Chapter 5

Microbial Metabolism

Metabolism

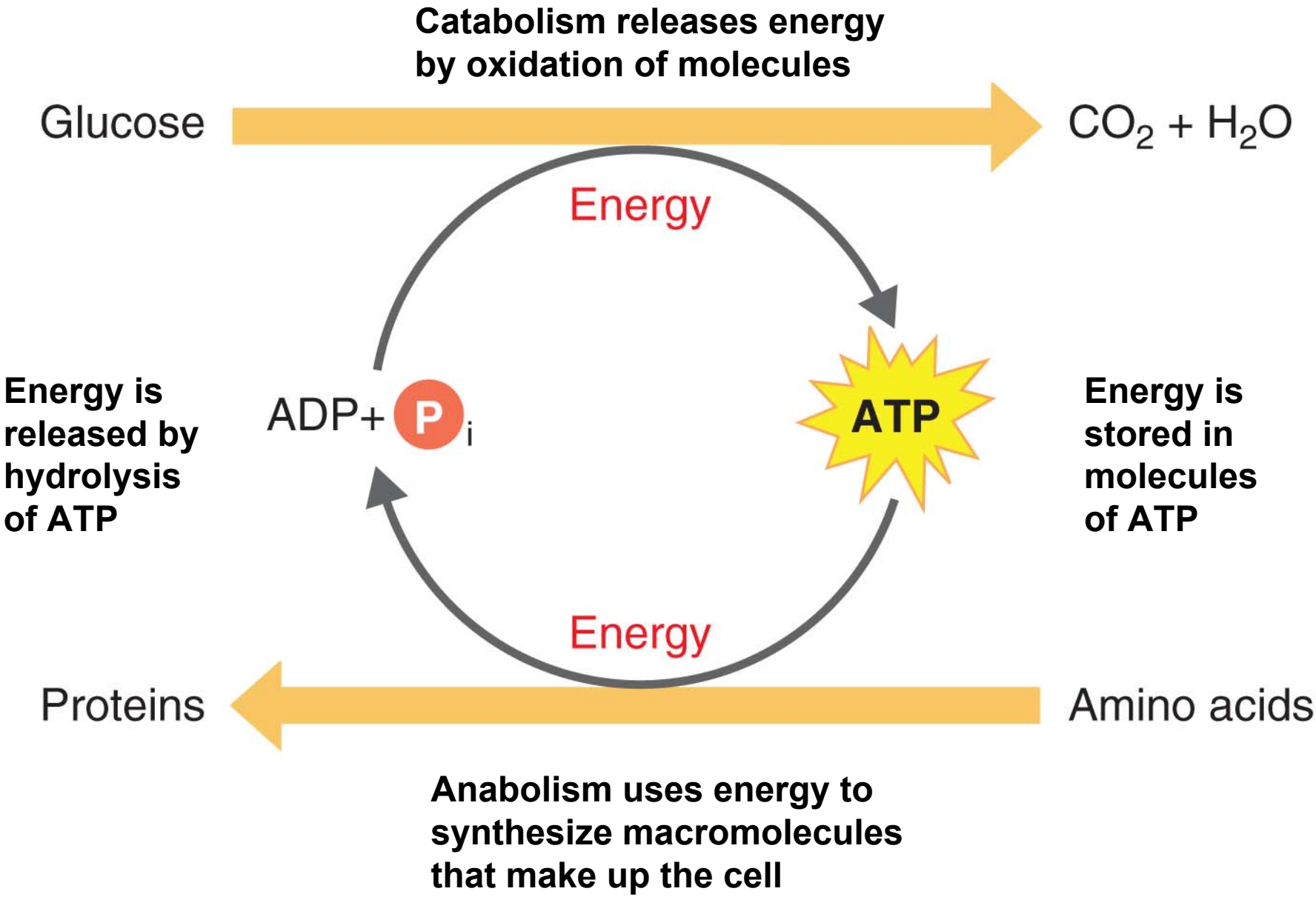
- **Metabolism**: the sum of all the chemical reactions in an organism
- A **metabolic pathway** is a sequence of chemical reactions catalyzed by enzymes // enzymes' name ends in "-ase"
  - Substrate 1 ---(E1)→ Substrate 2 ---(E2)→ Substrate 3
- Enzymes action influenced by temperature, pH, concentrations
- Enzymes are proteins // encoded by genes
  - DNA ---→ mRNA --→ Protein
- Proteins are "manufactured" "on" ribosomes (nickname = protein factories)
- Enzymes are not consumed in the reaction

Two Forms of Metabolism

- **Catabolism**: provides energy and building blocks for anabolism (e.g. protein to amino acids)

- **Anabolism**: uses energy and smaller building blocks to build larger molecules (e.g. amino acids to protein)

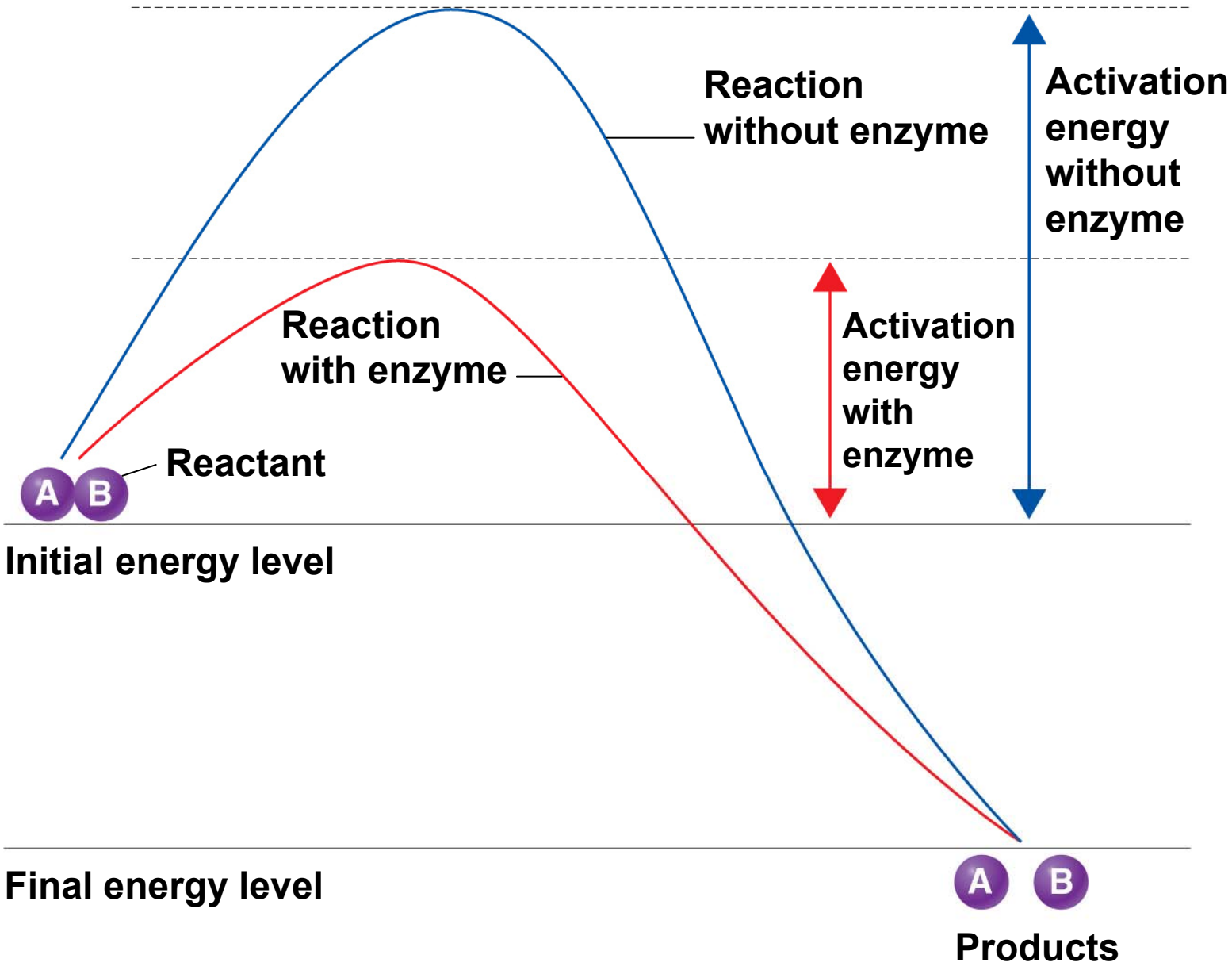
The role of ATP in coupling anabolic and catabolic reactions.



Collision Theory

- The **collision theory** states that chemical reactions can occur when atoms, ions, and molecules collide
- **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
- Enzymes reduce the activation energy required for a chemical reaction to occur

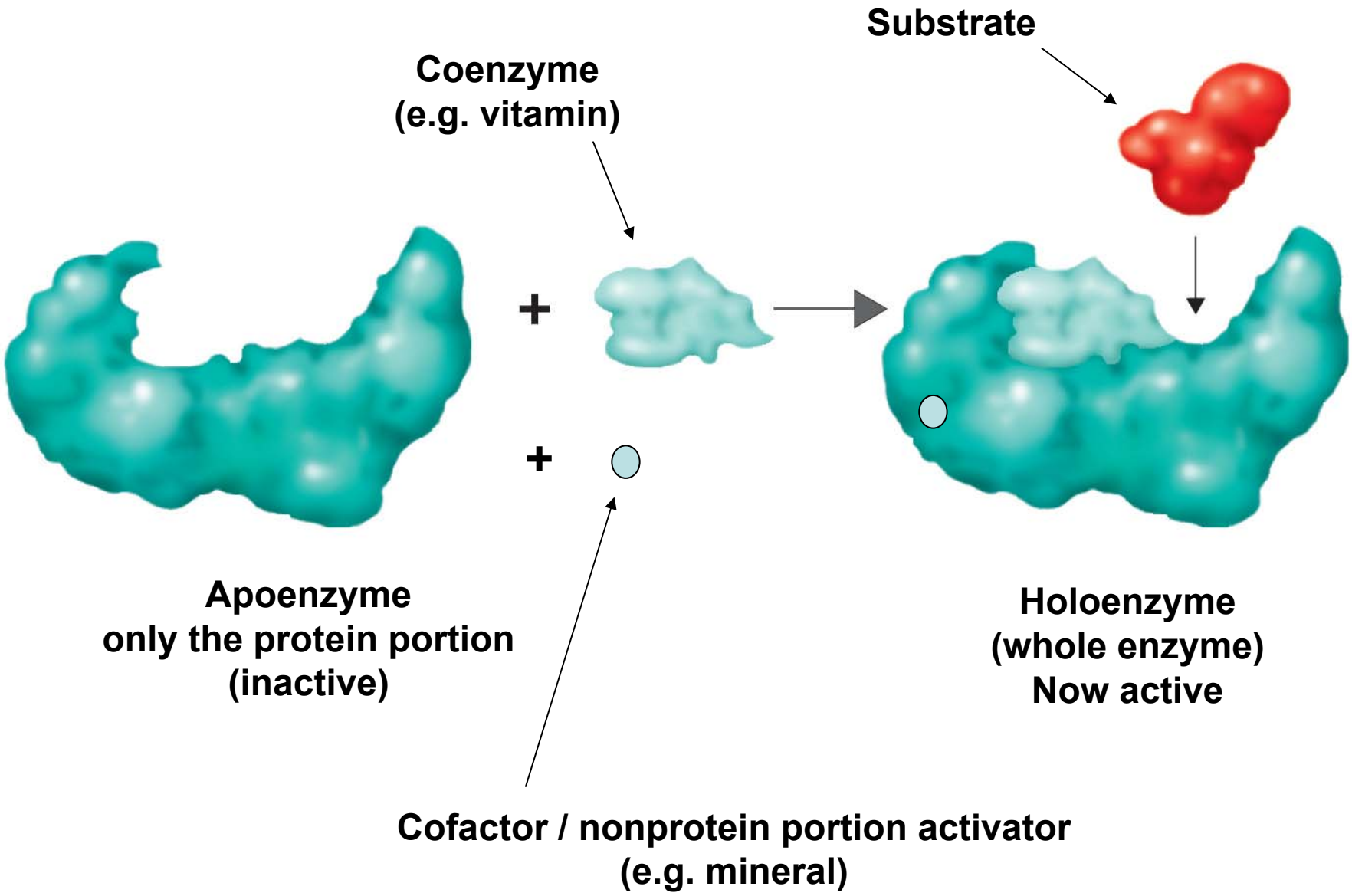
Energy requirements of a chemical reaction.



Enzyme Components

- Enzyme = Biological catalysts
- Specific for a chemical reaction // not consumed in reaction //   used over and over!
- **Apoenzyme**: protein portion of enzyme
- **Cofactor**: nonprotein component (e.g. mineral)
- **Coenzyme**: organic cofactor (e.g. vitamins are coenzymes)
- **Holoenzyme**: apoenzyme plus cofactor

Components of a Holoenzyme.

