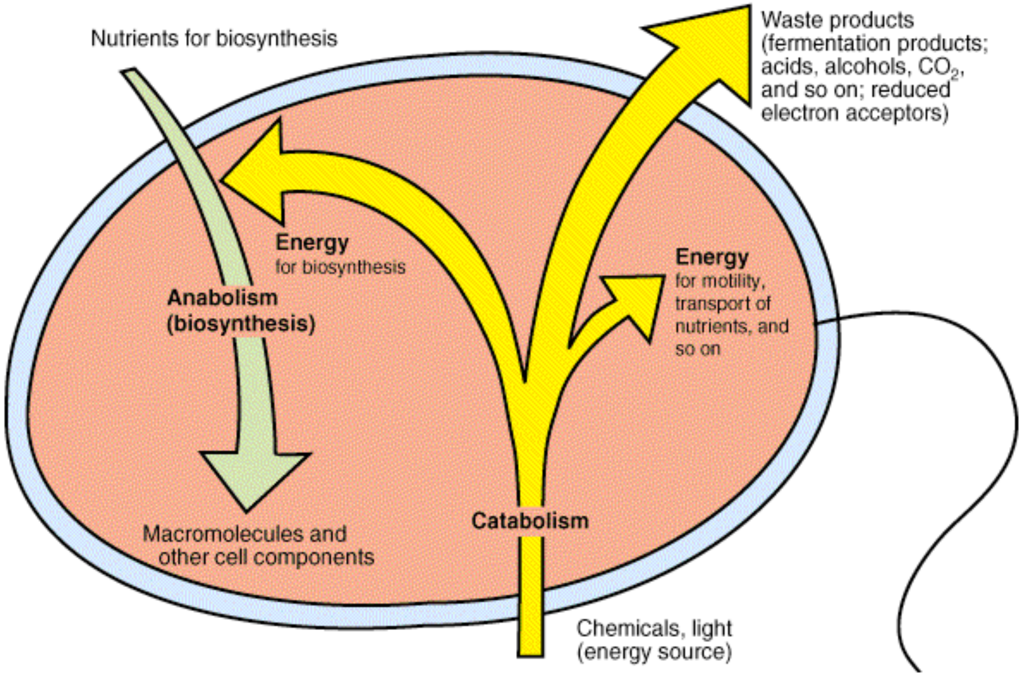
Lecture 12

BT 206

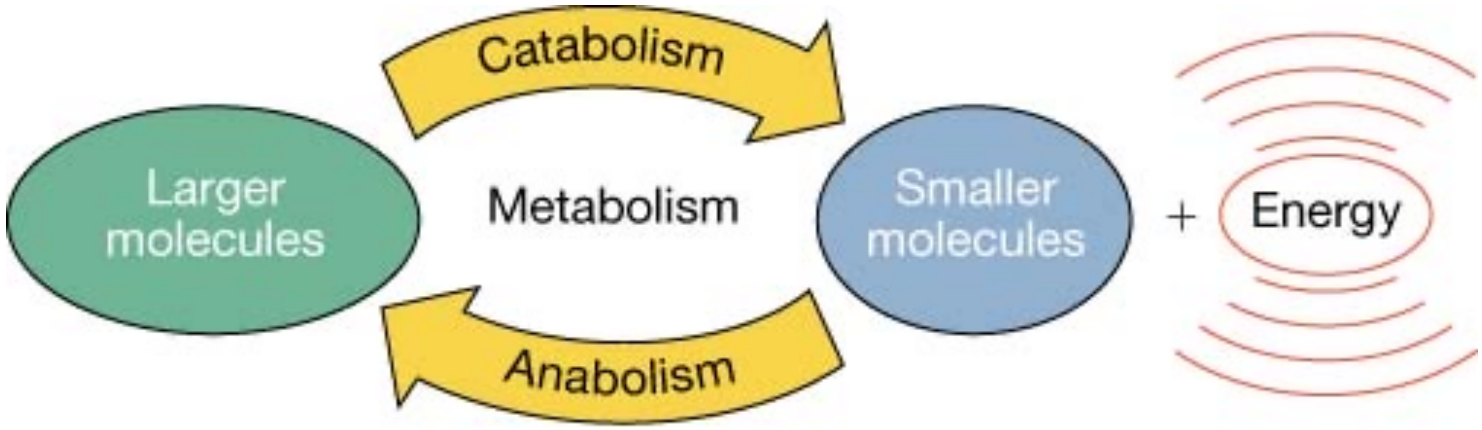
10/02/23

Microbial Metabolism

- Metabolism is the sum of the chemical reactions in an organism.
- **Catabolism** is the energy-releasing processes.
- **Anabolism** is the energy-using processes.



Metabolism Relationships



Microbial Metabolism

- Catabolism provides the building blocks and energy for anabolism.

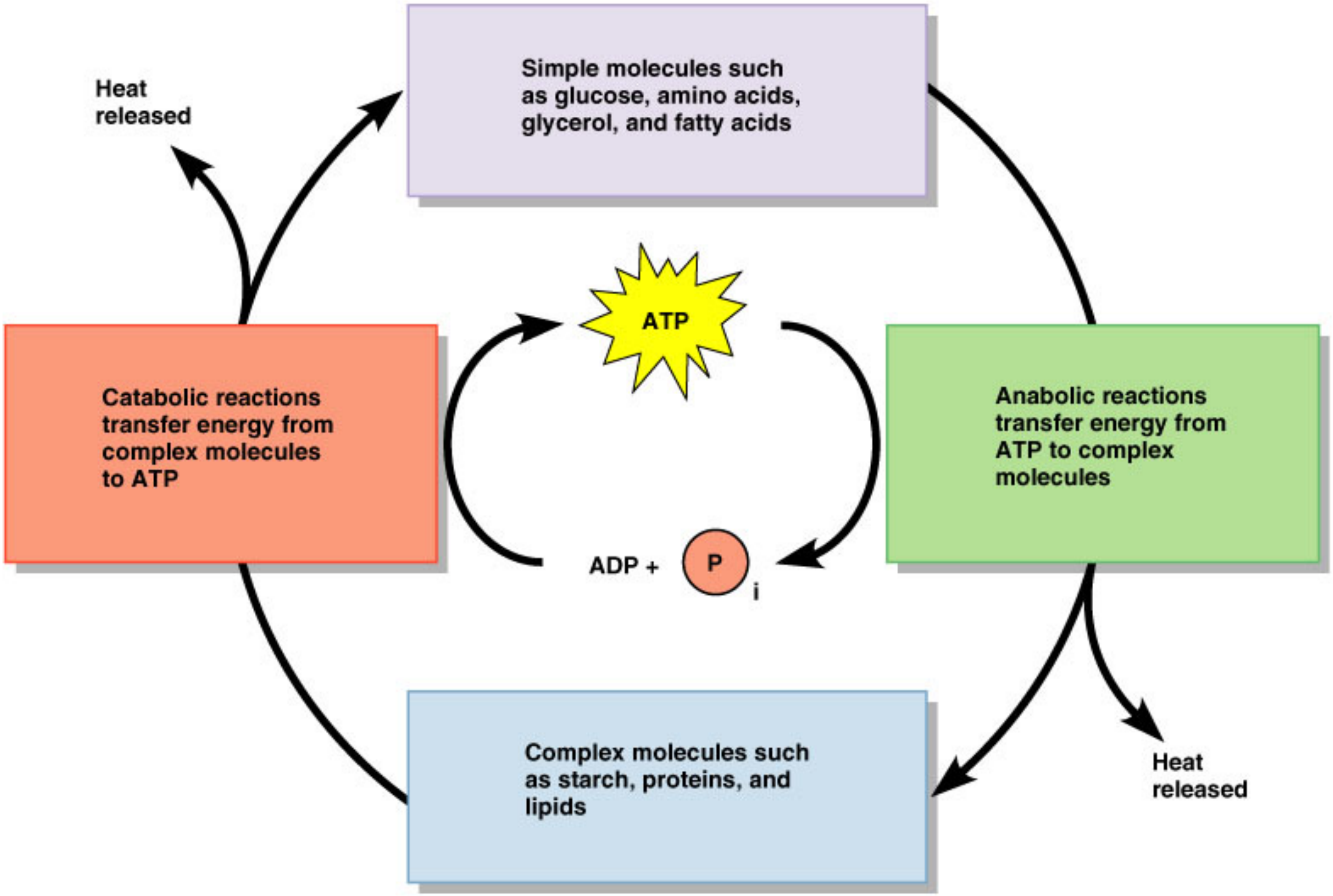
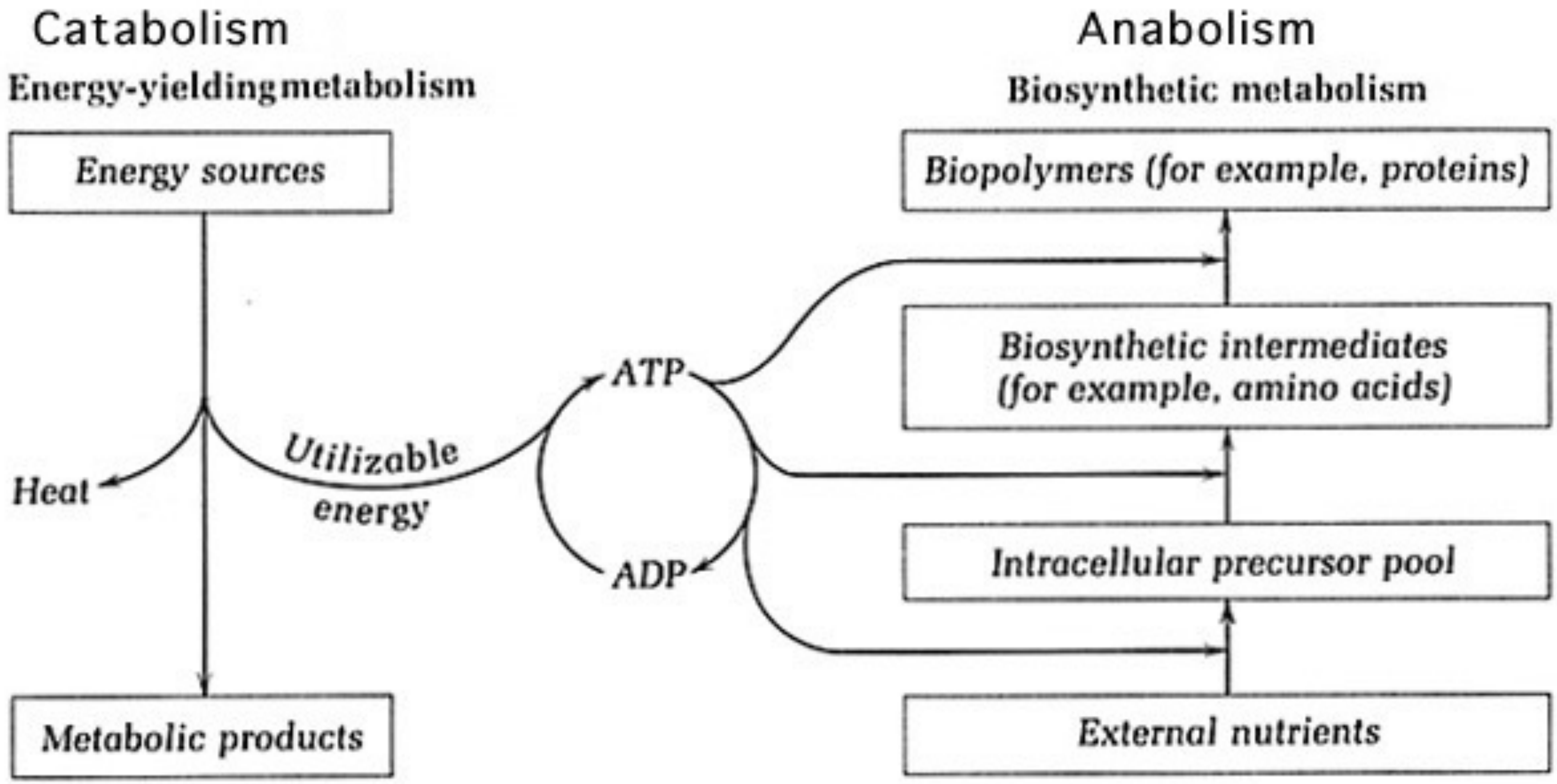


Figure 5.1

Metabolism Pathways



Energy

- **Activation energy** is needed to disrupt electronic configurations
- **Reaction rate** is the frequency of collisions with enough energy to bring about a reaction.
- Reaction rate can be increased by enzymes or by increasing temperature or pressure

Metabolism: Enzymes

- A metabolic pathway is a sequence of enzymatically catalyzed chemical reactions in a cell.
- Metabolic pathways are determined by enzymes.
- Enzymes are encoded by genes.

Enzymes

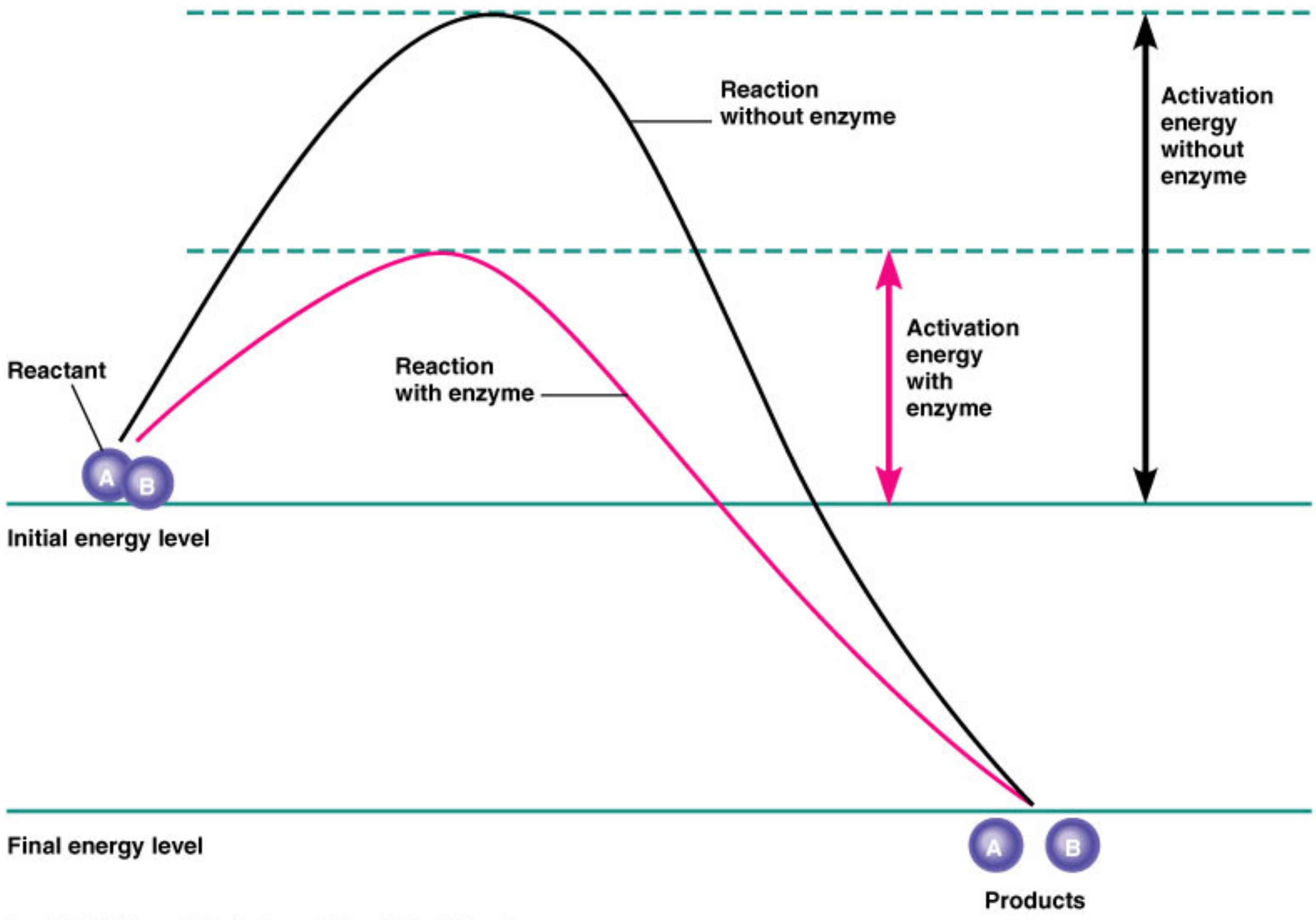
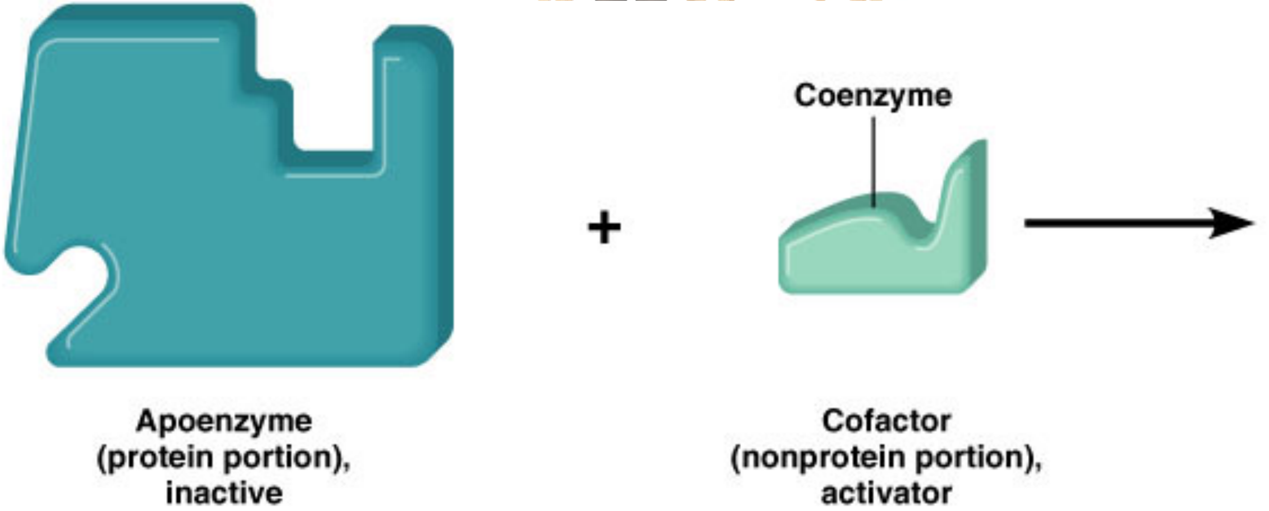


