MICROBIAL RESPIRATION

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Microbial Metabolism

A. Basic Concepts of Metabolism
B. Glycolytic Pathways
C. Fermentation
D. Respiration
E. Photosynthesis
F. Chemolithotrophy

Basic Concepts

- **Definitions**
  - **Metabolism**: The processes of catabolism and anabolism
  - **Catabolism**: The processes by which a living organism obtains its energy and raw materials from nutrients
  - **Anabolism**: The processes by which energy and raw materials are used to build macromolecules and cellular structures (biosynthesis)

Basic Concepts

Reduction and Oxidation

- An atom becomes more reduced when it undergoes a chemical reaction in which it
  - Gains electrons
  - By bonding to a less electronegative atom
  - And often this occurs when the atom becomes bonded to a hydrogen

Basic Concepts

Reduction and Oxidation

- An atom becomes more oxidized when it undergoes a chemical reaction in which it
  - Loses electrons
  - By bonding to a more electronegative atom
  - And often this occurs when the atom becomes bonded to an oxygen

Basic Concepts

Reduction and Oxidation

- In metabolic pathways, we are often concerned with the oxidation or reduction of carbon.
- Reduced forms of carbon (e.g. hydrocarbons, methane, fats, carbohydrates, alcohols) carry a great deal of potential chemical energy stored in their bonds.
- Oxidized forms of carbon (e.g. ketones, aldehydes, carboxylic acids, carbon dioxide) carry very little potential chemical energy in their bonds.

Basic Concepts

Reduction and Oxidation

- Reduction and oxidation always occur together. In a reduction-oxidation reaction (redox reaction), one substance gets reduced, and another substance gets oxidized. The thing that gets oxidized is called the electron donor, and the thing that gets reduced is called the electron acceptor.

Basic Concepts

Enzymatic Pathways for Metabolism

- Metabolic reactions take place in a step-wise fashion in which the atoms of the raw materials are rearranged, often one at a time, until the formation of the final product takes place.
- Each step requires its own enzyme.
- The sequence of enzymatically-catalyzed steps from a starting raw material to final end products is called an enzymatic pathway (or metabolic pathway)

Basic Concepts

Cofactors for Redox Reactions

- Enzymes that catalyze redox reactions typically require a cofactor to “**shuttle**” electrons from one part of the metabolic pathway to another part.

- There are two main redox cofactors: **NAD** and **FAD**. These are (relatively) small organic molecules in which part of the structure can either be reduced (e.g., **accept a pair of electrons**) or oxidized (e.g., **donate a pair of electrons**).

Basic Concepts

Cofactors for Redox Reactions

$$\mathbf{NAD_{(oxidized)} + H^+ + Pair\ of\ electrons \rightarrow NADH_{(reduced)}}$$

$$\mathbf{FAD_{(oxidized)} + H^+ + Pair\ of\ electrons \rightarrow FADH_{(reduced)}}$$

NAD and FAD are present only in small (catalytic) amounts – they cannot serve as the final electron acceptor, but must be regenerated (reoxidized) in order for metabolism to continue.

Basic Concepts

ATP: A "currency of energy" for many cellular reactions

- ATP stands for adenosine triphosphate. It is a nucleotide with three phosphate groups linked in a small chain.
- The last phosphate in the chain can be removed by hydrolysis (the ATP becomes ADP, or adenosine diphosphate).

  This reaction is energetically favorable: it has a DG°' of about **–7.5 kcal/mol**

ATP + H_2O ® ADP + Phosphate + Energy (7.5 kcal/mol)

Respiration

- **Features of respiratory pathways**
  - Pyruvic acid is oxidized completely to CO_2.
  - The final electron acceptor is usually an inorganic substance.
  - NADH is oxidized to form NAD: Essential for continued operation of the glycolytic pathways.
  - O_2 may or may not be required.
    - **Aerobic respiration**: O_2 is the final e^- acceptor.
    - **Anaerobic respiration**: An substance, usually inorganic, other than O_2 is the acceptor (eg nitrate, nitrite, sulfate)
  - A lot of additional ATP are made (up to 36 per glucose molecule).

Respiration

Stages of Respiration

- Preliminary reactions and the Krebs cycle (TCA or Citric Acid Cycle)
- Respiratory electron transport

Respiration in Bacteria

Respiration: Different electron acceptors in bacteria

Aerobic respiration
(many bacteria & eukaryotic mitochondria)

$$\frac{1}{2}O_2 \rightarrow H_2O$$

Anaerobic respiration

$$NO_3^{2-} \rightarrow NO_2^{-}$$

Nitrate reduction
(e.g., in several gram-negative enteric bacteria such as E. coli)

$$NO_2^{-} \rightarrow N_2$$

Nitrite reduction
(e.g., Pseudomonas, Bacillus, and related soil and aquatic genera)

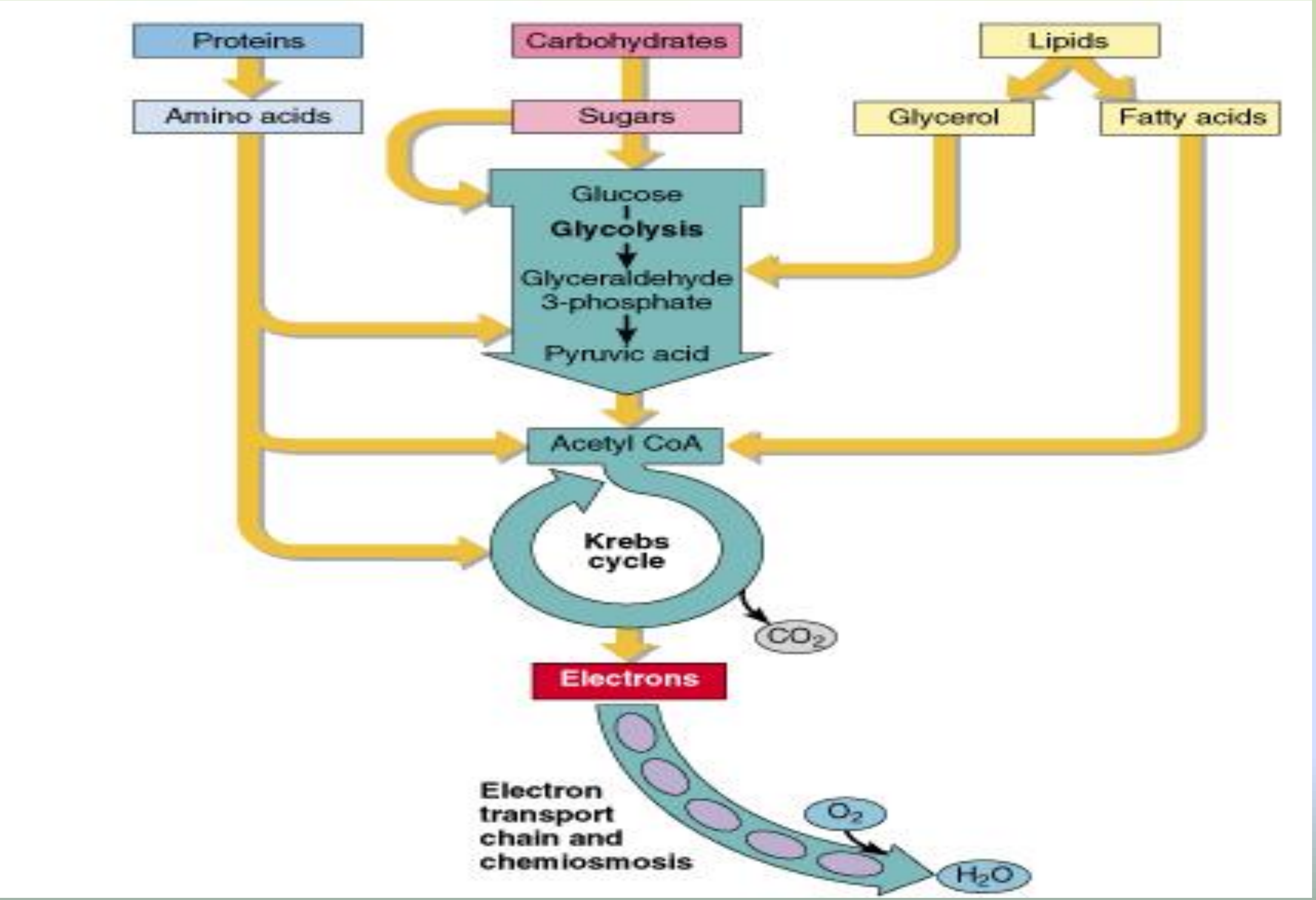
$$SO_4^{2-} \rightarrow H_2S$$

Sulfate reduction
(e.g., Desulfovibrio & Desulfotomaculatum)

$$S_o \rightarrow H_2S$$

Sulfur reduction
(Desulfuromonas)