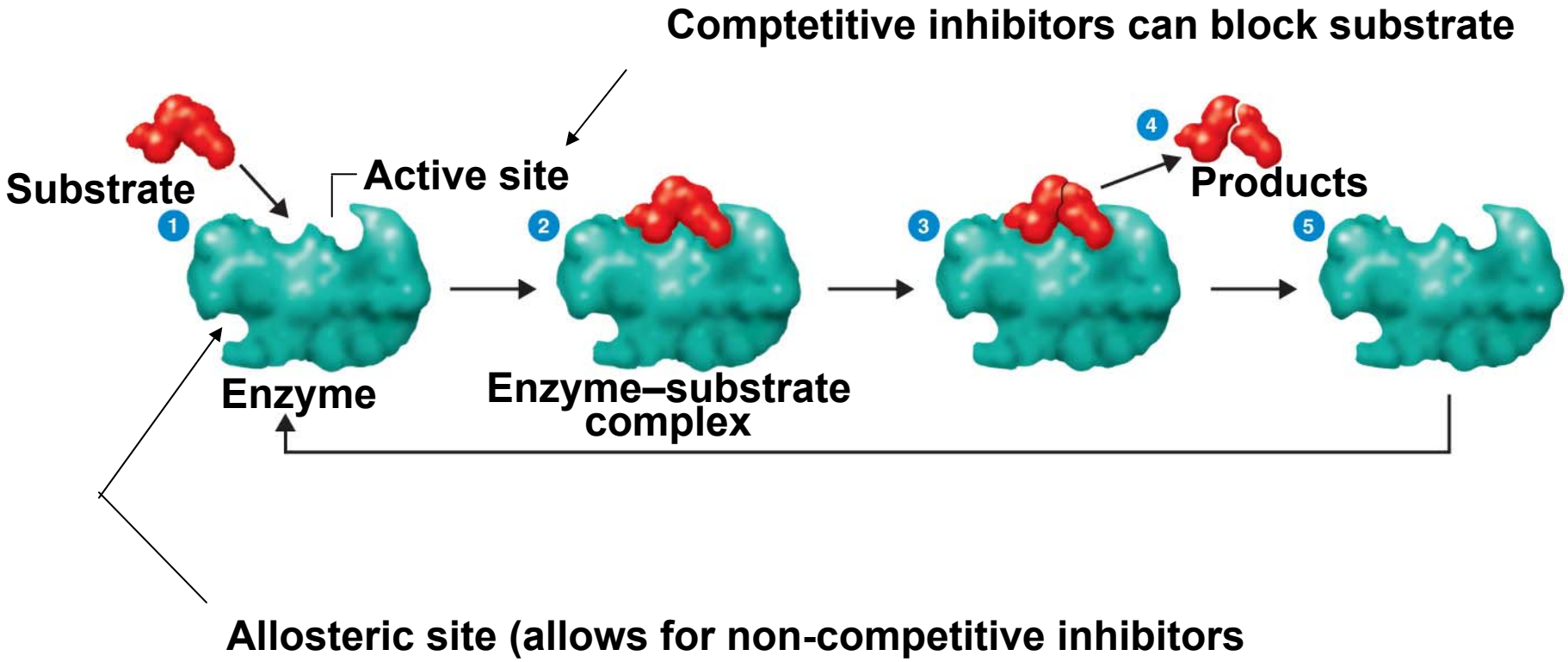


Important Coenzymes

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

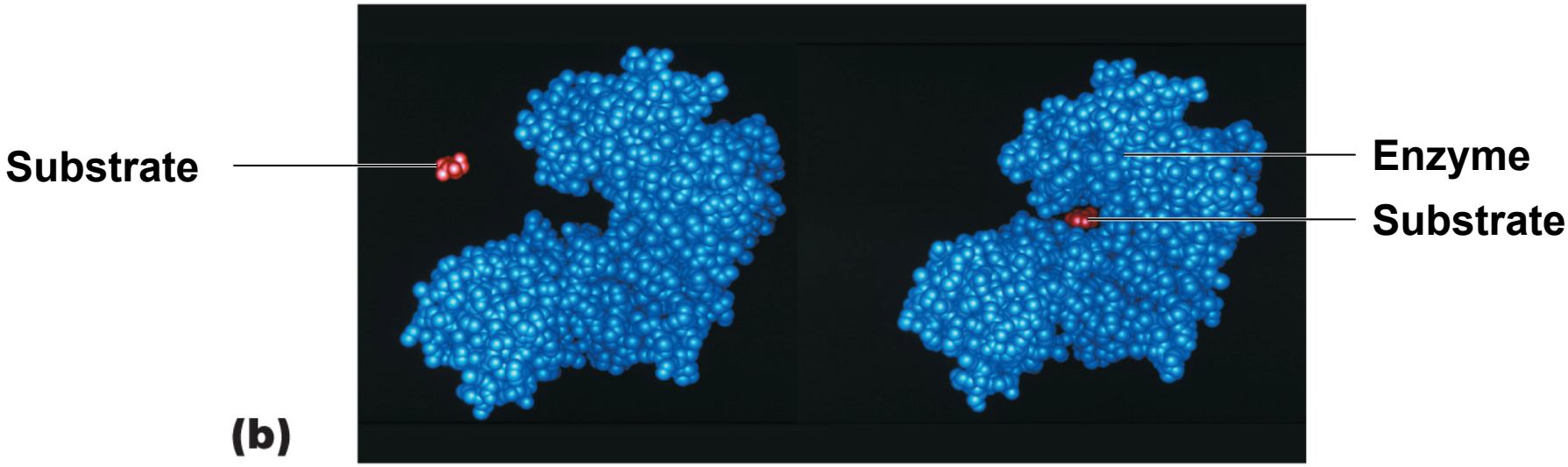
Note: These are all formed from vitamins.

Mechanism of Enzymatic Action.



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

The mechanism of enzymatic action.



Enzyme Classification

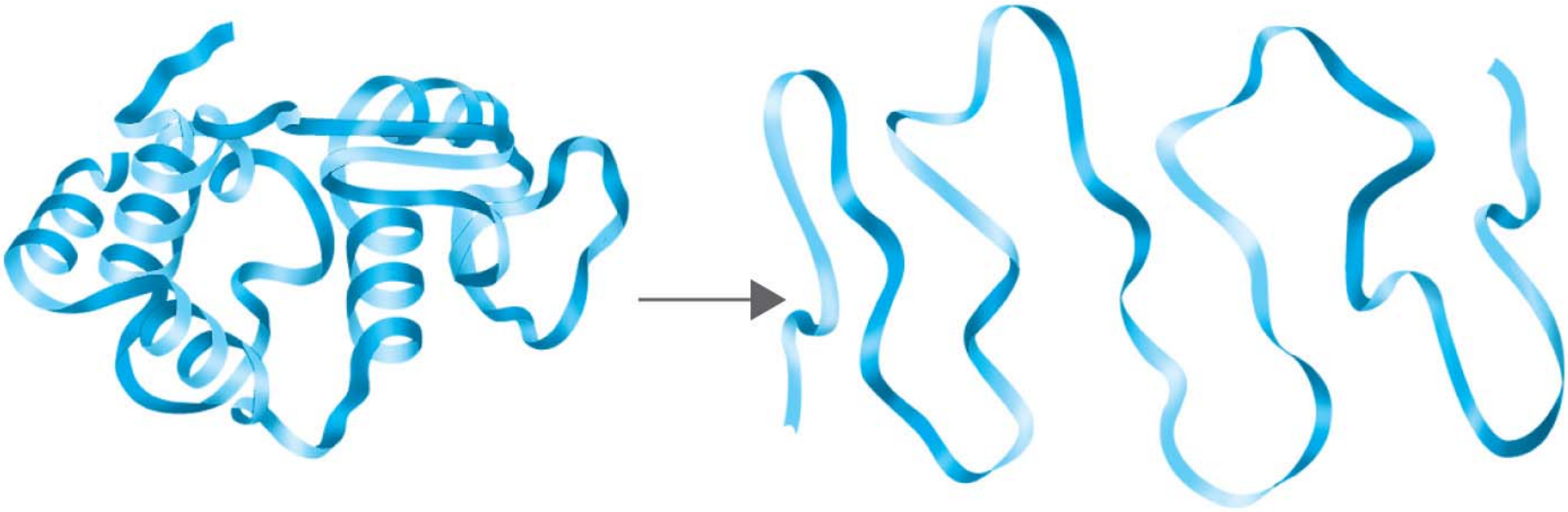
- **Oxidoreductase**: oxidation-reduction reactions
- **Transferase**: transfer functional groups
- **Hydrolase**: hydrolysis
- **Lyase**: removal of atoms without hydrolysis
- **Isomerase**: rearrangement of atoms
- **Ligase**: joining of molecules // uses ATP

Factors Influencing Enzyme Activity

- Temperature
- pH
- Substrate concentration
- Inhibitors

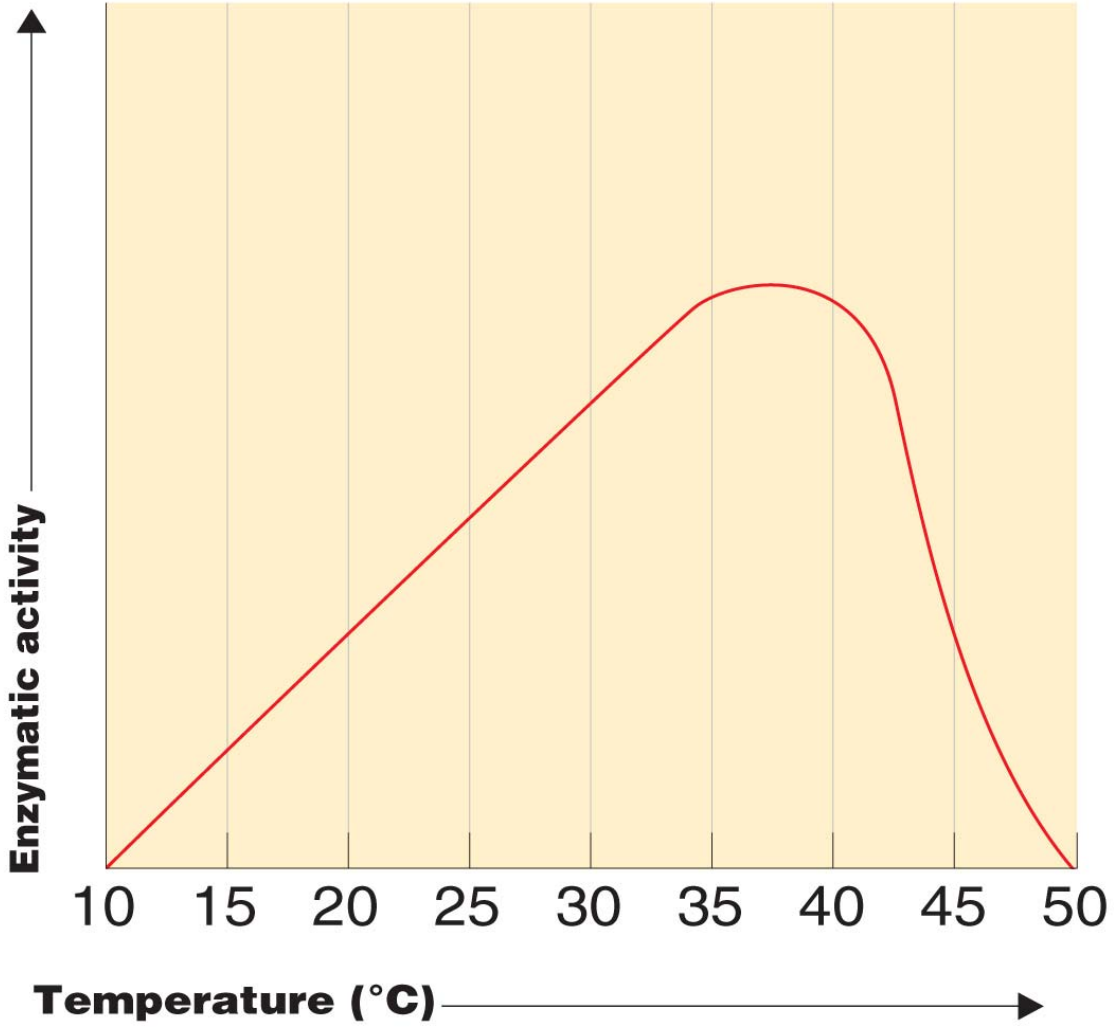
Factors Influencing Enzyme Activity

- Temperature and pH denature proteins (also whipping as in when you whip egg whites)