Figure 5.2

Enzymes

- Biological catalysts
  - Specific for a chemical reaction; not used up in that reaction
- Apoenzyme: protein
- Cofactor: Nonprotein component - metal - Fe
  - Coenzyme: Organic cofactor - vitamin
- Holoenzyme: Apoenzyme + cofactor

Enzymes



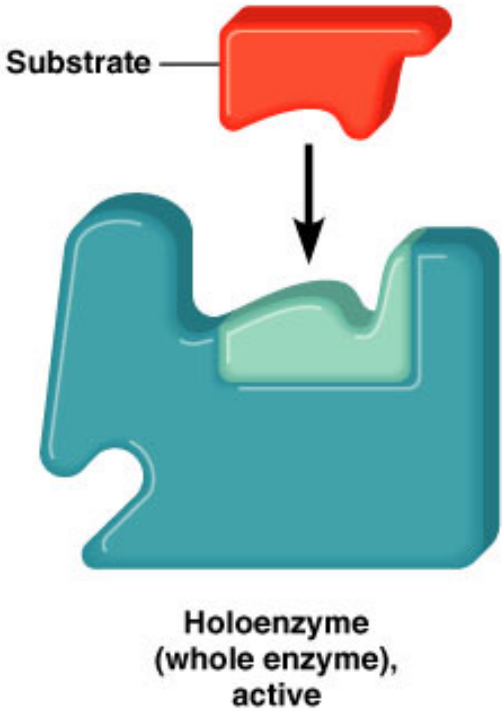


Figure 5.3

Important Coenzymes

- NAD^+
- $NADP^+$
- FAD
- Coenzyme A
- CoQ

Enzymes

- The turnover number is generally 1-10,000 molecules per second!

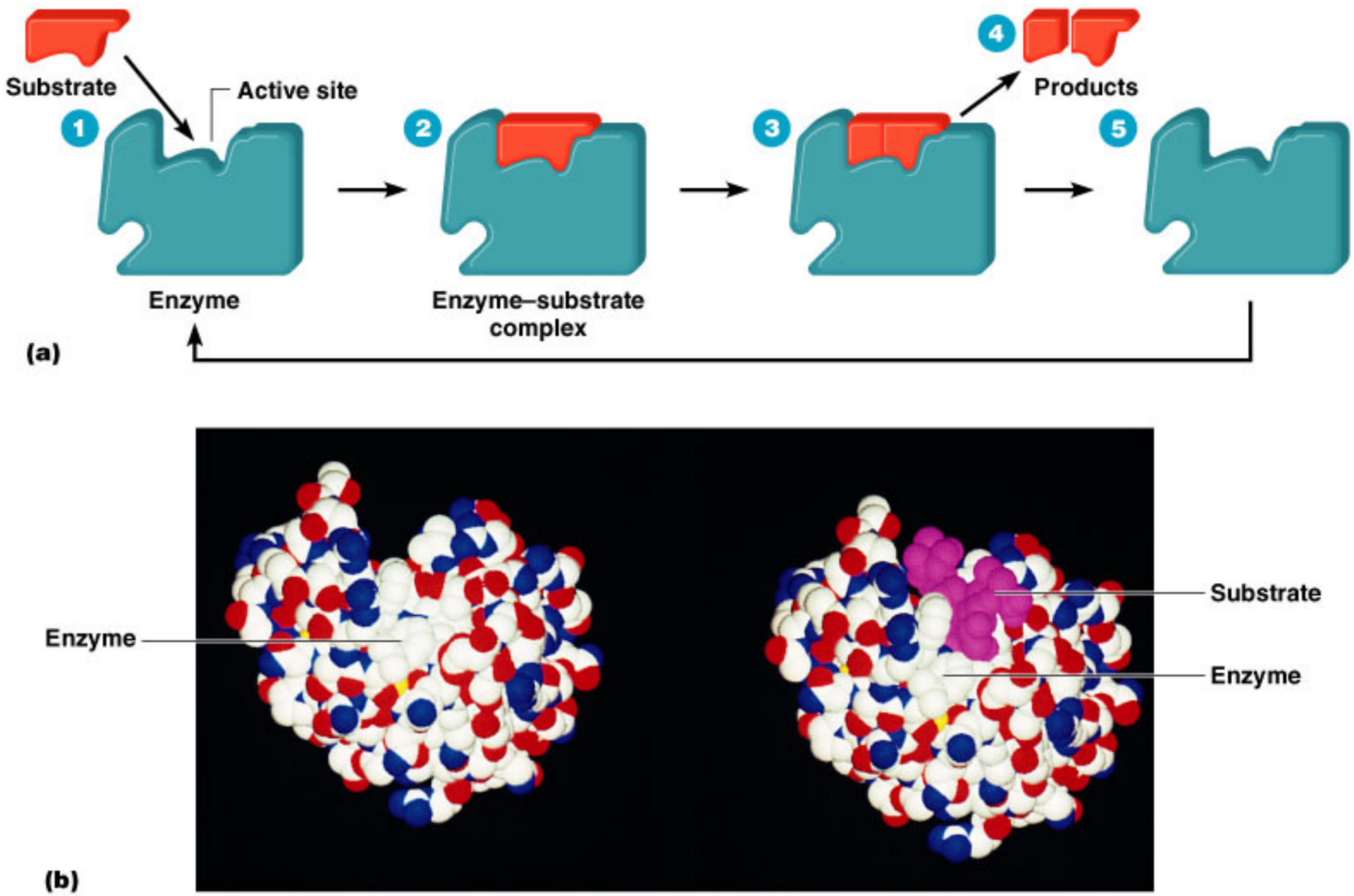


Figure 5.4

Enzyme Classification

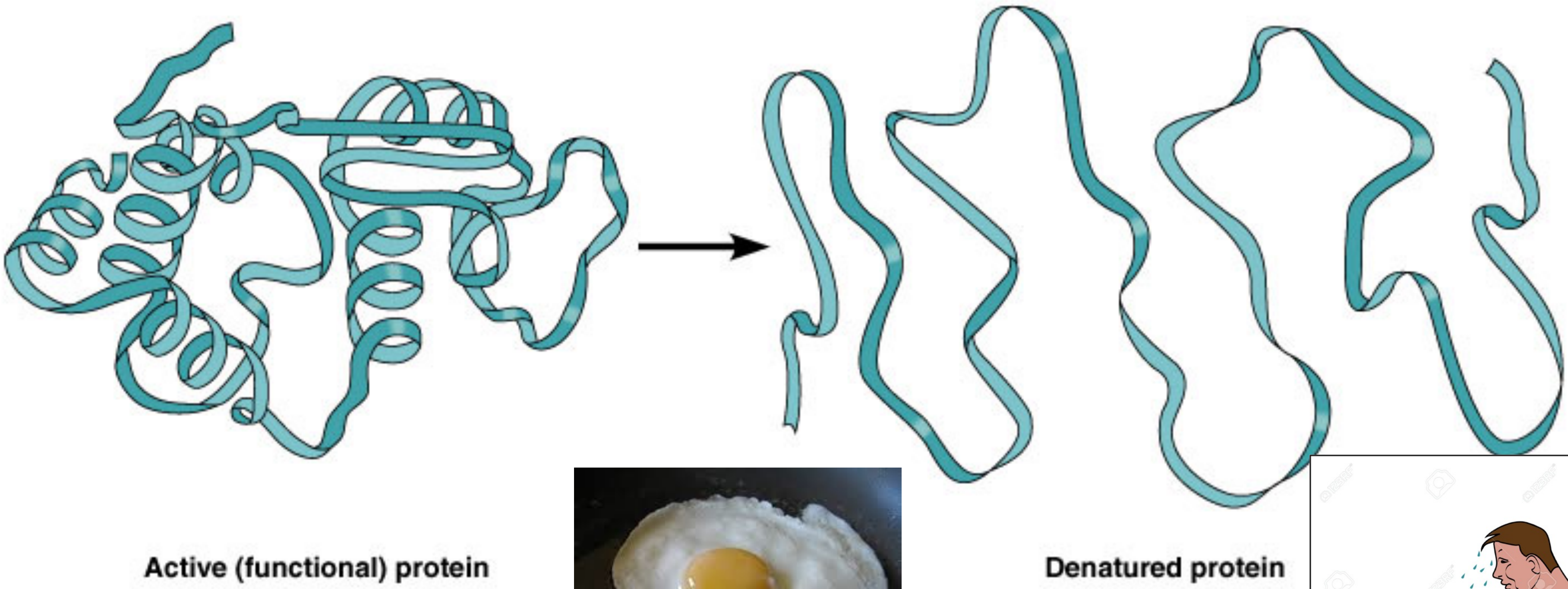
Sample enzyme name	Sample enzyme function
• Kinase	transfer a phosphate group
• Transferase	Transfer functional groups
• Protease	hydrolysis of proteins
• dehydrogenase	Removal of hydrogen
• Isomerase	Rearrangement of atoms
• Ligase	Joining of molecules, uses ATP
• Amylase	breaks down amylose (starch)
• Lipase	hydrolysis of lipids

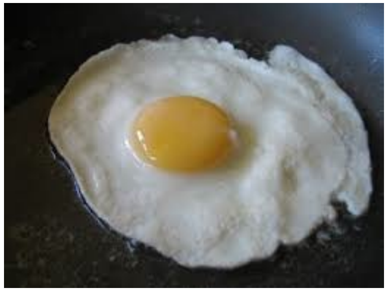
Factors Influencing Enzyme Activity

- Temperature
  - At high temperatures, enzymes undergo denaturation and lose their catalytic properties; at low temperatures, the reaction rate decreases.
- pH
  - optimum pH is the pH at which enzymatic activity is maximal
- Substrate concentration
  - Enzymatic activity increases as substrate concentration increases until the enzymes are saturated.
- Inhibitors
  - compete with the normal substrate for the active site of the enzyme & decrease the enzyme's ability to combine with the normal substrate.

Factors Influencing Enzyme Activity

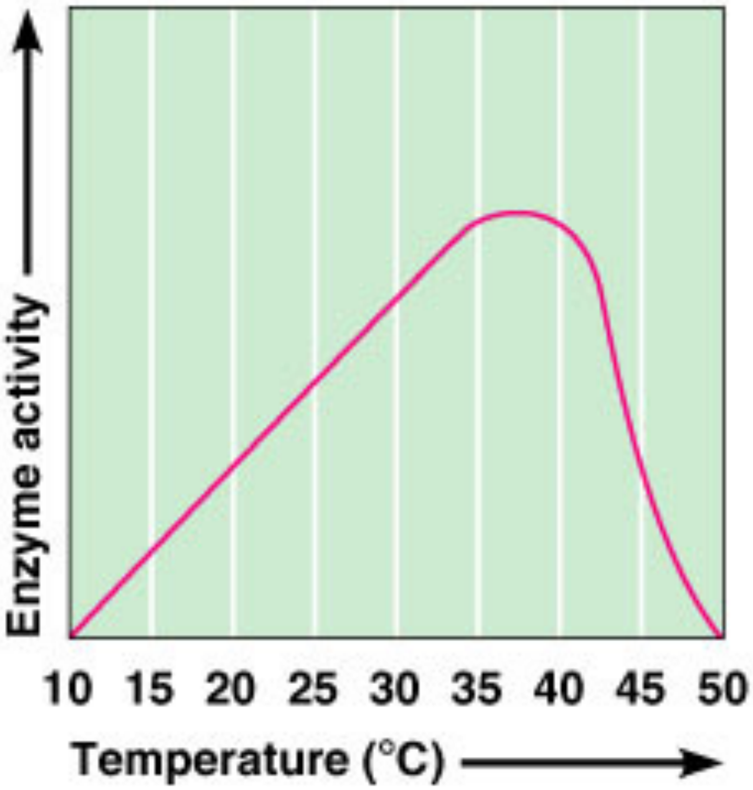
- Enzymes can be denatured by temperature and pH