Metabolism – An overview



Glycolytic Pathways

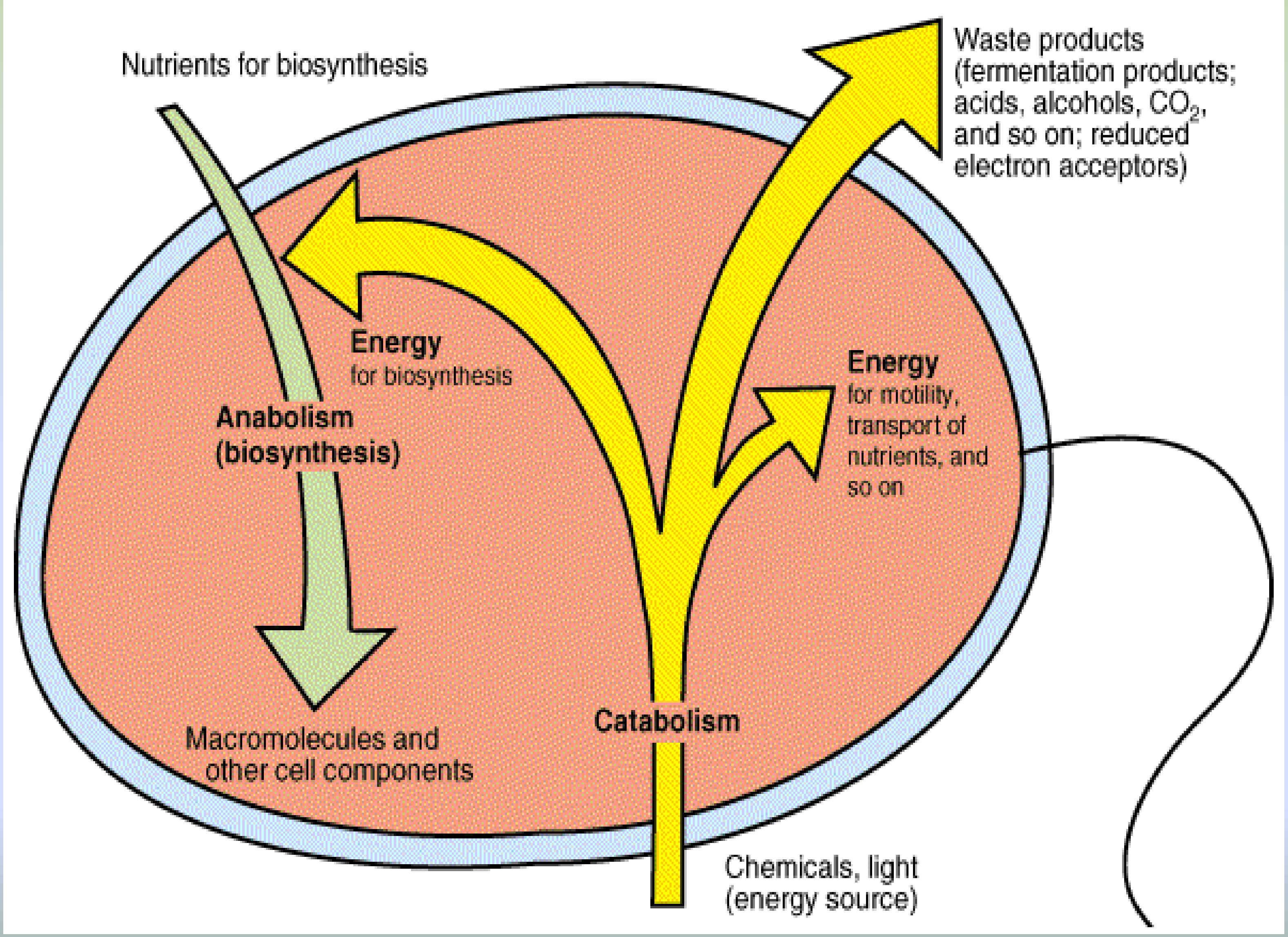
- **Features of glycolytic pathways**
  - Partial oxidation of glucose to form pyruvic acid
  - A small amount of ATP is made
  - A small amount of NAD is reduced to NADH

Glycolytic Pathways

Major glycolytic pathways found in different bacteria:

- **Embden-Meyerhoff-Parnas pathway**
  - “Classic” glycolysis
  - Found in almost all organisms
- **Hexose monophosphate pathway**
  - Also found in most organisms
  - Responsible for synthesis of pentose sugars used in nucleotide synthesis
- **Entner-Doudoroff pathway**
  - Found in ***Pseudomonas*** and related genera
- **Phosphoketolase pathway**
  - Found in ***Bifidobacterium*** and ***Leuconostoc***

Overview of Cell Metabolism



Energy Generating Patterns

- After Sugars are made or obtained, they are the major energy source of life.
- Breakdown of sugar (catabolism) different ways:
    - **Aerobic respiration**
    - **Anaerobic respiration**
    - **Fermentation**

Aerobic respiration

- Most efficient way to extract energy from glucose.
- Process: **Glycolysis**

  Kreb Cycle

  Electron transport chain
- **Glycolysis** : Several glycolytic pathways
- The most common one:

glucose-----> pyruvic acid + 2 NADH + 2ATP

General Outline of Aerobic Respiration

Glycolysis

↓ Transition Reaction

Krebs Cycle

↓

Electron Transport System

General Outline

Glucose

Glycolysis

Oxygen
Aerobic

Pyruvic Acid

No Oxygen
Anaerobic

Transition Reaction

Krebs Cycle

ETS

36 ATP

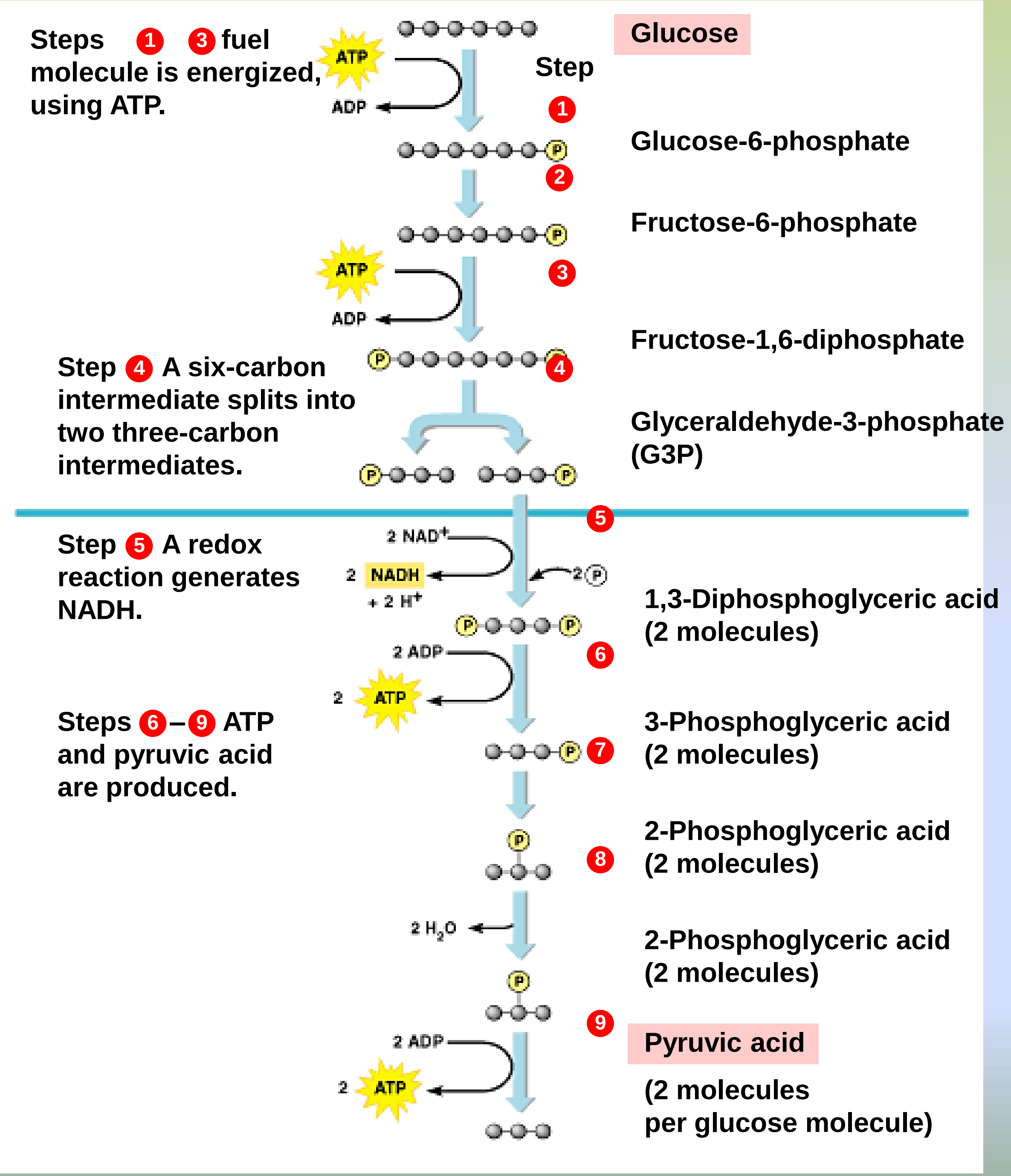
Fermentation

Glycolysis

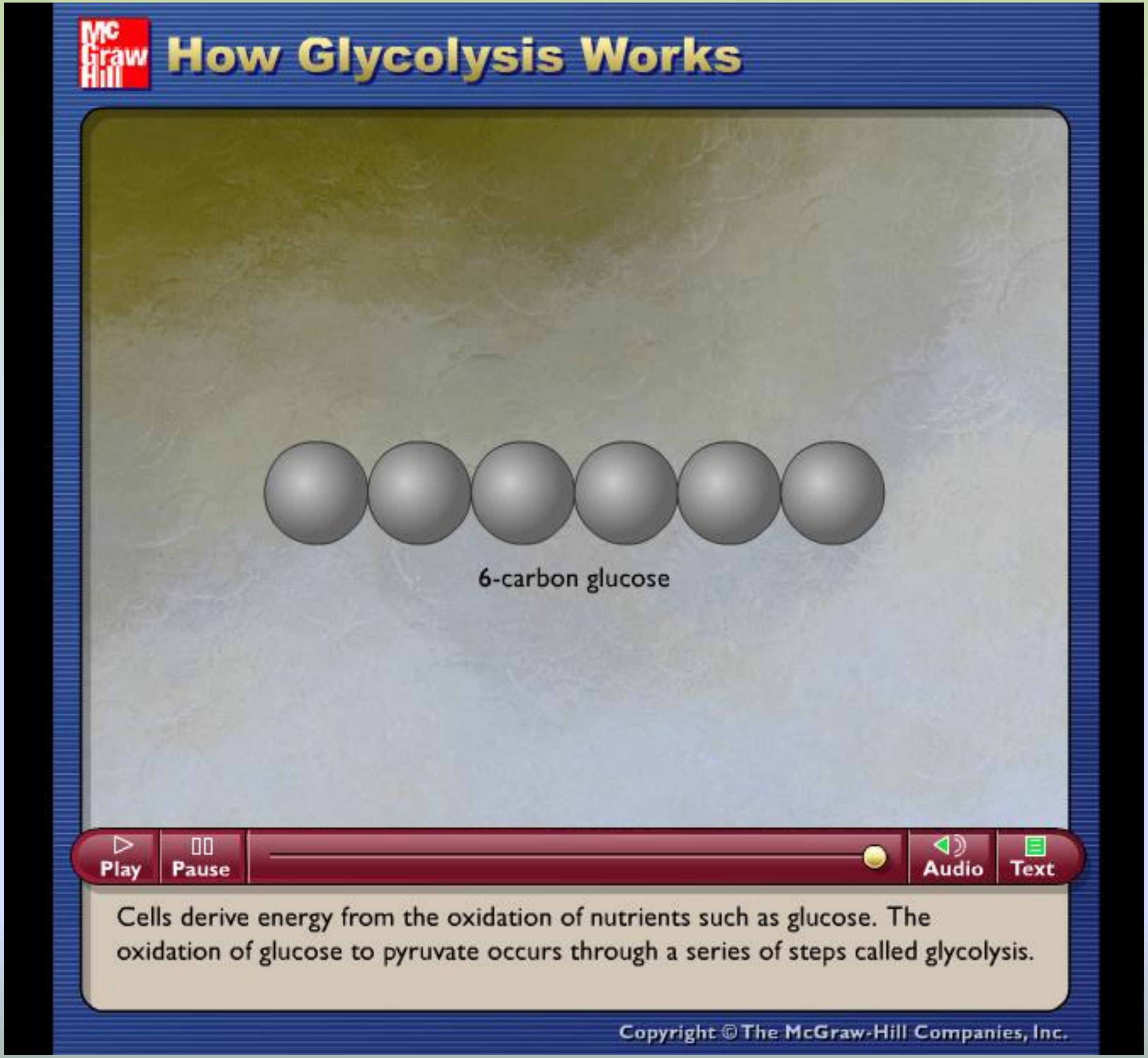
Energy In: 2 ATP

Energy Out: 4 ATP

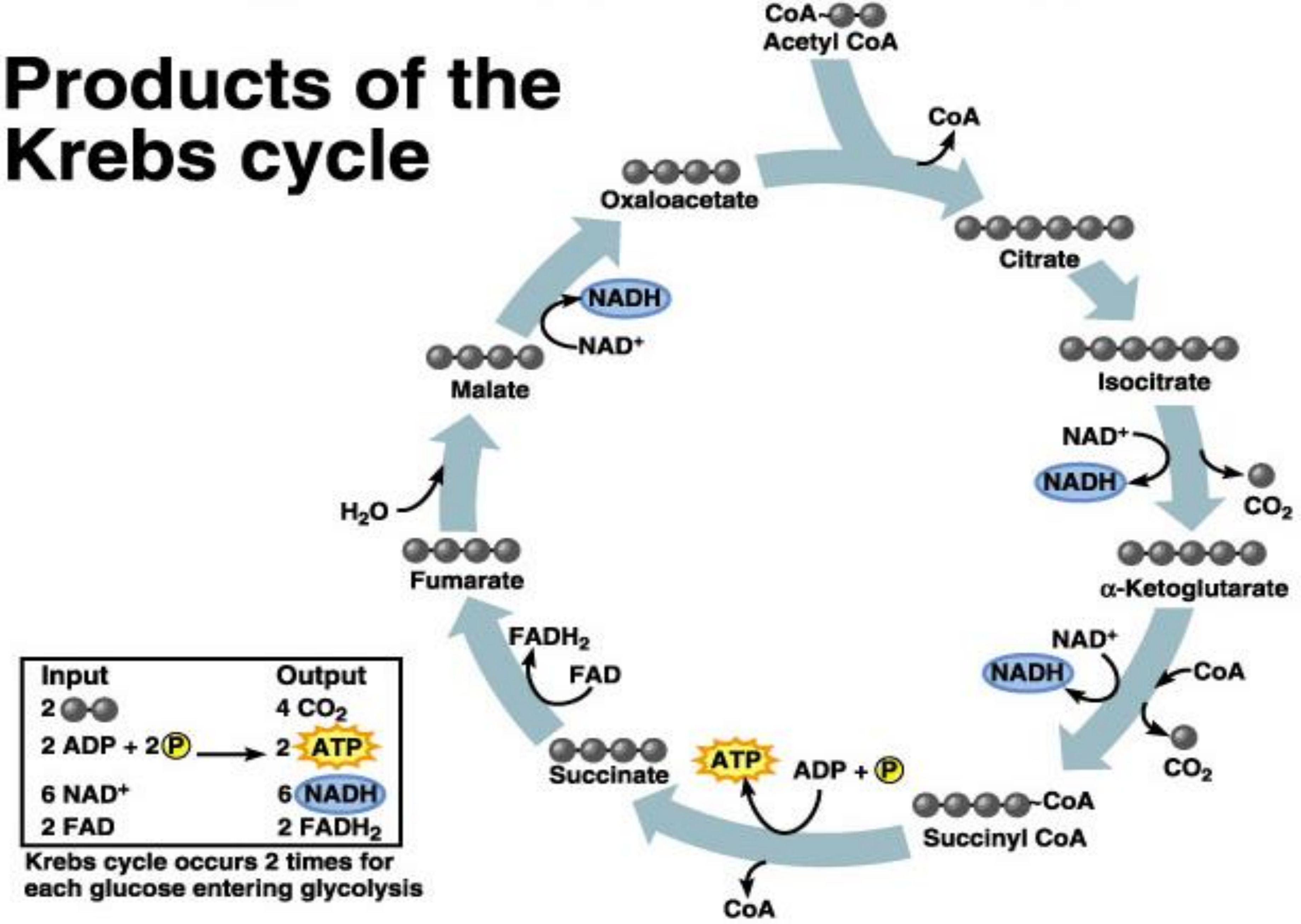
NET 2 ATP



How Glycolysis Works



Krebs Cycle

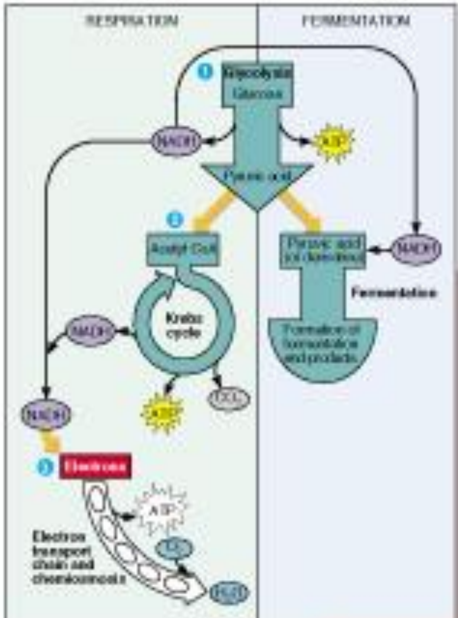


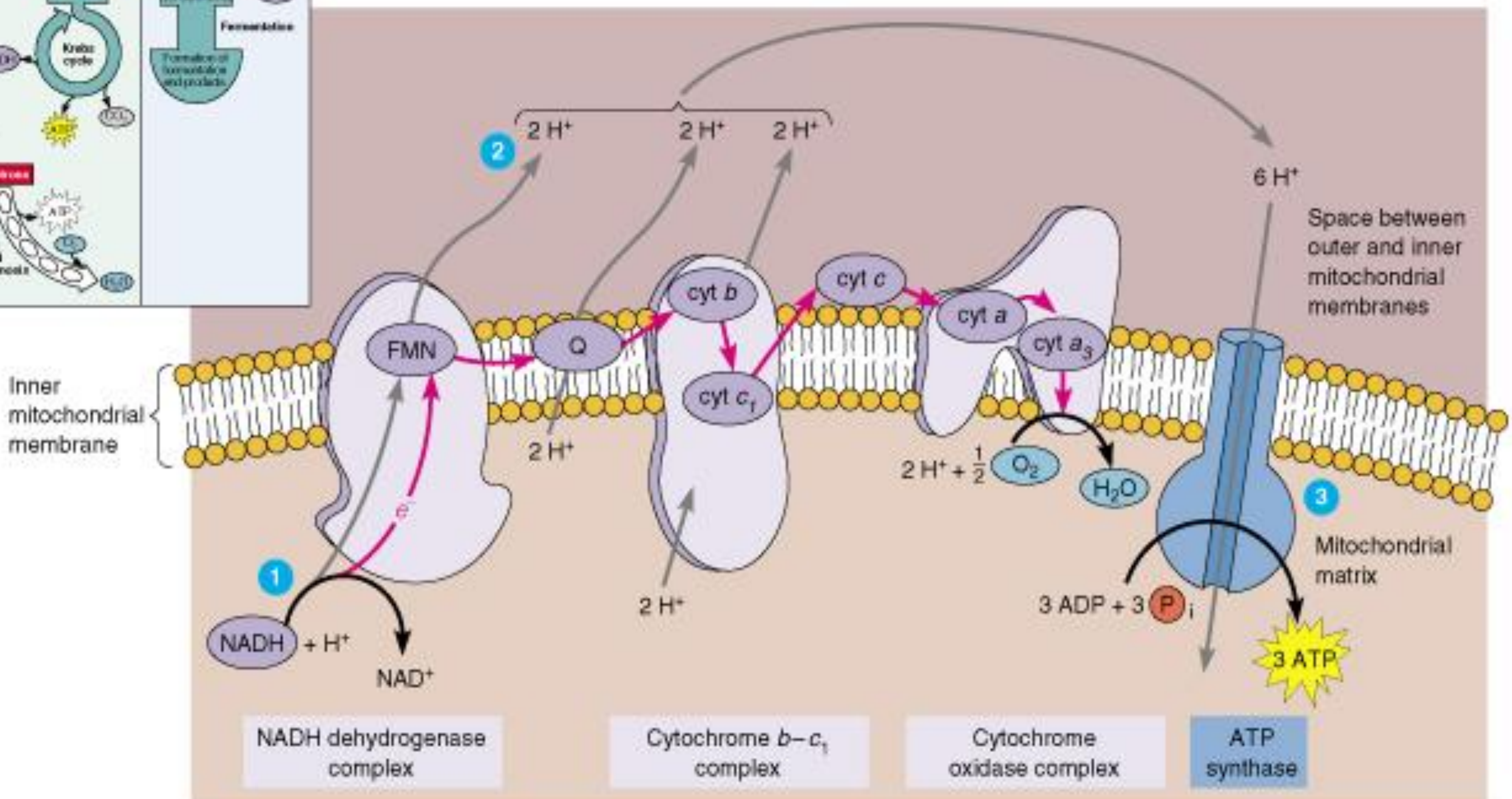
Products of the Krebs cycle
CoA
