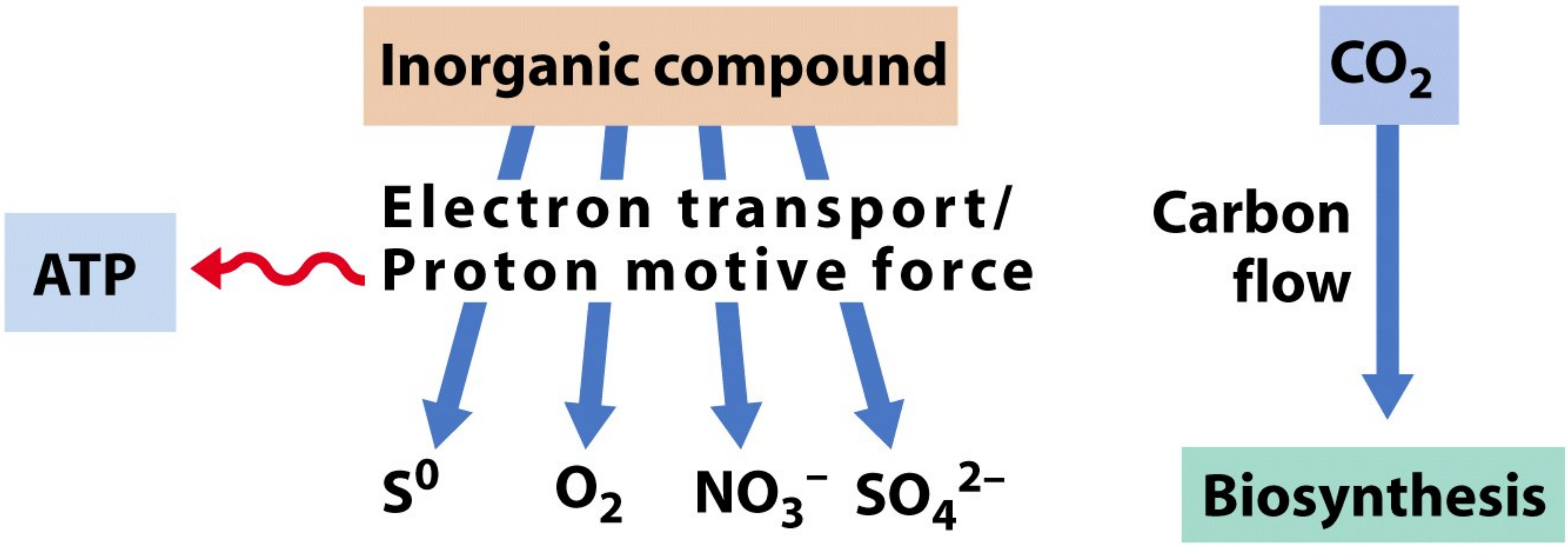
2. **<u>Chemoautotrophs</u>:** Require only CO_2 as a carbon source and obtain energy by oxidizing inorganic compounds. This mode of nutrition is unique only to certain prokaryotes.

3. **<u>Photoheterotrophs</u>:** Use light to generate ATP from an organic carbon source. This mode of nutrition is unique only to certain prokaryotes.

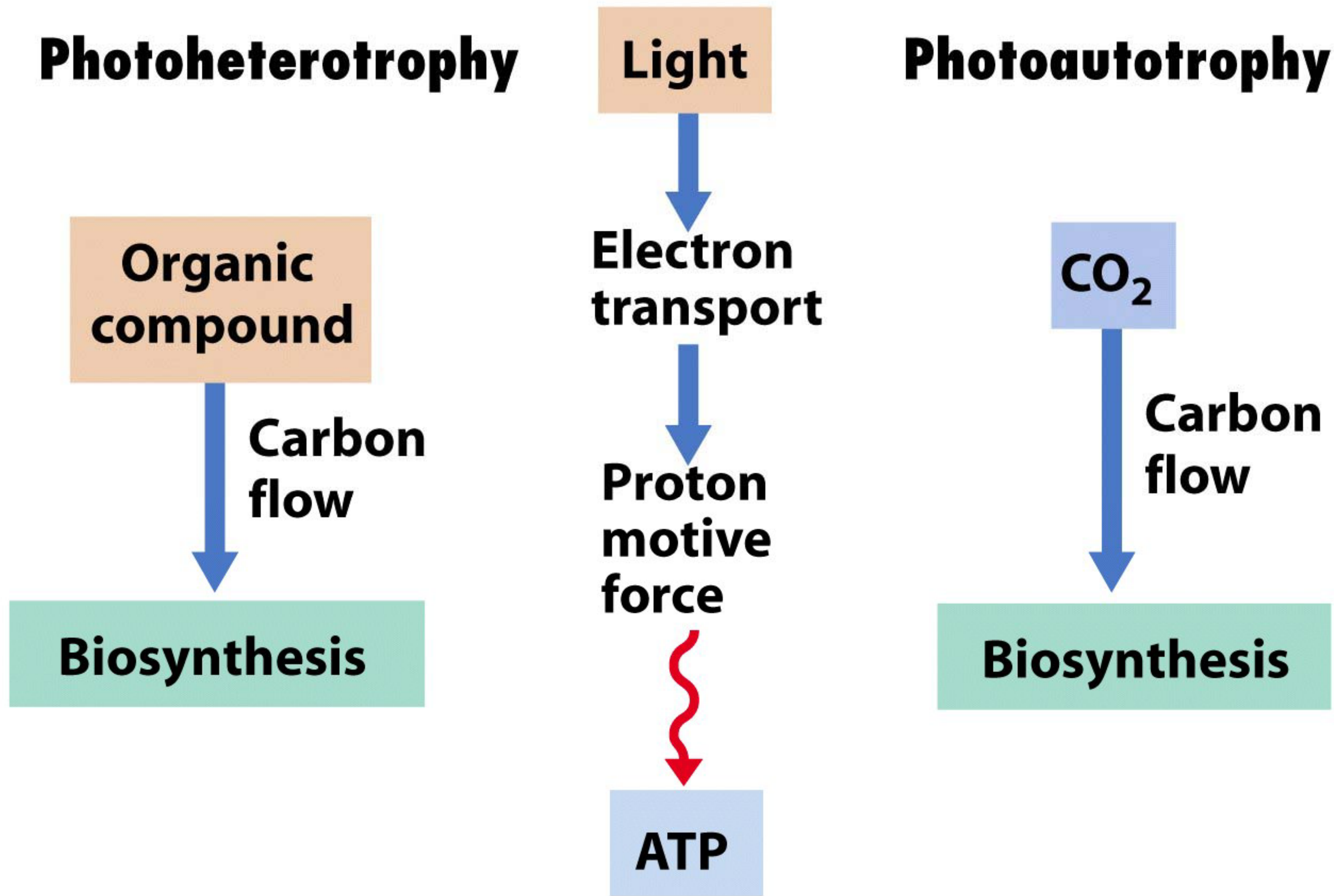
4. **<u>Chemoheterotrophs</u>:** Must obtain organic molecules for energy and as a source of carbon. Found in many bacteria as well as most eukaryotes.



Chemoorganotrophic metabolism



Chemolithotrophic metabolism



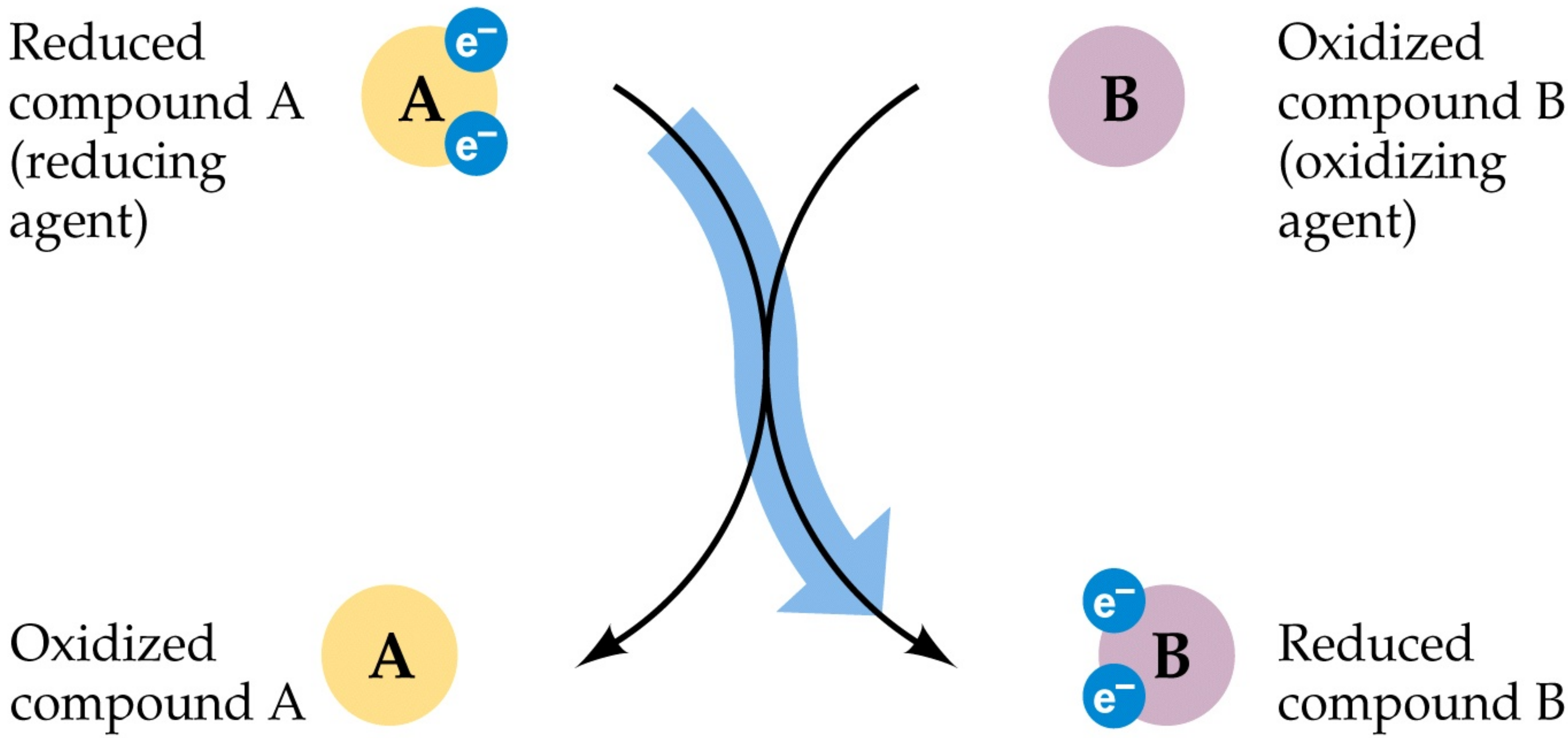
Phototrophic metabolism

Metabolic Menu For Chemotrophs

Potential Microbial Metabolic Processes:

	e- donor	e- acceptor	C source	Organisms
Autolithotrophy				
	H_2	O_2	CO_2	Hydrogen oxidizers
	$HS^-, S^0, S_2O_3^{-2}$	O_2	CO_2	Sulfur oxidizers
	Fe^{+2}	O_2	CO_2	Iron oxidizers
	Mn^{+2}	O_2	CO_2	Manganese oxidizers
	NH_4^+, NO_2^-	O_2	CO_2	Nitrifiers
	$HS^-, S^0, S_2O_3^{-2}$	NO_3^-	CO_2	Denitrifying/S-oxidizers
	H_2	NO_{3-}	CO_2	Hydrogen oxidizers
	H_2	S^0, SO_4^{-2}	CO_2	Sulfate Reducers (SRBs)
	H_2	CO_2	CO_2	Methanogens & Acetogens
Heteroorganotrophy				
	Org.C	O_2	Org.C	Aerobic Heterotrophy
	Org.C	NO_3^-	Org.C	Denitrifyers
	Org.C	S^0, SO_4^{-2}	Org.C	Sulfate Reducers (SRBs)
	Org.C	Org.C	Org.C	Fermenters
Methylotrophy				
	CH_4,(C-1's)	O_2, SO_4^{-2}	CH_4, CO_2,CO	Methane (C-1) oxidizers

Oxidation and Reduction are Coupled Reactions



A is oxidized, losing electrons

B is reduced, gaining electrons

Redox Rxns:

$$H_2 \rightarrow 2\,e^- + 2\,H^+$$

Electron-donating half reaction

$$\tfrac{1}{2}O_2 + 2\,e^- \rightarrow O^{2-}$$

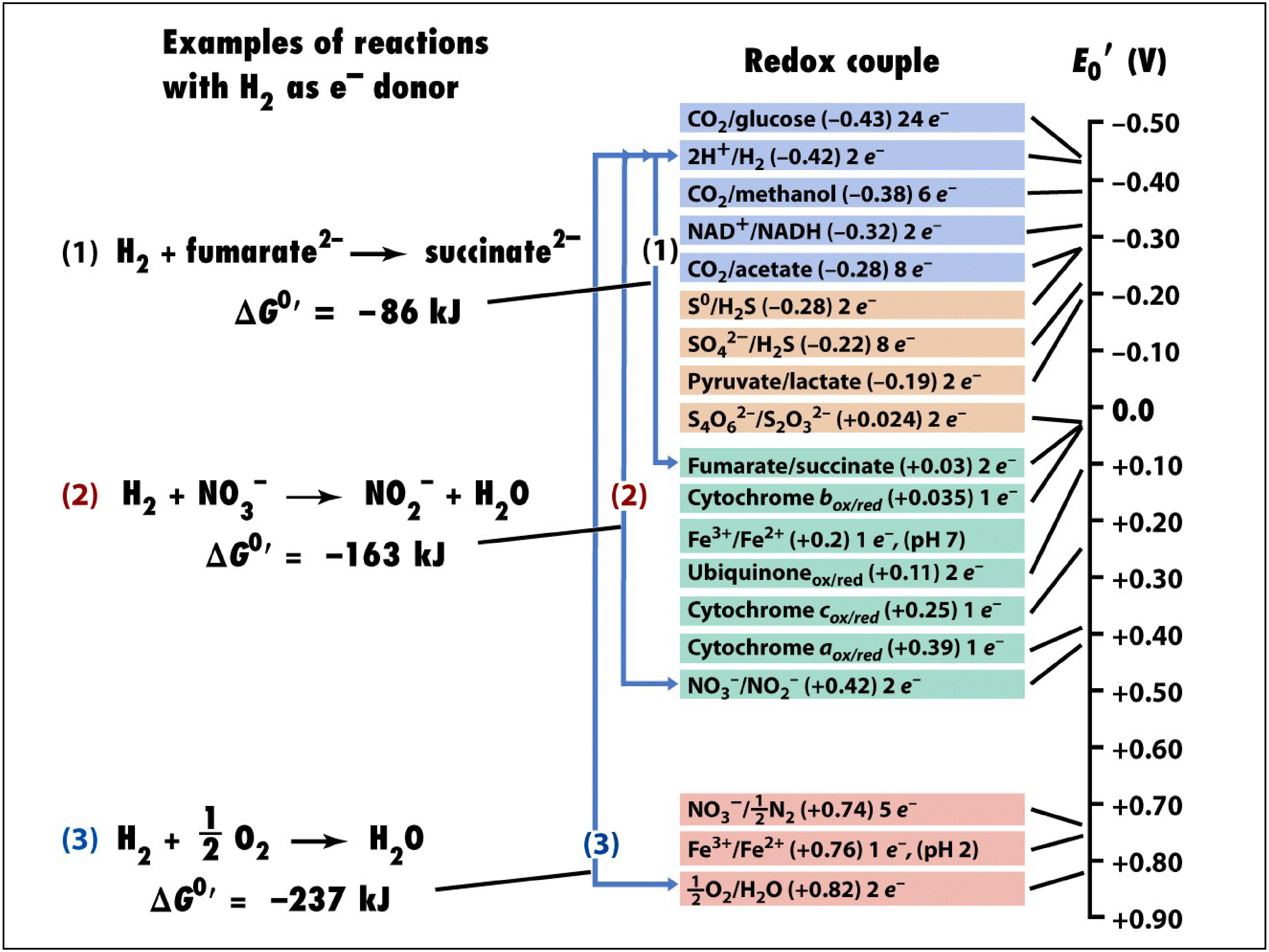
Electron-accepting half reaction

$$2\,H^+ + O^{2-} \rightarrow H_2O$$

Formation of water

Electron donor — $H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O$ — Electron acceptor

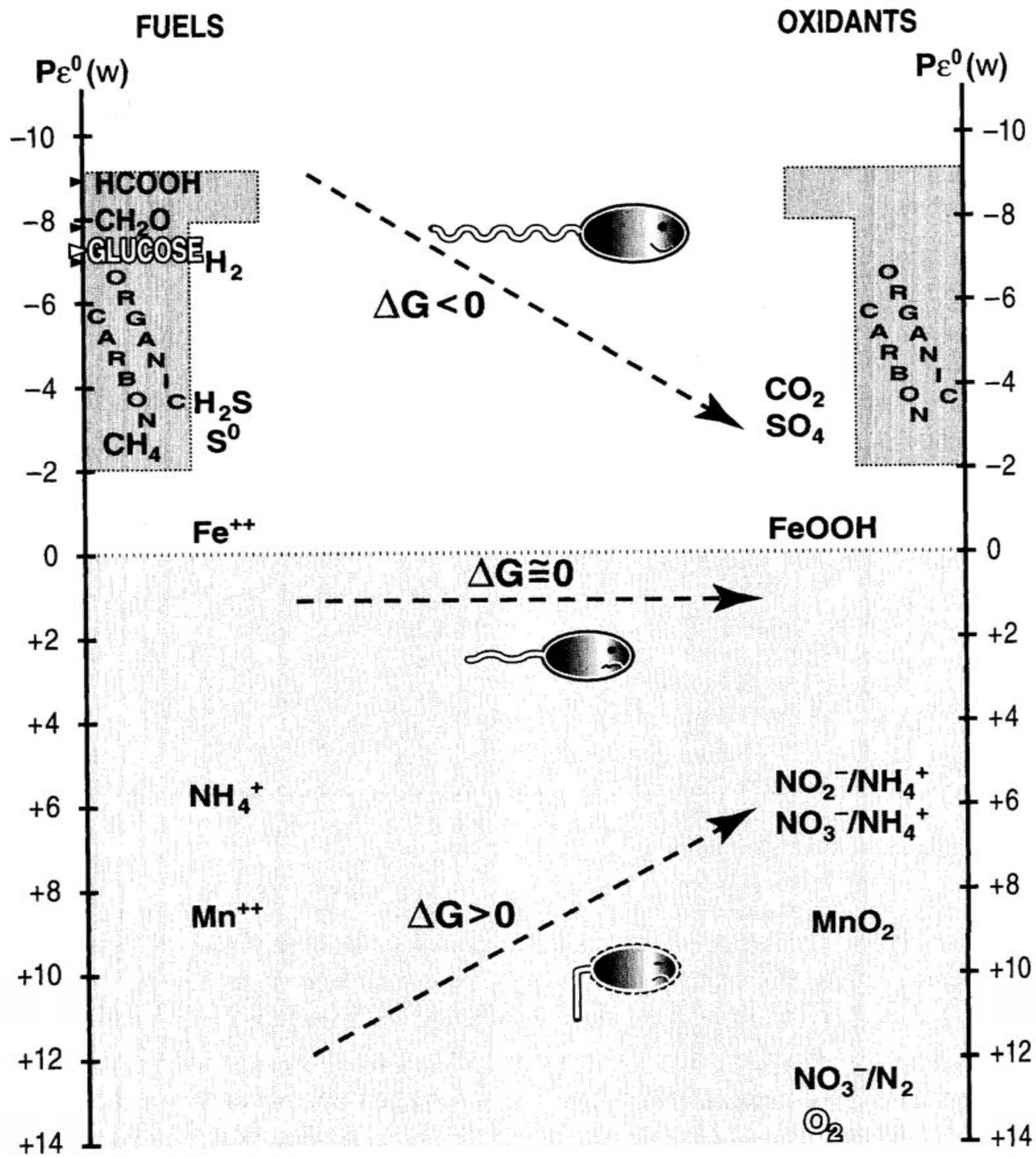
Net reaction



Examples of reactions with H_2 as e^- donor
(1) H_2 + fumarate^{2-} → succinate^{2-}
$\Delta G^{0\prime}$ = −86 kJ
(2) $H_2 + NO_3^- \rightarrow NO_2^- + H_2O$
$\Delta G^{0\prime}$ = −163 kJ
(3) $H_2 + \frac{1}{2} O_2 \rightarrow H_2O$
$\Delta G^{0\prime}$ = −237 kJ
Redox couple
E_0' (V)
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^-
−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
(1)
(2)
(3)