Factors Influencing Enzyme Activity

- Temperature

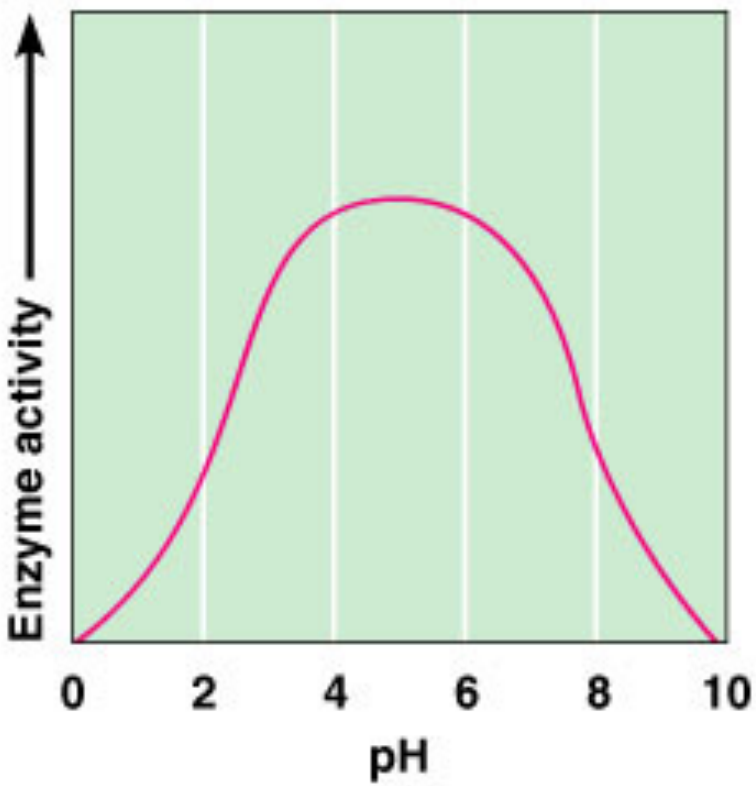


(a) Temperature

Figure 5.5a

Factors Influencing Enzyme Activity

- pH

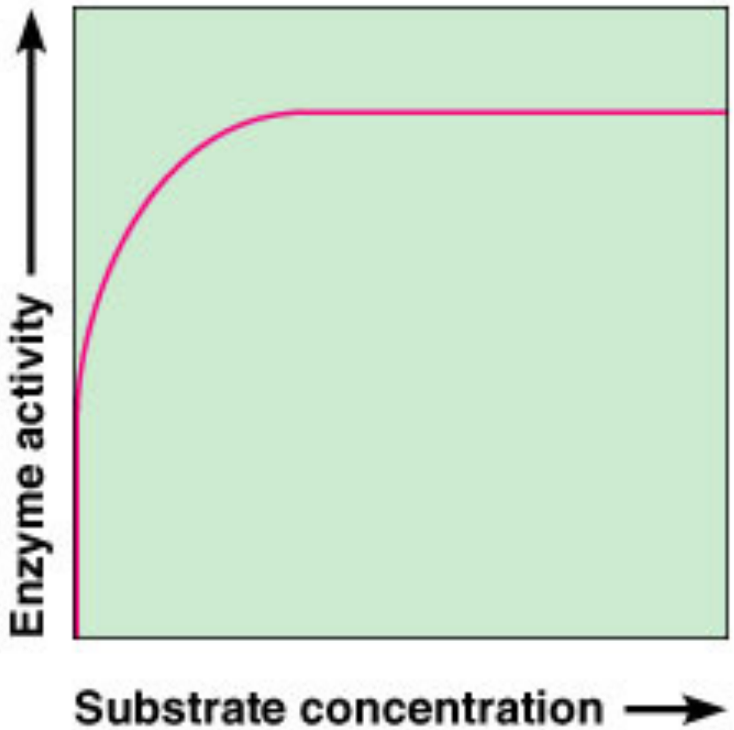


(b) pH

Figure 5.5b

Factors Influencing Enzyme Activity

- Substrate concentration



(c) Substrate concentration

Figure 5.5c

Factors Influencing and Control of Enzyme Activity

- Competitive inhibition : e.g PABA and folic acid

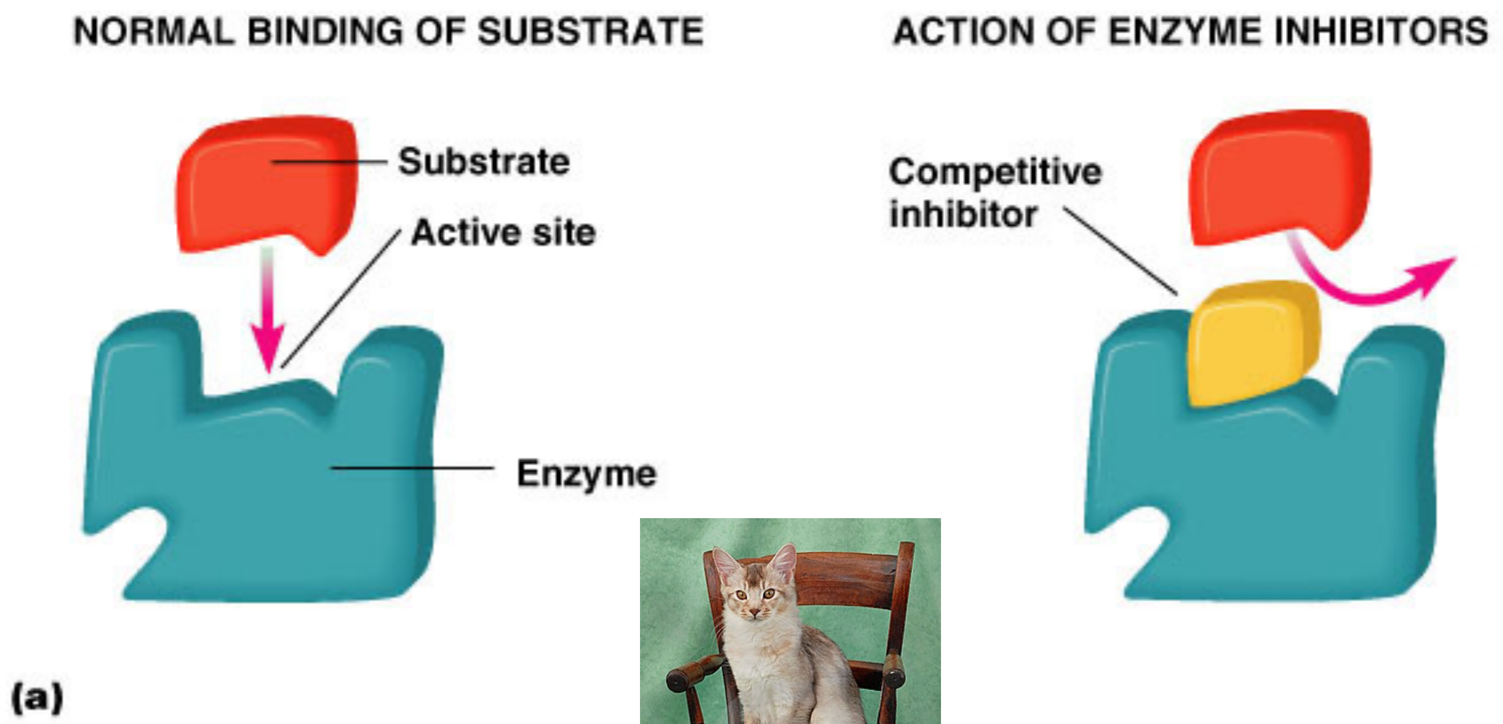
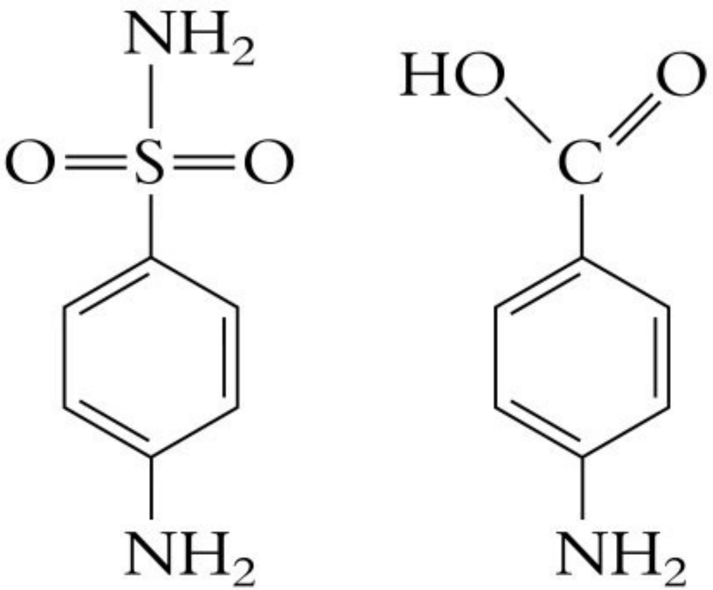


Figure 5.7a, b

Factors Influencing Enzyme Activity



Sulfanilamide PABA

- PABA essential nutrient used by bacteria to synthesize folic acid
- When antibiotic sulfanilamide is administered, enzymes responsible to synthesizing folic acid from PABA binds sulfanilamide. Therefore, no folic acid is synthesized by bacteria and it cannot grow.
- Sulfanilamide doesn’t harm humans as human doesn’t use PABA for synthesizing folic acid.

Factors Influencing Enzyme Activity

- **Noncompetitive inhibition: eg**

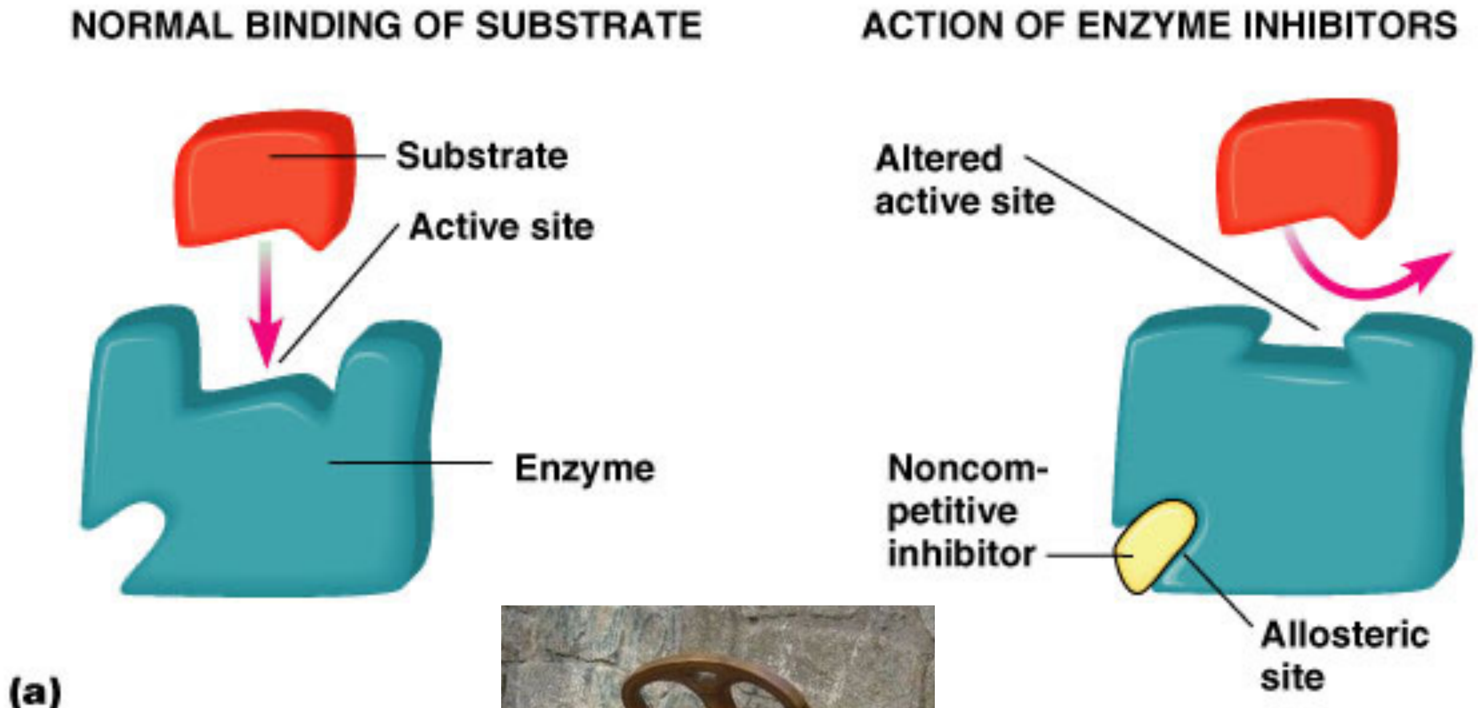


Figure 5.7a, c

Lecture 13

BT 206

13/02/23

Factors Influencing Enzyme Activity

- Feedback inhibition

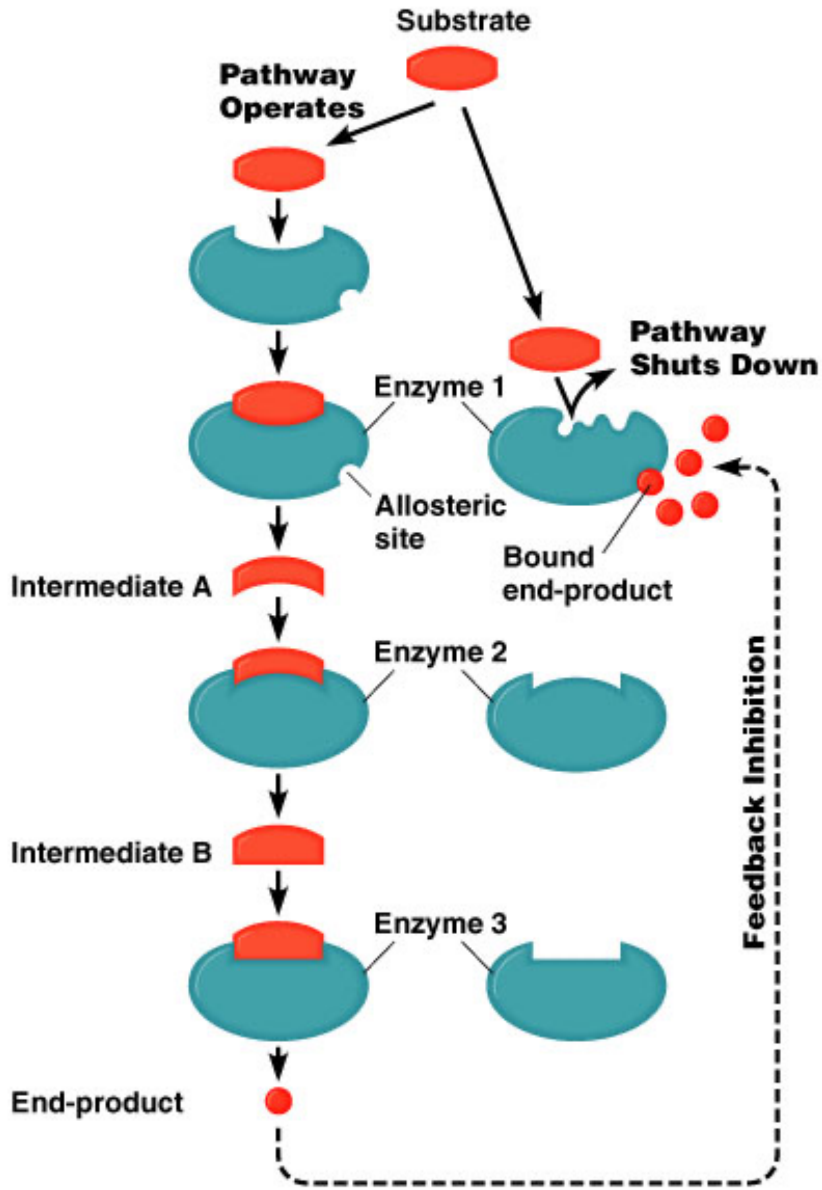


Figure 5.8

Ribozymes

- RNA that cuts and splices RNA
- Less diverse than proteins

Reactions of energy transfer

Oxidation-Reduction and Phosphorylation-Dephosphorylation reactions

Oxidation-Reduction involves removal and addition of electrons to molecules

- Oxidation is the removal of electrons.
- Reduction is the gain of electrons.
- Redox reaction is an oxidation reaction paired with a reduction reaction.