Acetyl CoA
CoA
Oxaloacetate
Citrate
Isocitrate
NAD+
NADH
CO_2
α-Ketoglutarate
NAD+
NADH
CoA
CO_2
CoA
Succinyl CoA
ADP + P
ATP
CoA
Succinate
FADH2
FAD
Fumarate
H_2O
Malate
NAD+
NADH
Input
2
2 ADP + 2 P
6 NAD+
2 FAD
Output
4 CO_2
2 ATP
6 NADH
2 $FADH_2$
Krebs cycle occurs 2 times for each glucose entering glycolysis

Electron Transport Chain

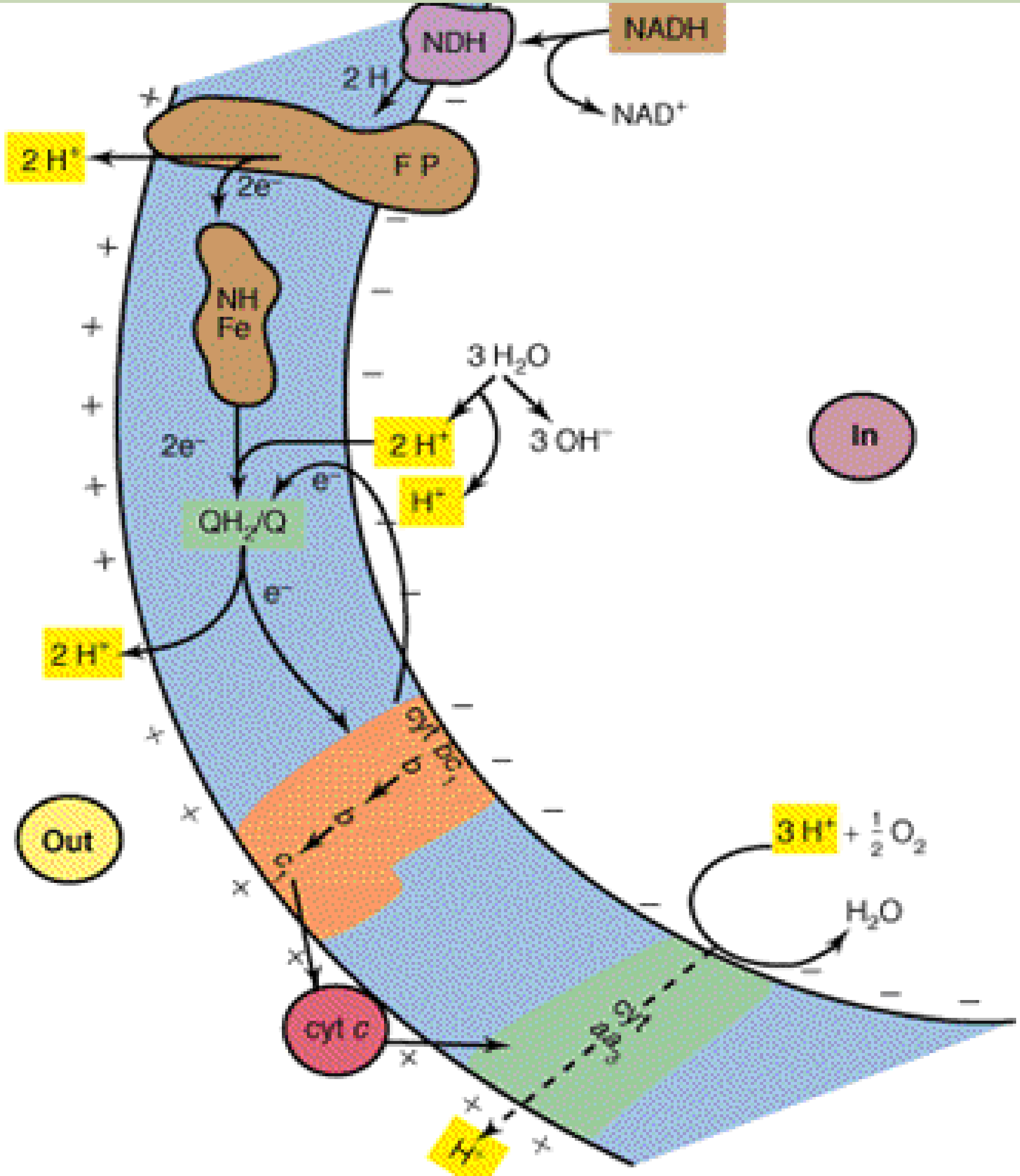
- Groups of redox proteins
  - On inner mitochondrial membrane
  - Binding sites for NADH and $FADH_2$
    - **On matrix side of membrane**
    - **Electrons transferred to redox proteins**
    - **NADH reoxidized to NAD^+**
    - **$FADH_2$ reoxidized to FAD**