Thermodynamics: The Chemical Fuels and Oxidants of Life

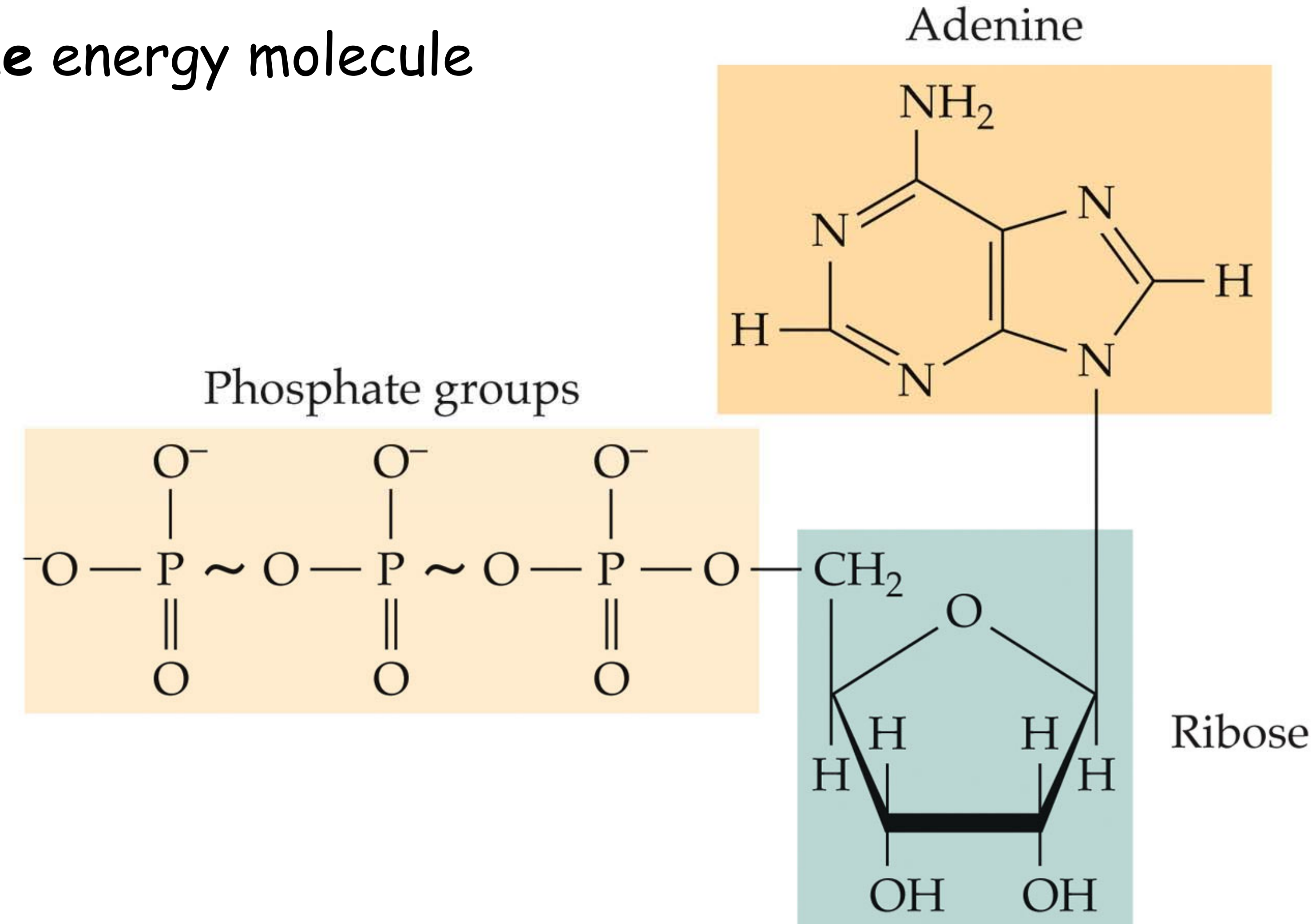
e- source



e- sink

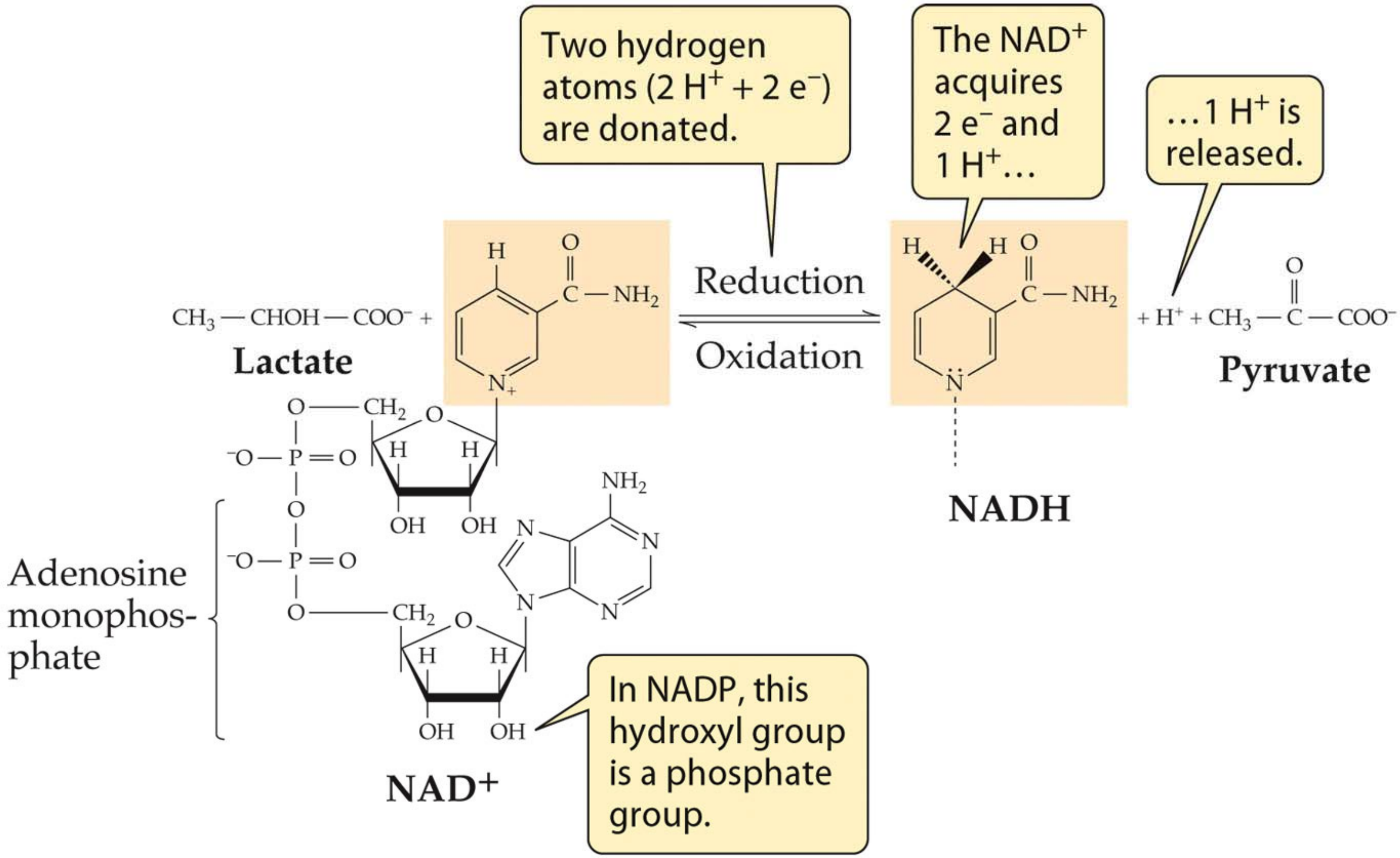
Adenosine-5′-triphosphate (ATP)

The energy molecule



Adenosine triphosphate

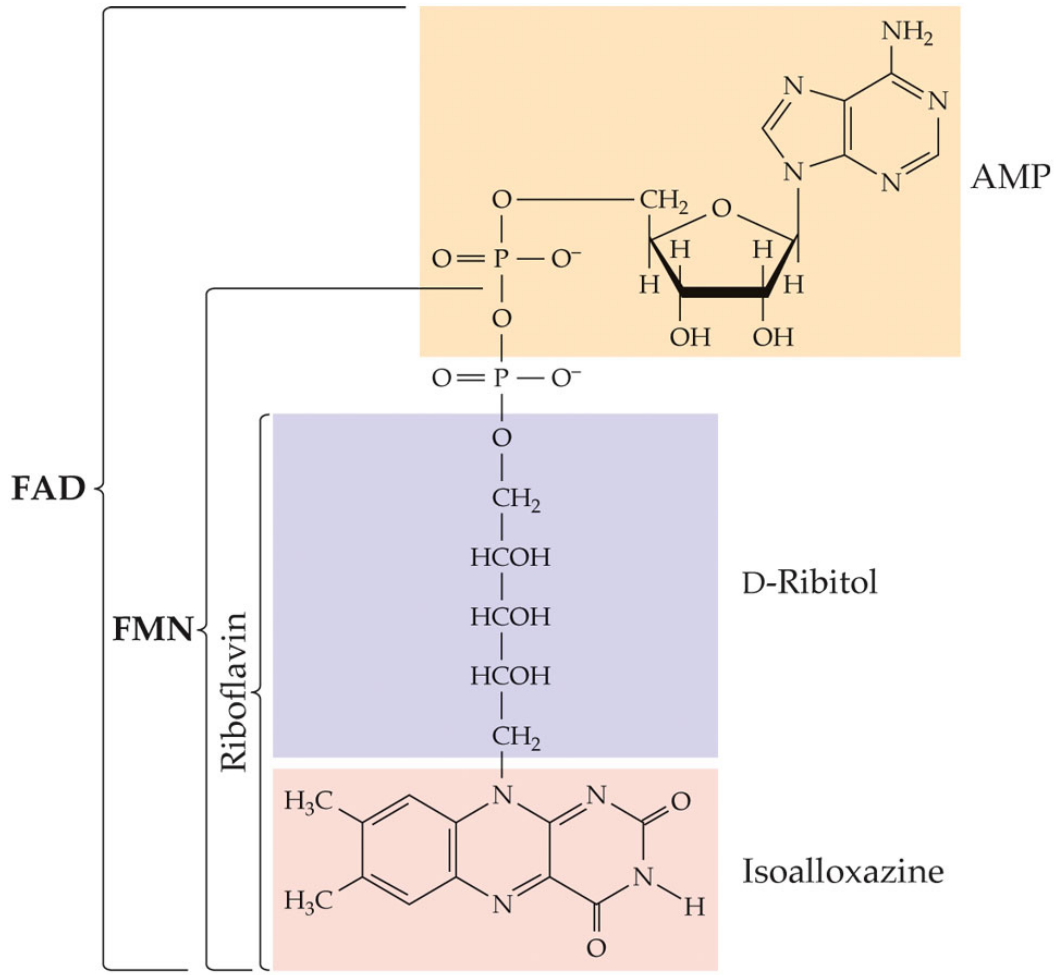
Nicotinamide adenine dinucleotide (NAD)



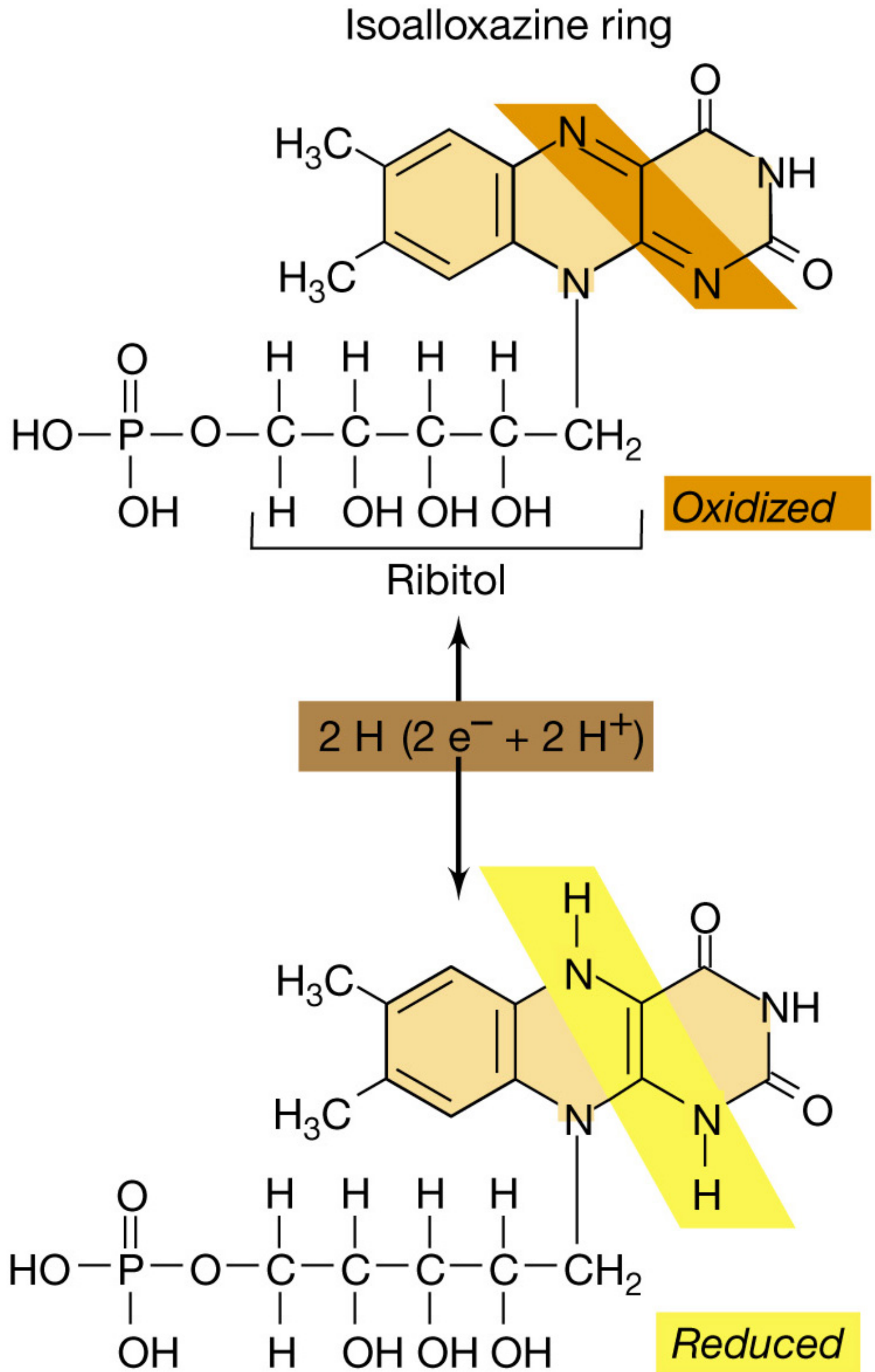
The redox molecule

Flavin nucleotides, components of flavoproteins

Specialty redox molecule

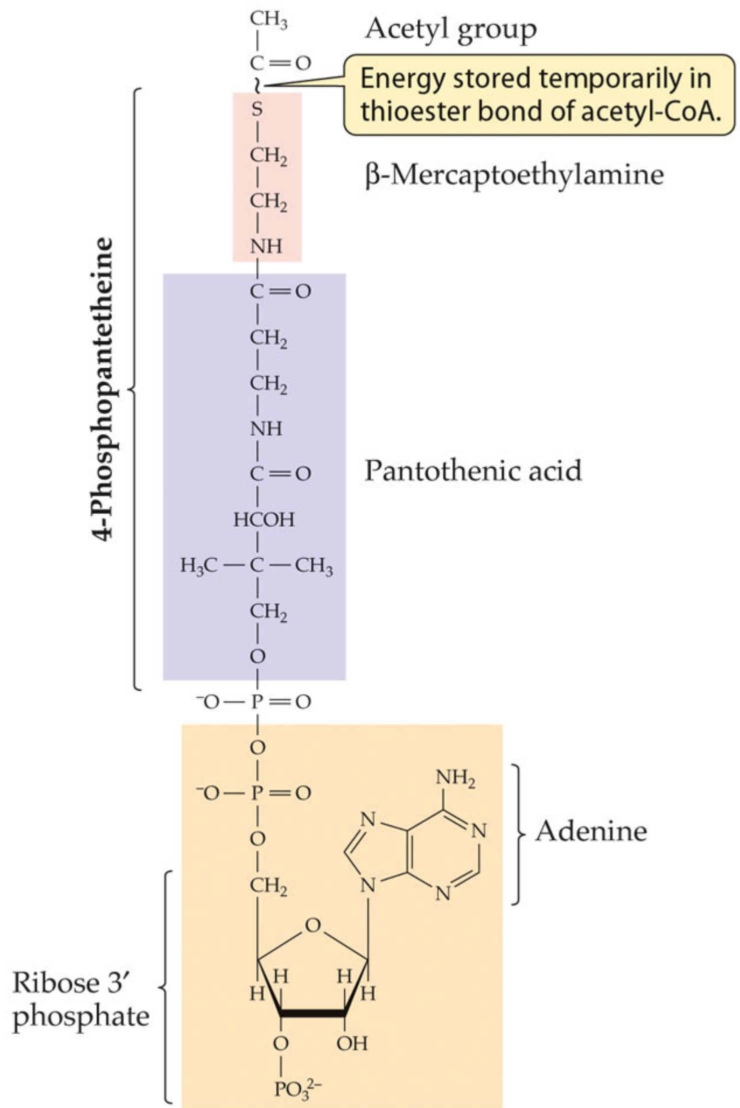


Flavin nucleotides

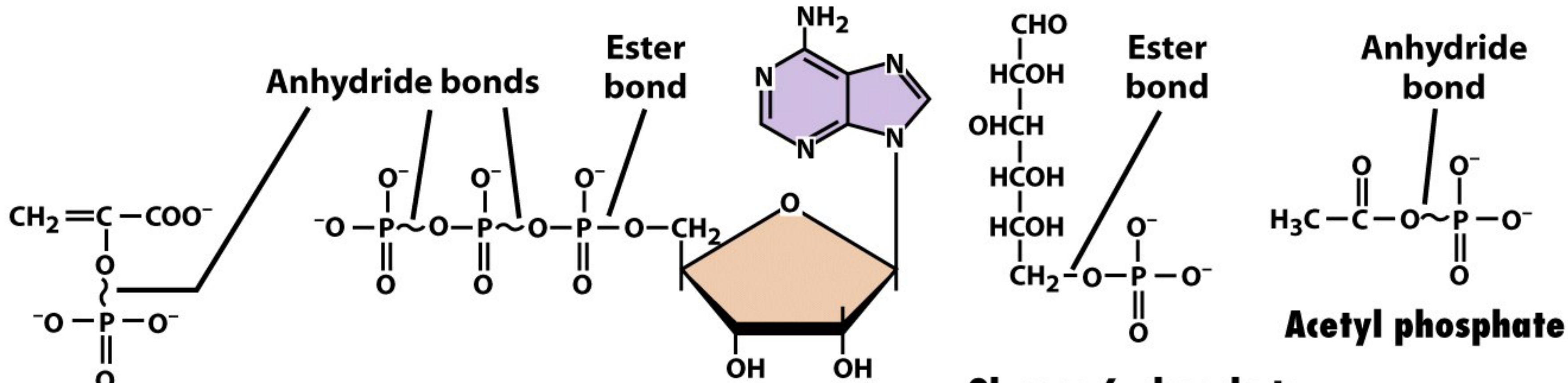

Isoalloxazine ring
Ribitol
Oxidized
2 H (2 e⁻ + 2 H⁺)
Reduced

Acetyl-coenzyme A (acetyl-CoA)