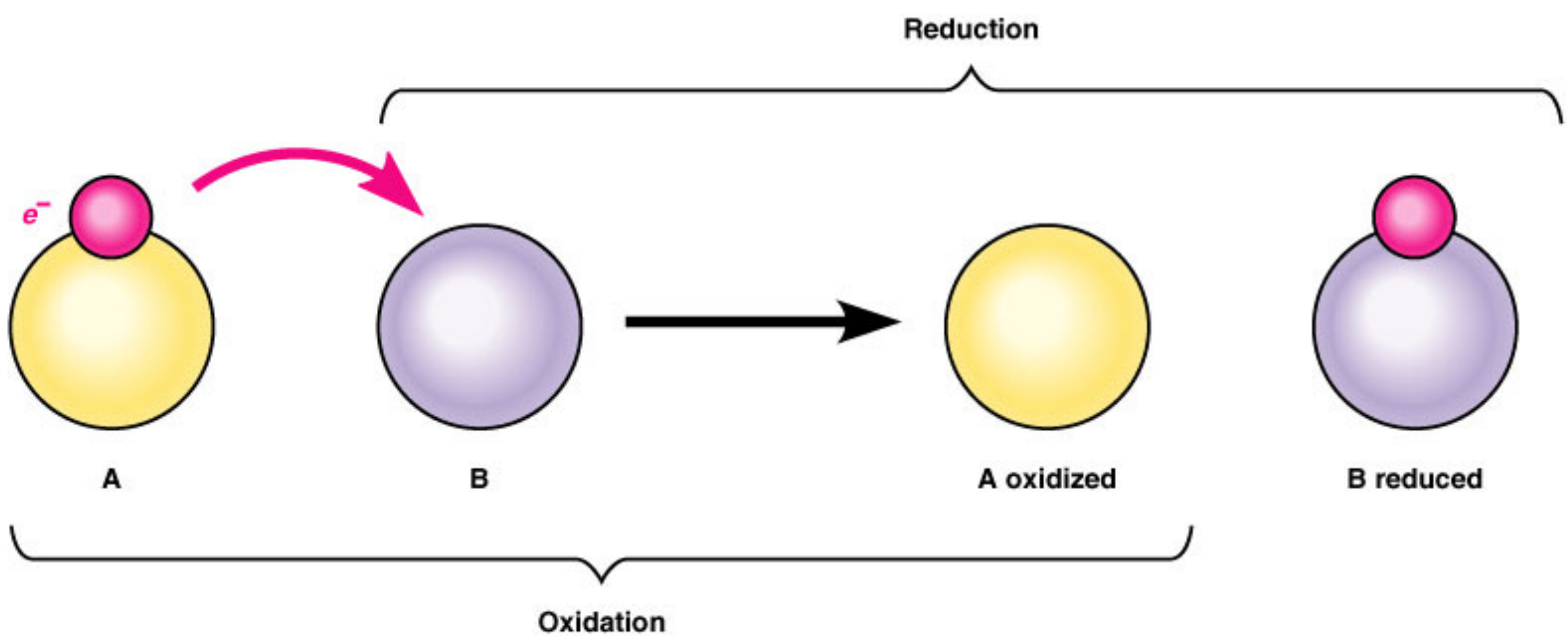


Figure 5.9

Oxidation-Reduction

- In biological systems, the electrons are often associated with hydrogen atoms. Biological oxidations are often dehydrogenations.

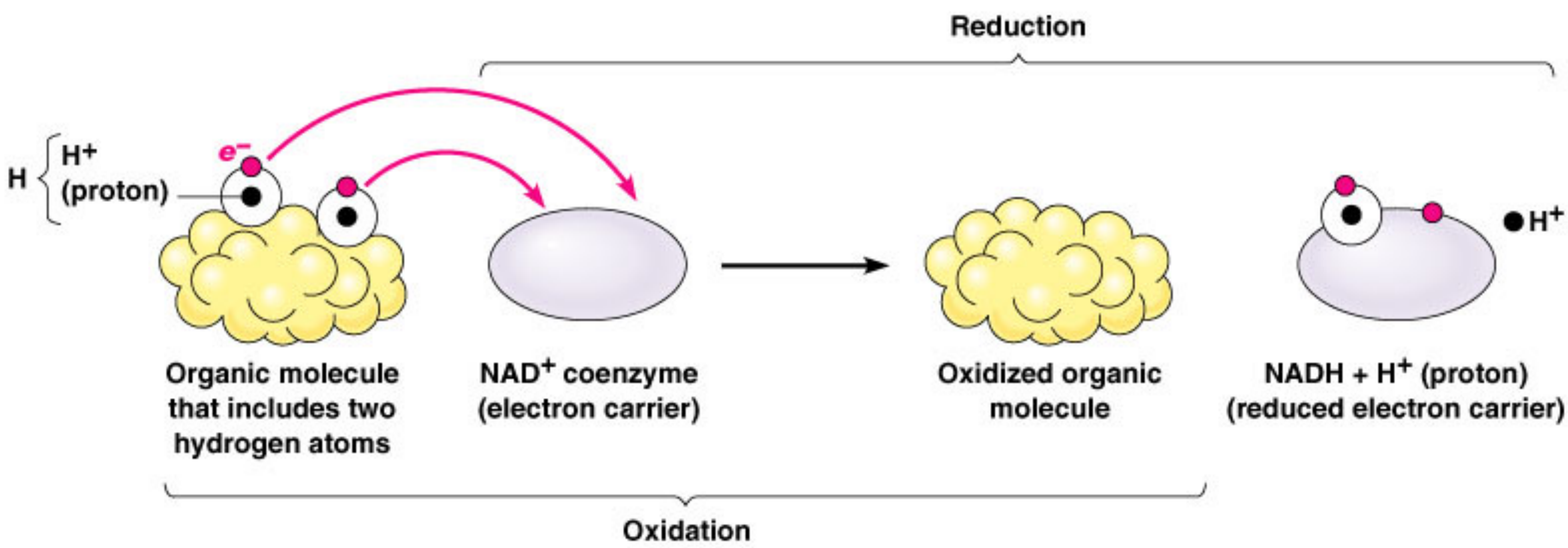


Figure 5.10

Phosphorylation and Dephosphorylation

Transferring a phosphate group.

- Adding is Phosphorylation - storing energy
- Removing is Dephosphorylation - releasing energy
    - ATP is generated by the phosphorylation of ADP.

$$\overbrace{\text{Adenosine}-P\sim P}^{\text{ADP}} + \text{Energy} + P \rightarrow \underbrace{\text{Adenosine}-P\sim P\sim P}_{\text{ATP}}$$

The Generation of ATP

Three main ways:

1. Photophosphorylation - using light energy to phosphorylate ADP to ATP
2. Substrate level phosphorylation - a transfer of a phosphate group from one molecule to another

   1,3-diphosphoglyceric acid + ADP $\rightarrow$ ATP + 3-phosphoglyceric acid
3. Oxidative phosphorylation - energy released from the transfer of electrons (oxidation) of one compound to another (reduction) can used to generate ATP by **chemiosmosis**.

Photophosphorylation

- Light causes chlorophyll to give up electrons. Energy released from the transfer of electrons (oxidation) of chlorophyll through a system of carrier molecules is used to generate ATP.

TABLE 5.6	**Photosynthesis Compared in Selected Eukaryotes and Prokaryotes**			
Characteristic	**Eukaryotes**	**Prokaryotes**		
	Algae, Plants	**Cyanobacteria**	**Green Bacteria**	**Purple Bacteria**
Substance that reduces CO_2	H atoms of H_2O	H atoms of H_2O	Sulfur, sulfur compounds, H_2 gas	Sulfur, sulfur compounds, H_2 gas
Oxygen production	Oxygenic	Oxygenic (and anoxygenic)	Anoxygenic	Anoxygenic
Type of chlorophyll	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll *a*	Bacteriochlorophyll *a* or *b*
Site of photosynthesis	Chloroplasts with thylakoids	Thylakoids	Chlorosomes	Intracytoplasmic membrane
Environment	Aerobic	Aerobic (and anaerobic)	Anaerobic	Anaerobic

Table 5.6

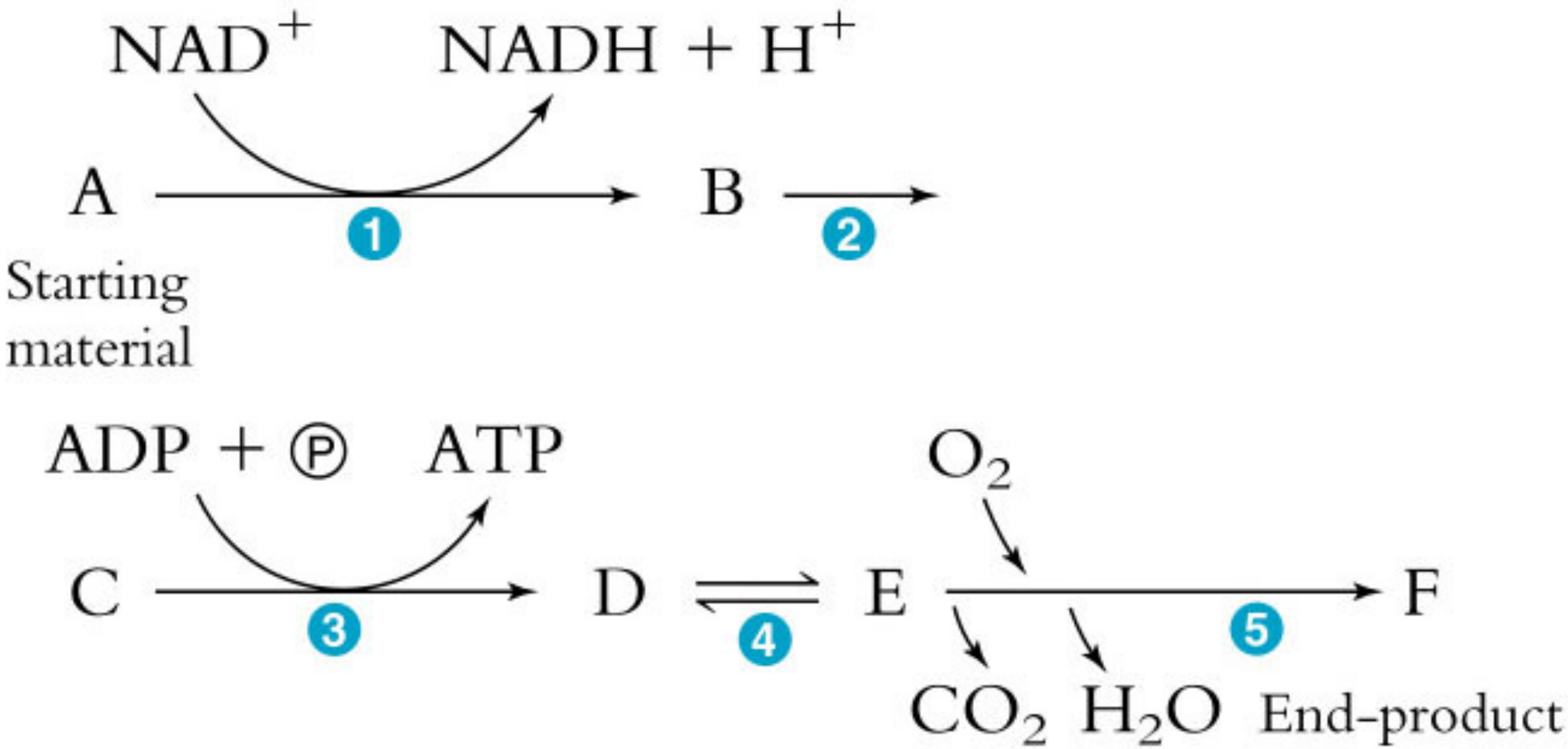
The Generation of ATP

- Substrate-level phosphorylation is the transfer of a high-energy PO_4^- to ADP.

$$C—C—C \sim P + ADP \longrightarrow C—C—C + ATP$$

Metabolic Pathways

Decarboxylation - CO_2 removal with electrons reduction



Carbohydrate Catabolism

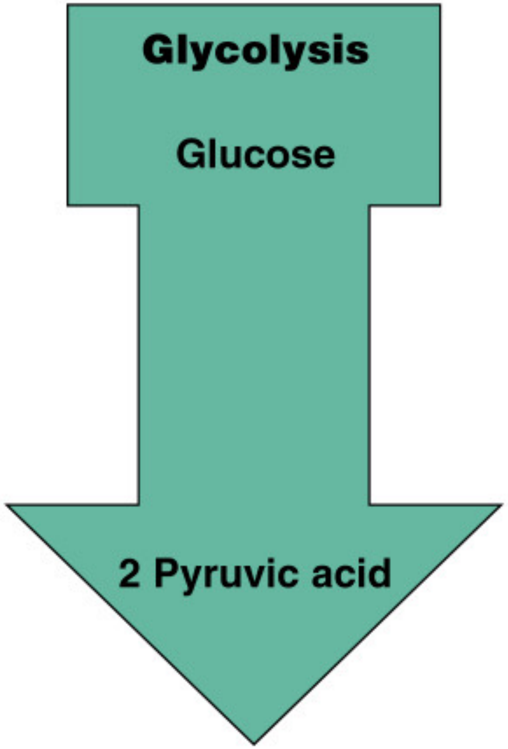
- The breakdown of carbohydrates to release energy
  - Glycolysis
    - Fermentation
  - Krebs cycle
  - Electron transport chain

Glycolysis

Glycolysis has three main stages:

1. Preparatory
2. splitting and
3. energy harvest or conserving.

- The oxidation of glucose to pyruvic acid, produces ATP and NADH.



Preparatory and Splitting Stages

1. **Preparatory**: 2 ATPs are used to prepare or activate glucose to form fructose 1,6 diphosphophosphate

2. **Splitting**: Glucose is split to form two glyceraldehyde-3-phosphate molecules

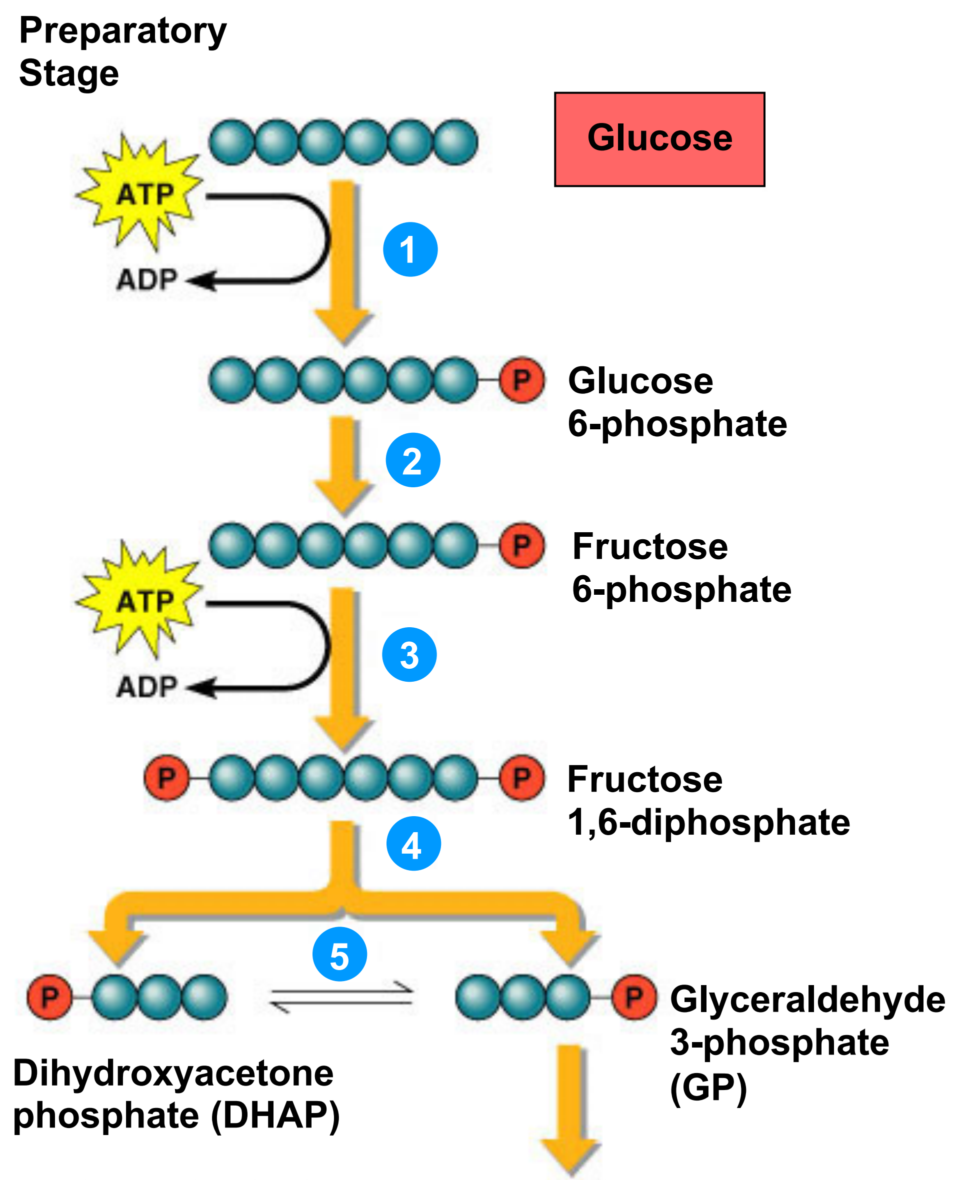


Figure 5.12.1

Energy-Conserving Stage

2 glyceraldehyde-3-phosphate molecules are oxidized into 2 Pyruvic acid molecules