ETC

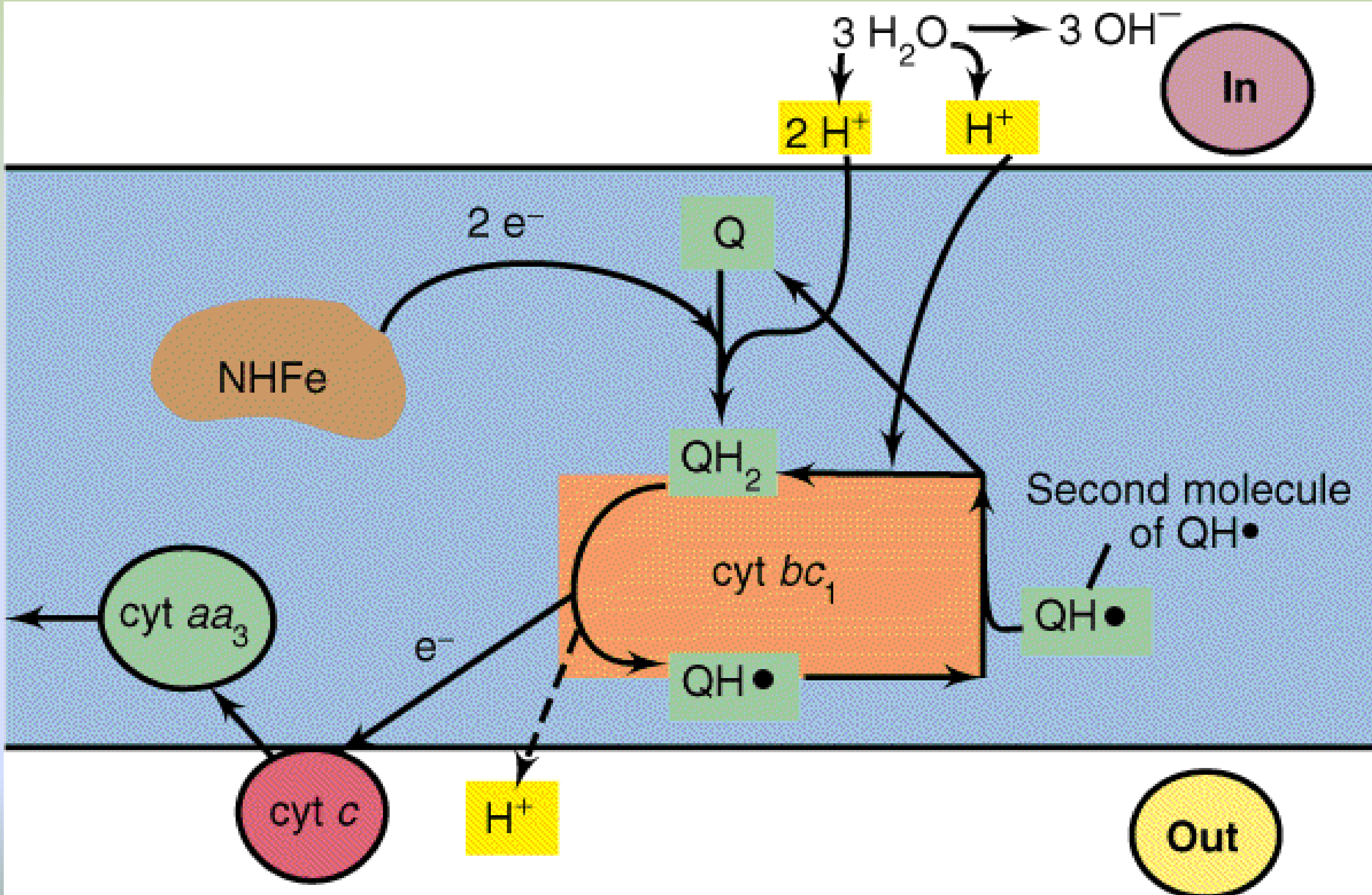

RESPIRATION
FERMENTATION
Krebs cycle
Fermentation


2 H⁺
2 H⁺
2 H⁺
6 H⁺
Space between outer and inner mitochondrial membranes
cyt c
cyt b
cyt a
cyt a3
FMN
Q
cyt c1
Inner mitochondrial membrane
2 H⁺
2 H⁺ + 1/2 O2
H2O
e⁻
Mitochondrial matrix
NADH + H⁺
NAD⁺
2 H⁺
3 ADP + 3 P i
3 ATP
NADH dehydrogenase complex
Cytochrome b–c1 complex
Cytochrome oxidase complex
ATP synthase

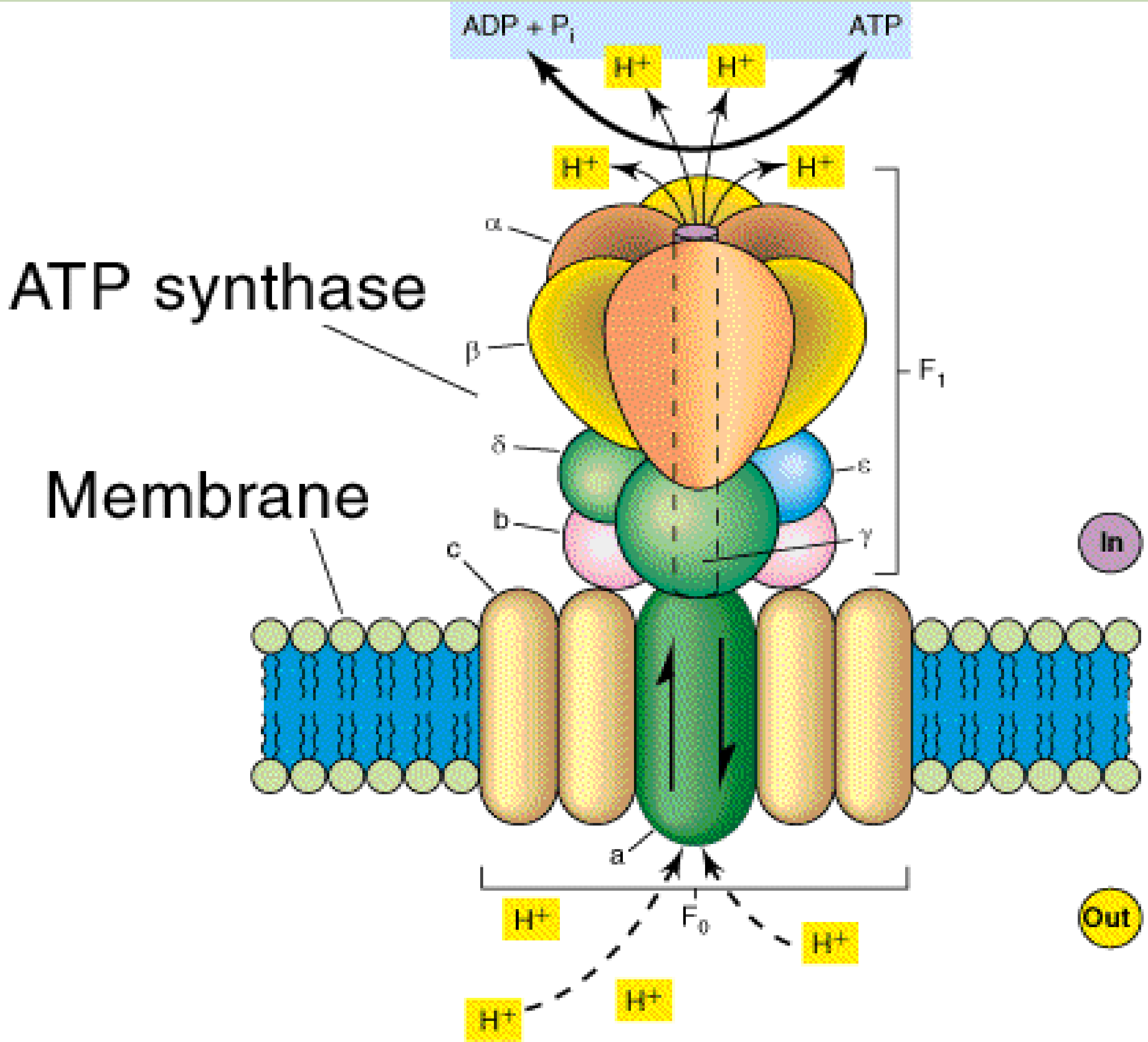
Generation of a proton-motive force(1)



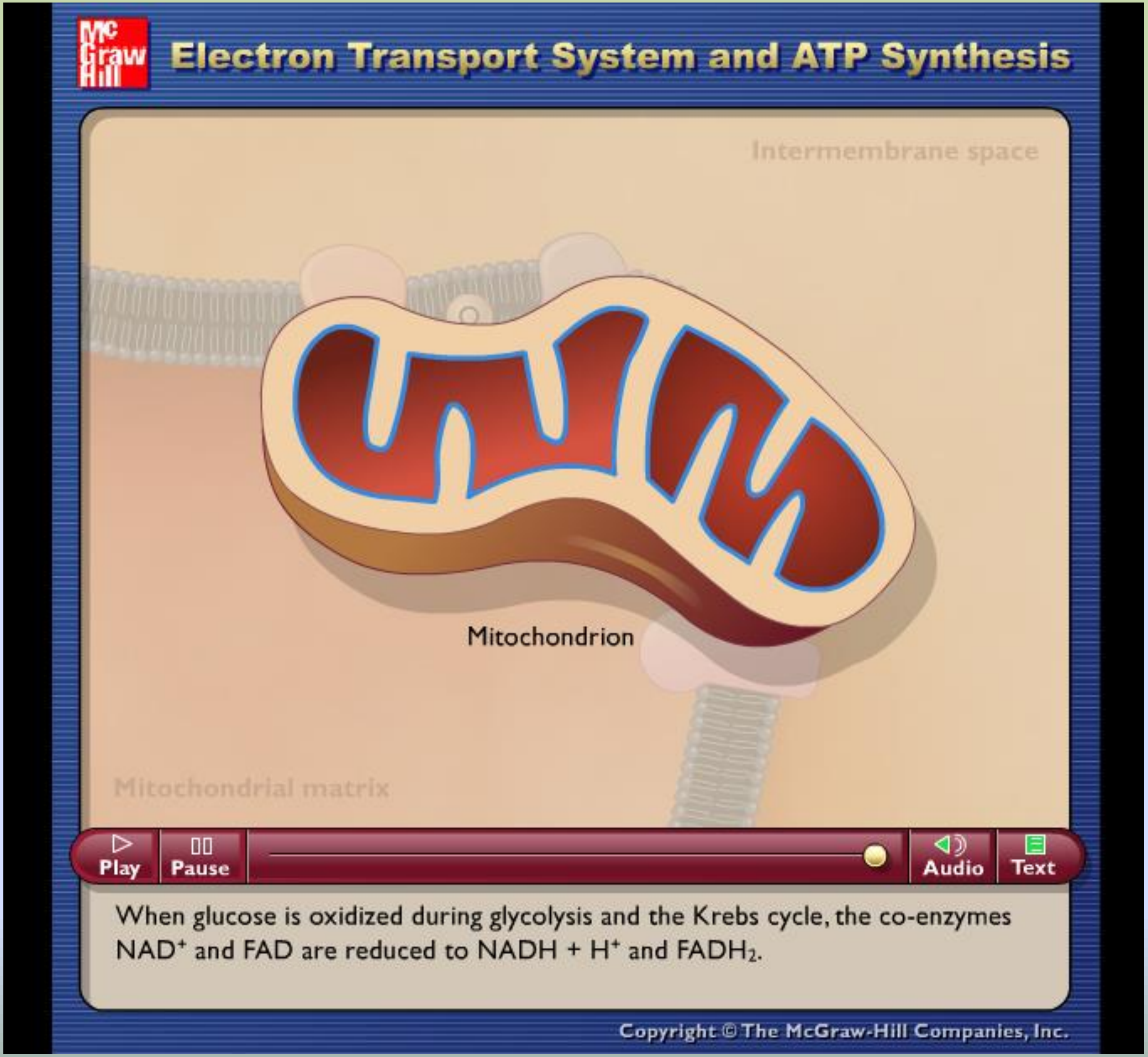
Generation of a proton-motive force(2)



Mechanism of ATPase



Electron Transport System and ATP Synthesis



Anaerobic respiration

– Final electron acceptor : **never be O2**

- **Sulfate reducer**: final electron acceptor is sodium sulfate (**Na_2SO_4**)
- **Methane reducer**: final electron acceptor is **CO_2**
- **Nitrate reducer** : final electron acceptor is sodium nitrate (**$NaNO_3$**)

O_2/H_2O coupling is the most oxidizing, more energy in aerobic respiration.