Specialty energy molecule



Acetyl-CoA

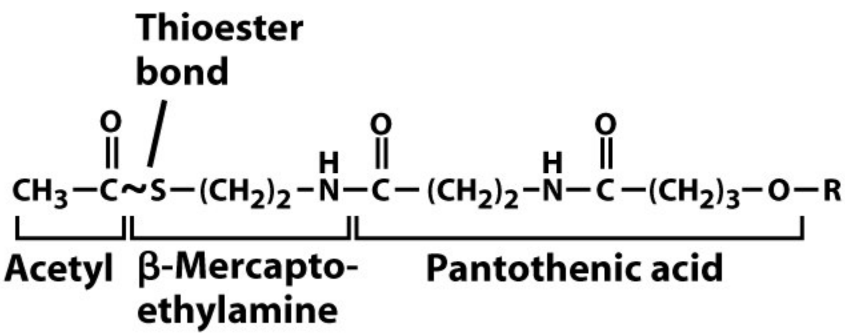


Phosphoenolpyruvate

Adenosine triphosphate (ATP)

Glucose 6-phosphate

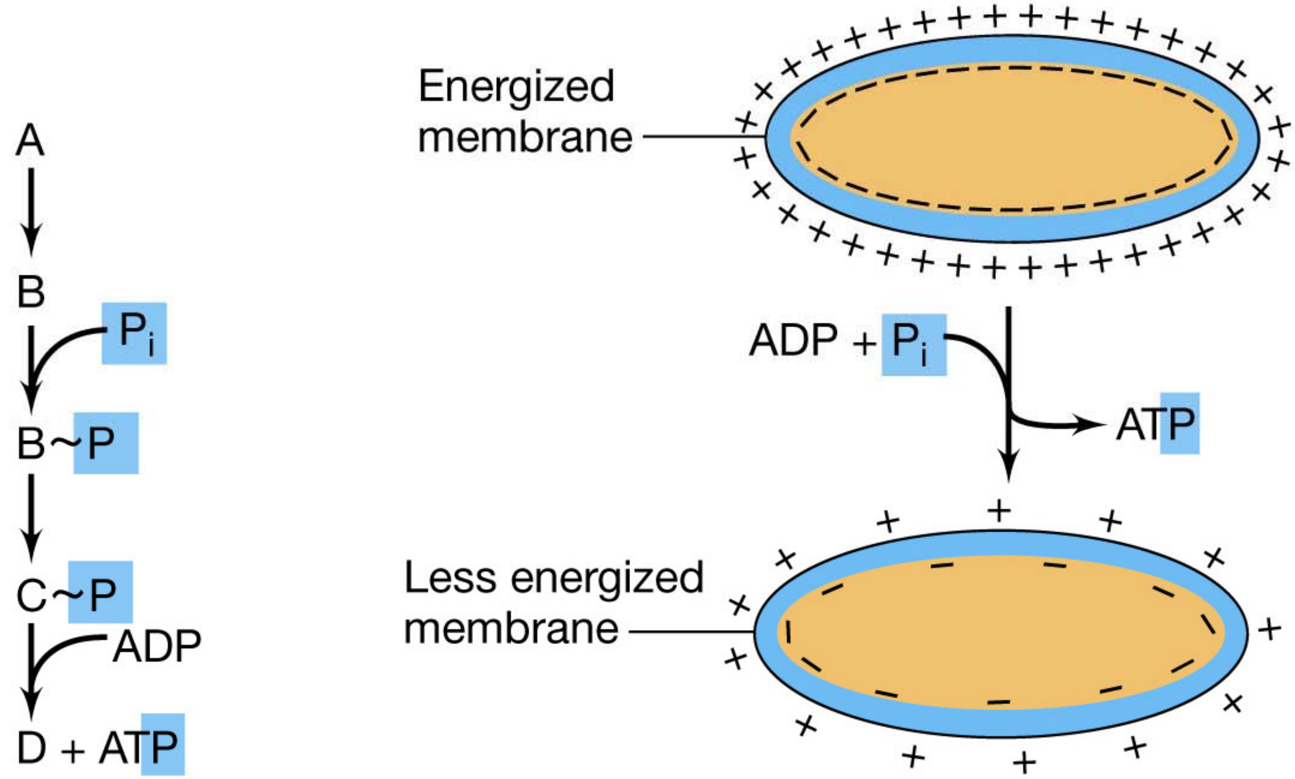
Acetyl phosphate



Acetyl-CoA

Compound	$G^{0\prime}$ kJ/mol
$\Delta G^{0\prime} > 30$kJ	
Phosphoenolpyruvate	−51.6
1,3-Bisphosphoglycerate	−52.0
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Acetyl CoA	−31
$\Delta G^{0\prime} < 30$kJ	
AMP	−14.2
Glucose 6-phosphate	−13.8

Two Ways to Make ATP: Quick & Dirty or Turbo-Charged

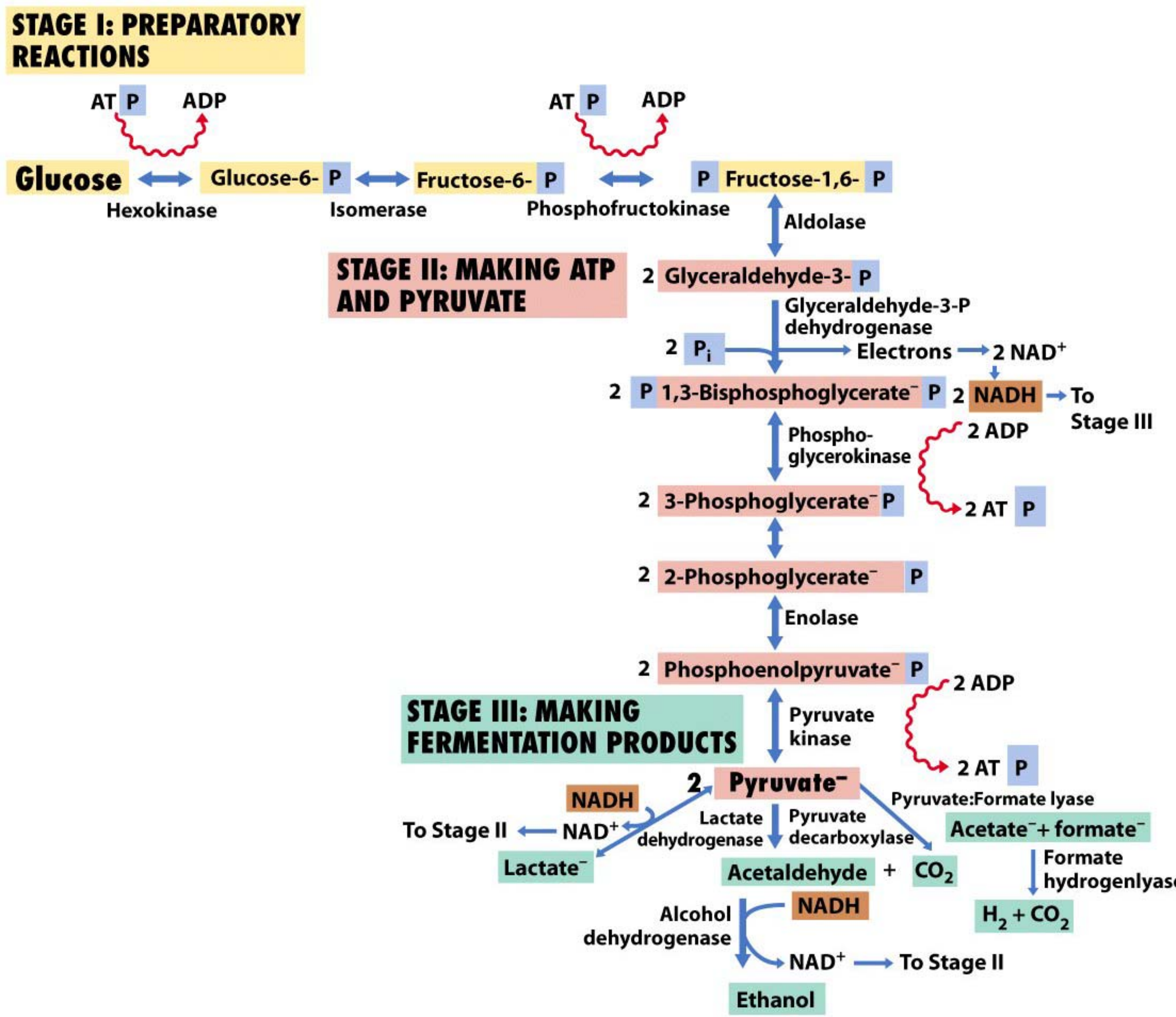


***(a)* Substrate-level phosphorylation**

***(b)* Oxidative phosphorylation**

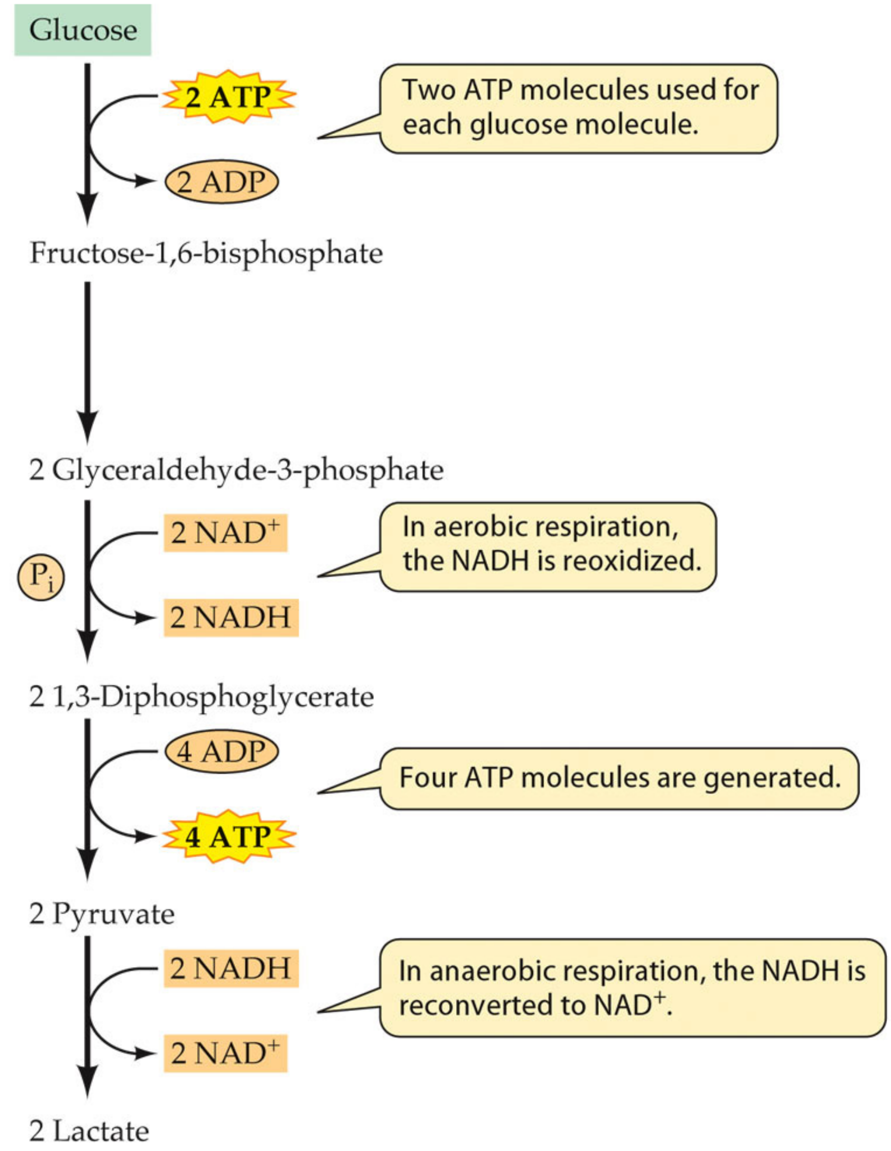
7.1 Cellular Locations for Energy Pathways in Eukaryotes and Prokaryotes

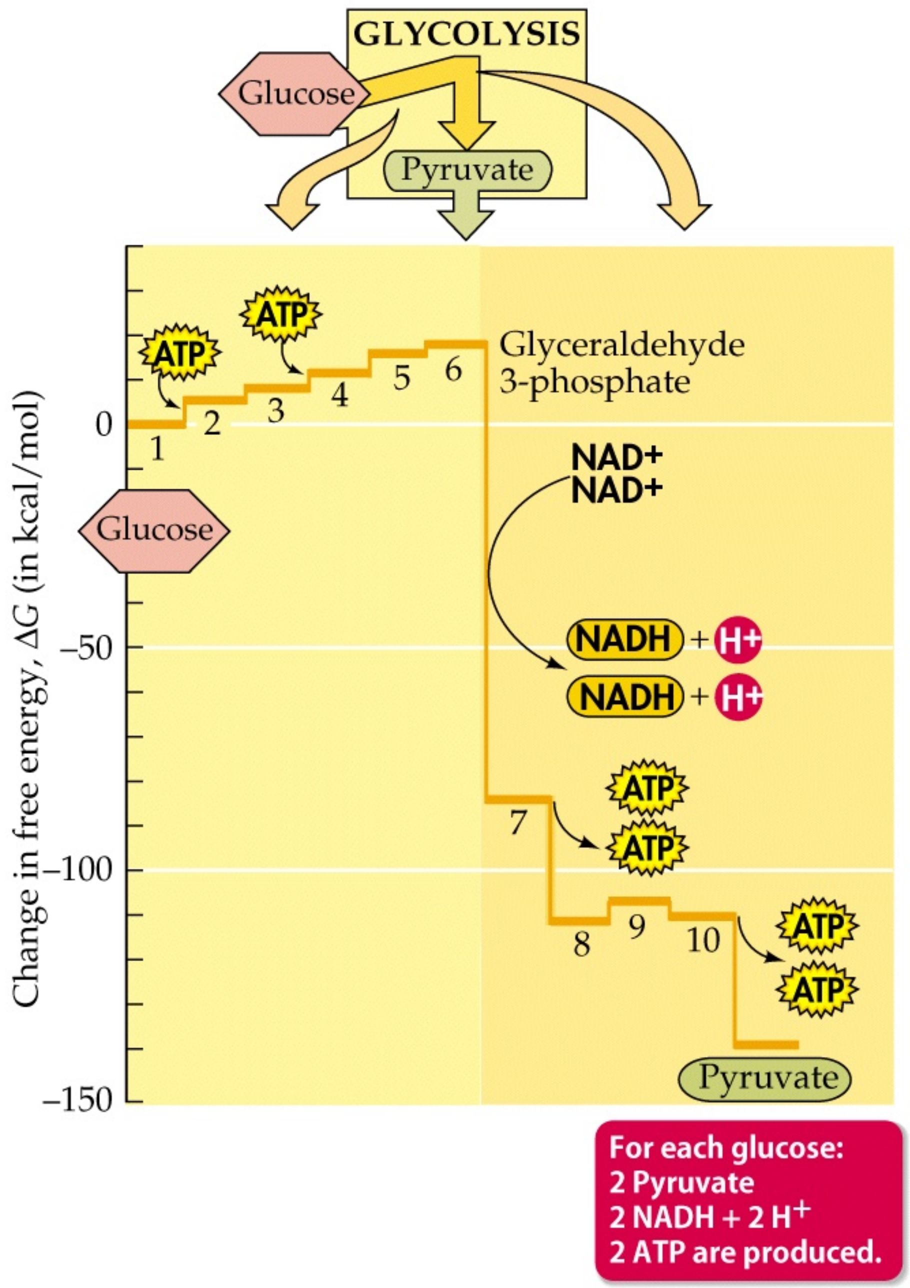
EUKARYOTES	PROKARYOTES
External to mitochondrion	**In cytoplasm**
Glycolysis	Glycolysis
Fermentation	Fermentation
	Citric acid cycle
Inside mitochondrion	**On inner face of plasma membrane**
Inner membrane	Pyruvate oxidation
Pyruvate oxidation	Respiratory chain
Respiratory chain	
Matrix	
Citric acid cycle	