Energy harvest

- 4 ATP produced*
- 2 NADH produced
  - * remember that preparatory stage required 2 ATP molecules to start

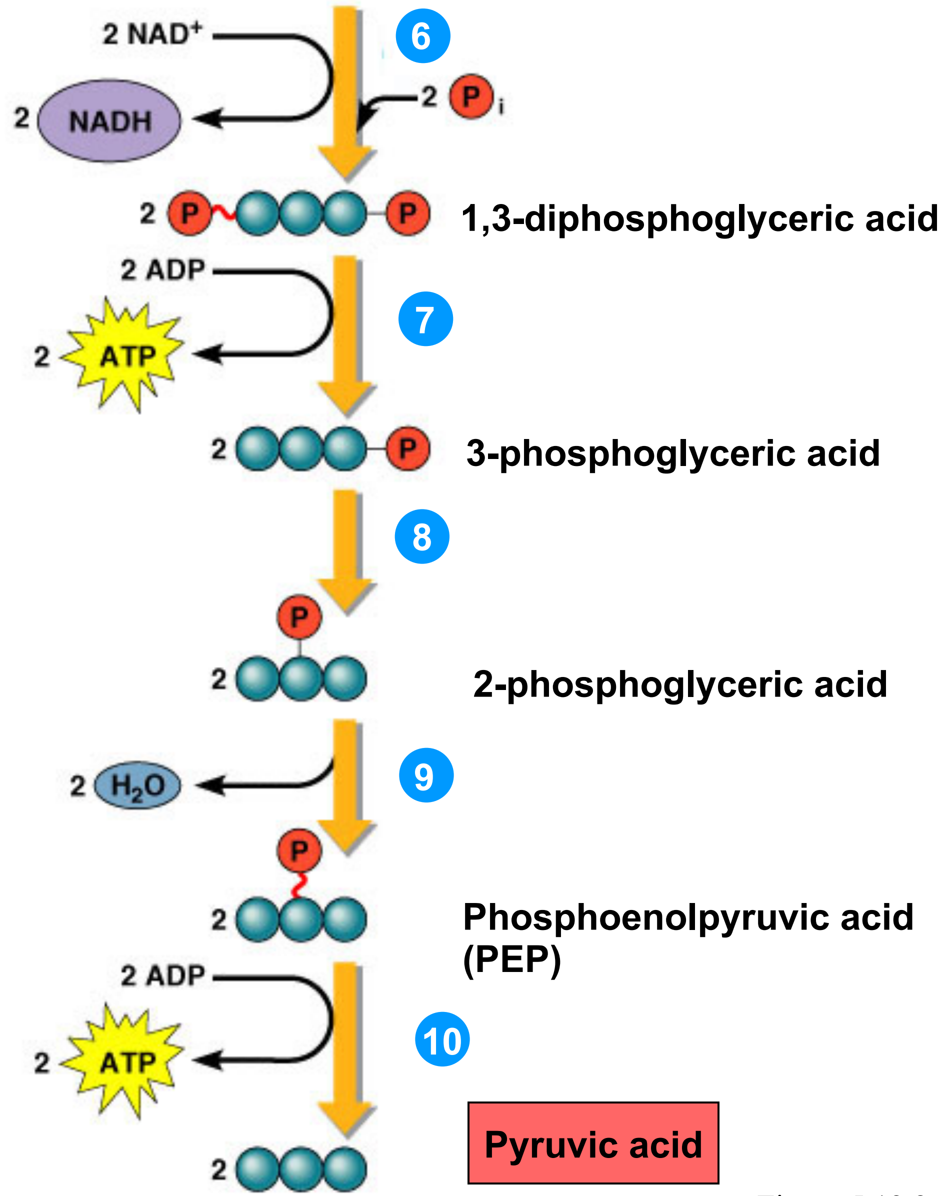


Figure 5.12.2

Glycolysis

Overall Net equation: Reactants and products

- Glucose + 2 ADP + 2 Pi + 2 NAD^+ $\rightarrow$

  2 pyruvic acid + 2 ATP + 2 NADH + $2H^+$

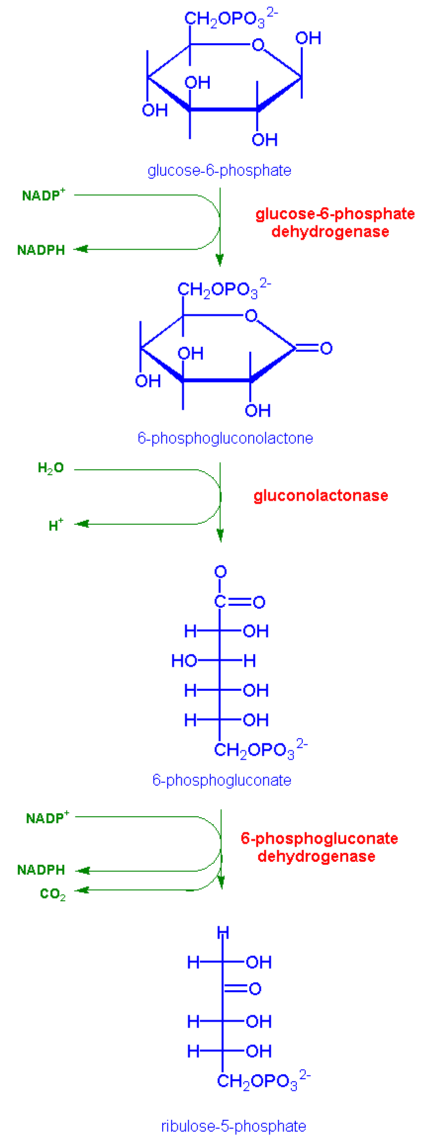
Alternatives to Glycolysis

- Pentose phosphate pathway:
  - Uses pentoses and NADPH
  - Operates with glycolysis
- Entner-Doudoroff pathway:
  - Produces NADPH and ATP
  - Does not involve glycolysis
  - *Pseudomonas, Rhizobium, Agrobacterium*

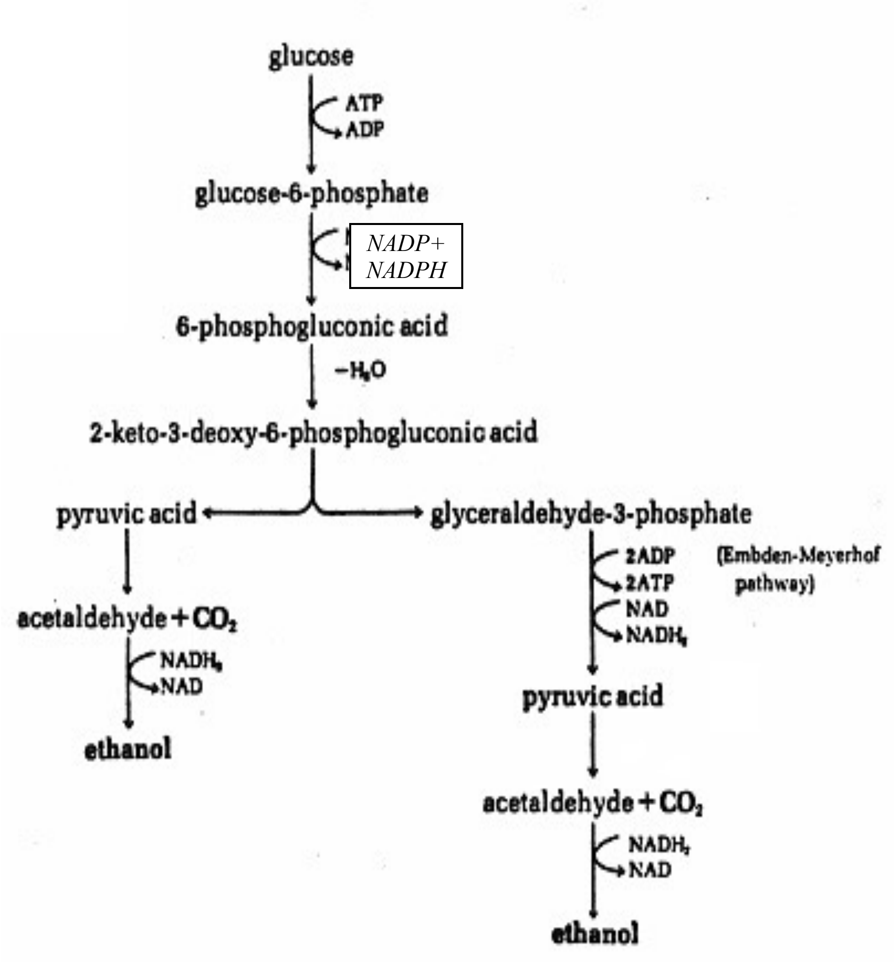
Glycolysis: PPP

- Breakdown 5-6 C
- Cytoplasm
- Anaerobic
- End products
  - 1 ATP
  - 2 NADPH
  - CO_2
  - 4,5,6,7 C
    - AA
    - Nucleotides
    - Glycolytic pathways
    - Photosynthesis

Oxidative Stage of Pentose Phosphate Pathway



Glycolysis: Entner-Duodoroff [E-D]



- Glycolytic
- Cytoplasm
- Anaerobic
- Different enzymes
  - *Pseudomonas*
  - *Enterococcus*
- End products
  - 2-1 = 1 net ATP
  - NADPH
  - NADH
  - 2 Pyruvic acids
  - H_20

Anaerobic Pathways Compared

Pathway	EM	PPP	ED
Location Substrate	Cytoplasm Glucose	Cytoplasm G6P	Cytoplasm Glucose
Steps	2 parts 6C → 3C	Different sugars (4-7C)	Glyceraldehyde
Products	2 Pyruvate 2 ATP 2 NADH 2 H^+	Pyruvate & Fructose 6 CO2 12 NADPH 12 H^+	2 Pyruvate 1 ATP 1 NADPH 1 NADH 2 H^+

Glycolytic Pathways used by various Bacteria

Bacterium	**E-M**	**PPP**	**E-D**
Acetobacter aceti	-	+	-
Bacillus subtilis	major	minor	-
E. coli	+	-	-
Lactobacillus acidophilus	+	-	-
Pseudomonas aeruginosa	-	-	+
Vibrio cholera	minor	-	major

Cellular Respiration

- Oxidation of molecules liberates electrons for an electron transport chain
- ATP generated by oxidative phosphorylation

Intermediate Step

- Pyruvic acid (from glycolysis) is oxidized and decarboxylated
- CO_2 is released
- NAD^+ is reduced to NADH

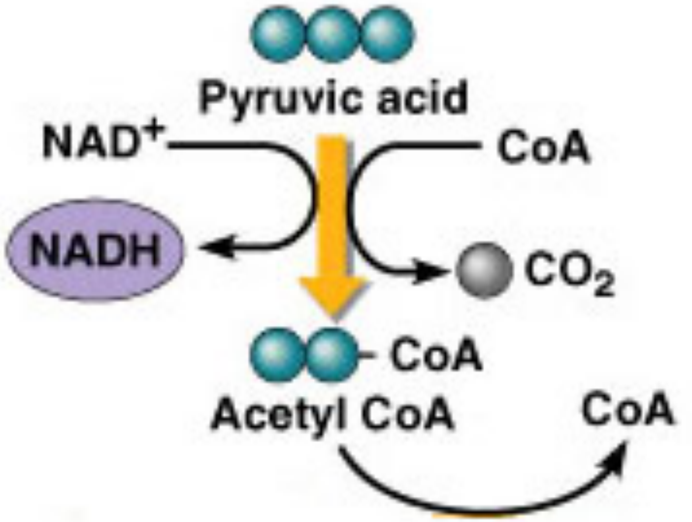


Figure 5.13.1

Krebs Cycle

- Oxidation of acetyl CoA produces NADH and $FADH_2$
- Summarize the reactants and products for the intermediate step and Kreb's cycle - keep energy harvest number straight!

Krebs Cycle

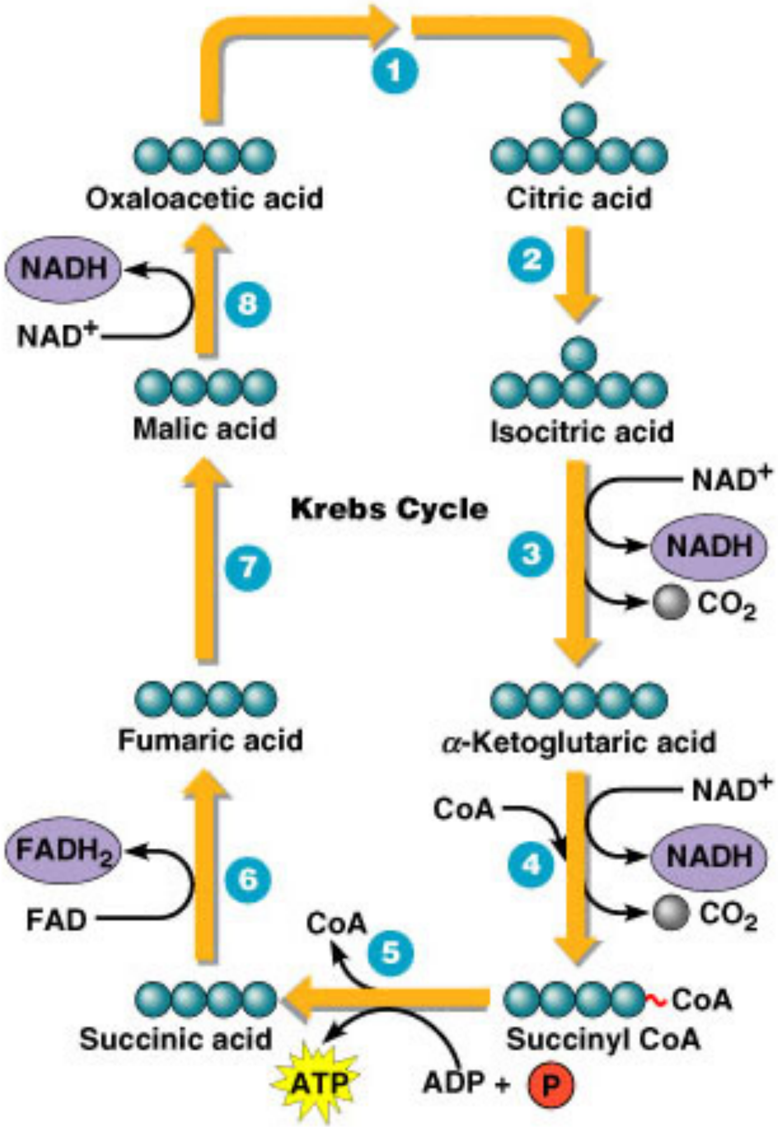


Figure 5.13.2

The Electron Transport Chain

- A series of carrier molecules that are, in turn, oxidized and reduced as electrons are passed down the chain.
- Energy released can be used to produce ATP by chemiosmosis.