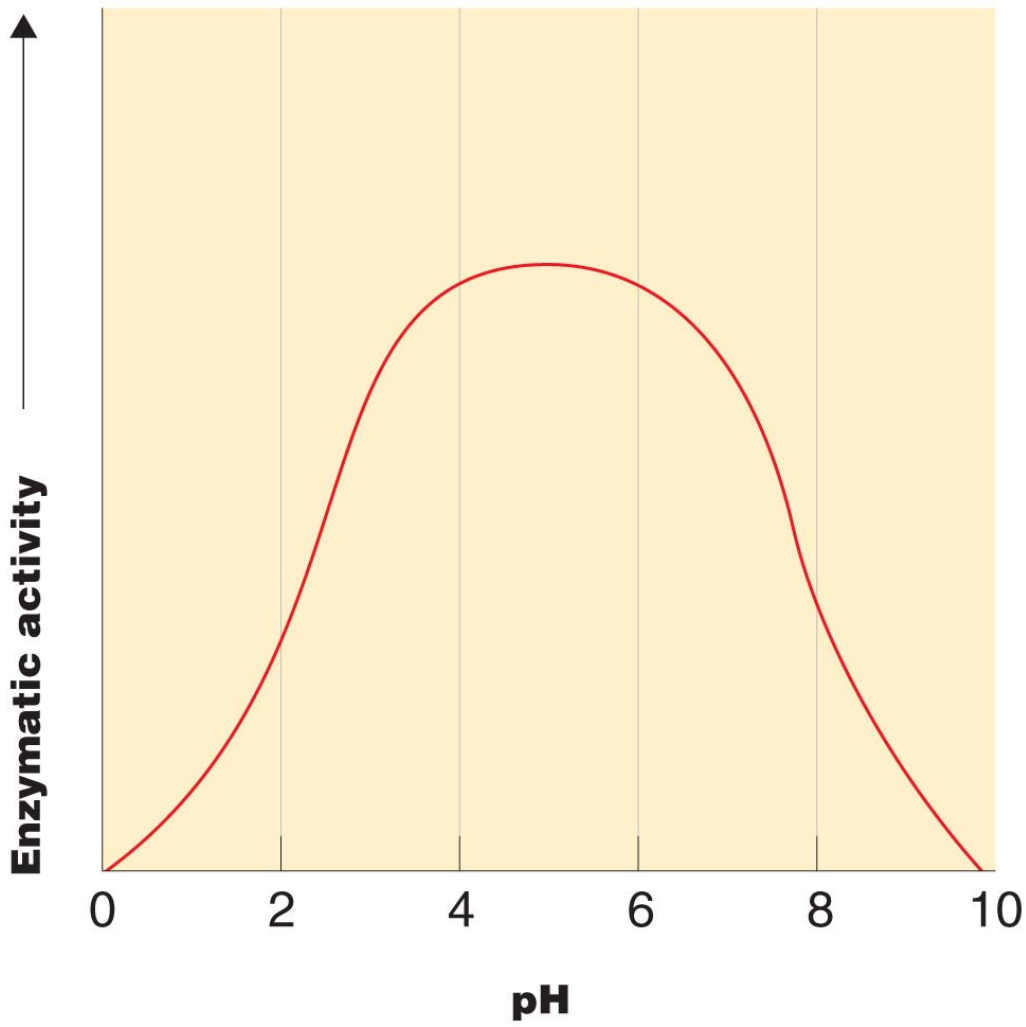
Active (functional) protein **Denatured protein**

Factors that influence enzymatic activity, plotted for a hypothetical enzyme.



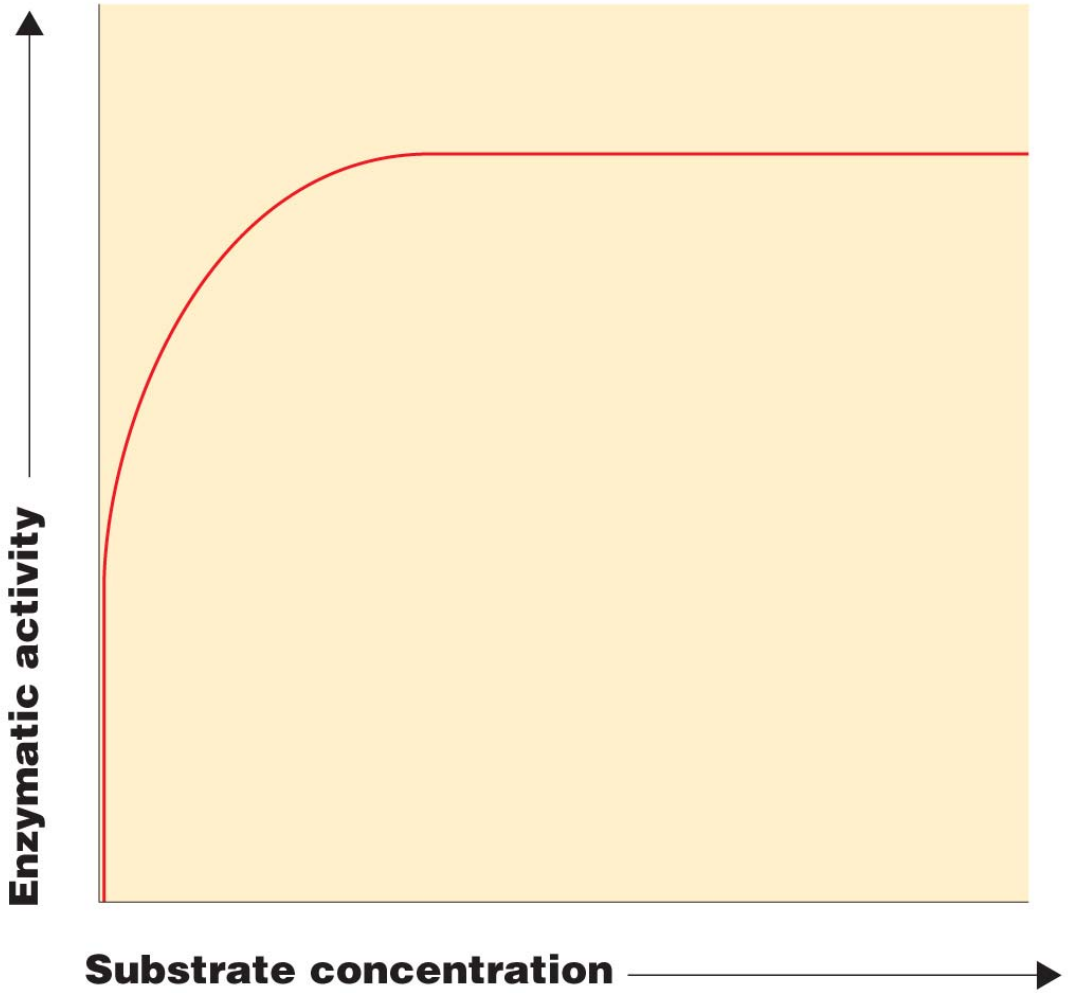
(a) Temperature. **The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.**

Factors that influence enzymatic activity, plotted for a hypothetical enzyme.



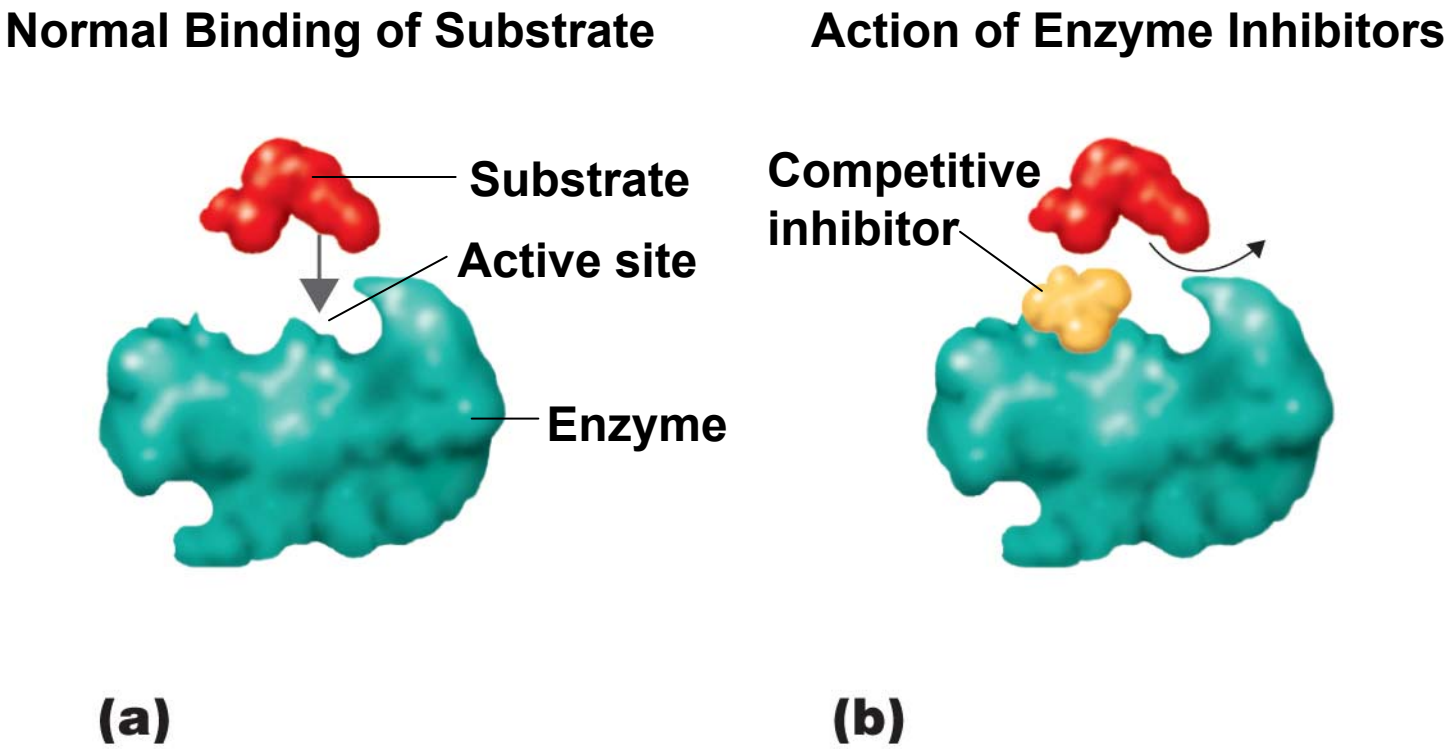
(b) pH. **The enzyme illustrated is most active at about pH 5.0.**

Factors that influence enzymatic activity, plotted for a hypothetical enzyme.

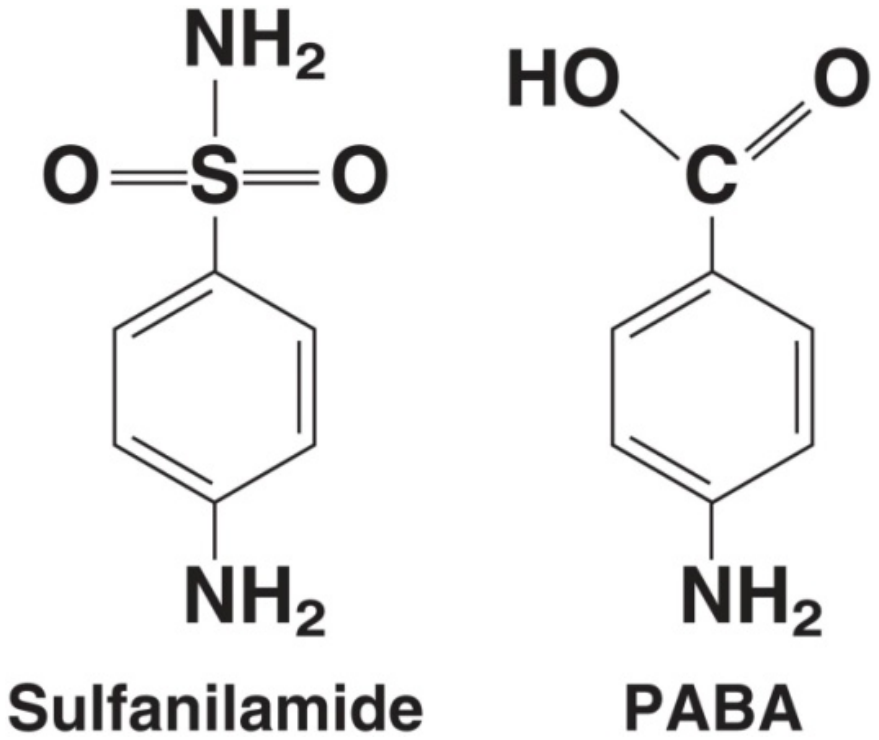


(c) Substrate concentration. With increasing concentration of substrate molecules, the rate of reaction increases until the active sites on all the enzyme molecules are filled, at which point the maximum rate of reaction is reached.