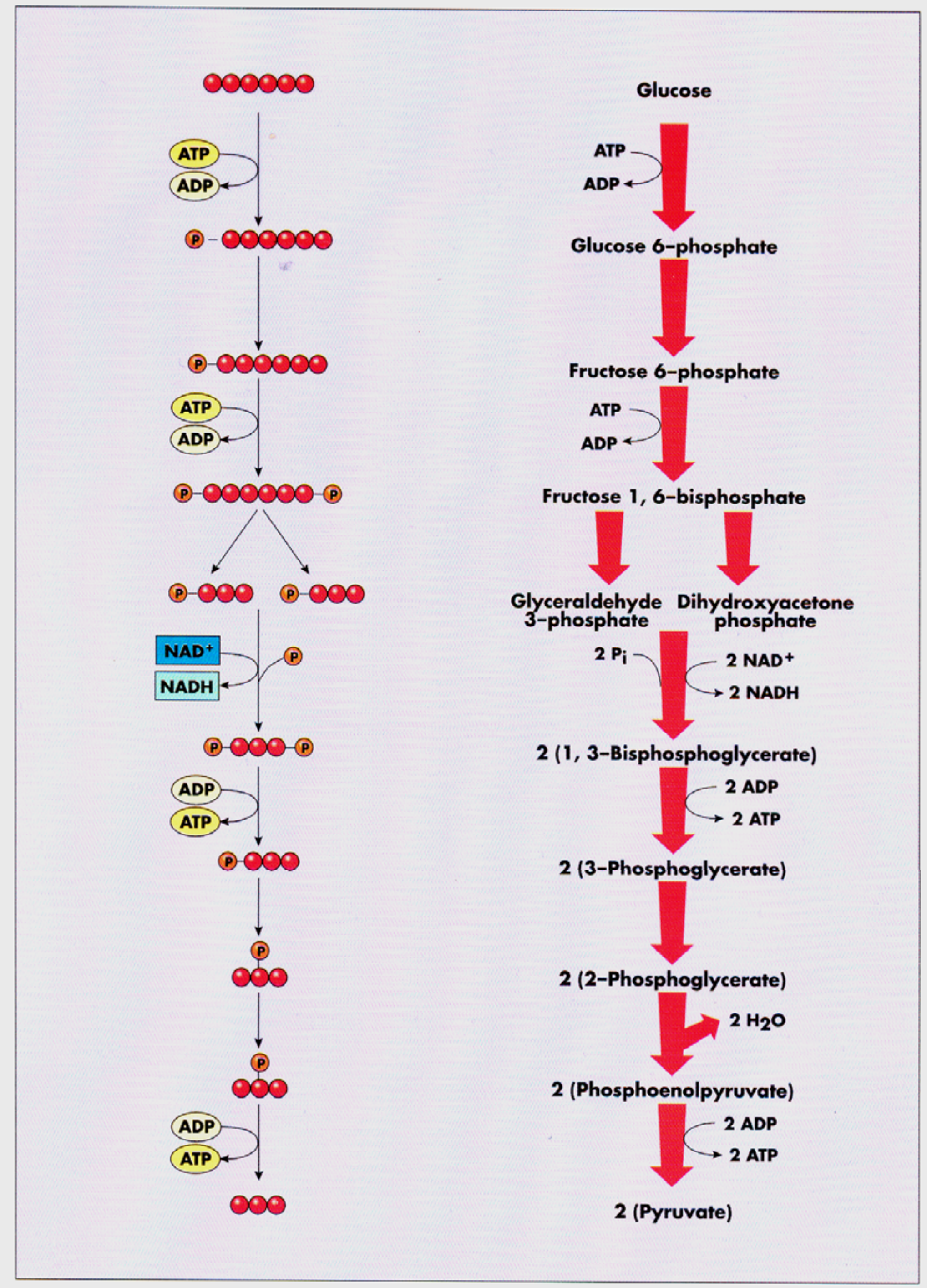


STAGE I: PREPARATORY REACTIONS
ATP
ADP
Glucose
Hexokinase
Glucose-6-P
Isomerase
Fructose-6-P
ATP
ADP
Phosphofructokinase
P Fructose-1,6-P
Aldolase
STAGE II: MAKING ATP AND PYRUVATE
2 Glyceraldehyde-3-P
Glyceraldehyde-3-P dehydrogenase
2 P_i
Electrons
2 NAD^+
2 P 1,3-Bisphosphoglycerate$^-$ P
2 NADH
To Stage III
Phospho-glycerokinase
2 ADP
2 ATP
2 3-Phosphoglycerate$^-$ P
2 2-Phosphoglycerate$^-$ P
Enolase
2 Phosphoenolpyruvate$^-$ P
2 ADP
Pyruvate kinase
2 ATP
STAGE III: MAKING FERMENTATION PRODUCTS
2 Pyruvate$^-$
NADH
To Stage II
NAD^+
Lactate dehydrogenase
Lactate$^-$
Pyruvate decarboxylase
Pyruvate:Formate lyase
Acetate$^-$ + formate$^-$
Formate hydrogenlyase
$H_2 + CO_2$
Acetaldehyde + CO_2
Alcohol dehydrogenase
NADH
NAD^+
To Stage II
Ethanol

Glycolysis: Short Form




GLYCOLYSIS
Glucose
Pyruvate
Change in free energy, ΔG (in kcal/mol)
0
–50
–100
–150
Glucose
ATP
ATP
1
2
3
4
5
6
Glyceraldehyde
3-phosphate
NAD+
NAD+
NADH + H+
NADH + H+
7
ATP
ATP
8
9
10
ATP
ATP
Pyruvate
For each glucose:
2 Pyruvate
2 NADH + 2 H+
2 ATP are produced.



Glycolysis aka Embden-Meyerhof

The short form!

4 ATP / 2 Net
2 NADH+H

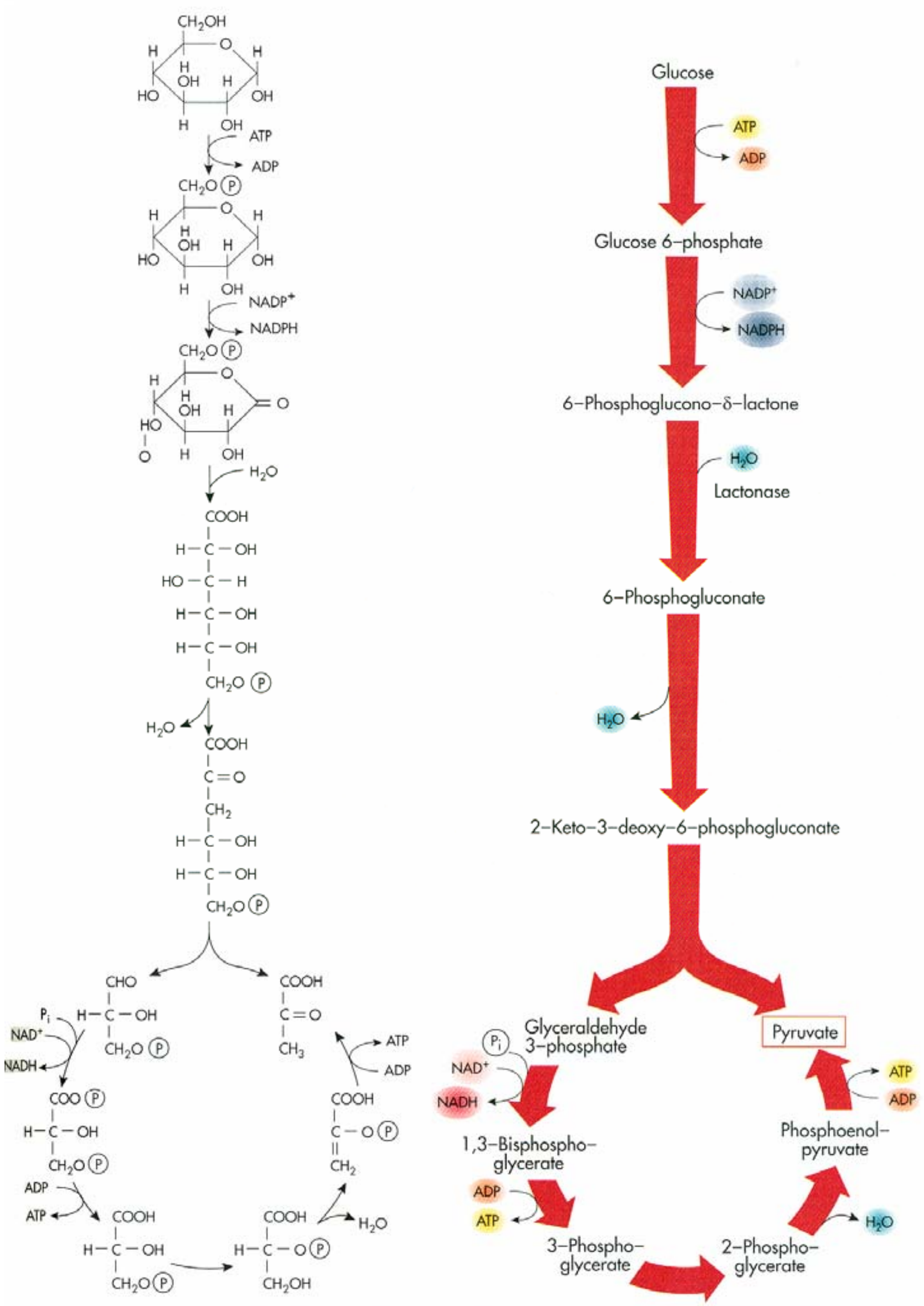


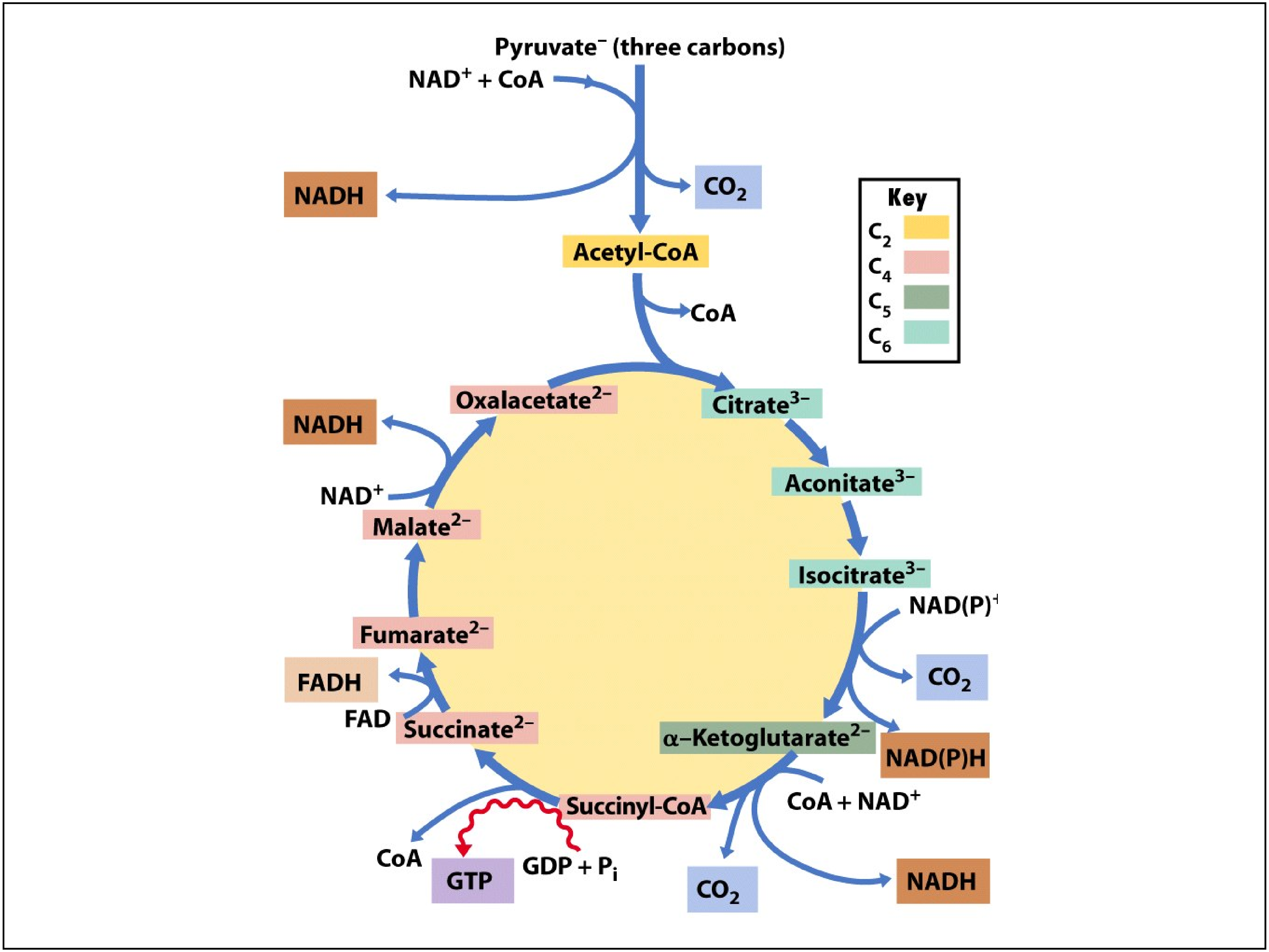
Fig. 4-5 Entner-Doudoroff Pathway of Glycolysis. The Entner-Doudoroff pathway is one of several types of glycolysis. Compared to the Embden-Myerhof pathway, less ATP is generated when this metabolic pathway is used.

Entner-Doudoroff

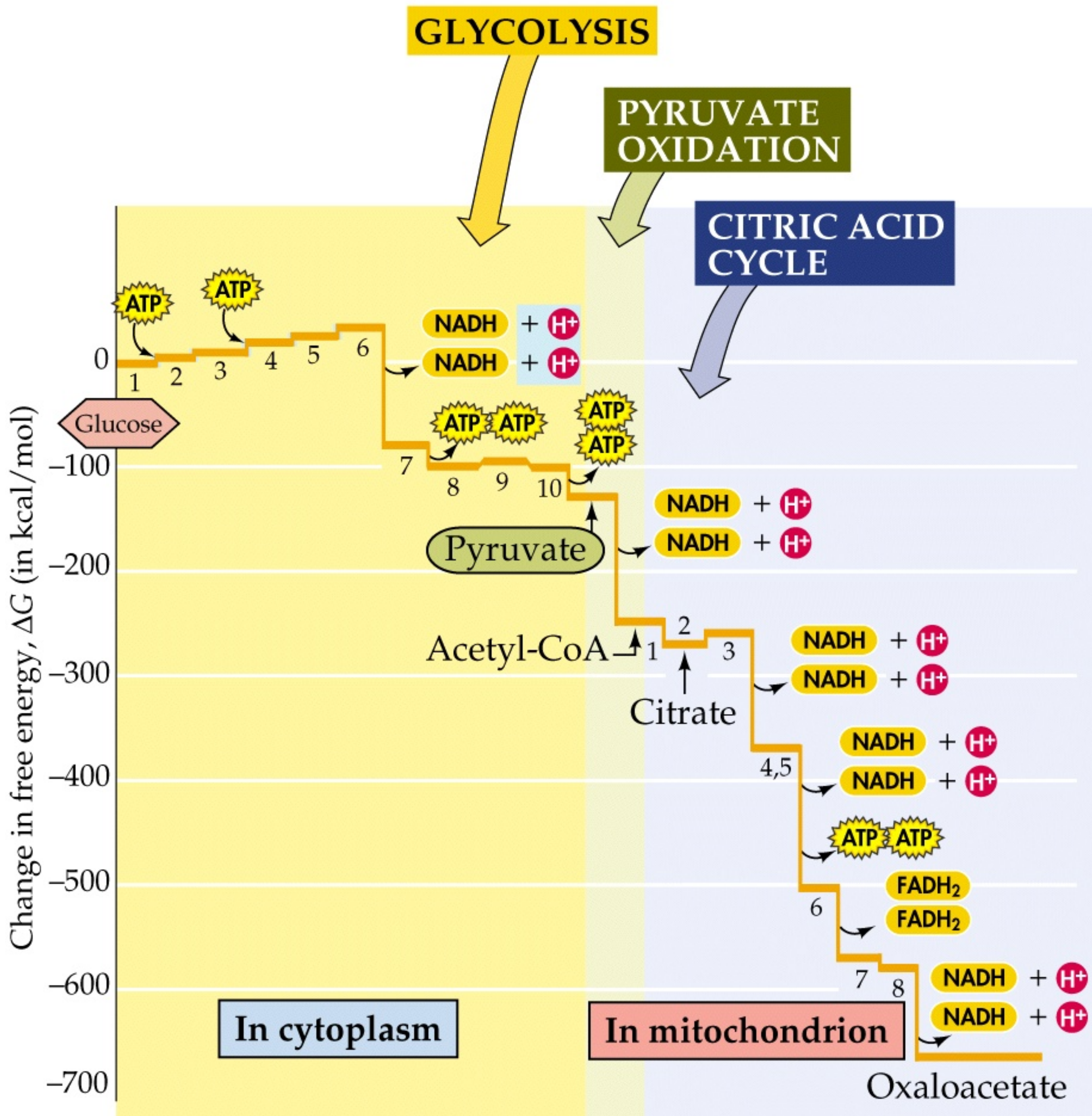
2 ATP / only 1 Net
2 NADH+H

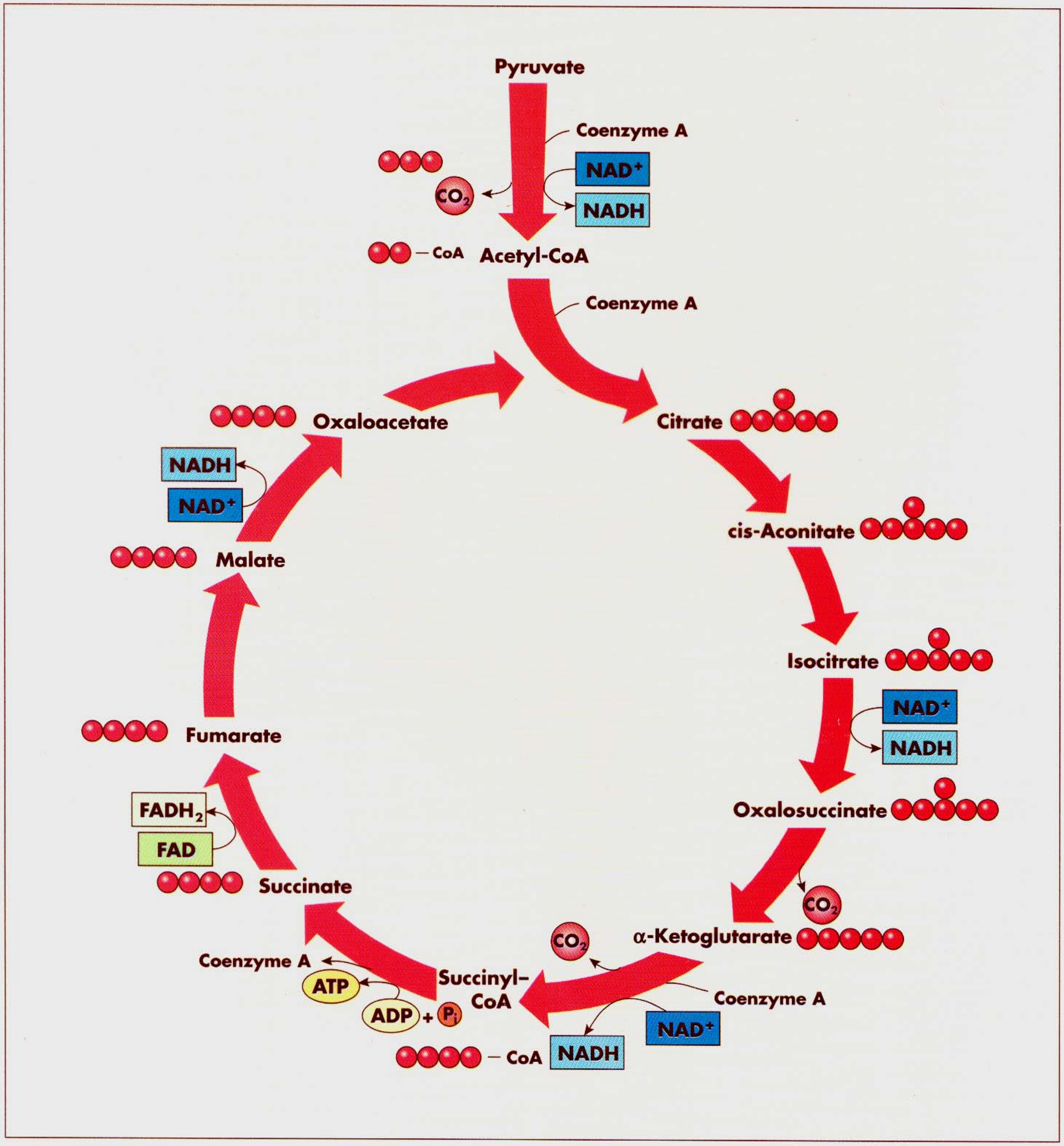
No PFK!!!