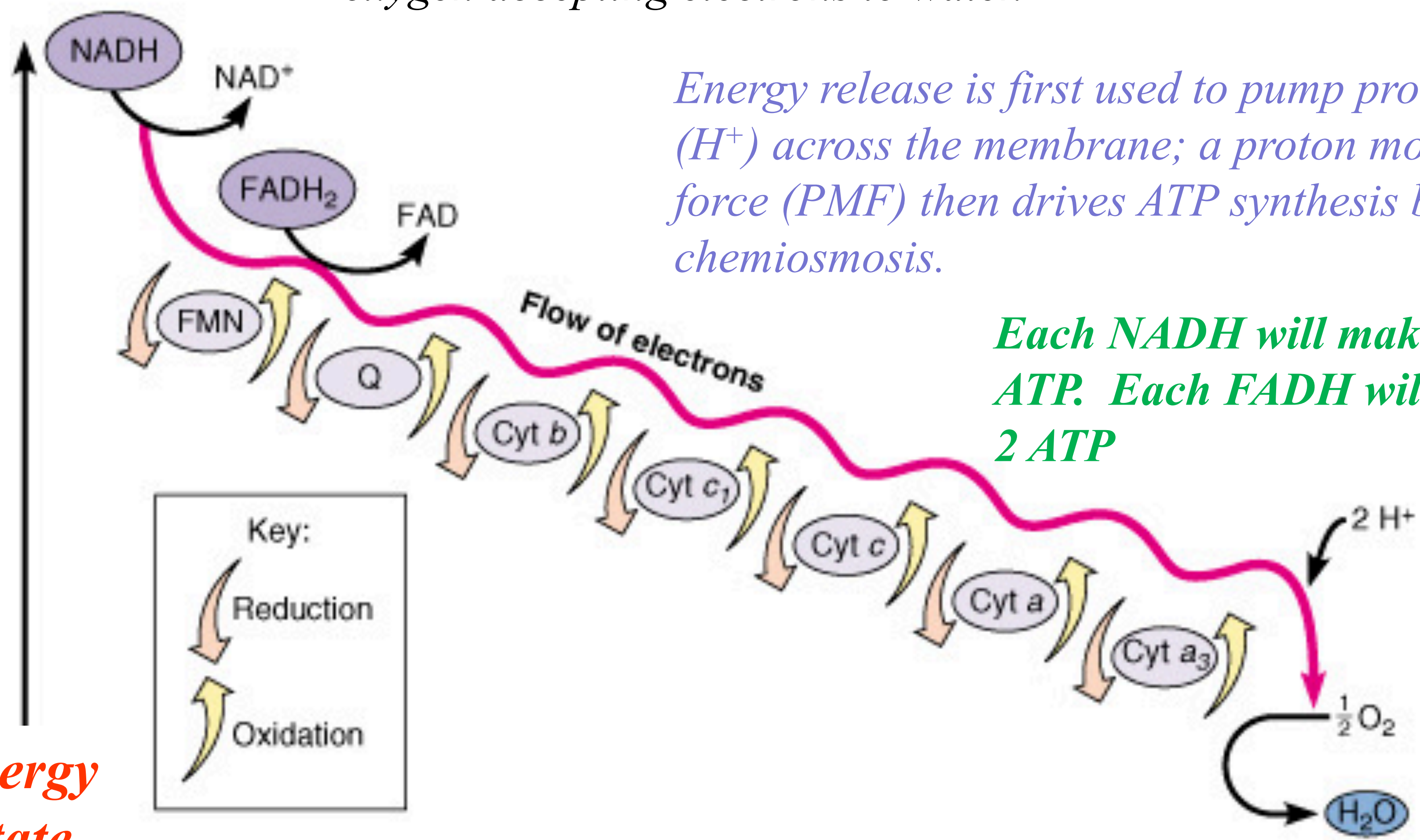
Lecture 14
BT 206
14/02/23

Electron Transport Chain (ETC) Function

The ETC is a series of membrane bound electron carriers that transports electrons from high to low energy state, ending with oxygen accepting electrons to water.

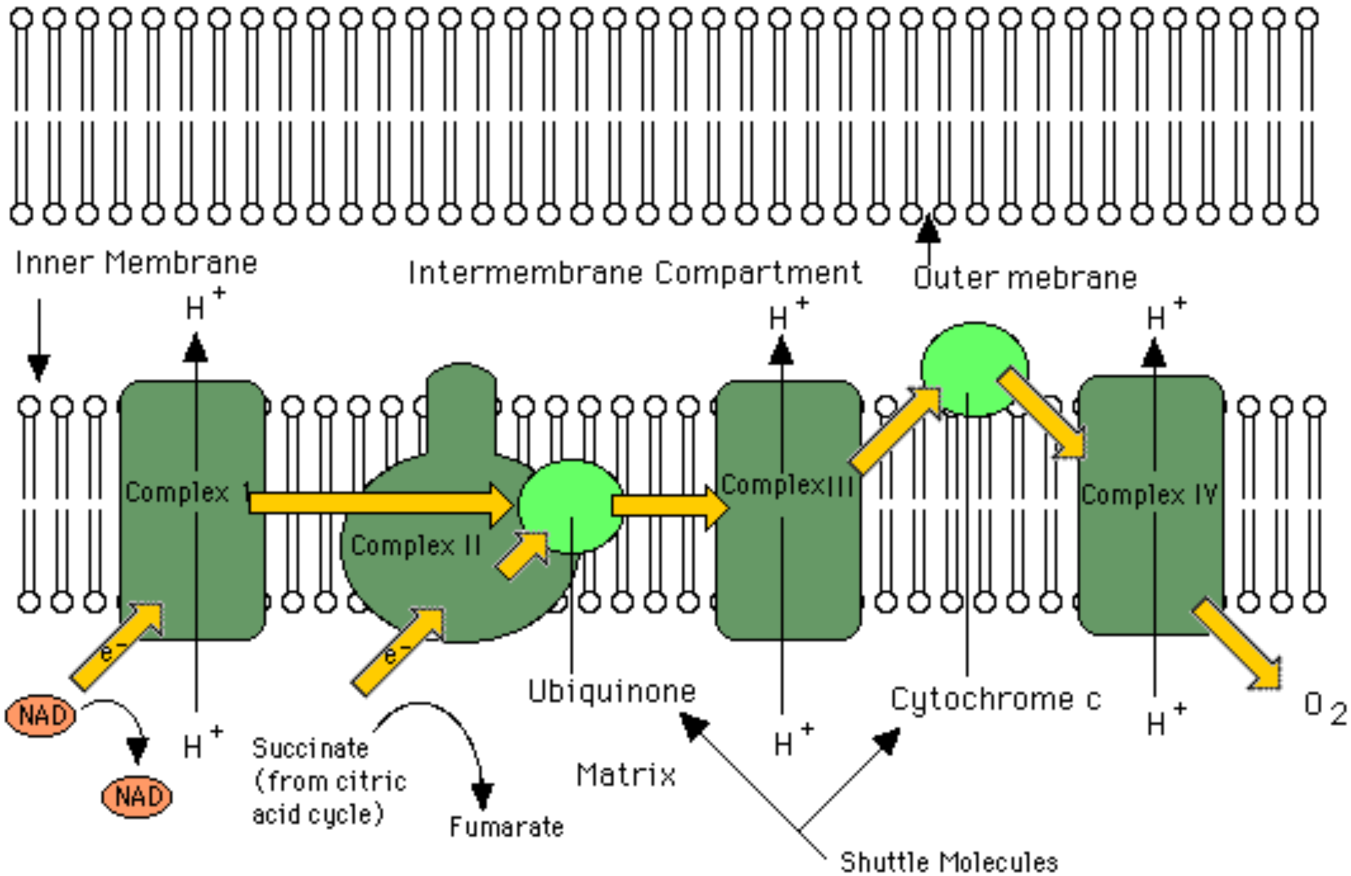
Energy release is first used to pump protons (H^+) across the membrane; a proton motive force (PMF) then drives ATP synthesis by chemiosmosis.

Each NADH will make 3 ATP. Each FADH will make 2 ATP



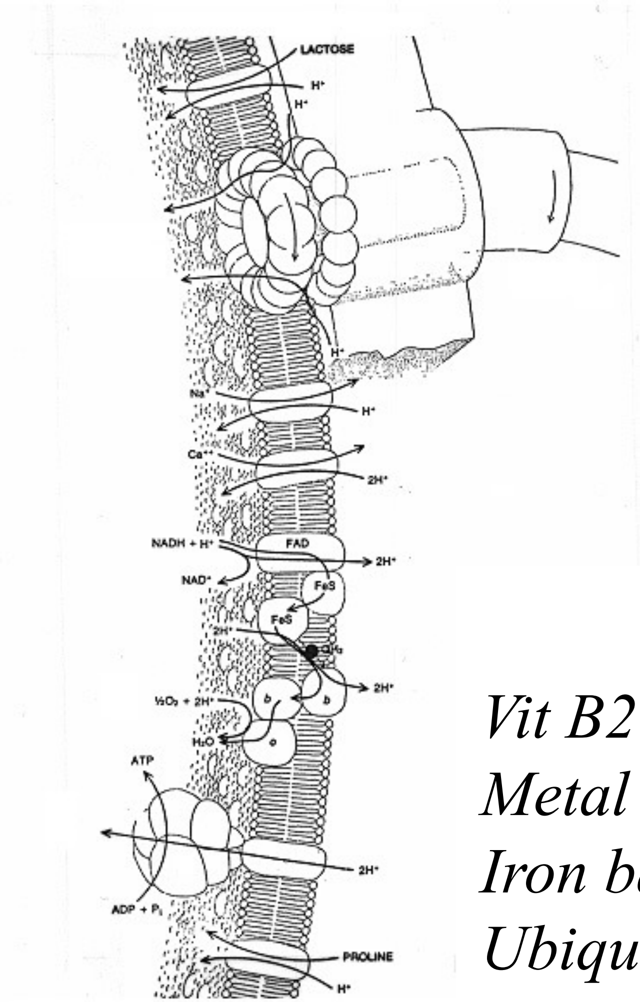
Energy State

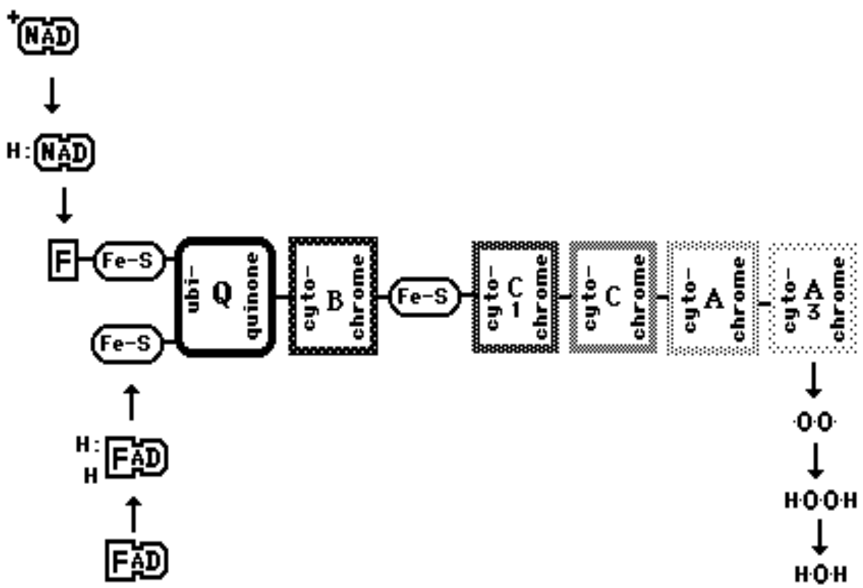
Eukaryotic ETC



Complex I = NADH-ubiquinone oxydoreductase
Complex II =Succinate-ubiquinone oxdoreductase
Comples III =ubiquinone-cytochrome c oxidoreductase
Complex IV =cytochrome c-O_2 oxidoreductase

Prokaryotic ETC



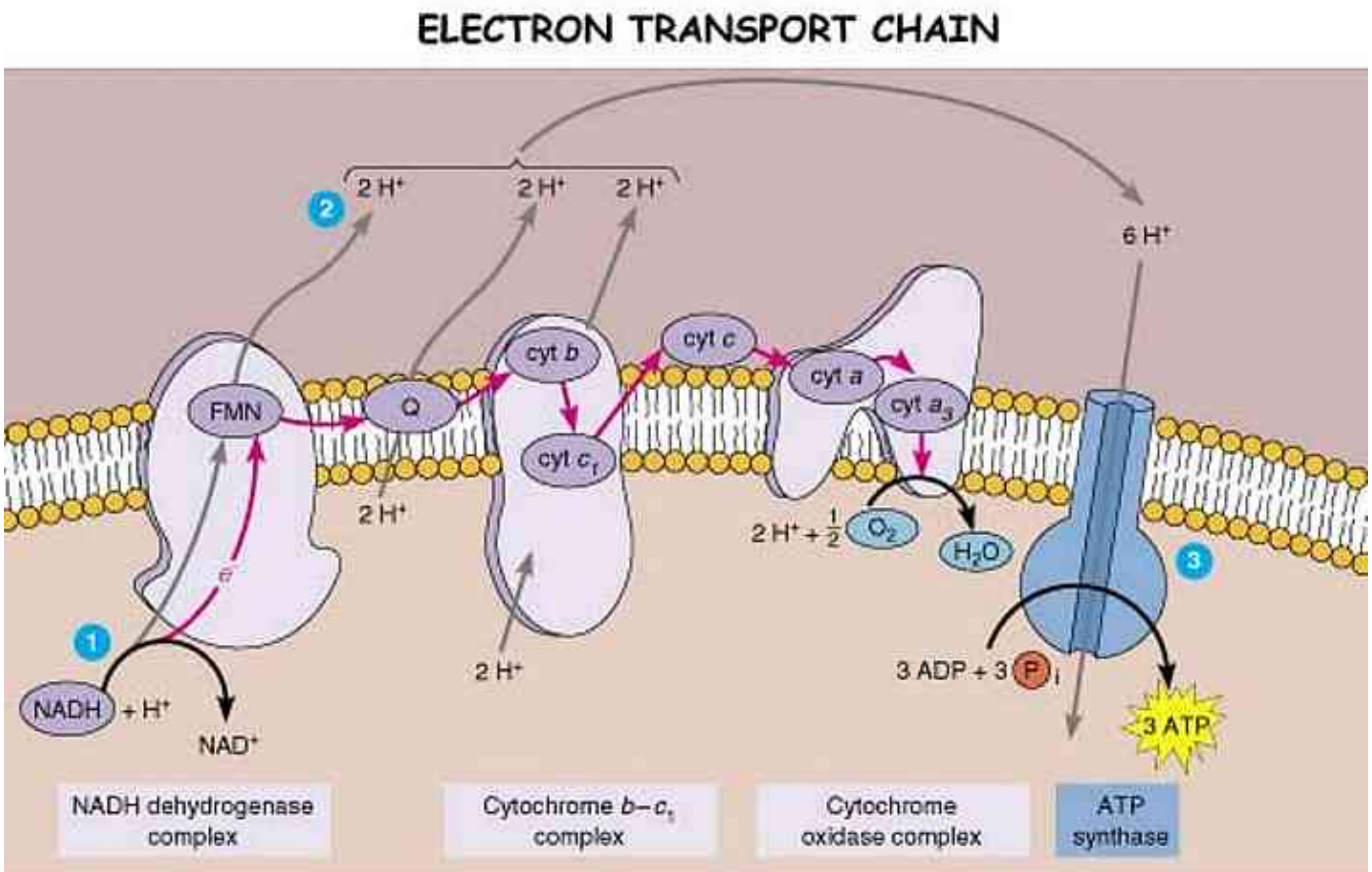


Vit B2 Riboflavin based Flavoproteins
Metal Proteins with ions (Fe, S, Cu)
Iron based Cytochromes (b,c1,c,a,a3)
Ubiquinones based on Vit K (CoEnzyme Q)