Regulation
Enzyme Competitive Inhibition



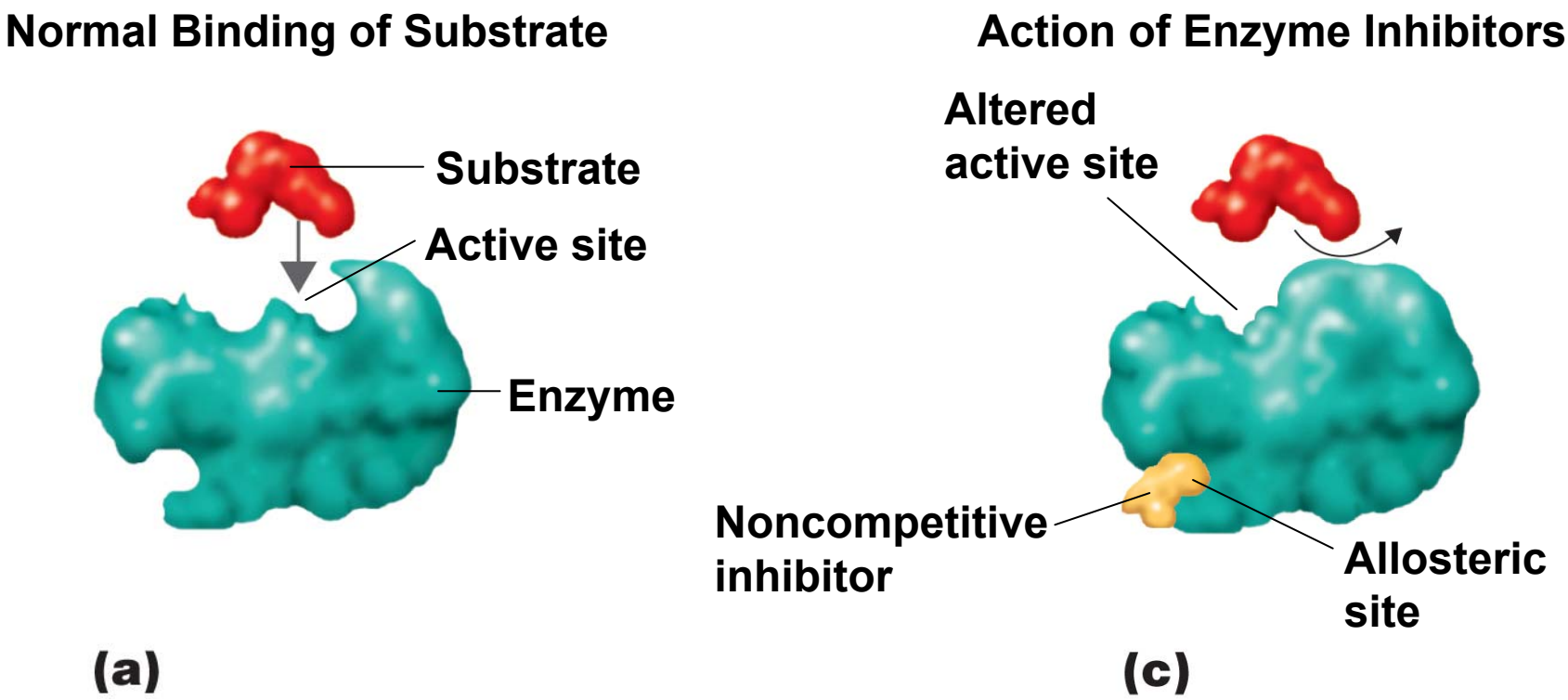
Regulation: Enzyme Competitive Inhibition



Para-aminobenzoic acid (PABA) = Essential nutrient for bacterial synthesis of folic acid (coenzyme)

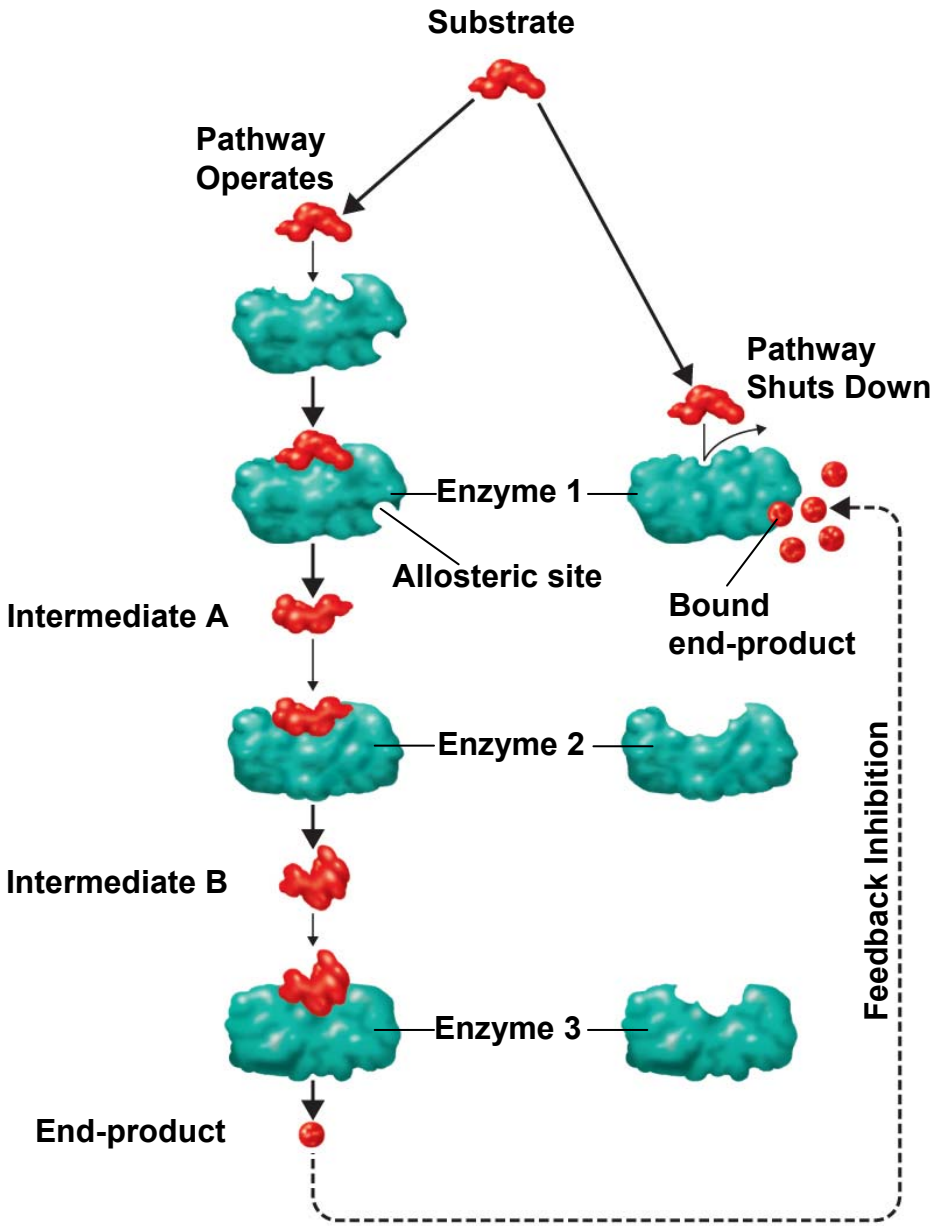
Sulfanilamide is a competitive inhibitor that blocks folic acid formation // How sulfa drugs inhibit bacterial growth.

Regulation // Enzyme Noncompetitive Inhibitor



Enzyme Regulation

Feedback Inhibitors Inhibition

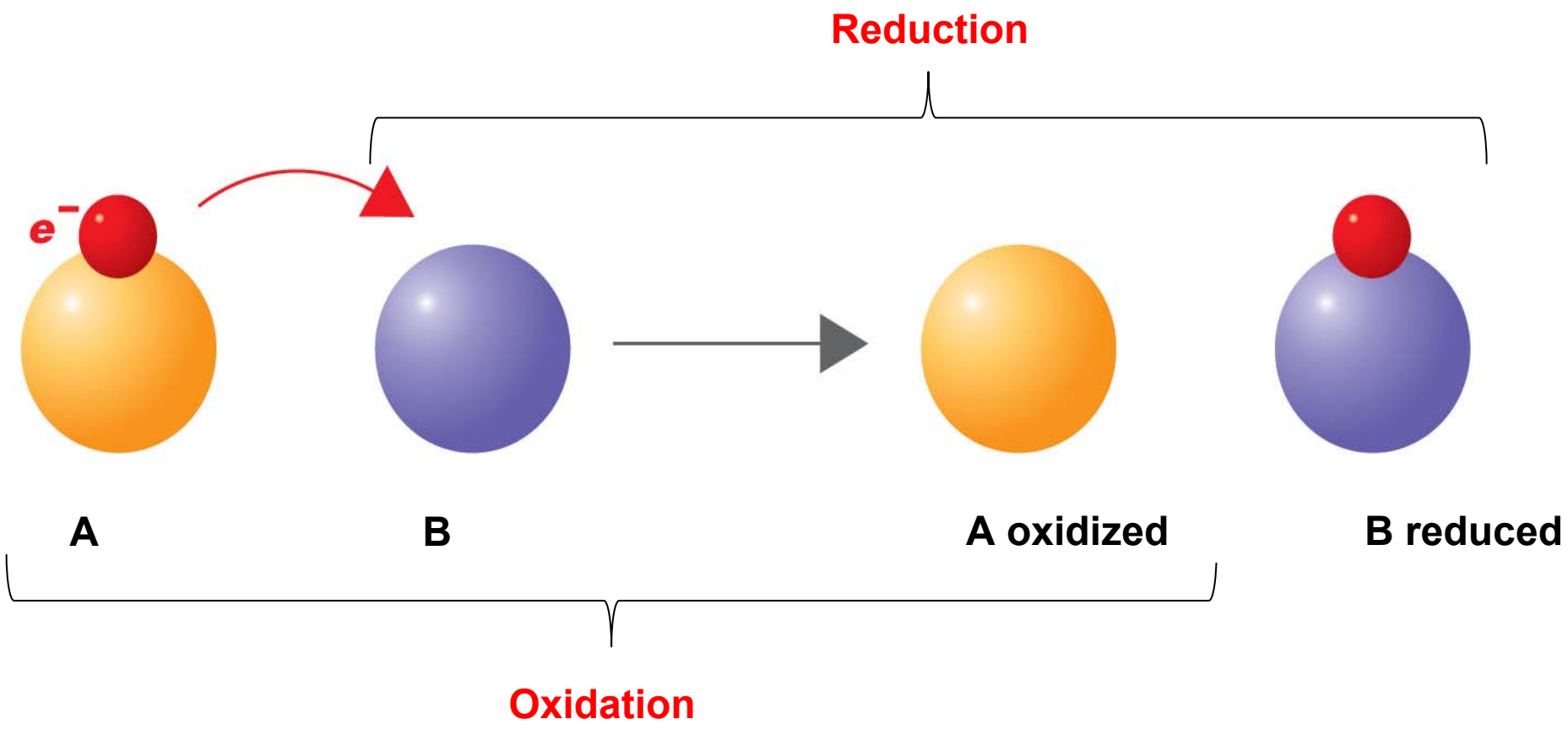


Ribozymes

- RNA is now understood to have greater role in cellular functions
- In the past, RNA believed to be only involved in protein synthesis (e.g. mRNA, tRNA, rRNA)
- Now RNA understood to also function like enzymes in many different mechanisms
- Micro RNA and other species of RNA able to cut and splice RNA
- Note: similar to enzymes // not consumed // reused // regulated

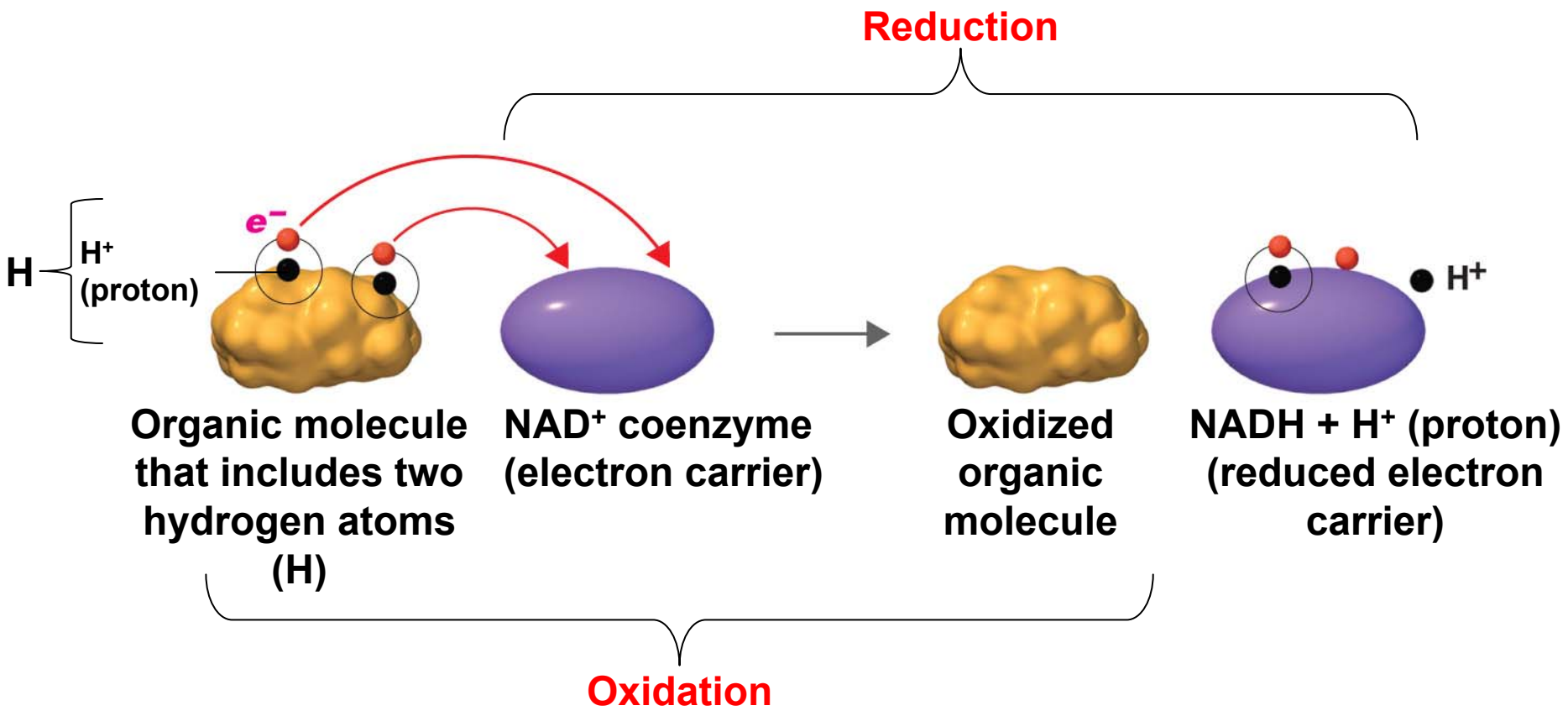
Oxidation-Reduction Reactions

- **Oxidation**: removal of electrons
- **Reduction**: gain of electrons
- **Redox reaction**: an oxidation reaction paired with a reduction reaction