Many Gram negatives use this pathway



Pyruvate⁻ (three carbons)
NAD⁺ + CoA
NADH
CO_2
Key
C_2
C_4
C_5
C_6
Acetyl-CoA
CoA
Oxalacetate^{2-}
Citrate^{3-}
NADH
NAD⁺
Aconitate^{3-}
Malate^{2-}
Isocitrate^{3-}
NAD(P)⁺
Fumarate^{2-}
CO_2
FADH
FAD
Succinate^{2-}
α–Ketoglutarate^{2-}
NAD(P)H
Succinyl-CoA
CoA + NAD⁺
CoA
GTP
GDP + P_i
CO_2
NADH


GLYCOLYSIS
PYRUVATE OXIDATION
CITRIC ACID CYCLE
Change in free energy, ΔG (in kcal/mol)
0
–100
–200
–300
–400
–500
–600
–700
Glucose
ATP
ATP
NADH + H+
NADH + H+
ATP ATP
ATP
ATP
Pyruvate
NADH + H+
NADH + H+
Acetyl-CoA
Citrate
NADH + H+
NADH + H+
NADH + H+
NADH + H+
ATP ATP
FADH2
FADH2
NADH + H+
NADH + H+
Oxaloacetate
In cytoplasm
In mitochondrion



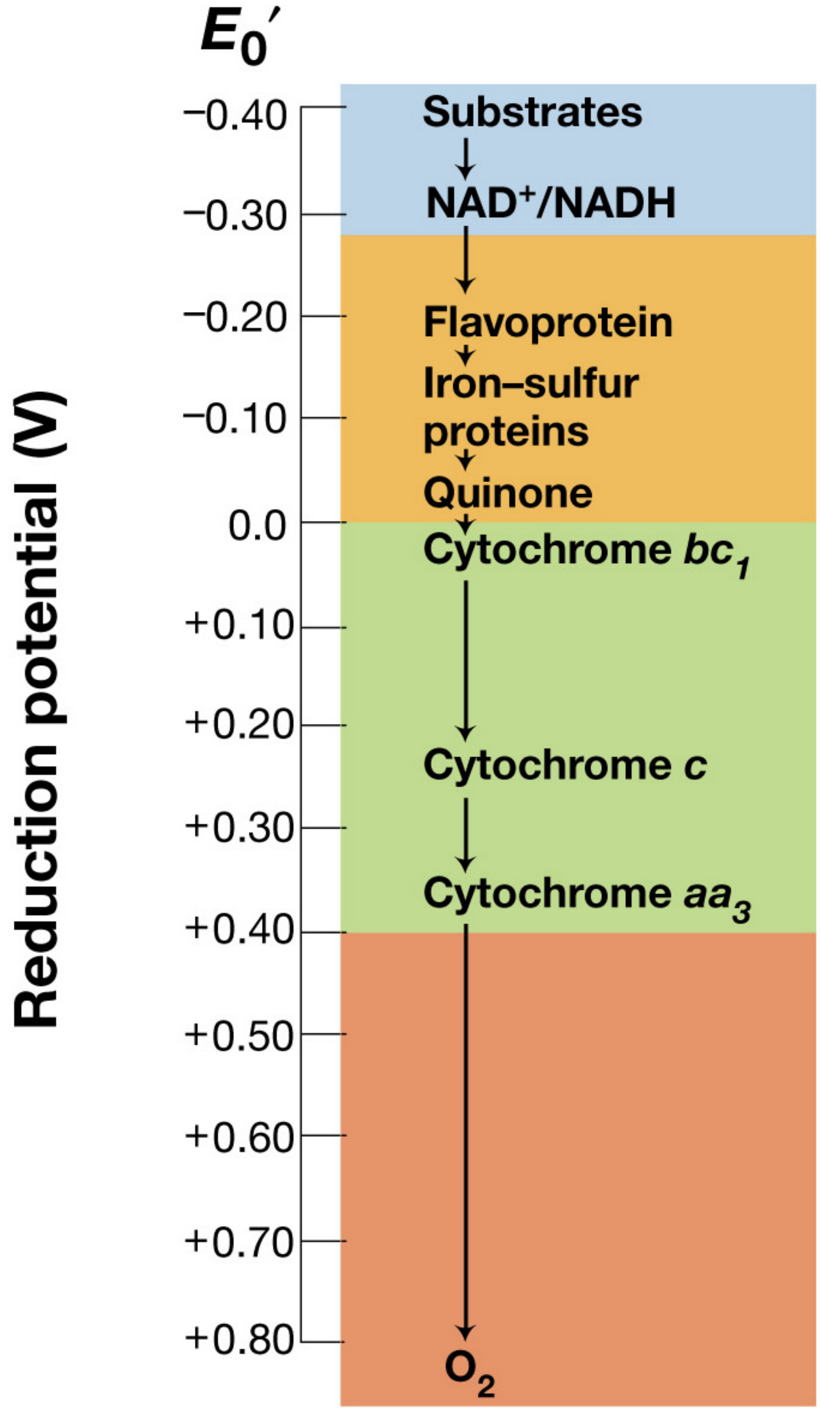
Citric Acid Cycle aka TCA cycle

The short form!

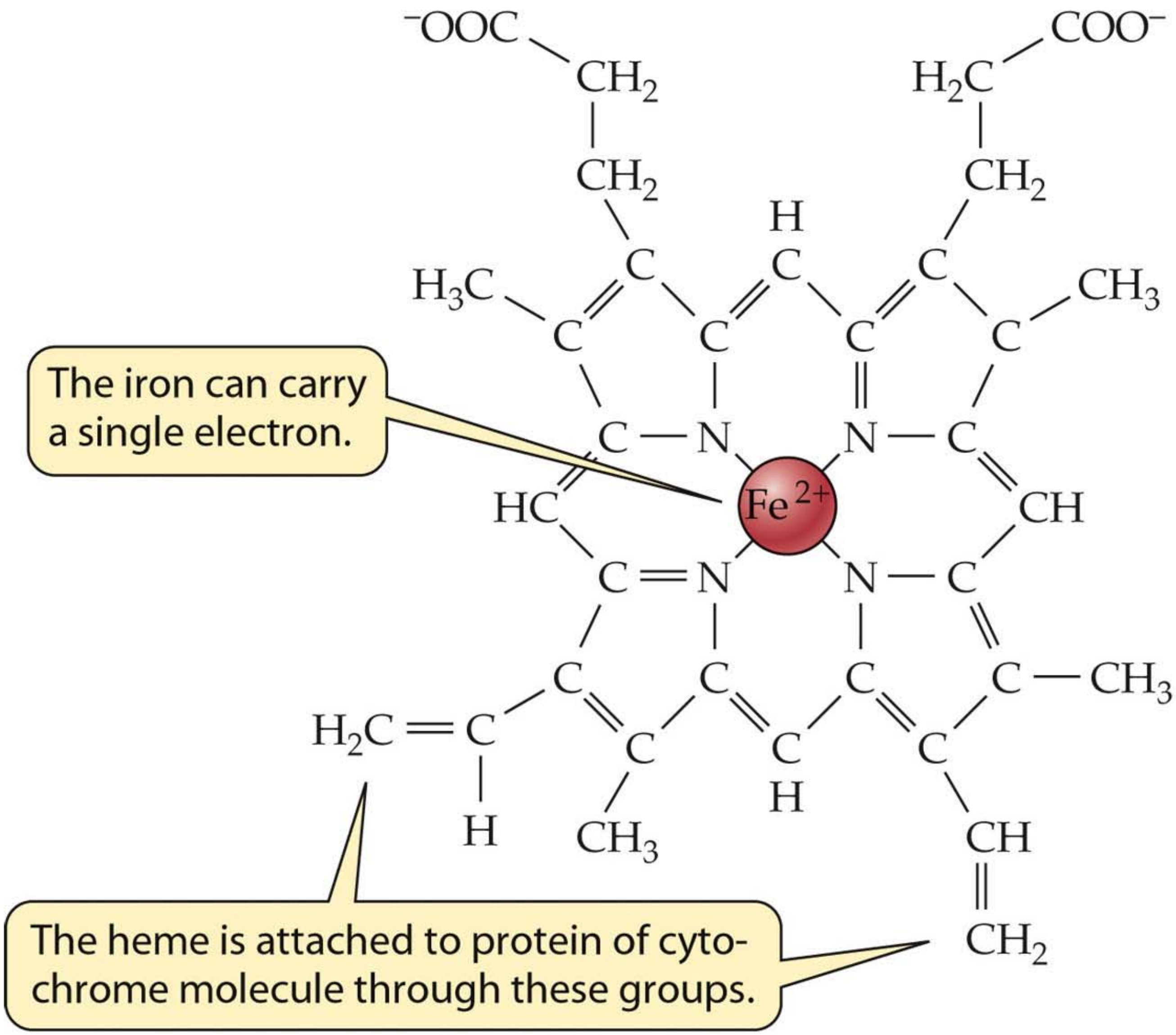
2 ATP (via GTP)
8 NADH+H
2 $FADH_2$

All Carbon to CO_2

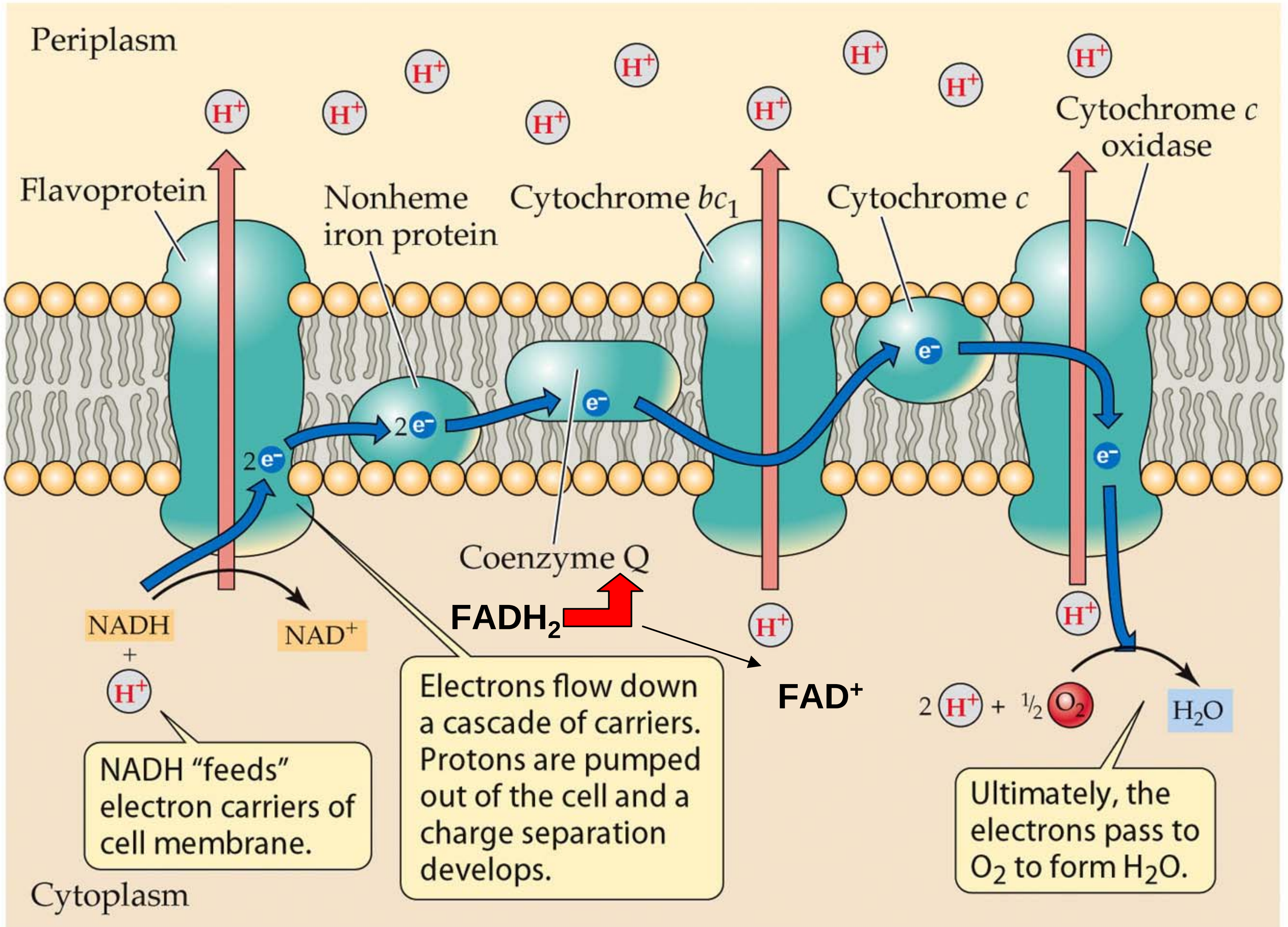
Electron Transport Chains and their Relative Potential



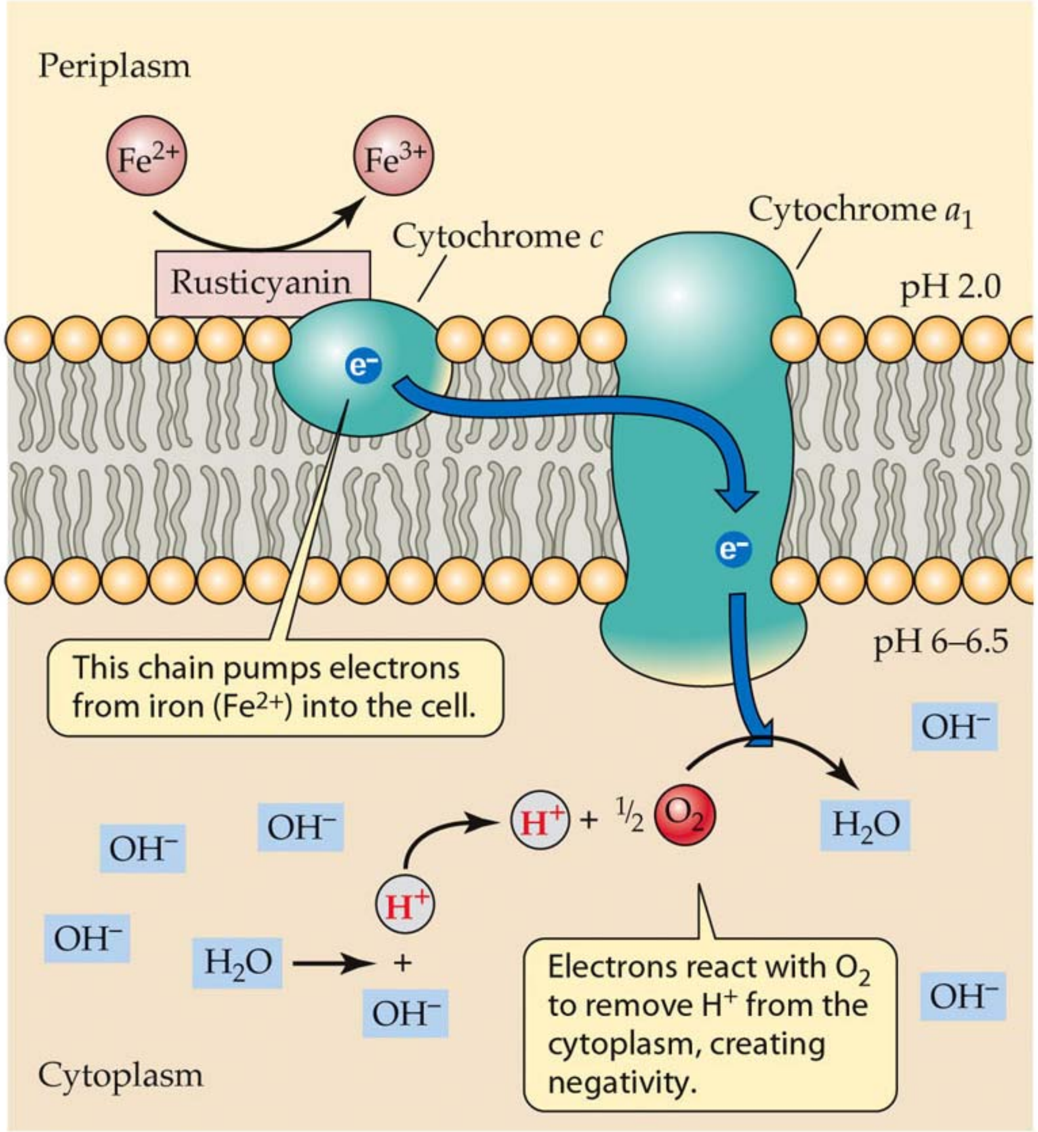
The heme part of a cytochrome, the elegant porphyrin ring!