ETC Steps

- Electrons from NADH or $FADH_2$ to flavoproteins (FMN)
- H^+ pumped into periplasm
- Electrons transported
  - To Iron-Sulfur proteins from NADH
  - To CoQ from $FADH_2$
- Cytochromes transfer electrons
- Final Electron Acceptor
  - O_2 if Aerobic
  - Other inorganic molecule if Anaerobic

ETC: Cytochromes

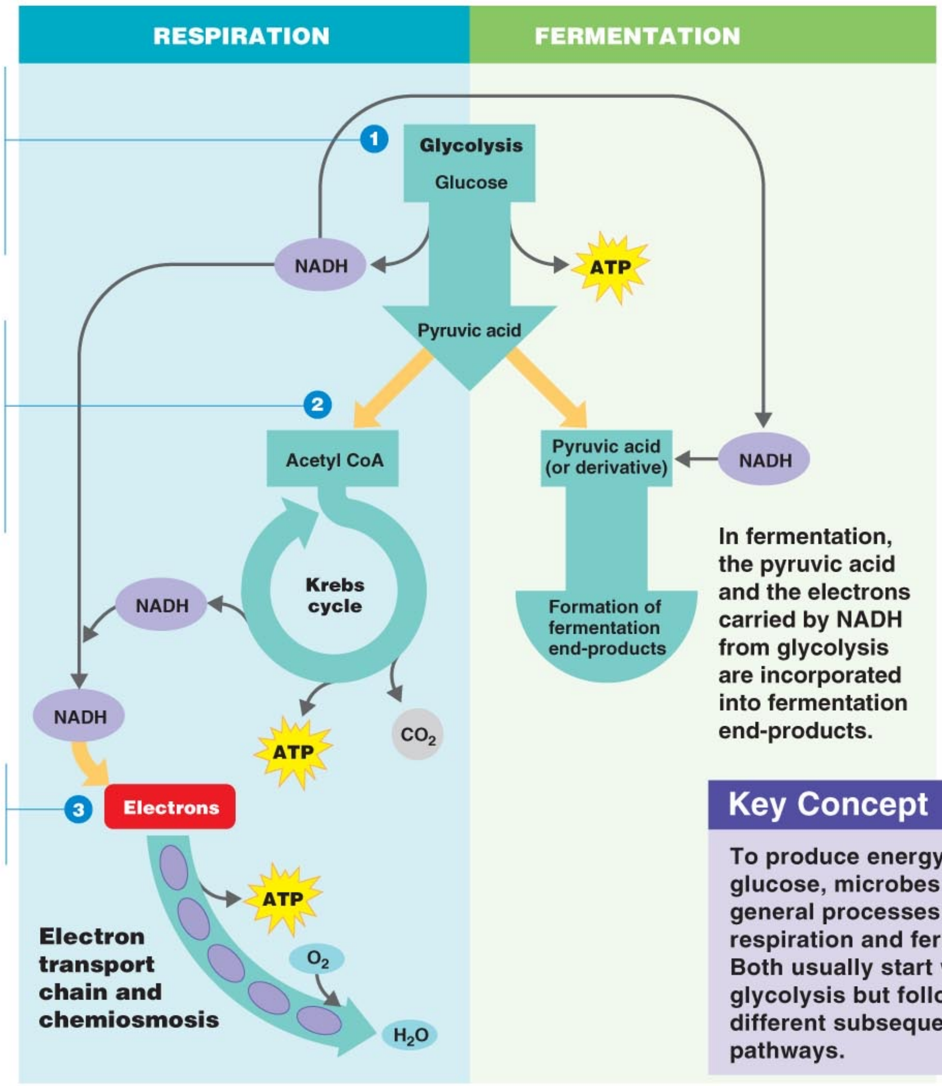


Overview of Respiration and Fermentation

1 Glycolysis produces ATP and reduces NAD^+ to NADH while oxidizing glucose to pyruvic acid. In respiration, the pyruvic acid is converted to the first reactant in the Krebs cycle.

2 The Krebs cycle produces ATP and reduces NAD^+ (and another electron carrier called $FADH_2$) while giving off CO_2. The NADH and $FADH_2$ from both processes carry electrons to the electron transport chain.

3 In the electron transport chain, the energy of the electrons is used to produce a great deal of ATP.



Key Concept

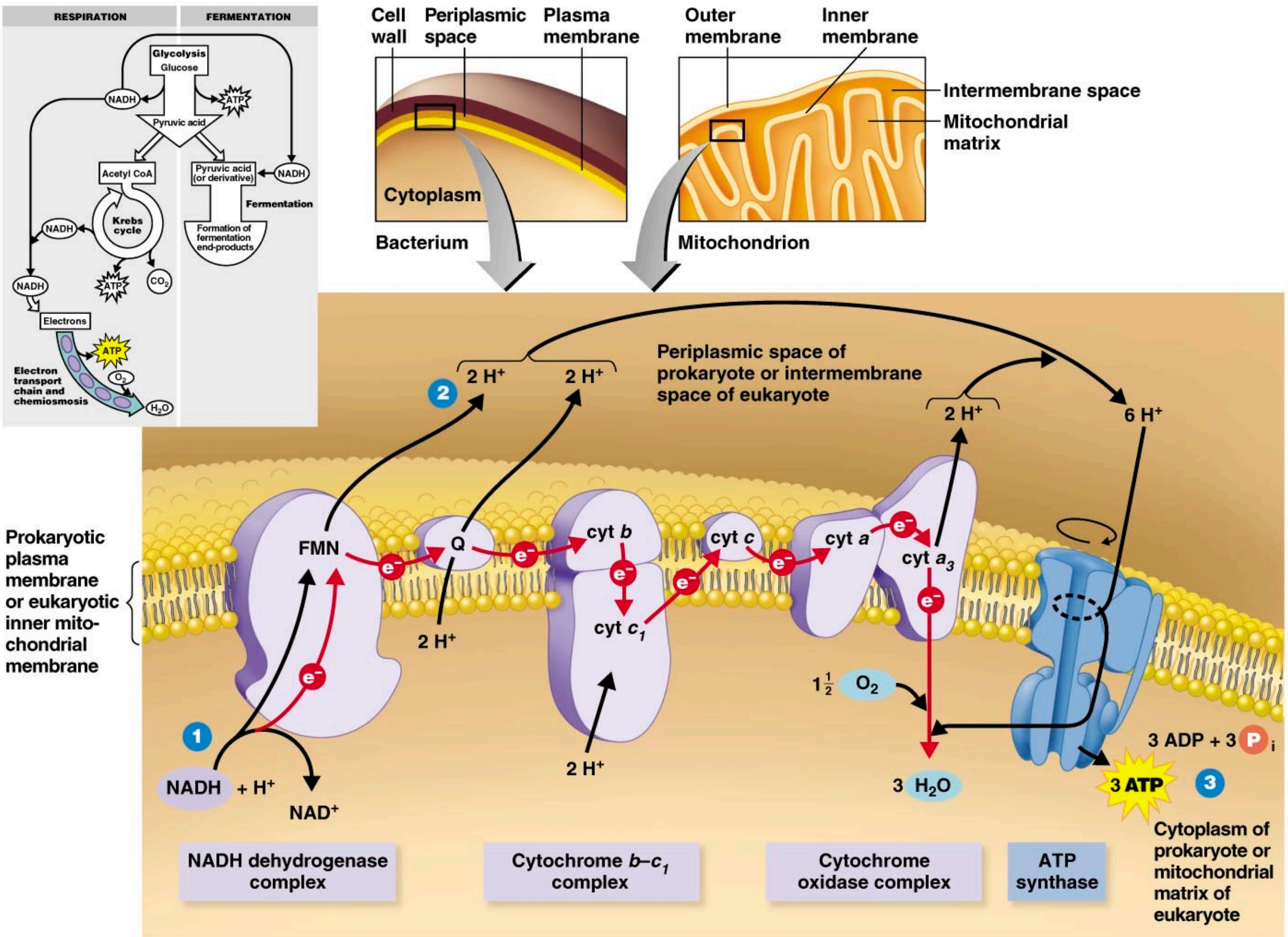
To produce energy from glucose, microbes use two general processes: respiration and fermentation. Both usually start with glycolysis but follow different subsequent pathways.

Figure 5.11

The Chemiosmotic Mechanism of ATP Generation

- In eukaryotes, electron carriers are located in the inner mitochondrial membrane; in prokaryotes, electron carriers are in the plasma membrane.
- Protons being pumped across the membrane generate a proton motive force as electrons move through a series of acceptors or carriers.

Chemiosmotic Generation of ATP



An overview of Chemiosmosis

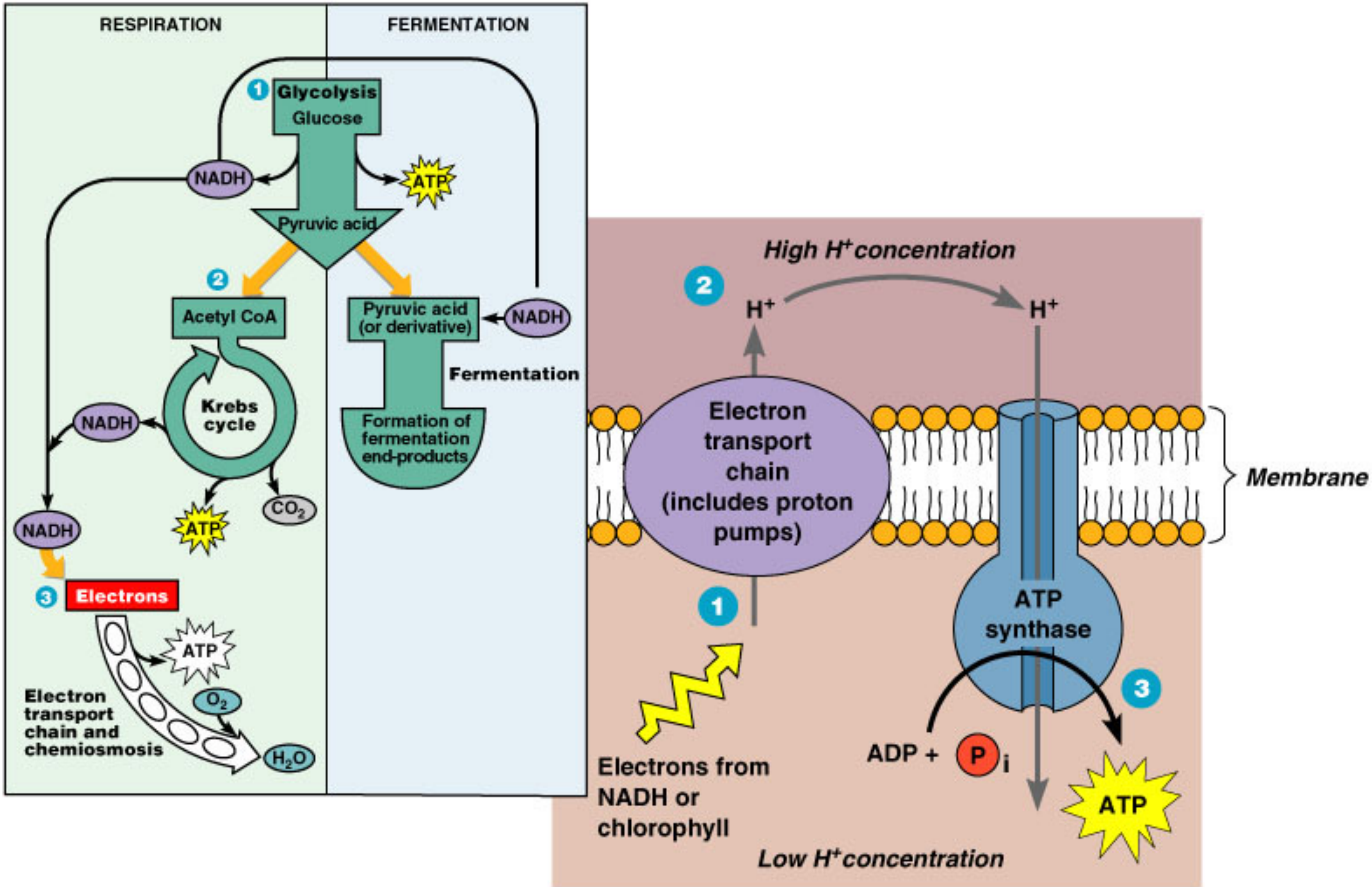


Figure 5.15

Chemiosmosis

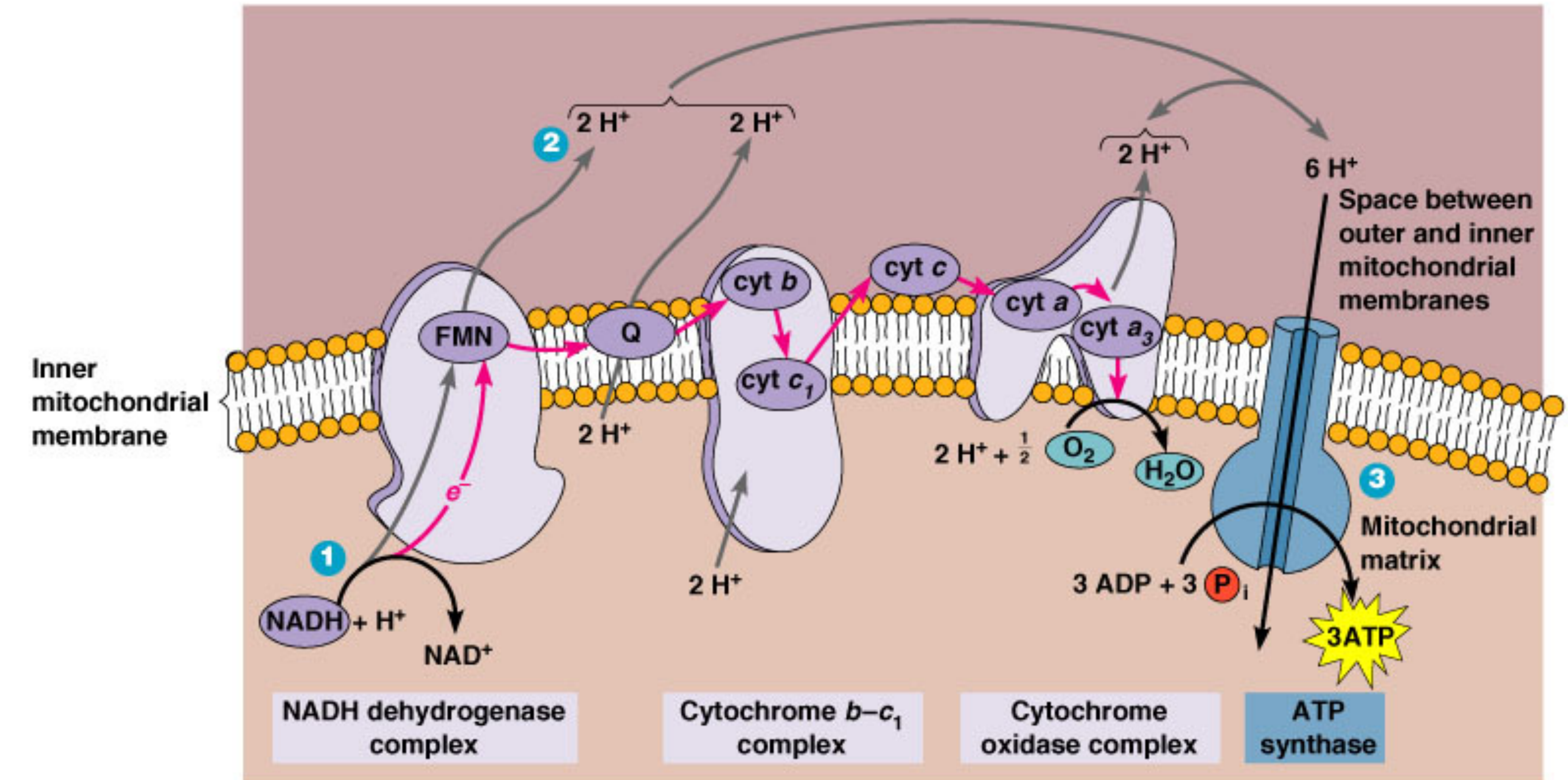


Figure 5.16.2

Types of Respiration

- Aerobic respiration: The final electron acceptor in the electron transport chain is molecular oxygen (O_2).
- Anaerobic respiration: The final electron acceptor in the electron transport chain is not O_2. Yields less energy than aerobic respiration because only part of the Krebs cycles operations under anaerobic conditions.