Therefore, anaerobic is less energy efficient.

Fermentation (F)

- **Glycosis:**

Glucose ----->2 Pyruvate (P.A)+ 2ATP + 2NADH

- **Fermentation pathways**

a. Homolactic acid F.

P.A -----> Lactic Acid

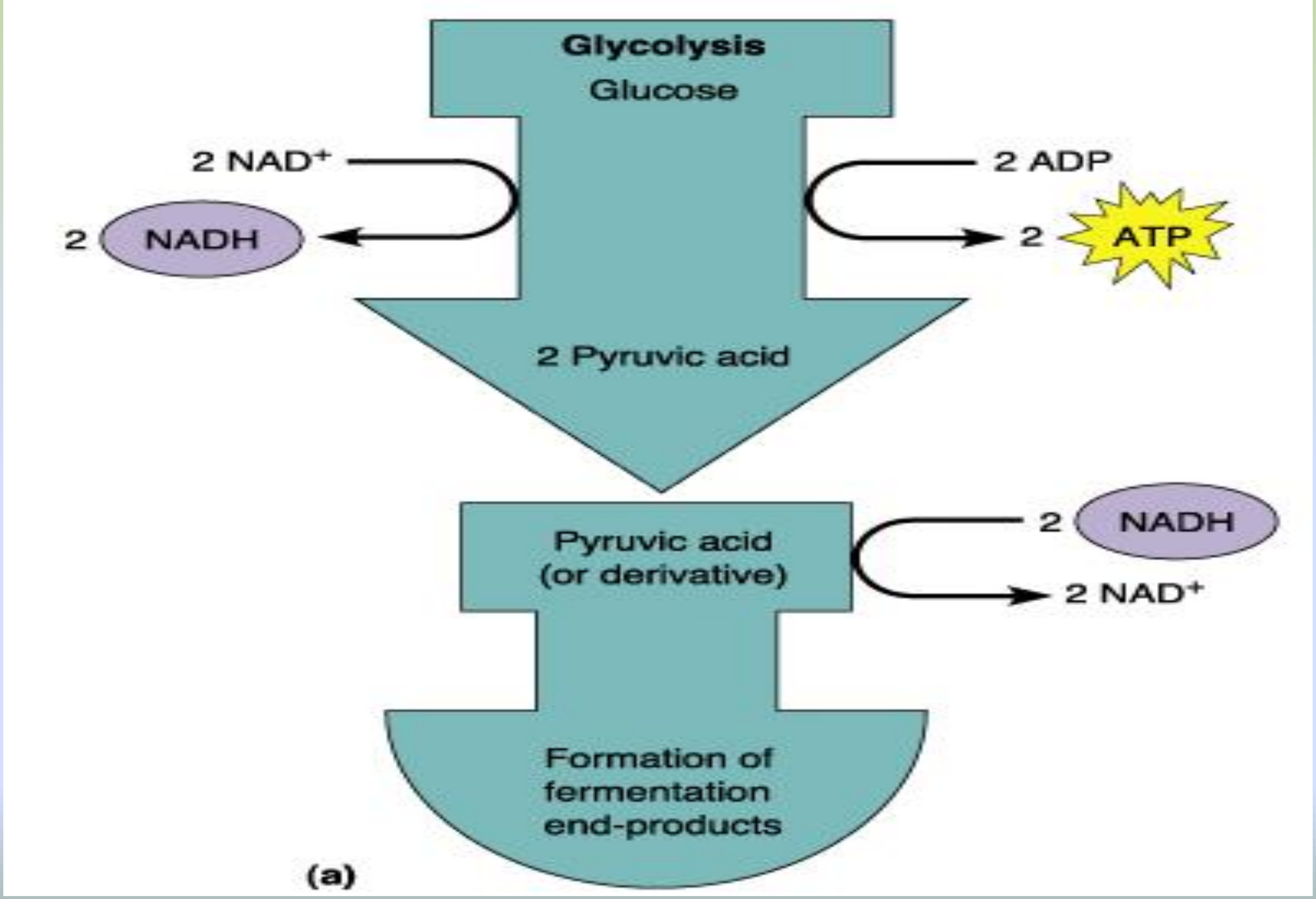
eg. *Streptococci*, *Lactobacilli*

b. Alcoholic F.