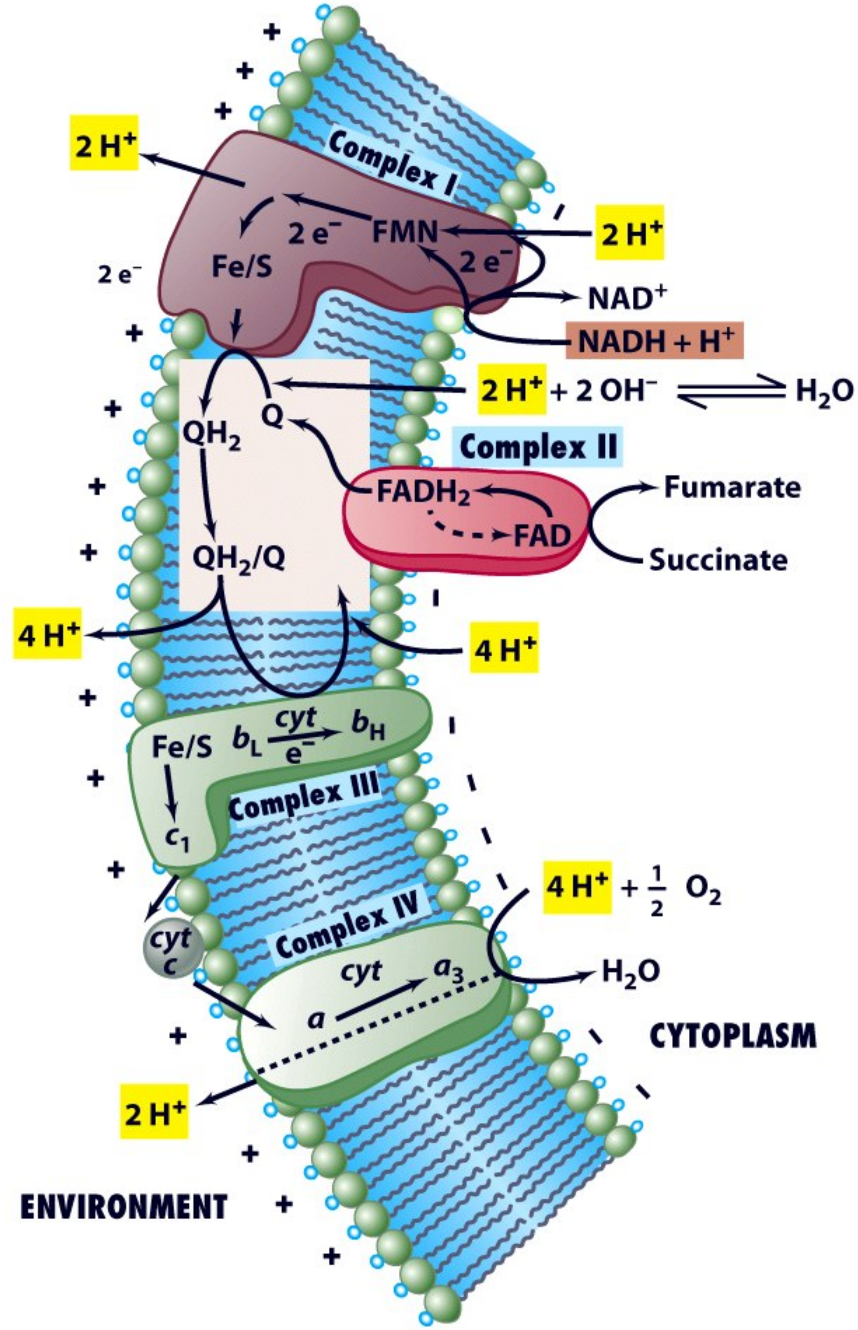
Electron transport chain in aerobic bacterium



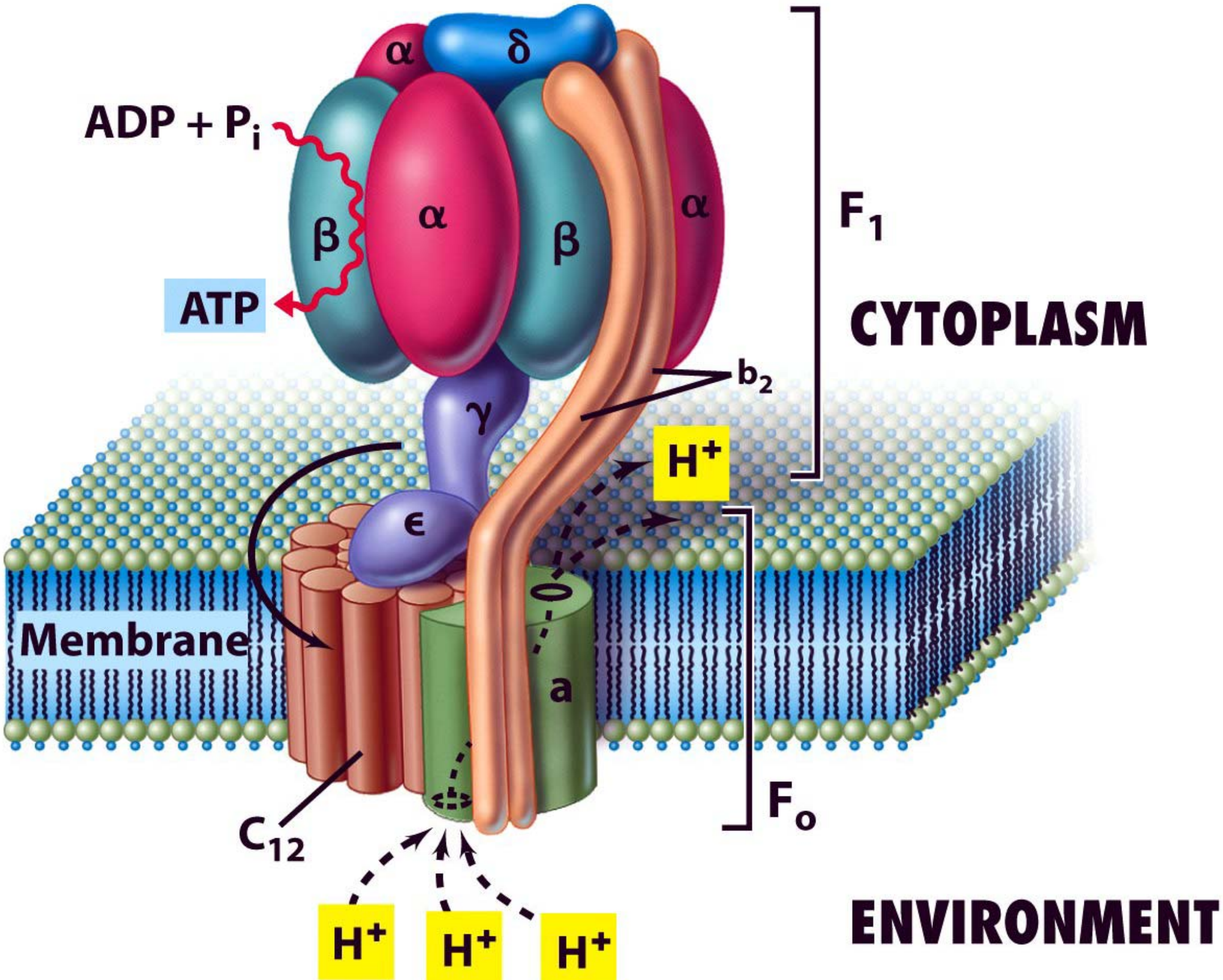
Abbreviated electron transport chain of an iron-oxidizing bacterium

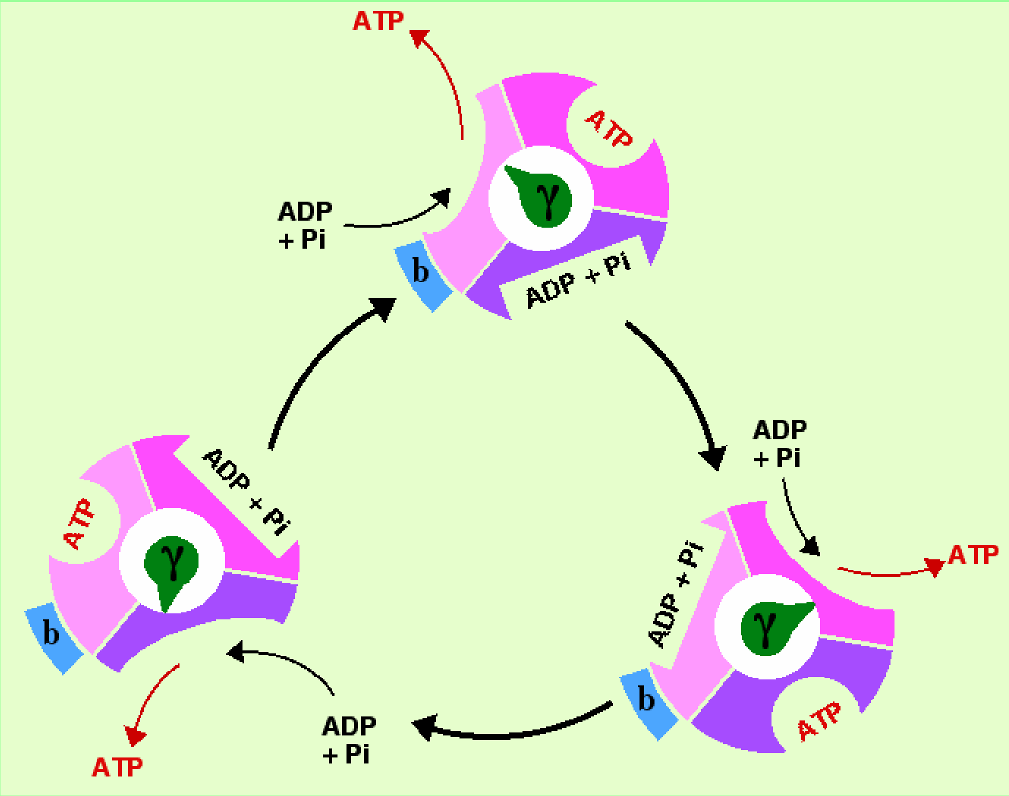


Generation of PMF



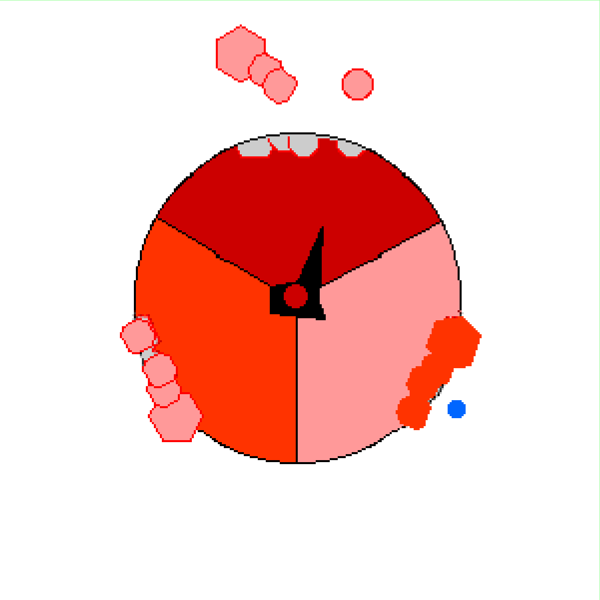
ATP Synthase
Structure & Function





F1 Subunit Topview

ATP Synthase acts as a rotary motor turning in 120 degree steps.



Energetics Balance Sheet for Aerobic Respiration

(1) **Glycolysis:** Glucose + 2NAD$^+$ + 2 ATP → 2 Pyruvate$^-$ + 4 ATP + 2 NADH
+ 4 ADP

Pyruvate$^-$ → to CAC; 2 NADH → to Complex I

(a) Substrate-level phosphorylation
2 ADP + Pi → 2 ATP (× 2)
(b) Oxidative phosphorylation
2 NADH → 6 ATP

8 ATP

(2) **CAC:** Pyruvate$^-$ + 4NAD$^+$ + GDP + FAD → 3 CO_2 + 4 NADH + FADH+ GTP

4 NADH → to Complex I; FADH → to Complex II

(a) Substrate-level phosphorylation
1 GDP + Pi → 1 GTP
1 GTP + 1 ADP → 1 ATP + 1 GDP
(b) Oxidative phosphorylation
4 NADH → 12 ATP
1 FADH → 2 ATP

15 ATP (×2)

(3) **Sum: Glycolysis plus CAC → 38 ATP per glucose**

Fermentation – Key Features

(1) Substrate-level phosphorylation is the rule*.

(2) Always anaerobic (even when some O_2 might be around).

(3) No externally supplied terminal electron acceptor.

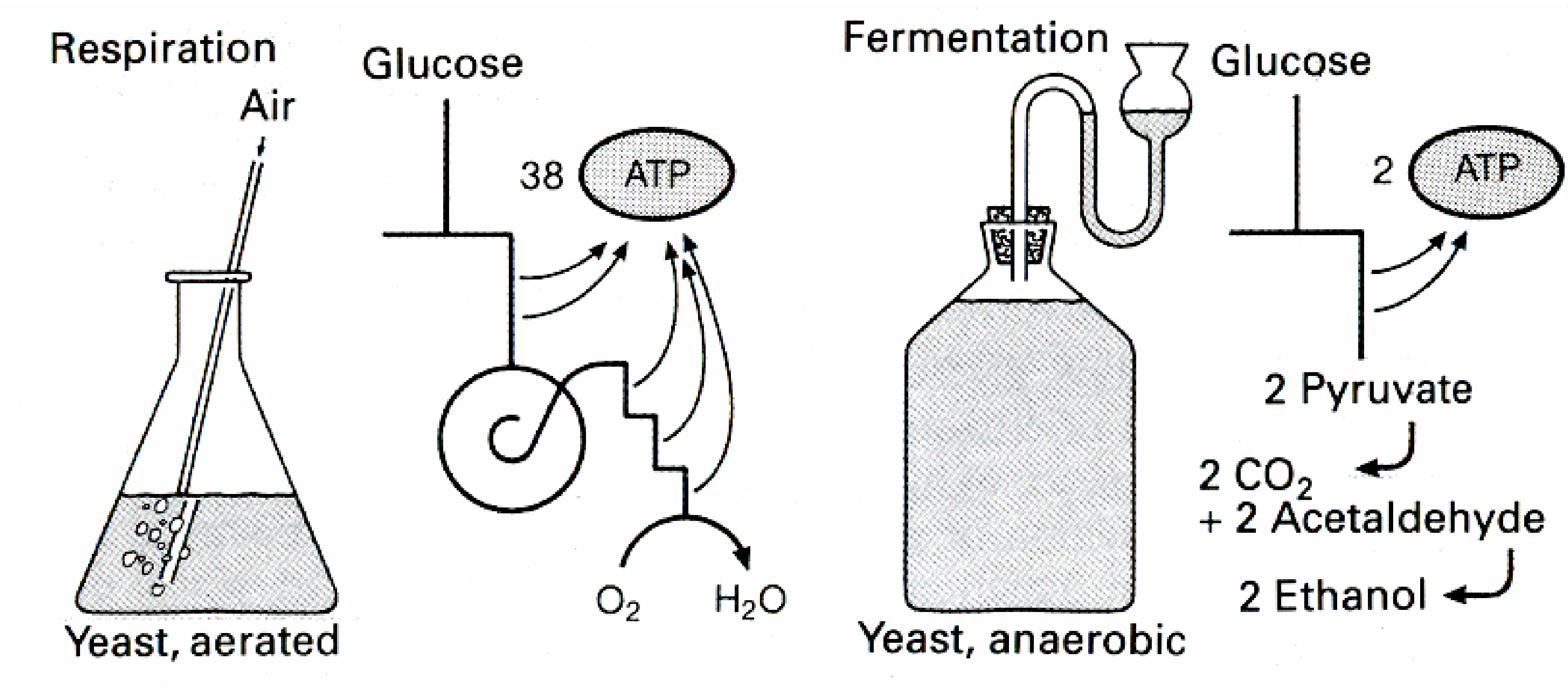
Many types.... 2 major themes

(1) $NADH+H^+$ gets oxidized to NAD^+

(2) Electron acceptor is usually **Pyruvate** or its derivative.

*Rules are always meant to be broken!