Oxidation-Reduction Reactions

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

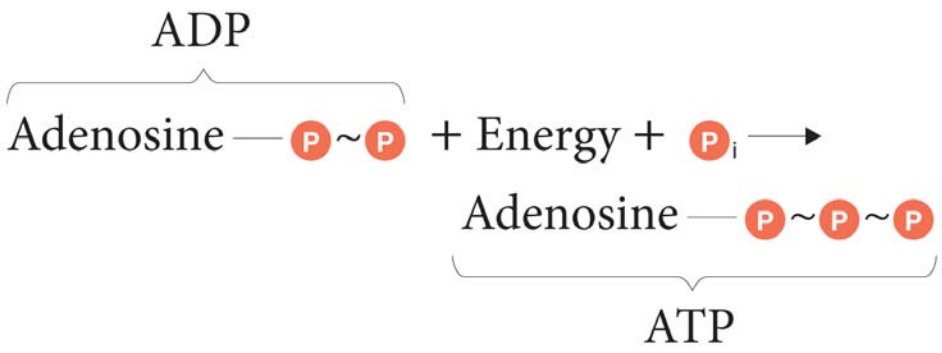


The Generation of ATP

- ATP's nickname is "molecular money"
- ATP is a universal molecule /// used by all cells to do cellular work!!!
- Three methods evolved to make ATP
  - Substrate-level Phosphorylation
  - Oxidative Phosphorylation
  - Photophosphorylation

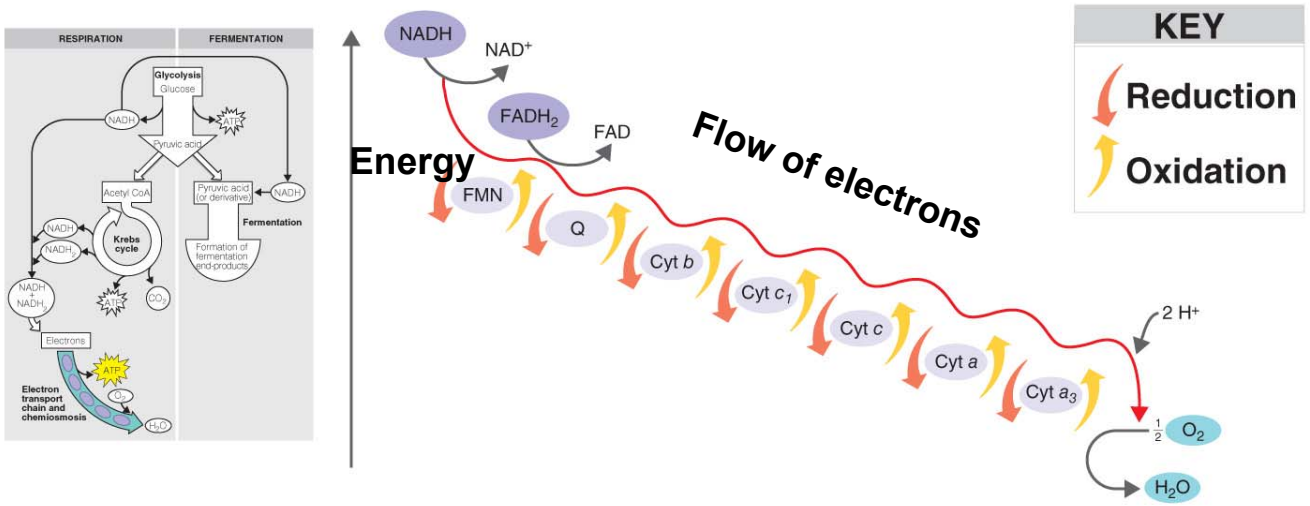
Substrate-Level Phosphorylation

- Energy from the transfer of a high-energy PO_4^- to ADP generates ATP
- The transferred phosphate comes from an organic molecule

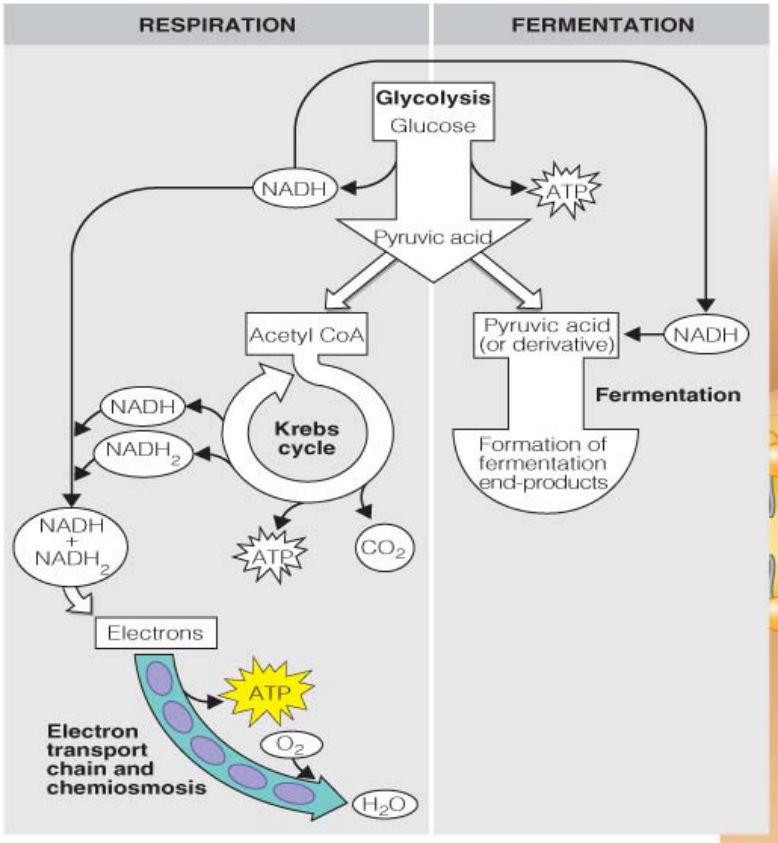


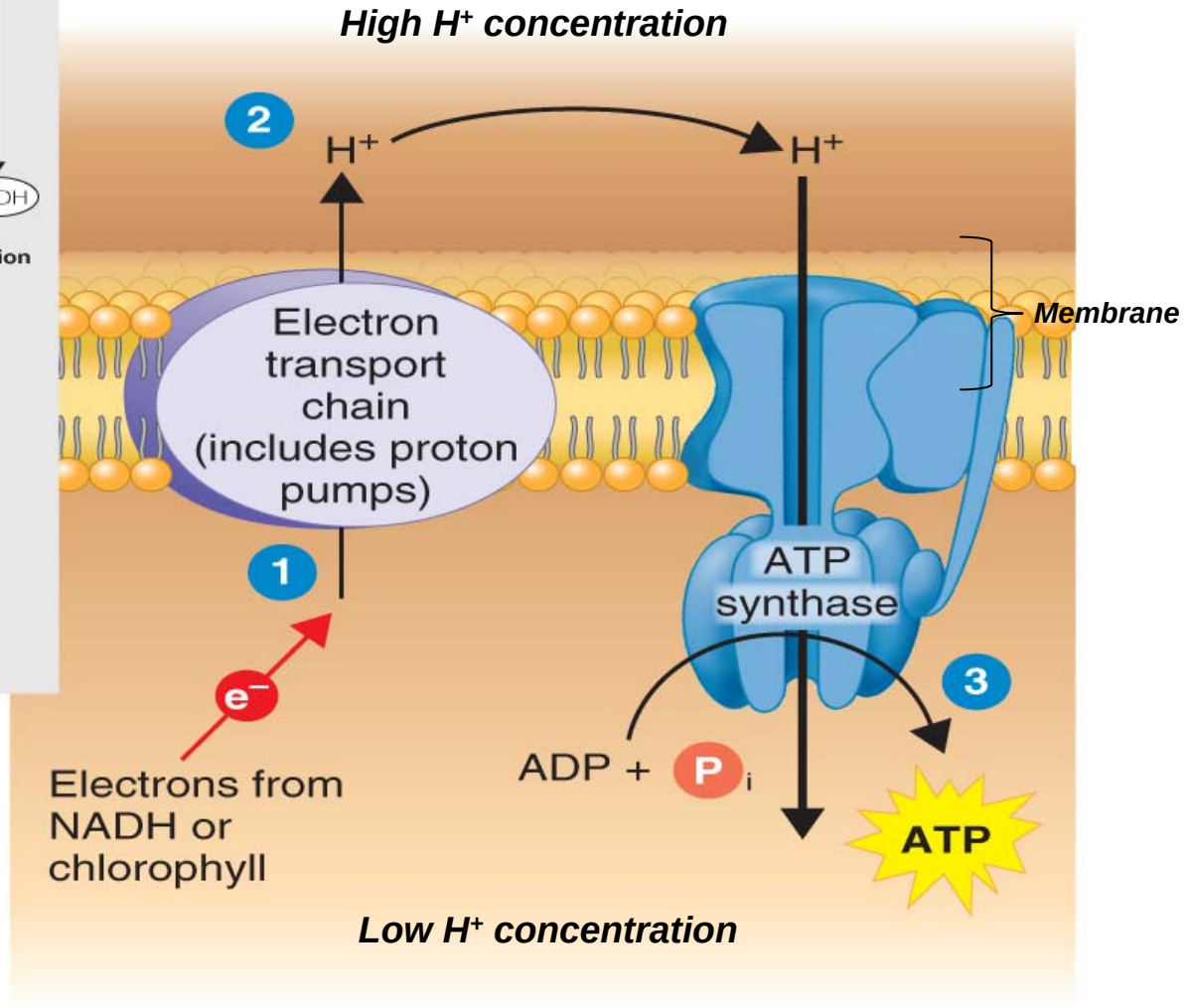
Oxidative Phosphorylation

- Electrons are transferred from organic compounds (oxidized) to electrons carriers like NAD or FAD (reduced to NADH and FADH)
- Electrons then transferred (i.e. oxidized-reduction) to a series of electron carriers built into membranes (i.e. the **electron transport chain)**
- ETC is series of oxidation/reduction reactions /// energy released at each step is used to concentrates H+ on one side of a membrane
- ATP produced in this process known as **"chemiosmosis"**



Oxidative Phosphorylation





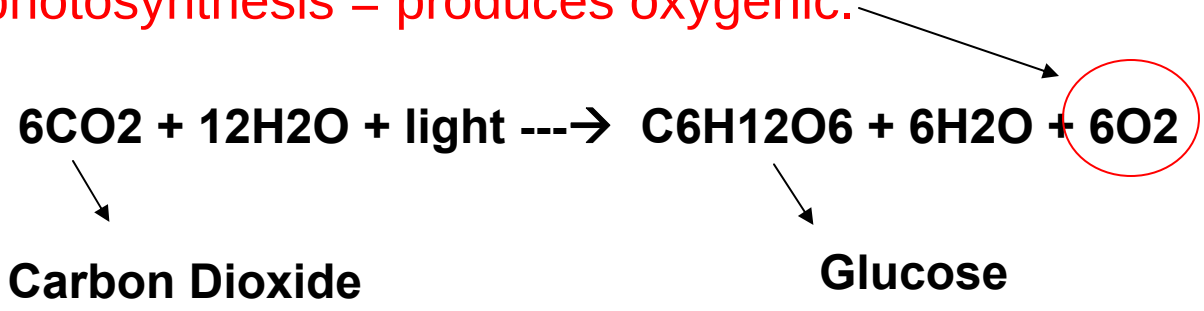
Chemiosmosis.

Photophosphorylation

- Many organisms obtain energy for cellular work by oxidizing organic compounds (glucose oxidized to carbon dioxide and energy released).
- Where do these organic compounds come from?
- Other organisms reduce inorganic molecules to make organic compounds!
- This is accomplished by using “Photosynthesis”. /// Carried out by plants and microbes.
- Photosynthesis essentially uses light energy of the sun to create chemical energy (ATP)
- This chemical energy is then used to reduce inorganic CO_2 into a more reduced organic carbon compound glucose (carbon fixation).