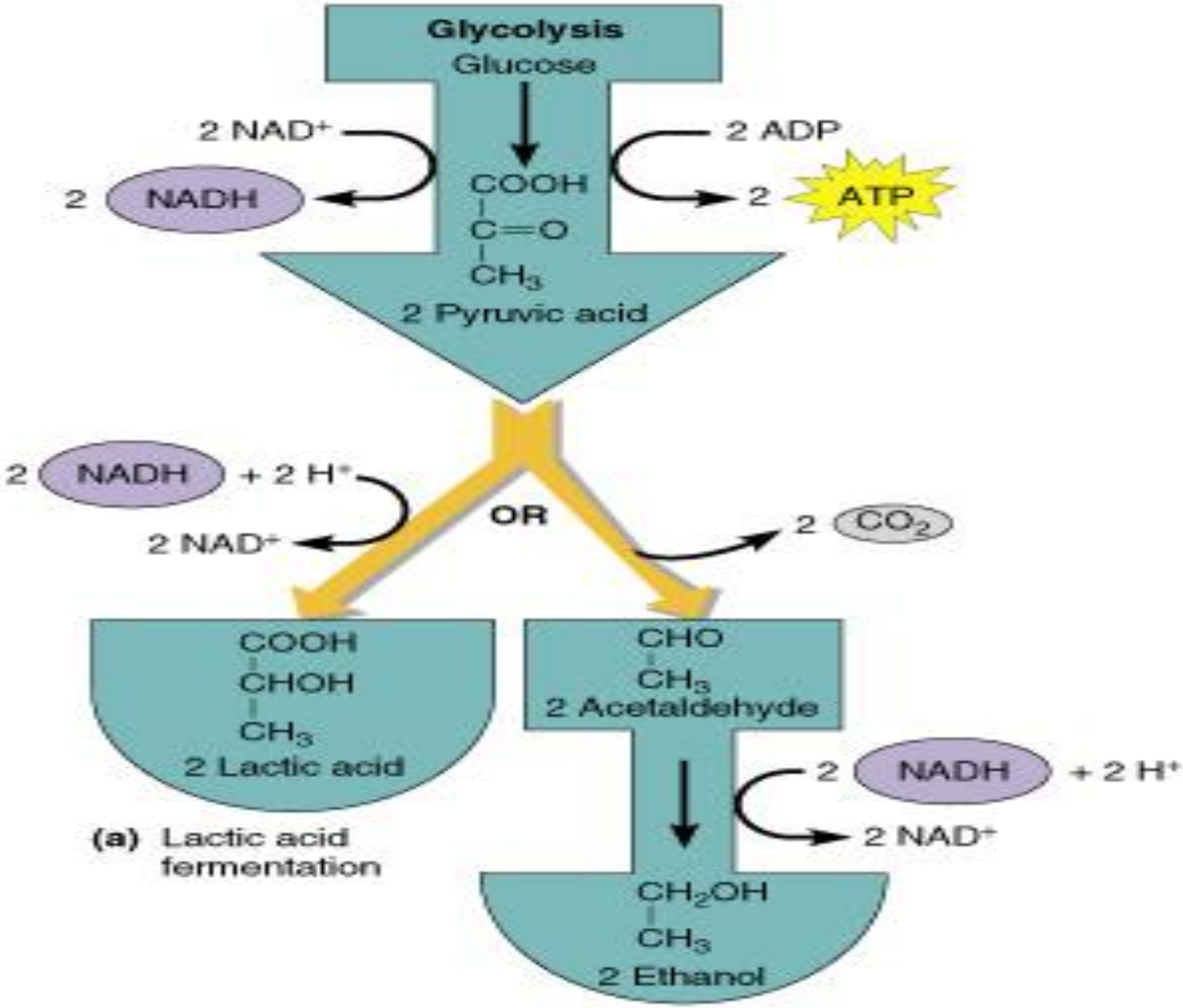
P.A -----> Ethyl alcohol

eg. yeast

Fermentation – An overview



Alcoholic fermentation



(a) Lactic acid fermentation

(b) Alcohol fermentation

c. **Mixed acid fermentation**

P.A -----> lactic acid

acetic acid

$H_2 + CO_2$

succinic acid

ethyl alcohol

eg. ***E.coli*** and some **Enterobacter**

d. **Butylene-glycol F.**

P.A -----> 2,3, butylene glycol

eg. ***Pseudomonas***

e. **Propionic acid F.**

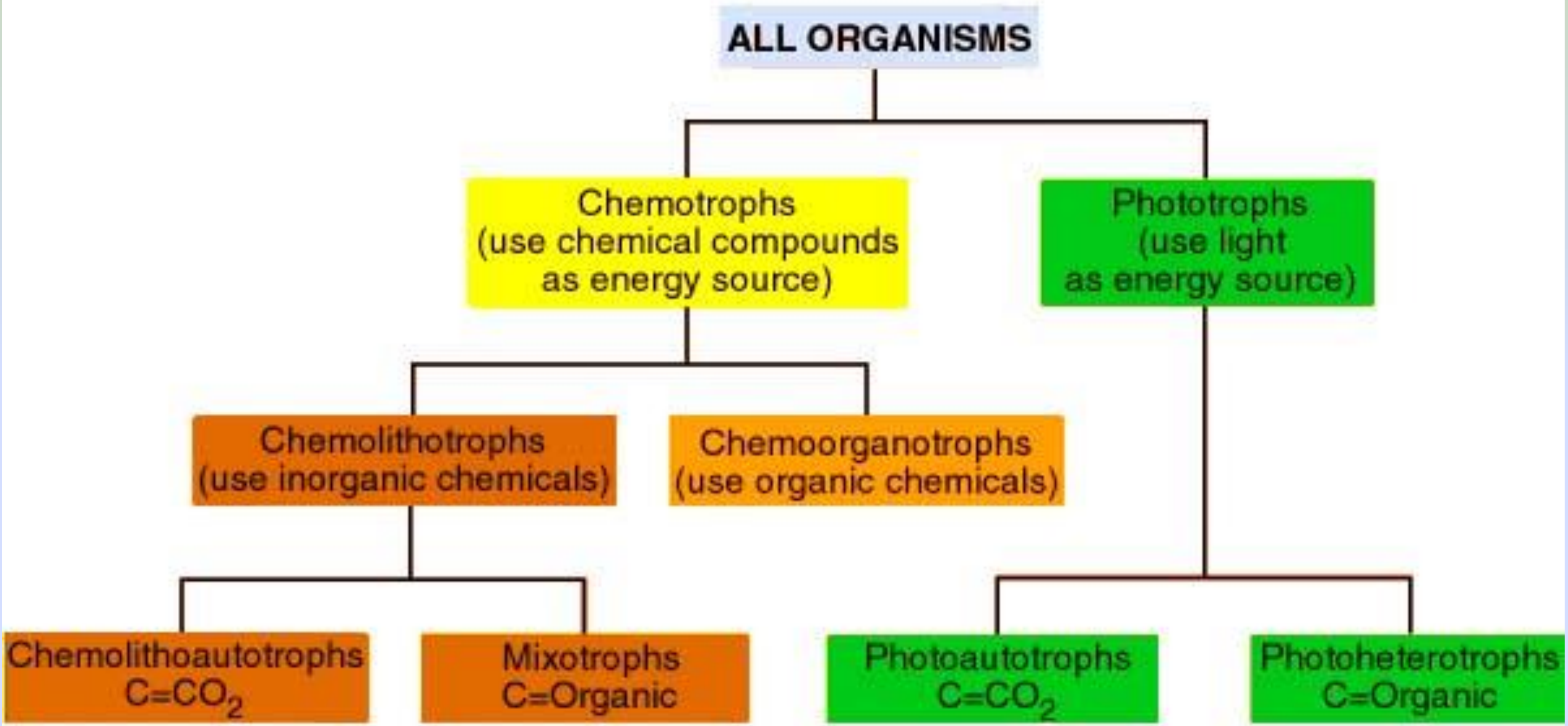
P.A -----> 2 propionic acid

eg. ***Propionibacterium***

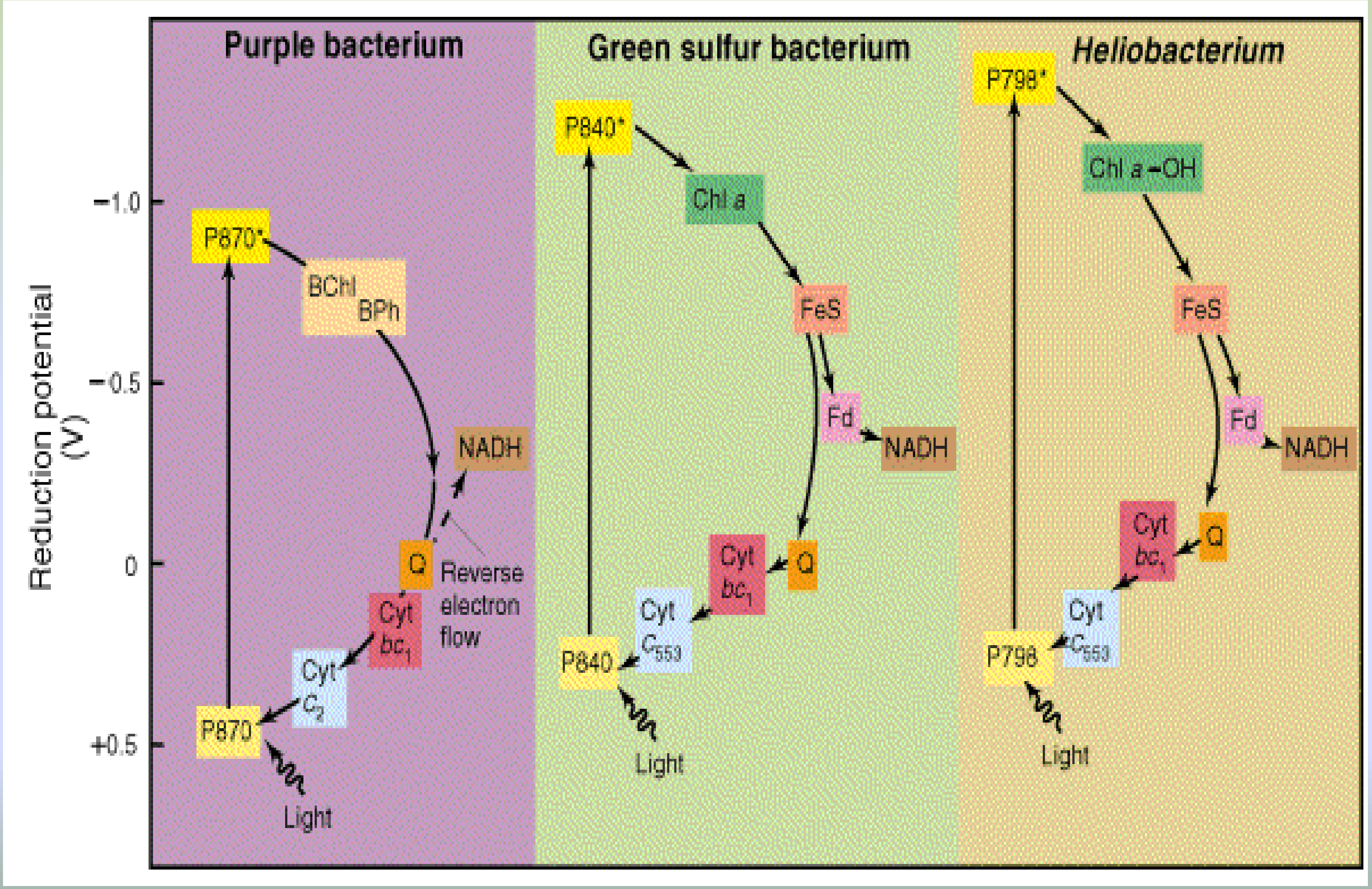
Metabolic strategies

	Pathways involved	Final e- acceptor	ATP yield
Aerobic respiration	Glycolysis, TCA, ET	O_2	38
Anaerobic respiration	Glycolysis, TCA, ET	NO_3^-, SO_4^{-2}, CO_3^{-3}	variable
Fermentat ion	Glycolysis	Organic molecules	2

Energy/carbon classes of organisms



Comparison of reaction centers of anoxyphototrophs



Photosynthesis

- **Overview of Photosynthesis**
  - **Light-dependent Reactions:**
    - Light energy is harvested by photosynthetic pigments and transferred to special reaction center (photosystem) chlorophyll molecules.
    - The light energy is used to strip electrons from an electron donor (the electron donor goes from a reduced to an oxidized state).
    - The electrons are shuttled through a series of electron carriers from high energy state to a low energy state.
    - During this process, ATP is formed.
    - In the cyclic pathway of electron transport, electrons are returned to the electron transport chain
    - In the noncyclic pathway, the electrons are used to reduce NAD (or NADP) to NADH (or NADPH)

Photosynthesis

Light-independent Reactions:

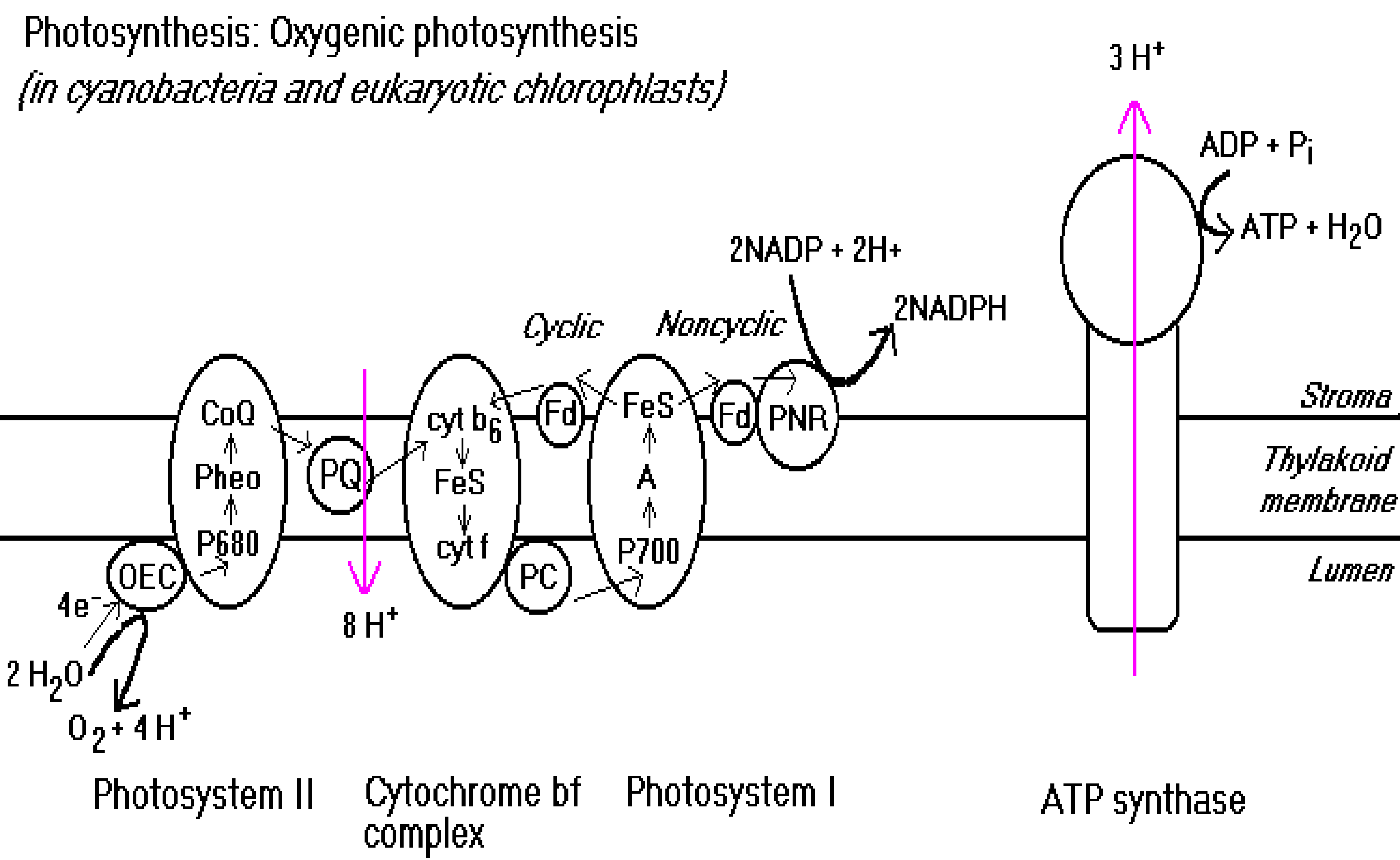
- ATP and NADH (NADPH) from the light-dependent reactions are used to reduce CO_2 to form organic carbon compounds (carbon fixation).
- The reduced organic carbon is usually converted into glucose or other carbohydrates.

Photosynthesis

Oxygenic photosynthesis

- Found in cyanobacteria (blue-green algae) and eukaryotic chloroplasts
- Electron donor is H_2O: Oxidized to form O_2
- Two photosystems: PSII and PSI
- Major function is to produce NADPH and ATP for the carbon fixation pathways

Oxygenic photosynthesis



Photosynthesis

Anoxygenic photosynthesis

– Found in:
  - Green sulfur bacteria (e.g. *Chlorobium*)
  - Green nonsulfur bacteria (e.g. *Chloroflexus*)
  - Purple sulfur bacteria (e.g. *Chromatium*)
  - Purple nonsulfur bacteria (e.g. *Rhodobacter*)

Photosynthesis

Anoxygenic photosynthesis (cont.)

b) **Electron donors vary:**

- H_2S or S_o in the green and purple sulfur bacteria
- H_2 or organic compounds in the green and purple nonsulfur bacteria

c) Only one photosystem

- In green bacteria, the photosystem is similar to PSI
- In purple bacteria, the photosystem is similar to PSII

d) Primary function is ATP production, chiefly via cyclic photophosphorylation

Photosynthetic bacteria

(1) **Chlorobium-green sulfur bacteria**

Use green pigment chlorophyll

Use H_2S (hydrogen sulfide), S (sulfur), $Na_2S_2O_3$ (sodium thiosulfate) and H_2 as e- donors.

(2) **Chromatium-purple sulfur bacteria**

Use purple carotenoid pigment, same e-donors

(3) ***Rhodospirillum***-non sulfur purple bacteria

Use H_2 and other organic compounds such as isopropanol etc, as e-donors.

Reaction: $CO_2 + 2H_2\mathbf{A} \text{ -----> } CH_20 + H_20 + 2\mathbf{A}$

- A is not O

Chemolithotrophy

- **Features of Chemolithotrophy**
  - Electrons are removed from a reduced inorganic electron donor
  - The electrons are passed through a membrane-bound electron transport pathway, often coupled to the synthesis of ATP and NADH
  - The electrons are ultimately passed to a final electron acceptor
  - ATP and NADH may be used to convert CO_2 to carbohydrate

Chemolithotrophy

Examples of electron donors

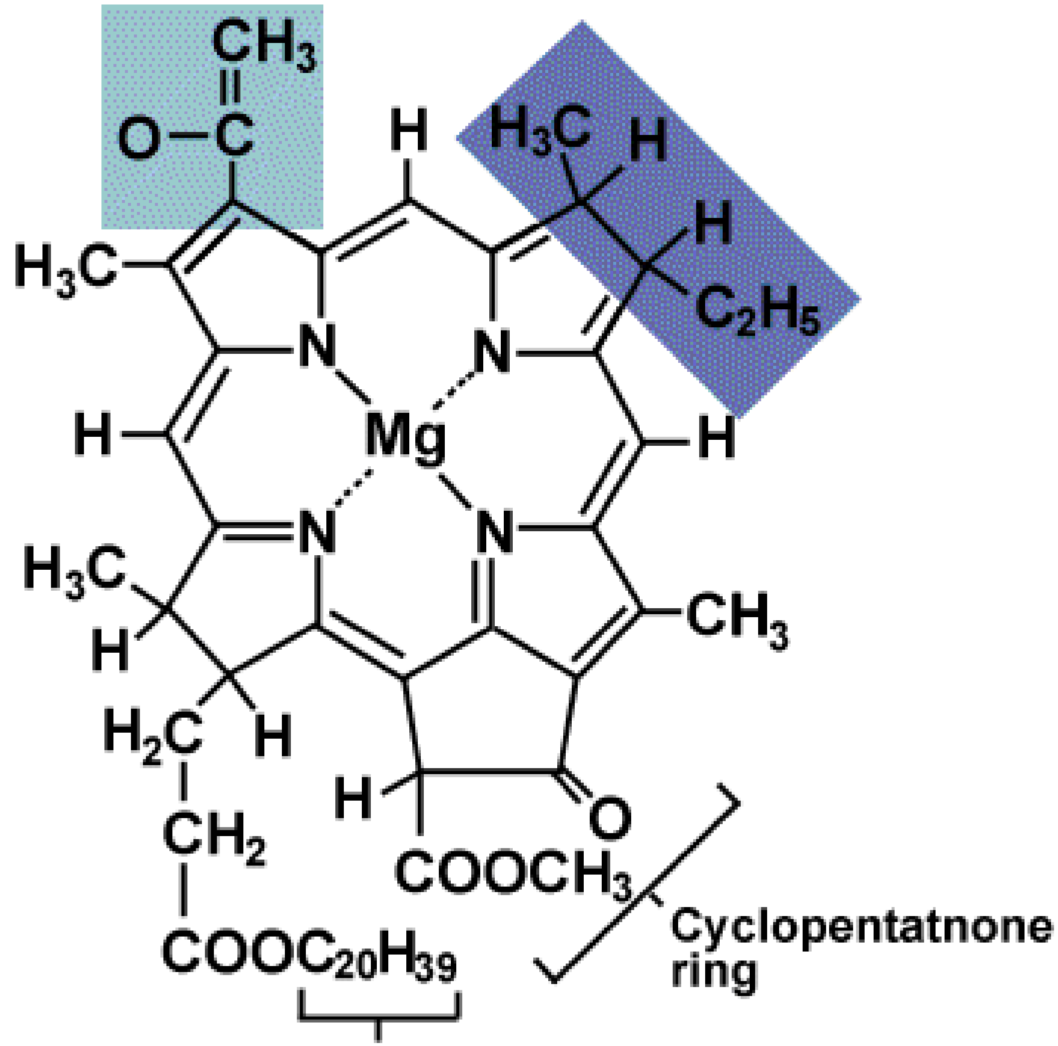
- **Ammonia (NH_4^+)** $\rightarrow$ Nitrite (NO_2^-) in *Nitrosomonas*
- **Nitrite (NO_2^-)** $\rightarrow$ Nitrate (NO_3^{2-}) in *Nitrobacter*
- **Hydrogen sulfide (H_2S)** $\rightarrow$ Sulfur (S_o) in *Thiobacillus* and *Beggiatoa*
- **Sulfur (S_o)** $\rightarrow$ Sulfate (SO_4^{2-}) in *Thiobacillus*
- **Hydrogen (H_2)** $\rightarrow$ Water (H_2O) in *Alcaligenes*

Chemolithotrophy

Examples of electron acceptors

- **Oxygen** (O_2) → Water (H_2O)
  in many organisms
- **Carbon dioxide** (CO_2) → Methane (CH_4)
  in the methanogenic bacteria

Chlorophyll a and bacteriochlophyll a



Bacteriochlorophyll *a*

Chemoautotroph

- Some bacteria use O_2 in the air to oxidize inorganic compounds and produce ATP (energy). The energy is enough to convert CO_2 into organic material needed for cell growth.
- Examples:

  Thiobacillus (sulfur S)

  Nitrosomonas (ammonia)

  Nitrobacter (nitrite)
- Various genera (hydrogen etc.)

THANK YOU