Pasteur Effect: ~20X more biomass when aerated



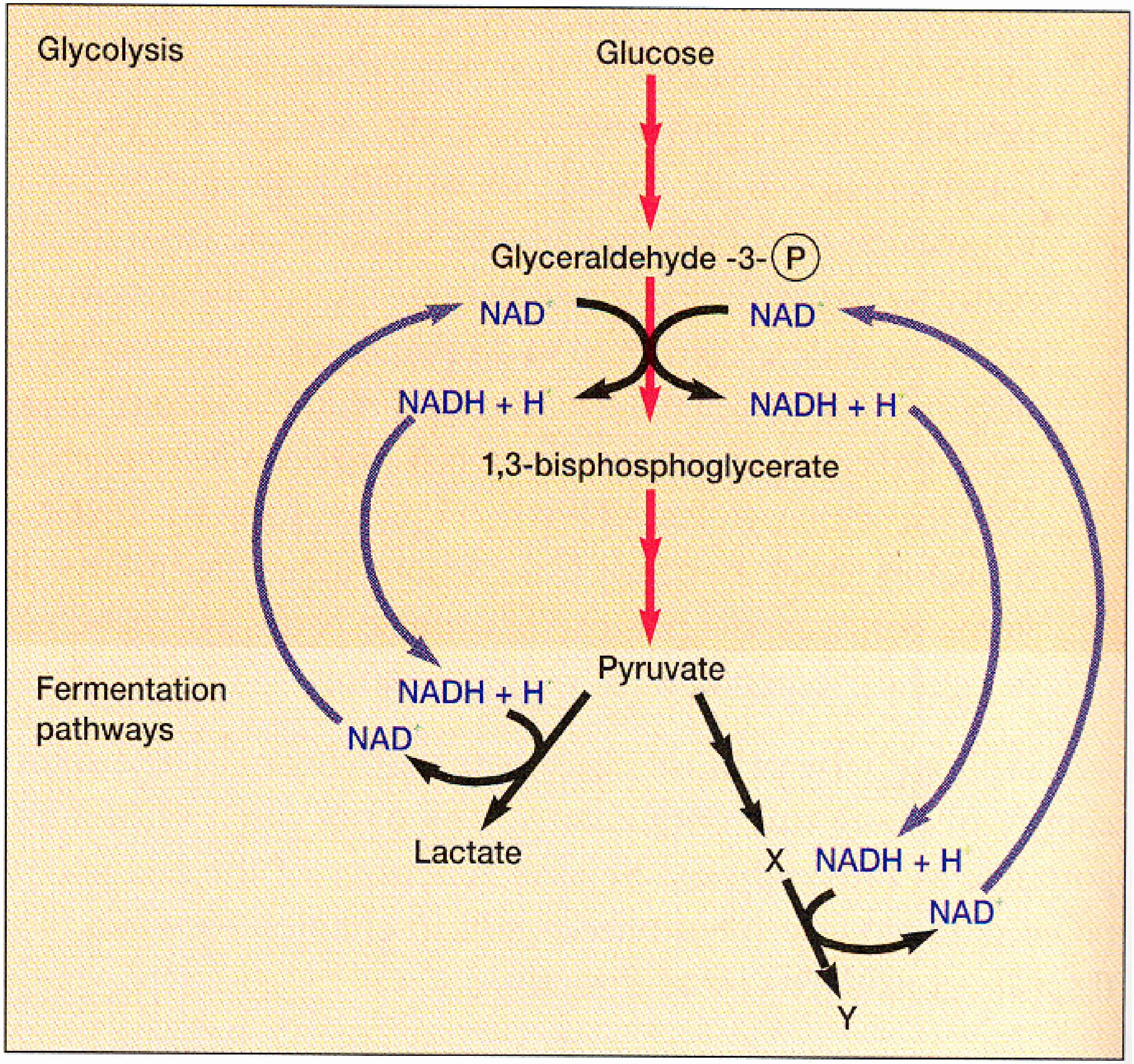
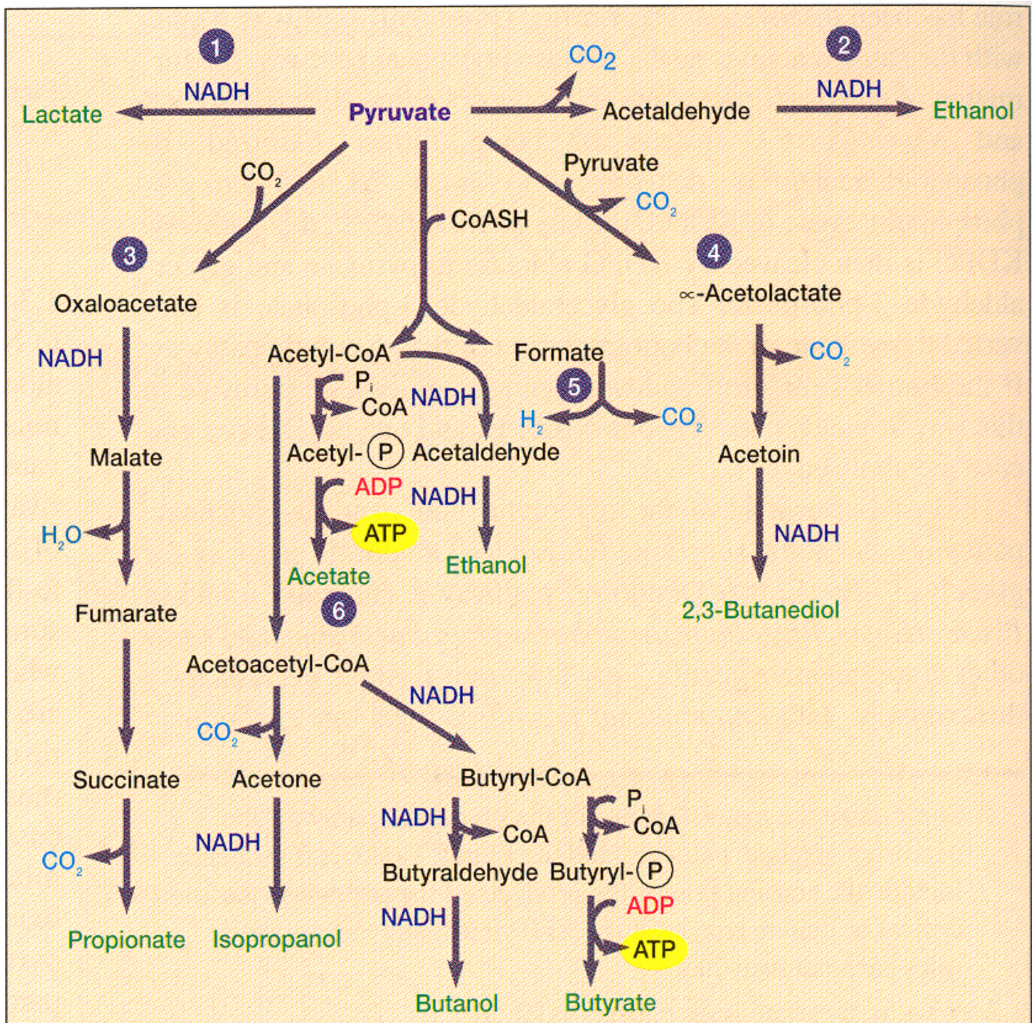
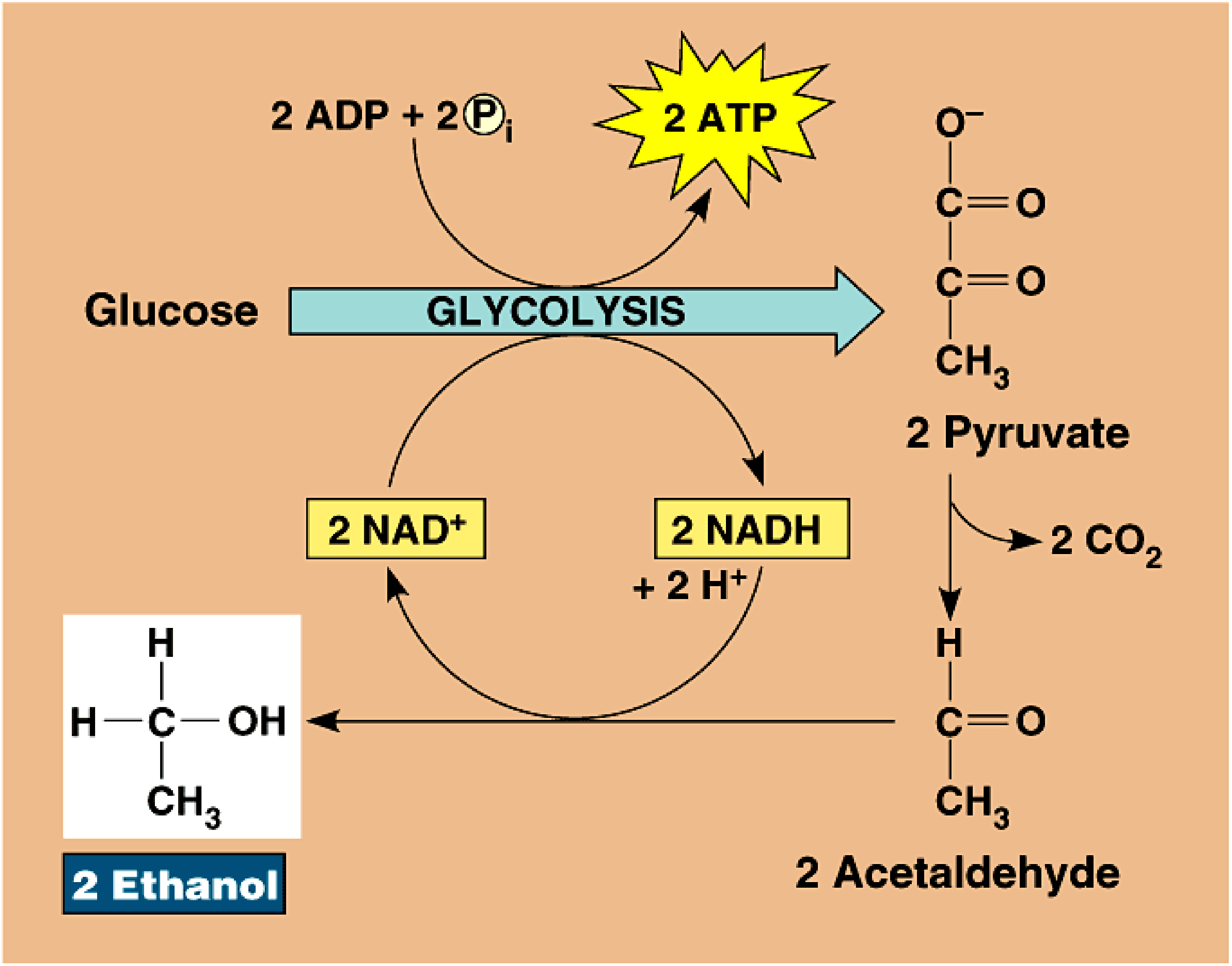


Figure 9.9 Reoxidation of NADH During Fermentation. NADH from glycolysis is reoxidized by being used to reduce pyruvate or a pyruvate derivative (X). Either lactate or reduced product Y result.

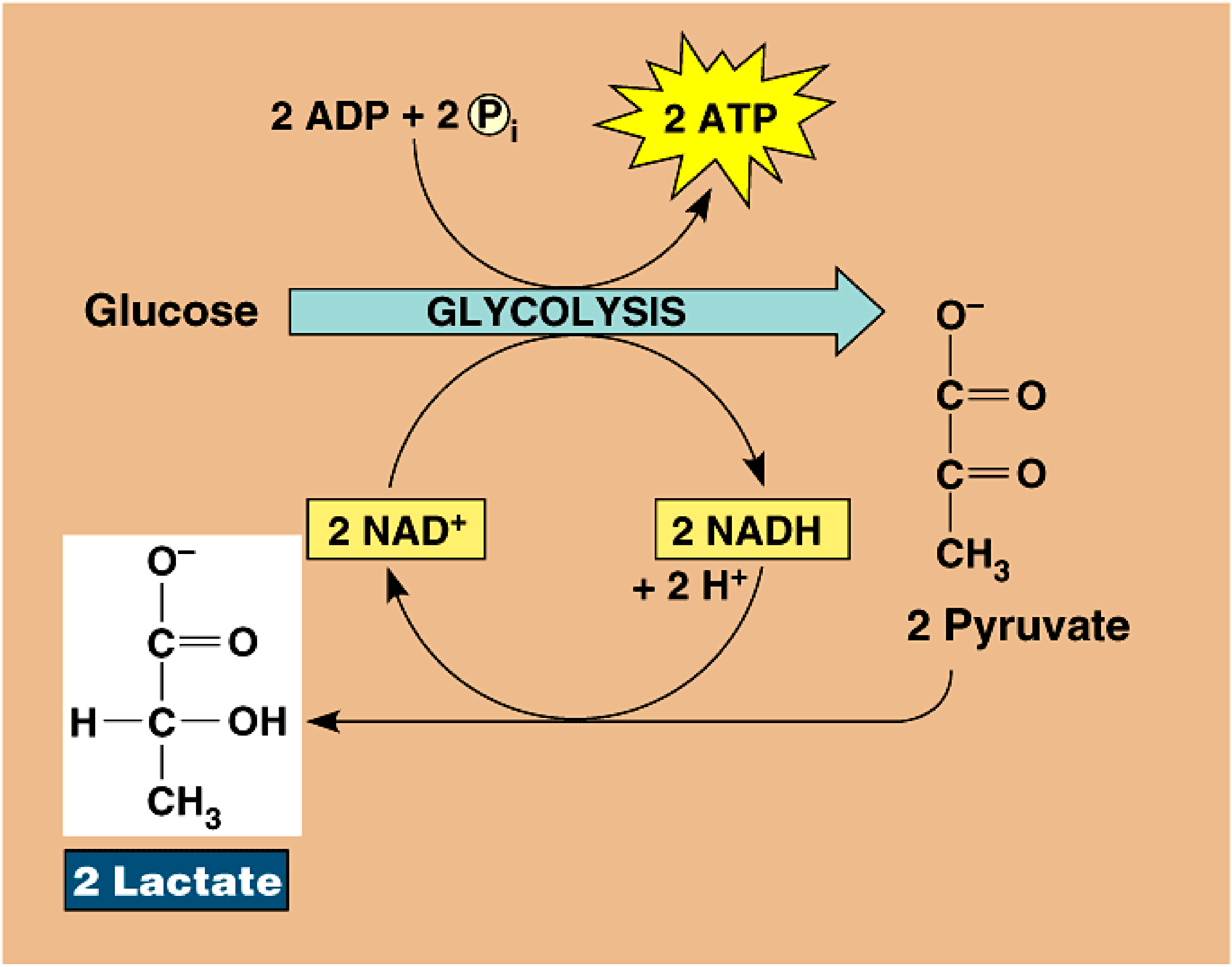
Figure 9.10 Some Common Microbial Fermentations. Only pyruvate fermentations are shown for the sake of simplicity; many other organic molecules can be fermented. Most of these pathways have been simplified by deletion of one or more steps and intermediates. Pyruvate and major end products are shown in color.



1. Lactic acid bacteria (*Streptococcus, Lactobacillus*), *Bacillus*
2. Yeast, *Zymomonas*
3. Propionic acid bacteria (*Propionibacterium*)
4. *Enterobacter, Serratia, Bacillus*
5. Enteric bacteria (*Escherichia, Enterobacter, Salmonella, Proteus*)
6. *Clostridium*



(a) Alcohol fermentation



(b) Lactic acid fermentation

Propionic Acid Fermentation

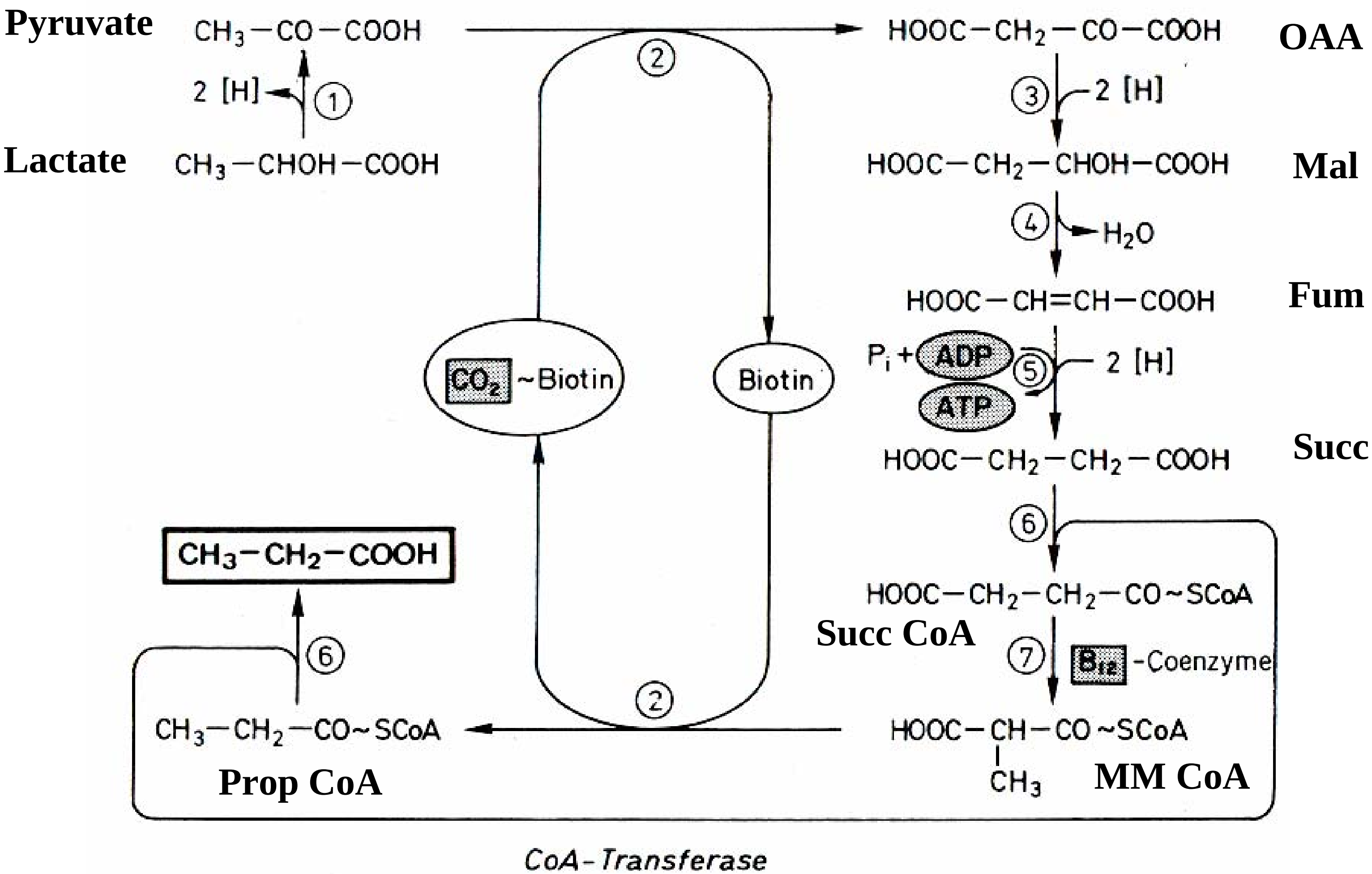
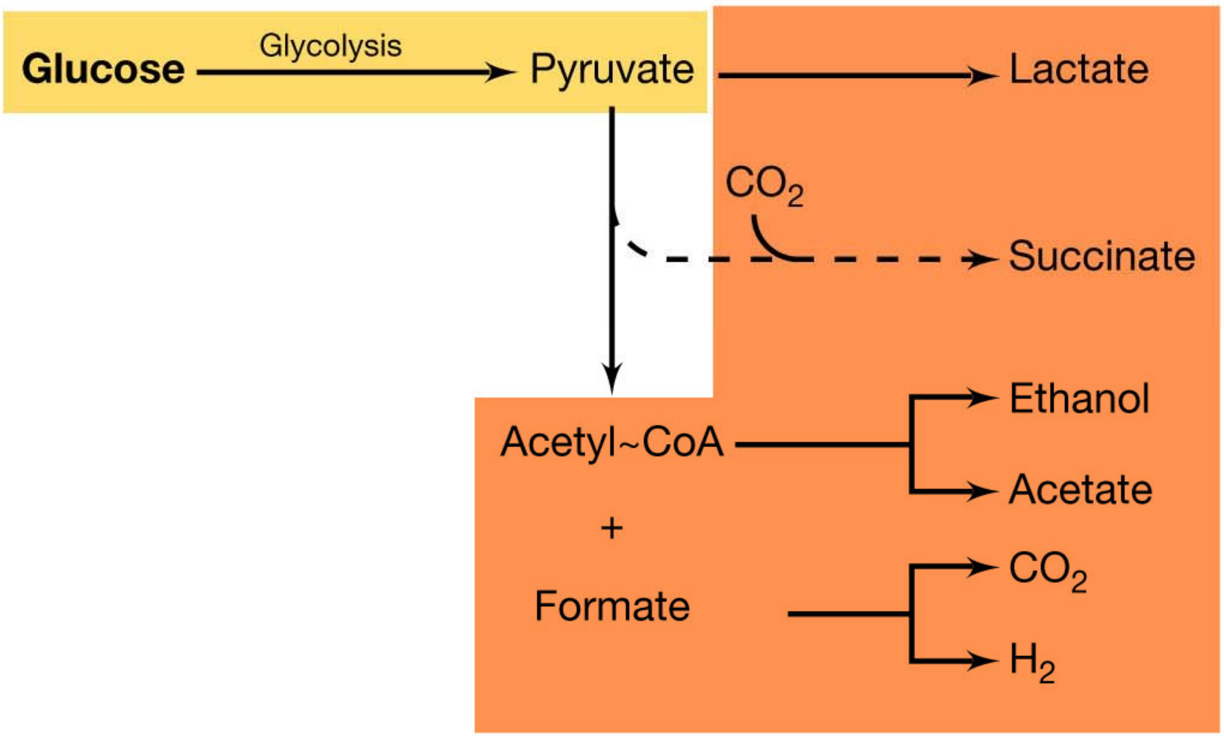


Fig. 8.3. *Methylmalonyl-CoA pathway of propionate formation.*

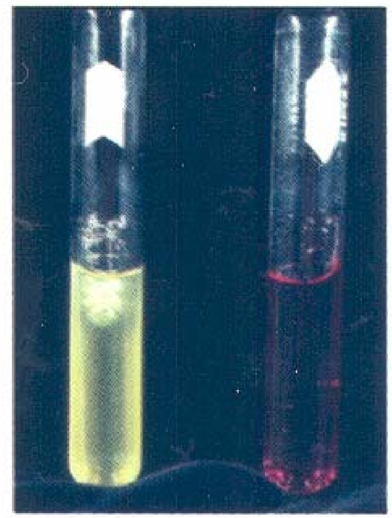
Enzymes: (1) lactate dehydrogenase; (2) methylmalonyl-CoA carboxy-transferase; (3) malate dehydrogenase; (4) fumarase; (5) fumarate reductase (leading to regeneration of ATP by proton translocation); (6) CoA transferase; (7) methylmalonyl-CoA mutase.