Two Types of Photophosphorylation

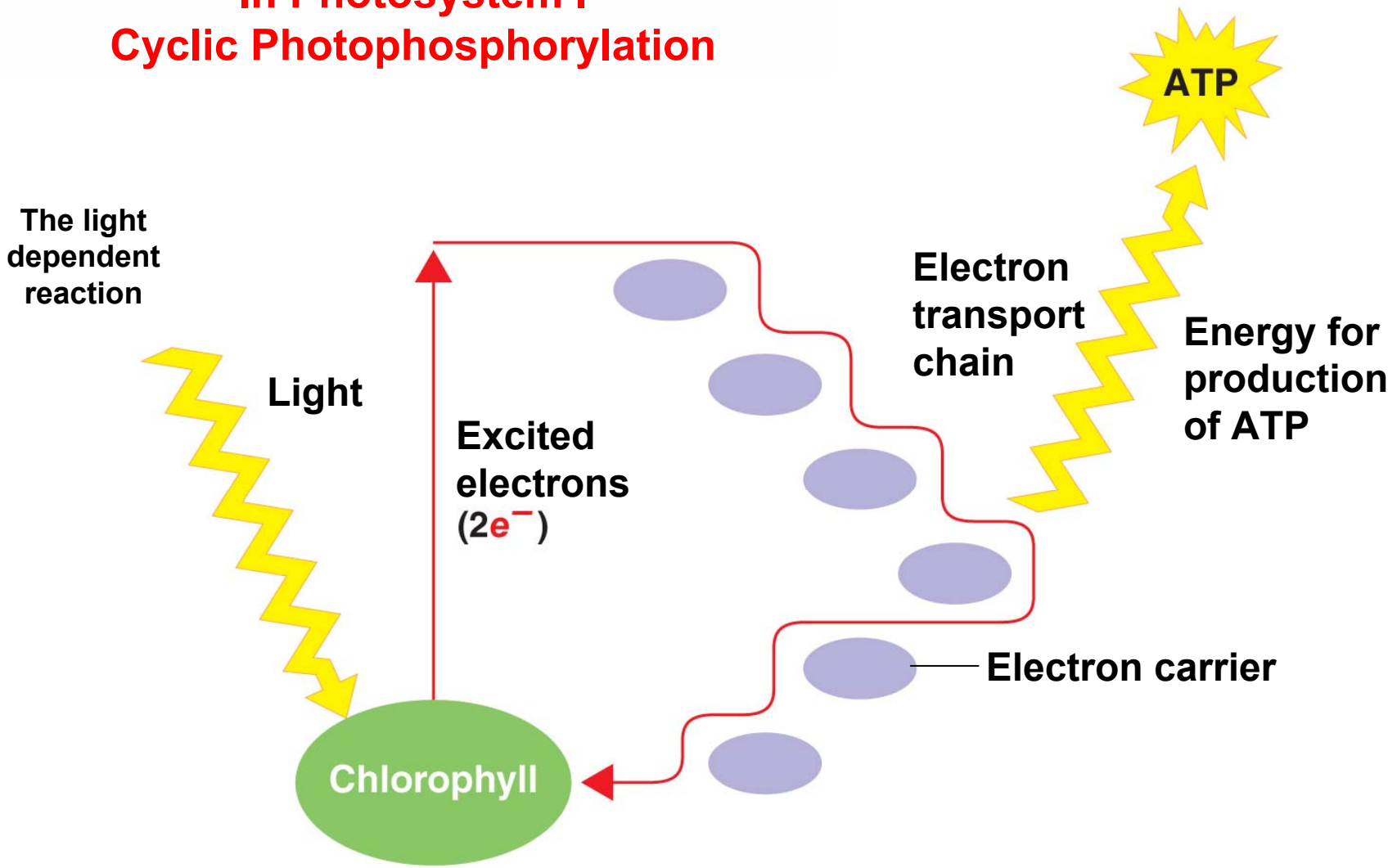
- Plants, algae, and cyanobacteria use water as a hydrogen donor, releasing oxygen. /// Non-Cyclic photosynthesis = produces oxygenic.



- Purple sulfur and greeen sulfur bacteria use H2S as a hydrogen donor, producing sulfur granules. Cyclic photosynthesis = anoxygenic.

6CO2 + 12H2S + light ---→ C6H12O6 + 6H2O +12S

In Photosystem I
Cyclic Photophosphorylation

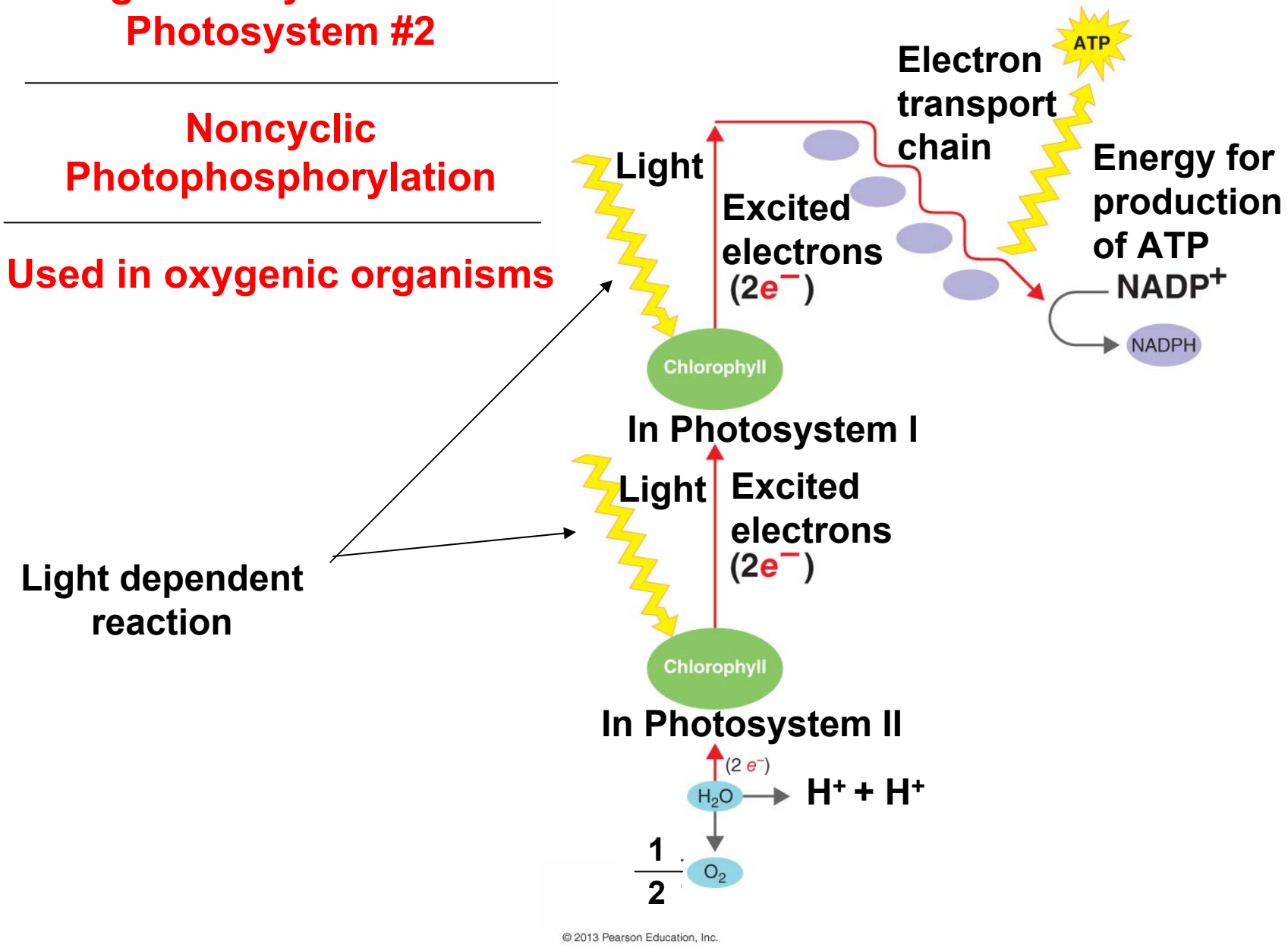


No Oxygen Produced
Only ATP Produced

Using Photosystem #1 and Photosystem #2

Noncyclic Photophosphorylation

Used in oxygenic organisms



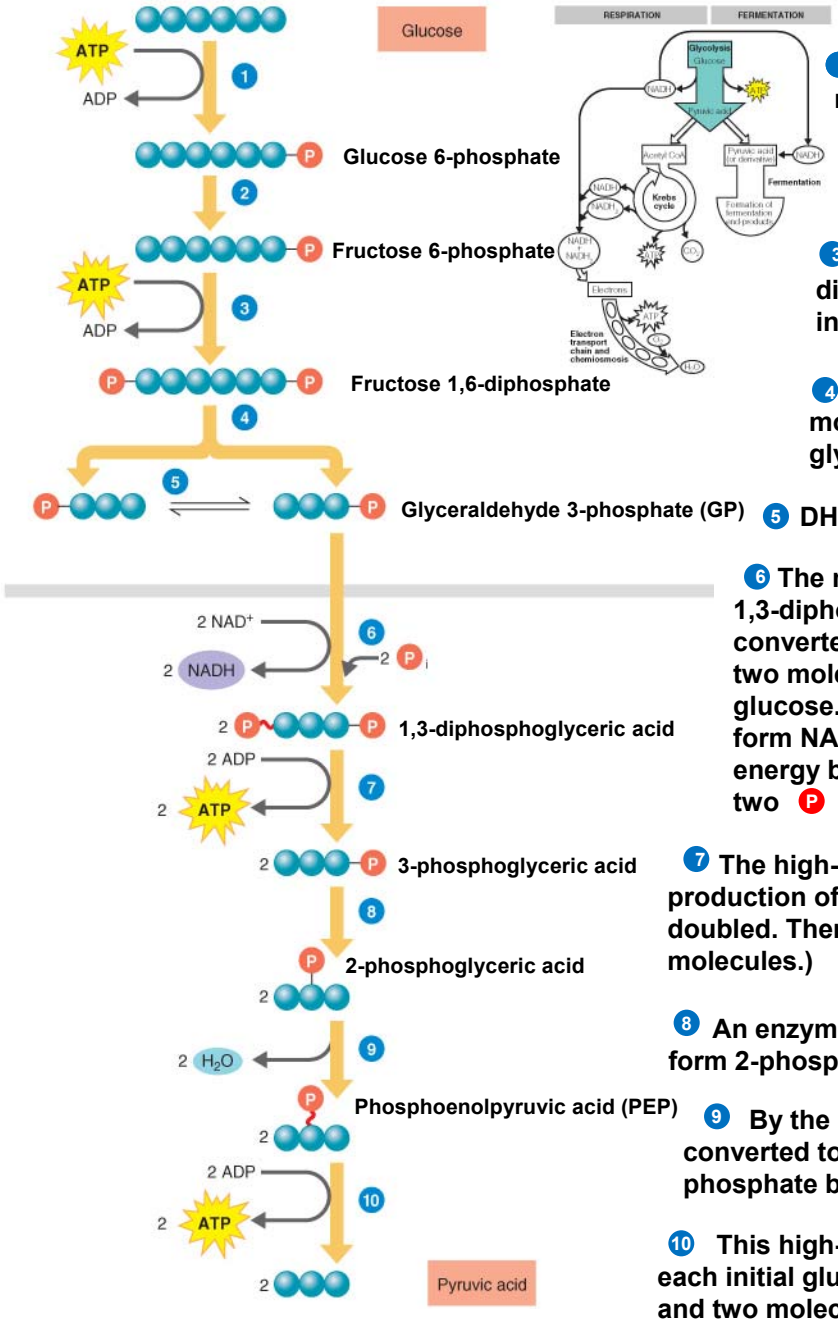
Carbohydrate Catabolism

- The breakdown of carbohydrates to release energy
- Three Stages (pathways) Are Required to Complete Glucose Catabolism
  - **Glycolysis**
  - **Krebs cycle**
  - **Electron transport chain**

Glycolysis

- The oxidation of glucose to pyruvic acid produces ATP and NADH
- Preparatory phase:
  - 2 ATP are used
  - Glucose is split to form 2 glucose-3-phosphate
  - No oxygen required

An outline of the reactions of glycolysis (Embden-Meyerhof pathway).



1 Glucose enters the cell and is phosphorylated. A molecule of ATP is invested. The product is glucose 6-phosphate.

2 2 Glucose 6-phosphate is rearranged to form fructose 6-phosphate.

3 The P from another ATP is used to produce fructose 1,6-diphosphate, still a six-carbon compound. (Note the total investment of two ATP molecules up to this point.)

4 An enzyme cleaves (splits) the sugar into two three-carbon molecules: dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GP).

5 DHAP is readily converted to GP (the reverse action may also occur).

6 The next enzyme converts each GP to another three-carbon compound, 1,3-diphosphoglyceric acid. Because each DHAP molecule can be converted to GP and each GP to 1,3-diphosphoglyceric acid, the result is two molecules of 1,3-diphosphoglyceric acid for each initial molecule of glucose. GP is oxidized by the transfer of two hydrogen atoms to NAD^+ to form NADH. The enzyme couples this reaction with the creation of a high-energy bond between the sugar and a P. The three-carbon sugar now has two P groups.