Anaerobic respiration

Electron acceptor	Products
NO_3^-	NO_2^-, $N_2 + H_2O$
SO_4^-	$H_2S + H_2O$
CO_3^{2-}	$CH_4 + H_2O$

Pathway	Eukaryote	Prokaryote
Glycolysis	Cytoplasm	Cytoplasm
Intermediate step	Cytoplasm	Cytoplasm
Krebs cycle	Mitochondrial matrix	Cytoplasm
ETC	Mitochondrial inner membrane	Plasma membrane

- Energy produced from complete oxidation of 1 glucose using aerobic respiration

Pathway	ATP produced	NADH produced	$FADH_2$ produced
Glycolysis	2	2	0
Intermediate step	0	2	
Krebs cycle	2	6	2
Total	**4**	**10**	**2**

- ATP produced from complete oxidation of 1 glucose using aerobic respiration

Pathway	By substrate-level phosphorylation	By oxidative phosphorylation	
		From NADH	From FADH
Glycolysis	2	6	0
Intermediate step	0	6	0
Krebs cycle	2	18	4
Total	**4**	**30**	**4**

- 36 ATPs are produced in eukaryotes.

Fermentation

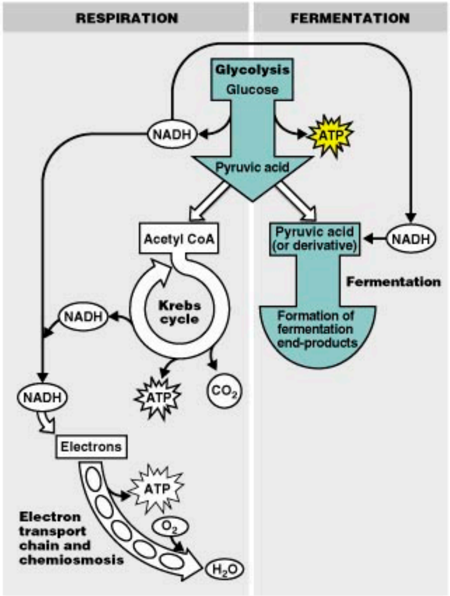
- Any spoilage of food by microorganisms (general use)
- Any process that produces alcoholic beverages or acidic dairy products (general use)
- Any large-scale microbial process occurring with or without air (common definition used in industry)

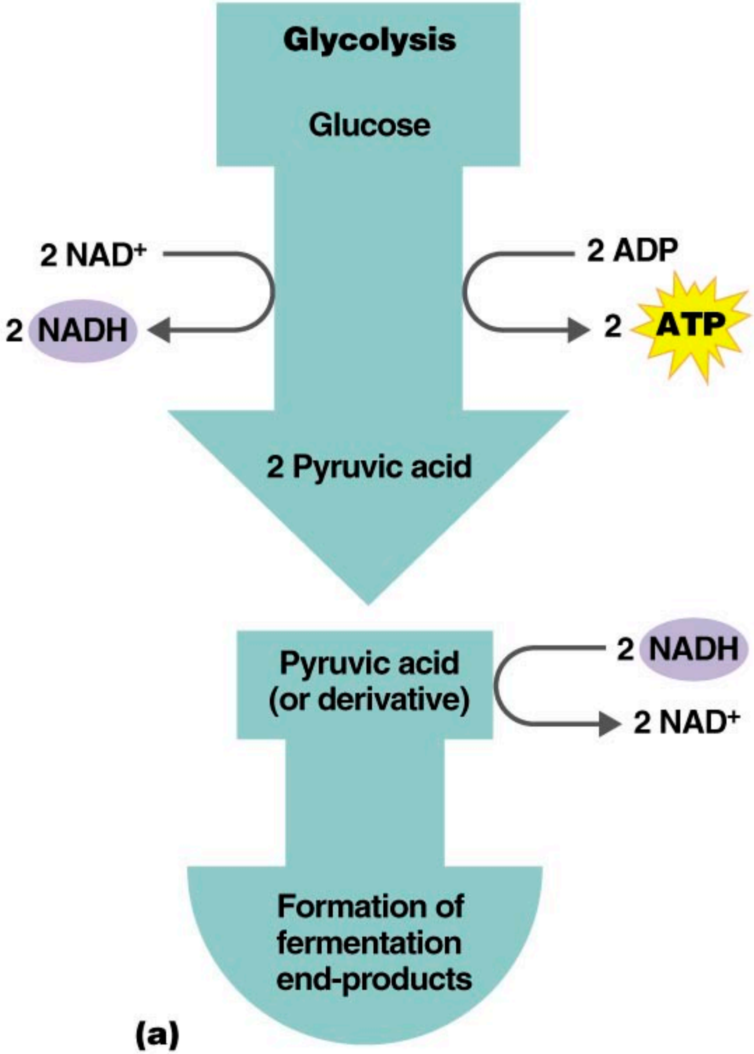
Fermentation

- Scientific definition:
  - Releases energy from oxidation of organic molecules
  - Does not require oxygen
  - Does not use the Krebs cycle or ETC
  - Uses an organic molecule as the final electron acceptor

Glucose fermentation is one test used to differentiate between Escherichia coli and Pseudomonas aeruginosa. Pseudomonas does NOT ferment glucose & E. coli does.

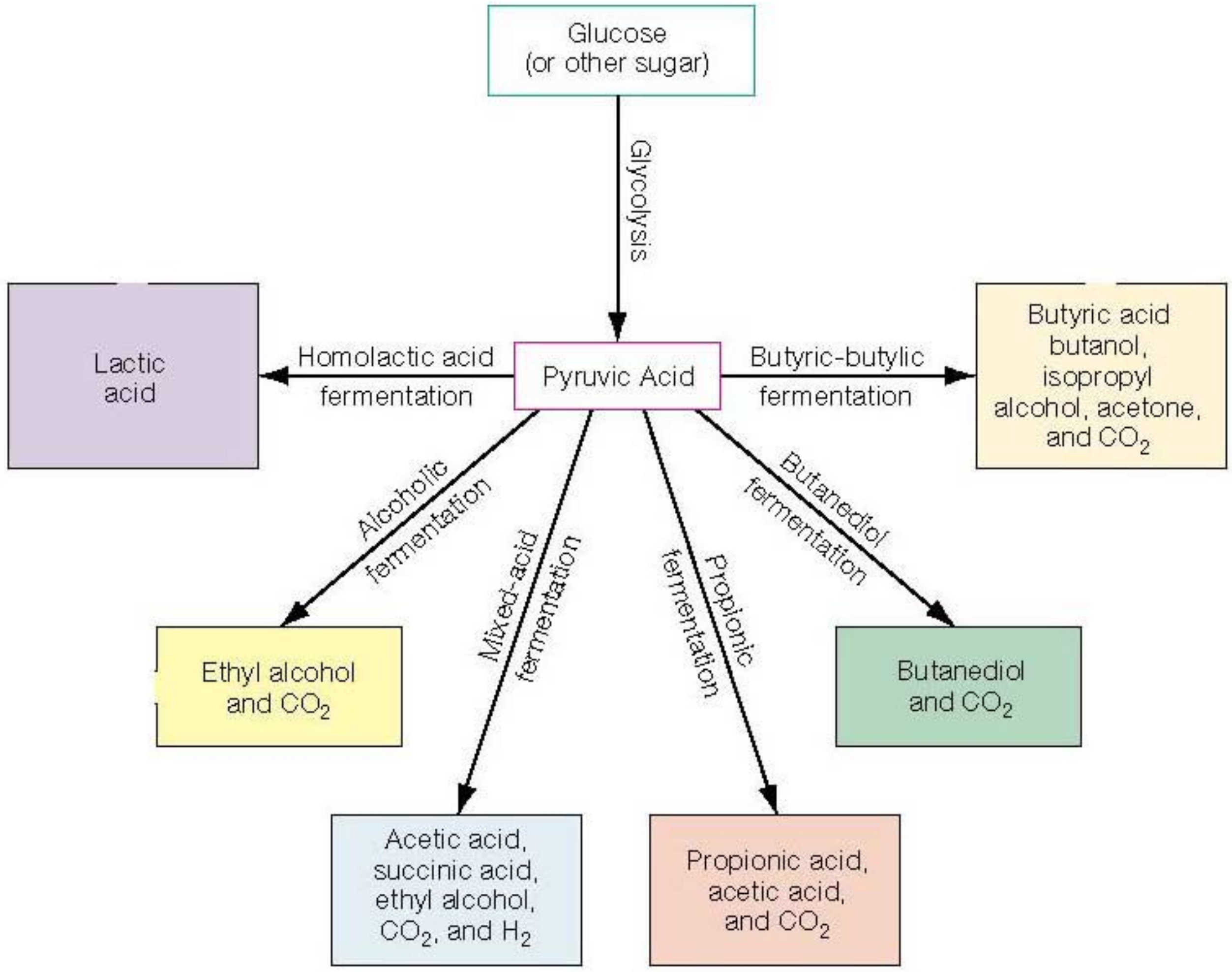
An Overview of Fermentation





(a)

Fermentation



End-Products of Fermentation

Pyruvic acid

Organism	*Streptococcus, Lactobacillus, Bacillus*	*Saccharomyces* (yeast)	*Propionibacterium*	*Clostridium*	*Escherichia, Salmonella*	*Enterobacter*
Fermentation end-product(s)	Lactic acid	Ethanol and CO_2	Propionic acid, acetic acid, CO_2, and H_2	Butyric acid, butanol, acetone, isopropyl alcohol, and CO_2	Ethanol, lactic acid, succinic acid, acetic acid, CO_2, and H_2	Ethanol, lactic acid, formic acid, butanediol, acetoin, CO_2, and H_2

(b)

Figure 5.18b

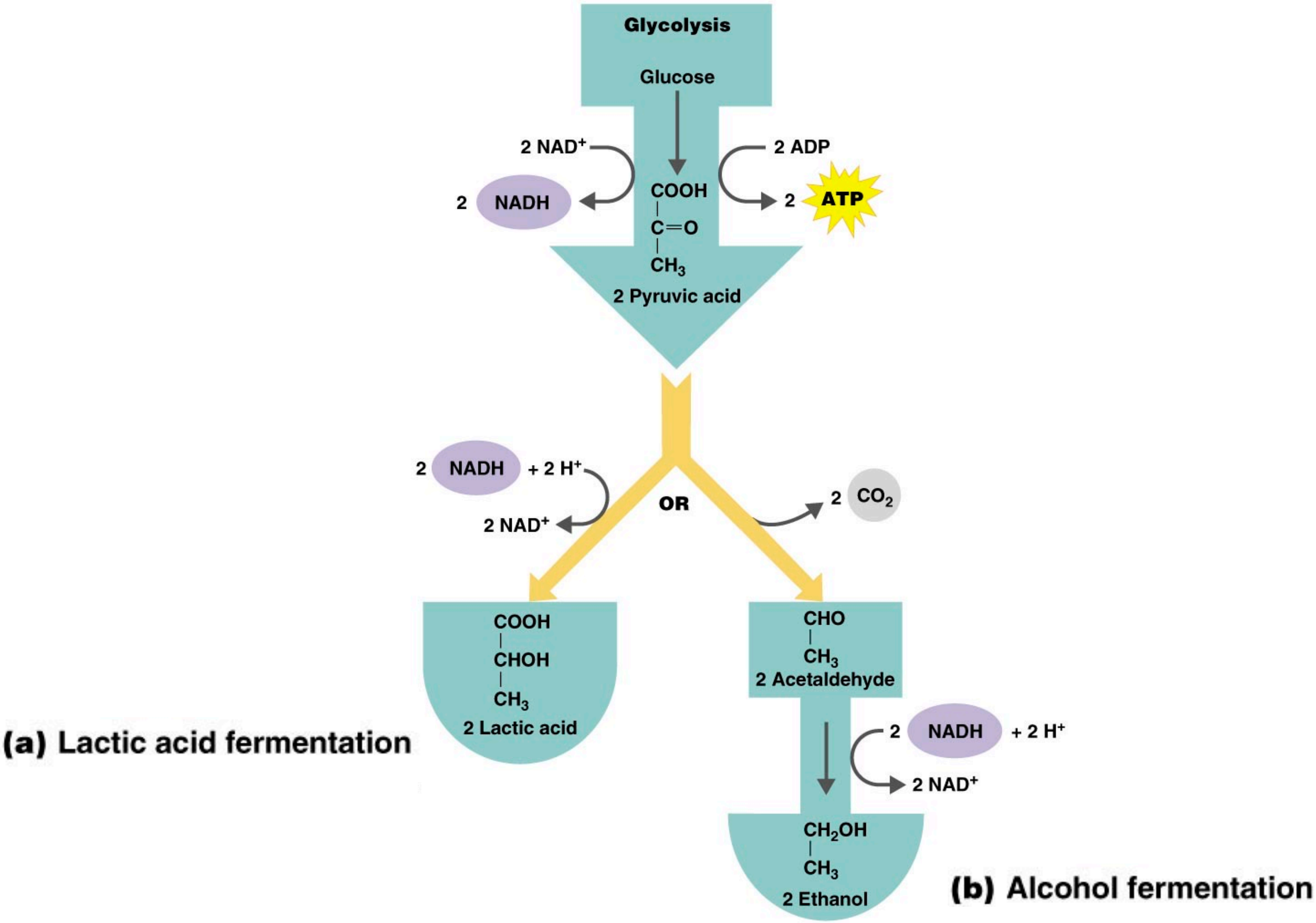
Fermentation

Two most important fermentation process are:

- **Alcohol fermentation**: Produces ethanol + CO_2

- **Lactic acid fermentation**: Produces lactic acid
  - Homolactic fermentation: Produces lactic acid only
  - Heterolactic fermentation: Produces lactic acid and other compounds

Types of Fermentation



Fermentation Summary

- Anaerobic
- Cytoplasm
- Partial Oxidation
- Small amounts of ATP generated via substrate level phosphorylation
- Organic intermediaries as final electron acceptors
- End products
  - Acid: Lactic Acid, Acetic Acid, Butyric Acid, Acetone
  - Alcohol: Ethanol, Isopropyl
  - Gas : CO_2, H_2
  - Contaminants

Till now we have discussed about carbohydrate catabolism…

Now we move to other macromolecules catabolism…