(*a*) **Mixed acid fermentation** (for example, *Escherichia coli*)



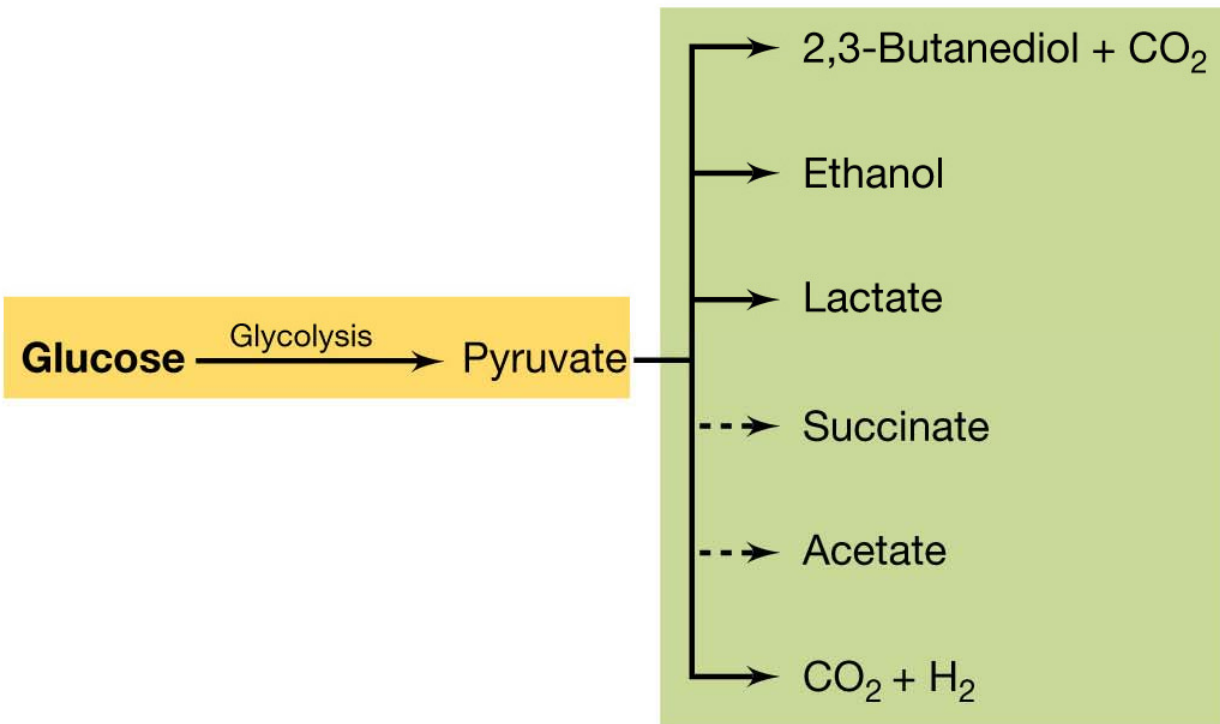
Typical products (molar amounts)

Acidic : neutral
4 : 1
$CO_2 : H_2$
1 : 1



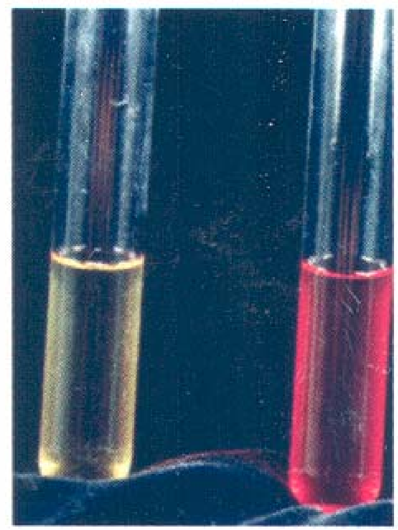
Methyl Red Test

(*b*) **Butanediol fermentation** (for example, *Enterobacter*)



Typical products (molar amounts)

Acidic : neutral
1 : 6
CO_2 : H_2
5 : 1



Voges-Proskauer Test

Clostridial Fermentations

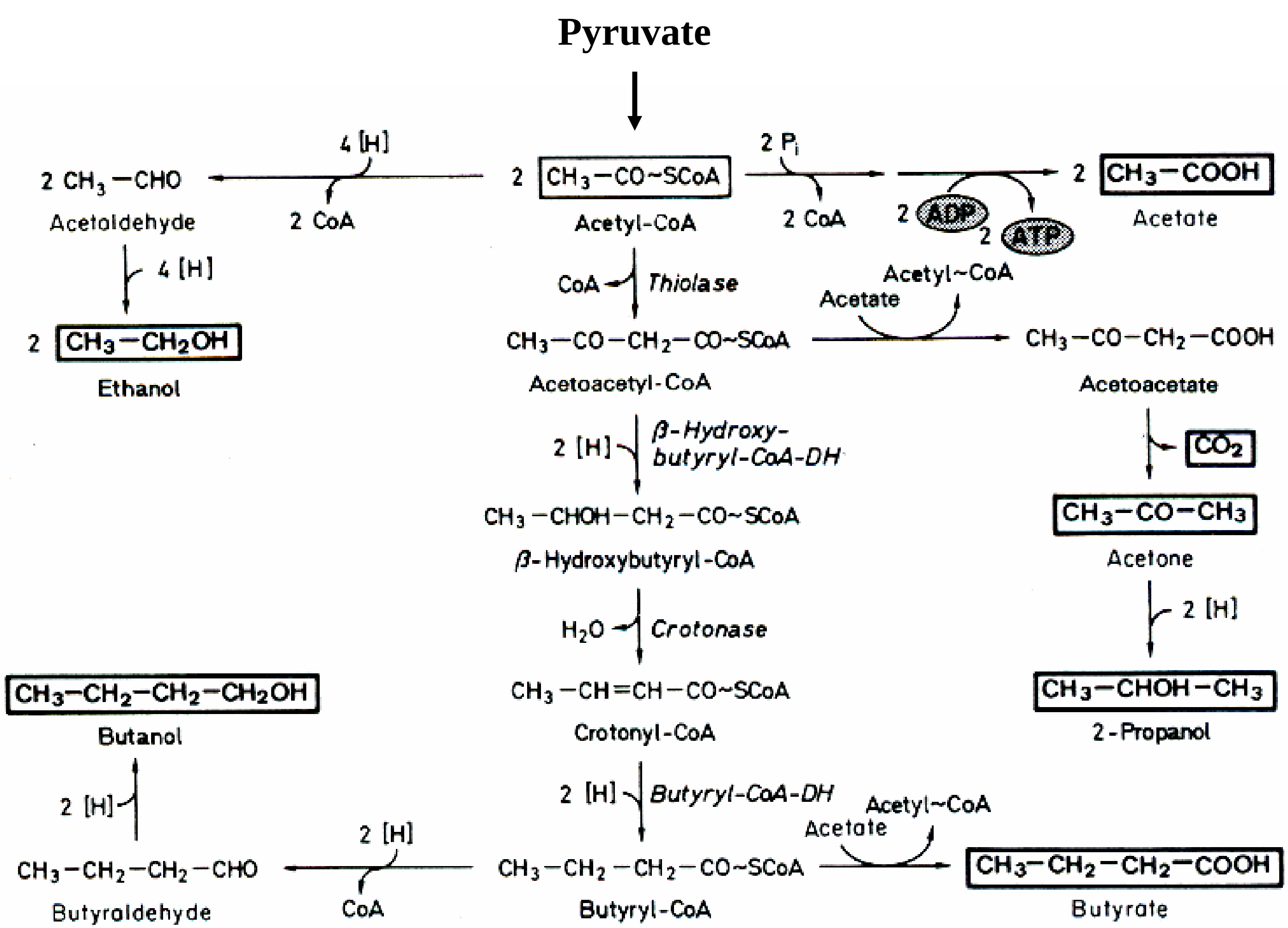


Fig. 8.4. *The formation of acetate, ethanol,* n-*butanol, butyrate, acetone, and 2-propanol during clostridial fermentations.*

Table 17.7 Examples of common bacterial fermentations and some of the organisms carrying them out

Type	Overall reaction[a]	Organisms
Alcoholic	Hexose $\rightarrow$ 2 Ethanol + 2 CO_2	Yeast *Zymomonas*
Homolactic	Hexose $\rightarrow$ 2 $Lactate^-$ + 2 H^+	*Streptococcus* Some *Lactobacillus*
Heterolactic	Hexose $\rightarrow$ $Lactate^-$ + Ethanol + CO_2 + H^+	*Leuconostoc* Some *Lactobacillus*
Propionic acid	$Lactate^-$ $\rightarrow$ $Propionate^-$ + $Acetate^-$ + CO_2	*Propionibacterium* *Clostridium propionicum*
Mixed acid	Hexose $\rightarrow$ Ethanol + 2, 3-Butanediol + $Succinate^{2-}$ + $Lactate^-$ + $Acetate^-$ + $Formate^-$ + H_2 + CO_2	Enteric bacteria[b] *Escherichia* *Salmonella* *Shigella* *Klebsiella* *Enterobacter*
Butyric acid	Hexose $\rightarrow$ $Butyrate^-$ + $Acetate^-$ + H_2 + CO_2	*Clostridium butyricum*
Butanol	Hexose $\rightarrow$ Butanol + $Acetate^-$ + Acetone + Ethanol + H_2 + CO_2	*Clostridium acetobutylicum*
Caproate	Ethanol + $Acetate^-$ + CO_2 $\rightarrow$ $Caproate^-$ + $Butyrate^-$ + H_2	*Clostridium kluyveri*
Homoacetogenic	Fructose $\rightarrow$ 3 $Acetate^-$ + 3 H^+ 2 H_2O 4 H_2 + 2 CO_2 + H^+ $\rightarrow$ $Acetate^-$ +	*Clostridium aceticum* *Acetobacterium*
Methanogenic	$Acetate^-$ + H_2O $\rightarrow$ CH_4 + HCO_3^-	*Methanosaeta* *Methanosarcina*

[a] Reactions are intended as an overview of the process and are not necessarily balanced.

[b] Not all organisms produce all products. In particular, butanediol production is limited to only certain enteric bacteria.

Table 8.2 **Examples of products generated during fermentation of glucose and the microorganism involved**

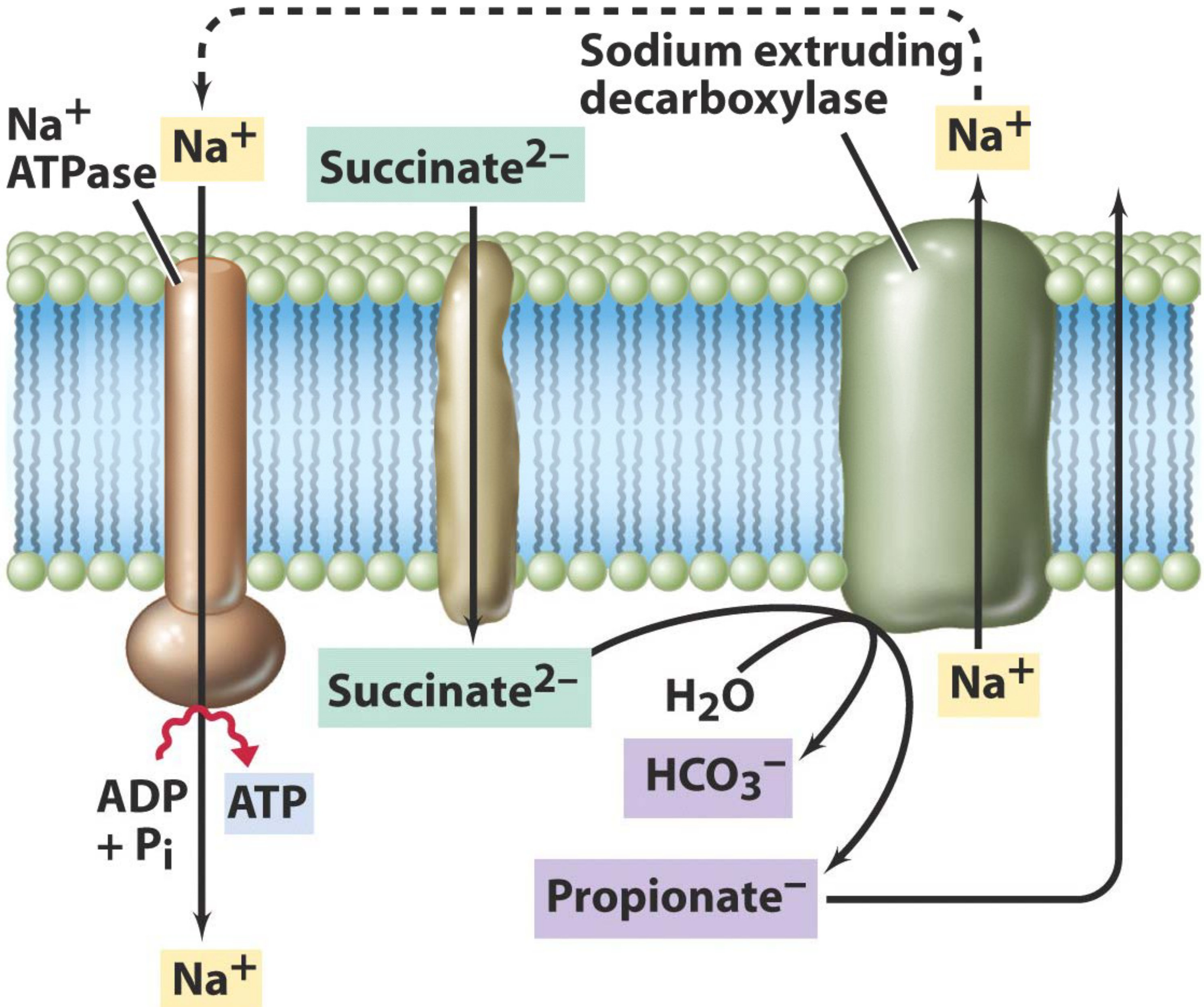
Type	Nongaseous Product	Micro-organism
Mixed acid	ethanol + acetate + lactate	*Escherichia coli*
Butanediol (neutral)	2,3-butanediol + ethanol	*Enterobacter aerogenes*
Alcoholic	ethanol	*Zymomonas mobilis*
Homolactic	lactate	*Lactobacillus acidophilus*
Heterolactic	lactate + ethanol	*Lactobacillus brevis*
Butanol/acetone	acetone + butanol	*Clostridium butyricum*

The short list

Table 17.8 Some unusual bacterial fermentations

Type	Overall balanced reaction	Organisms
Acetylene	$2\ C_2H_2 + 3\ H_2O \rightarrow$ Ethanol + Acetate$^-$ + H^+	*Pelobacter acetylenicus*
Glycerol	4 Glycerol + $2\ HCO_3^- \rightarrow 7$ Acetate$^-$ + $5\ H^+ + 4\ H_2O$	*Acetobacterium* sp.
Resorcinol (an aromatic compound)	$2\ C_6H_4(OH)_2 + 6\ H_2O \rightarrow 4$ Acetate$^-$ + Butyrate$^-$ + $5\ H^+$	*Clostridium* sp.
Phloroglucinol (an aromatic compound)	$C_6H_6O_3 + 3\ H_2O \rightarrow 3$ Acetate$^-$ + $3\ H^+$	*Pelobacter massiliensis* *Pelobacter acidigallici*
Putrescine	$10\ C_4H_{12}N_2 + 26\ H_2O \rightarrow 6$ Acetate$^-$ + 7 Butyrate$^-$ + $20\ NH_4^+ + 16\ H_2 + 13\ H^+$	Unclassified gram-positive nonsporing anaerobes
Citrate	Citrate^{3-} + $2\ H_2O \rightarrow$ Formate$^-$ + 2 Acetate$^-$ + $HCO_3^- + H^+$	*Bacteroides* sp.
Aconitate	Aconitate^{3-} + $H^+ + 2\ H_2O \rightarrow 2\ CO_2$ + 2 Acetate$^-$ + H_2	*Acidaminococcus fermentans*
Glyoxylate	4 Glyoxylate$^-$ + $3\ H^+ + 3\ H_2O \rightarrow 6\ CO_2 + 5\ H_2$ + Glycolate$^-$	Unclassified gram-negative bacterium
Succinate	Succinate^{2-} + $H_2O \rightarrow$ Propionate$^-$ + HCO_3^-	*Propionigenium modestum*
Oxalate	Oxalate^{2-} + $H_2O \rightarrow$ Formate$^-$ + HCO_3^-	*Oxalobacter formigenes*
Malonate	Malonate^{2-} + $H_2O \rightarrow$ Acetate$^-$ + HCO_3^-	*Malonomonas rubra* *Sporomusa malonica*
Benzoate	2 Benzoate$^-$ $\rightarrow$ Cyclohexane carboxylate$^-$ + 3 Acetate$^-$ + HCO_3^- + $3H^+$	*Syntrophus aciditrophicus*

The unusual fermentations of succinate and oxalate



The unusual fermentations of succinate and oxalate