7 The high-energy P is moved to ADP, forming ATP, the first ATP production of glycolysis. (Since the sugar splitting in step 4, all products are doubled. Therefore, this step actually repays the earlier investment of two ATP molecules.)

8 An enzyme relocates the remaining P of 3-phosphoglyceric acid to form 2-phosphoglyceric acid in preparation for the next step.

9 By the loss of a water molecule, 2-phosphoglyceric acid is converted to phosphoenolpyruvic acid (PEP). In the process, the phosphate bond is upgraded to a high-energy bond.

10 This high-energy P is transferred from PEP to ADP, forming ATP. For each initial glucose molecule, the result of this step is two molecules of ATP and two molecules of a three-carbon compound called pyruvic acid.

Energy-Conserving Stage of Glycolysis

- 2 glucose-3-phosphate are oxidized to form 2 pyruvic acid
- 4 ATP are produced
- 2 NADH are produced
- Note: used two ATP to start process

Glycolysis

- Glucose + 2 ATP + 2 ADP + 2 PO_4^- + 2 NAD^+ $\rightarrow$

  $\rightarrow$ 2 pyruvic acid + 4 ATP + 2 NADH + $2H^+$

Two Alternatives to Glycolysis
1st = Pentose Phosphate Pathway

- Uses pentoses and produces only one ATP and one NADPH
- Operates simultaneous with glycolysis // ability to break down both five and six carbon sugars
- Able to make important intermediates for necessary molecules // plays important role in anabolic pathways
  - Nucleic acids
  - Form glucose from carbon dioxide
  - Make certain amino acids
- Bacillus subtilis, E. coli, Enterococcus faecalis

Two Alternatives to Glycolysis
2nd = Entner-Doudoroff Pathway

- Produces 2 NADPH and 1 ATP
- Bacteria with these enzymes can metabolize glucose and produce ATP without glycolysis or pentose phosphate pathway
- Some gram negative bacteria use this pathway (gram positive generally do not use this pathway)
- *Pseudomonas, Rhizobium, Agrobacterium*

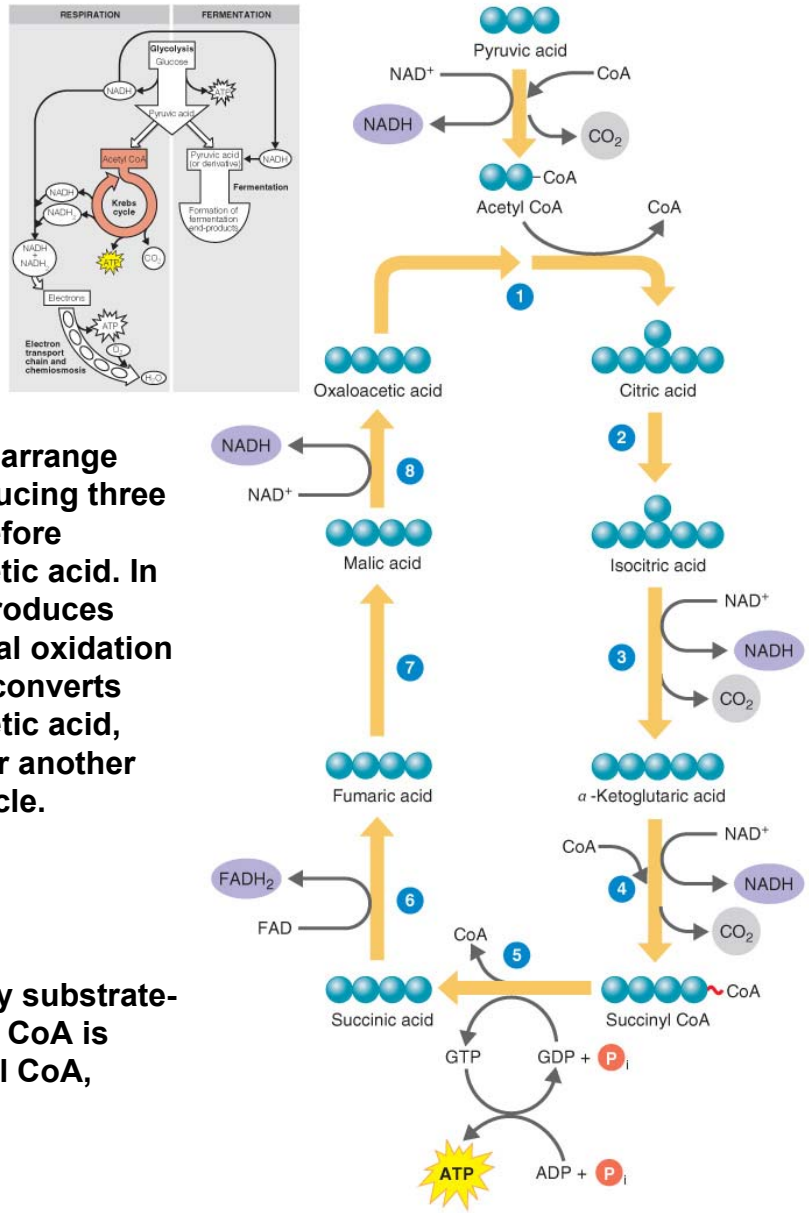
Cellular Respiration

- Oxidation of molecules liberates electrons which are transferred to NAD or FAD.
- These “reduced” electron carriers transport electrons to the electron transport chain
- ATP is then generated by mechanism called oxidative phosphorylation

The Intermediate Step

- Occurs between glycolysis and entry into the Krebs Cycle
- Pyruvic acid (from glycolysis) is oxidized and decarboxylated to produce acetyl which is attached to CoA
  - Acetyl-CoA enters the Krebs Cycle
  - Oxidation of acetyl CoA produces NADH and $FADH_2$
  - Note: beta oxidation of fatty acids produce Acetyl-CoA which then may also enter Krebs Cycle

The Krebs Cycle



1 **A turn of the cycle begins as enzymes strip off the CoA portion from acetyl CoA and combine the remaining two-carbon acetyl group with oxaloacetic acid. Adding the acetyl group produces the six-carbon molecule citric acid.**

2 **–** **4** **Oxidations generate NADH. Step 2 is a rearrangement. Steps 3 and 4 combine oxidations and decarboxylations to dispose of two carbon atoms that came from oxaloacetic acid. The carbons are released as CO_2, and the oxidations generate NADH from NAD^+. During the second oxidation (step 4), CoA is added into the cycle, forming the compound succinyl CoA.**

6 **–** **8** **Enzymes rearrange chemical bonds, producing three different molecules before regenerating oxaloacetic acid. In step 6, an oxidation produces $FADH_2$. In step 8, a final oxidation generates NADH and converts malic acid to oxaloacetic acid, which is ready to enter another round of the Krebs cycle.**

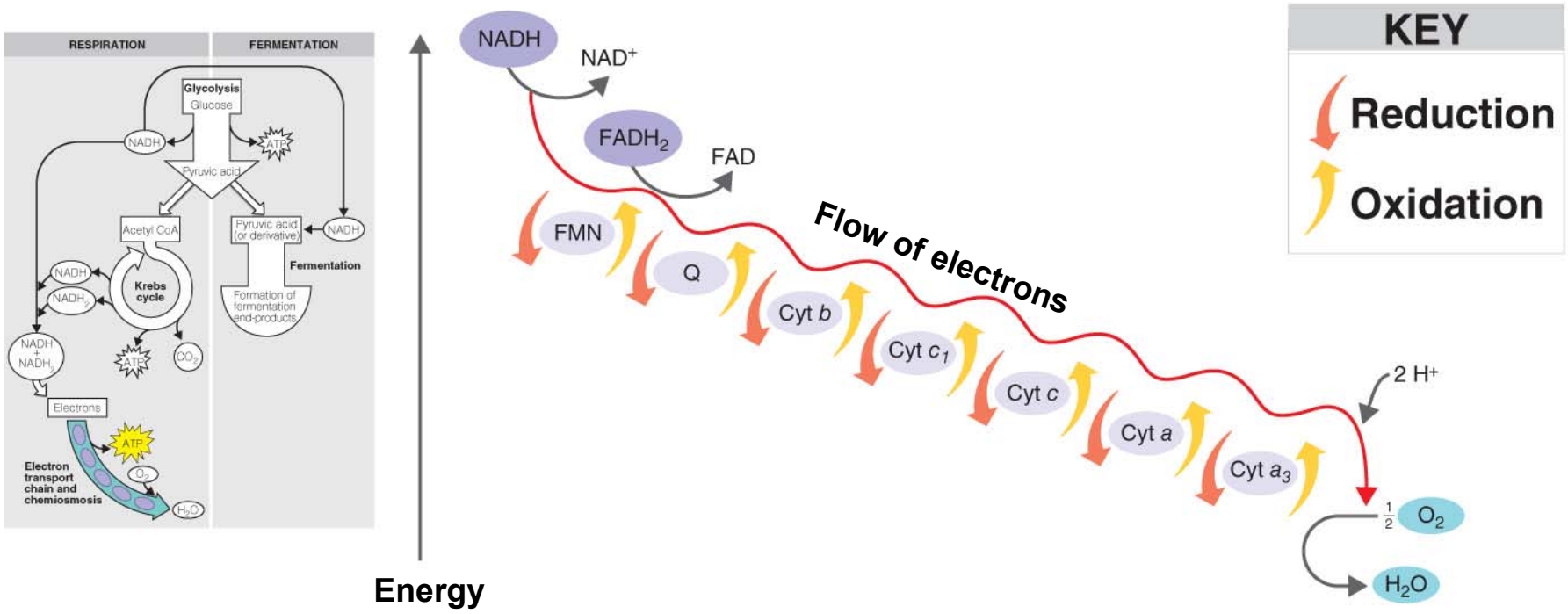
5 **ATP is produced by substrate-level phosphorylation. CoA is removed from succinyl CoA, leaving succinic acid.**

The Electron Transport Chain

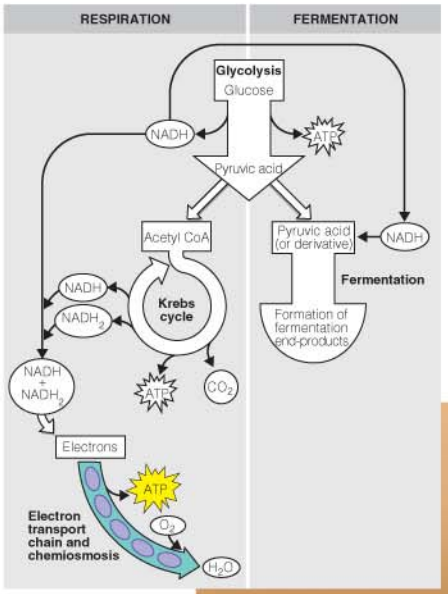
- A series of carrier molecules that are, in turn, oxidized and reduced as electrons are passed down the chain

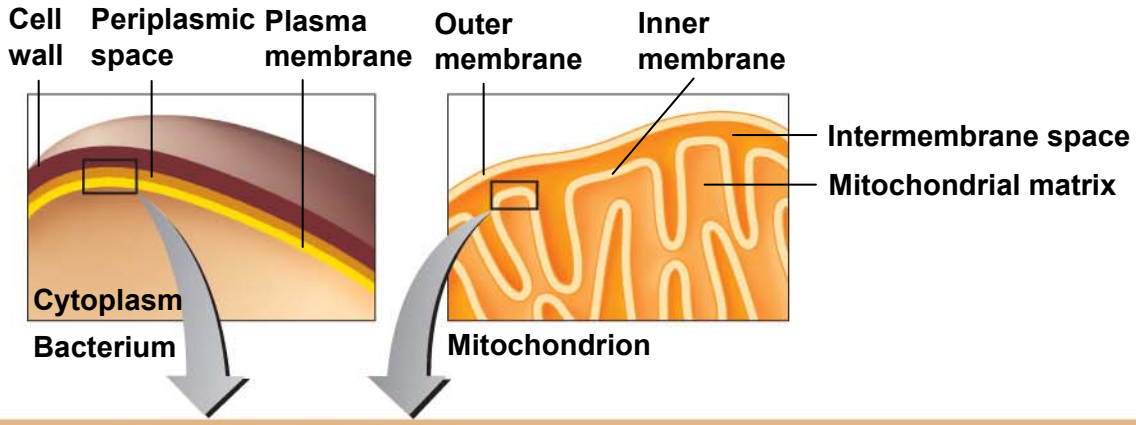
- Energy released along the chain can be used to produce ATP by **chemiosmosis**

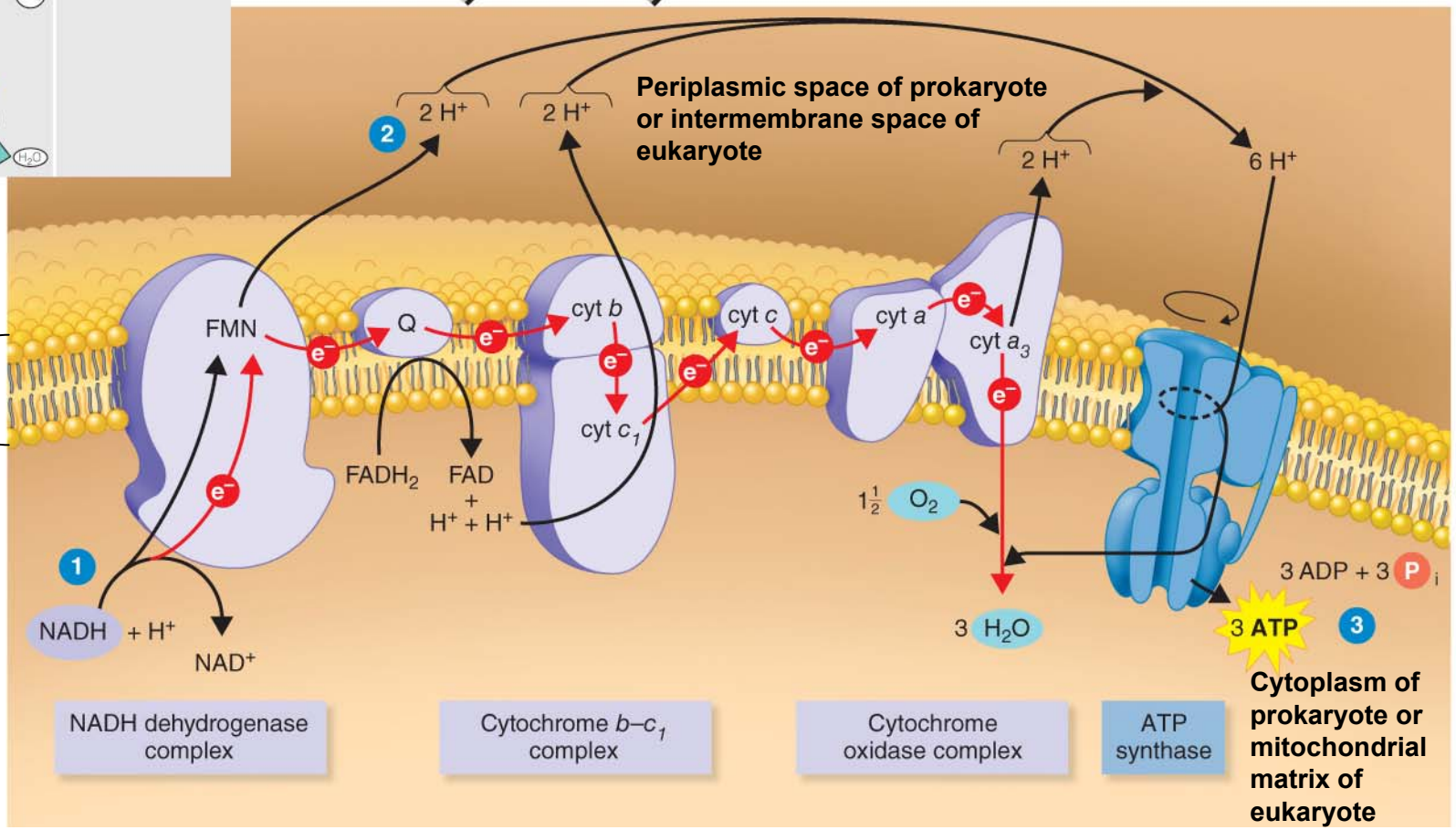
An electron transport chain (system)



Electron transport and the chemiosmotic generation of ATP.

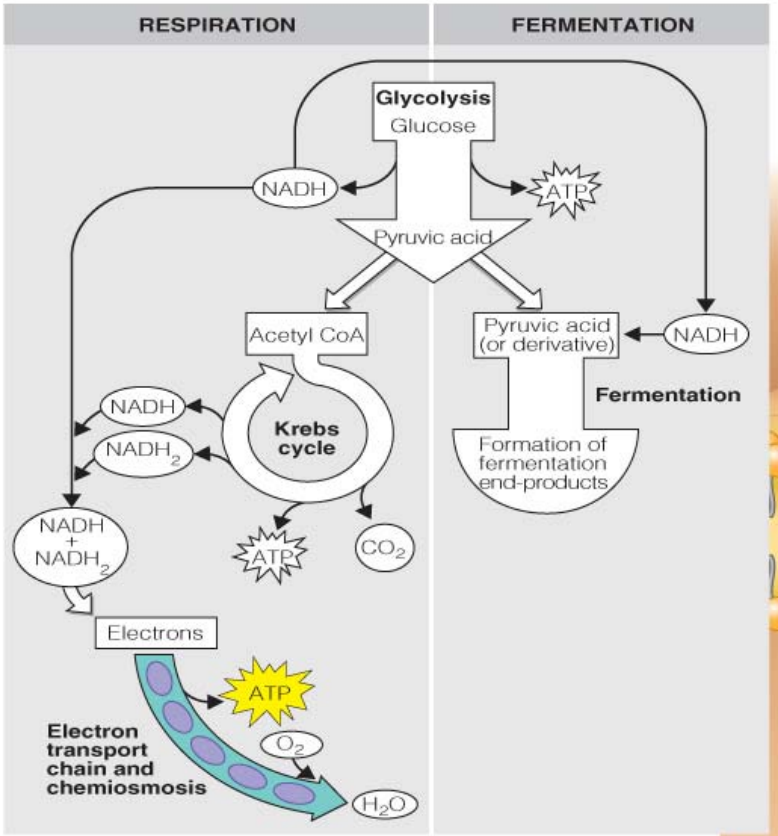


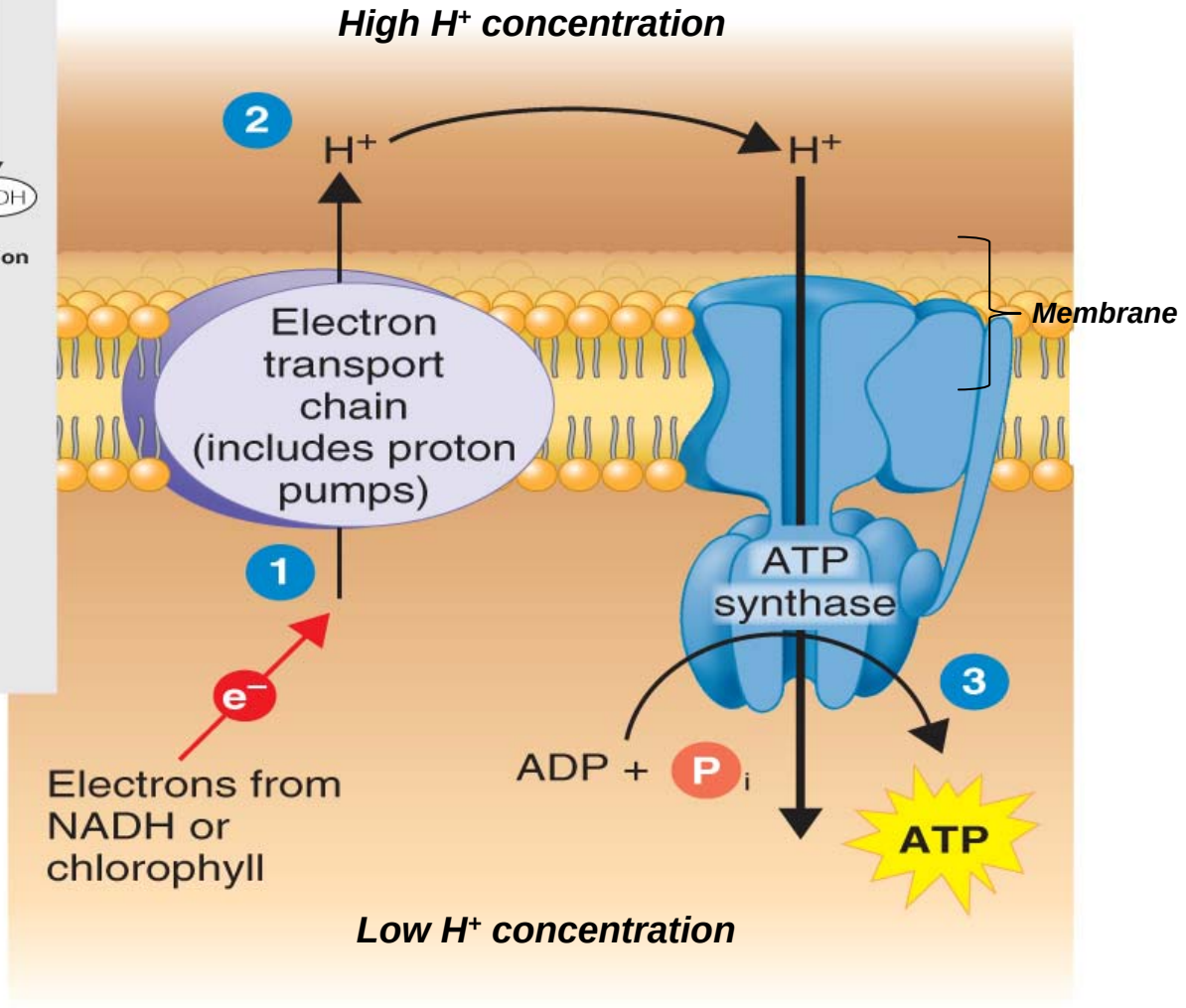




Prokaryotic plasma membrane or eukaryotic inner mitochondrial membrane

Chemiosmosis.





A Summary 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 cycle operates under anaerobic conditions
  - Therefore anaerobes grow slower than aerobic organisms !!!

Anaerobic Respiration

Some anaerobic bacteria use Nitrate ion (NO_3^-) as final electron acceptor

Reduced to either nitrite ion (NO_2^-) or nitrous oxide (N_2O) or nitrogen gas (N_2)

Some anaerobic bacteria use sulfate (SO_4^{2-}) to form hydrogen sulfide (H_2S)

Some anaerobic bacteria use carbonate (CO_3^{2-}) to form methane

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Note: in all cases electrons are transferred to or accepted by a molecule other than oxygen // these are reduced // accept the electrons

Anaerobic Respiration

Electron Acceptor		Products
NO_3^-	Nitrate	NO_2^-, N_2 + H_2O
SO_4^-	Sulfate	H_2S + H_2O
CO_3^{2-}	Carbonate	CH_4 + H_2O

Carbohydrate Catabolism

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

Carbohydrate Catabolism

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

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

Carbohydrate Catabolism

- ATP produced from complete oxidation of one 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**

Carbohydrate Catabolism

- 36 ATPs are produced in eukaryotes

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**

Fermentation

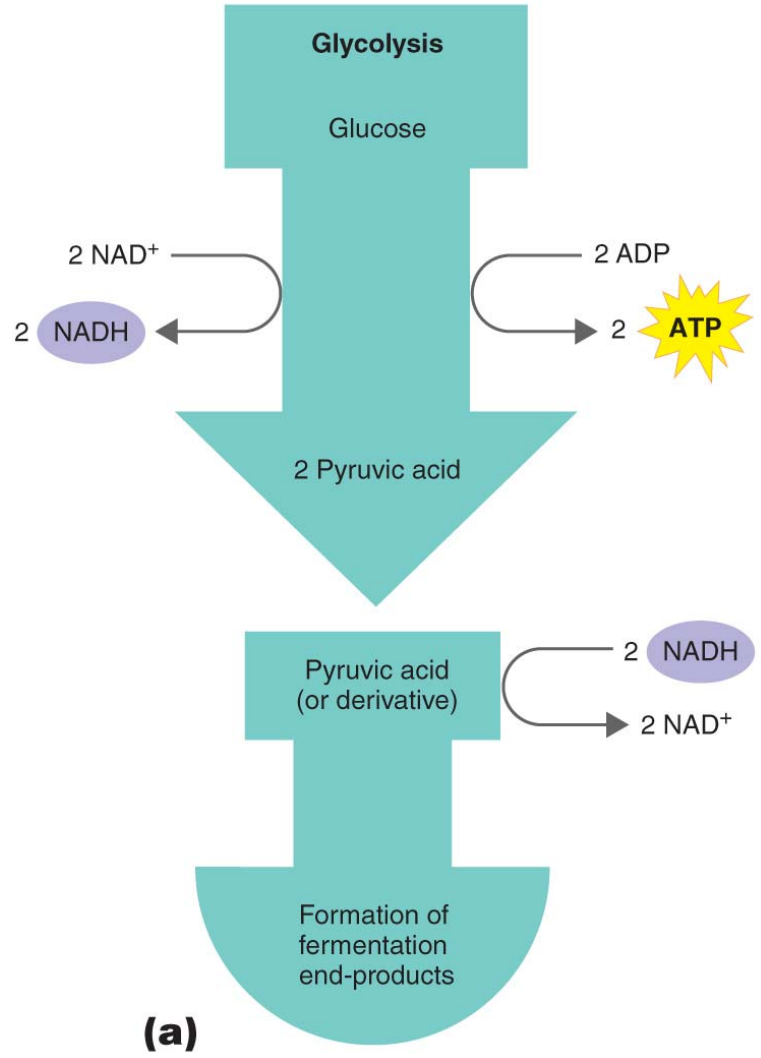
- Any spoilage of food by microorganisms (general use)
- Any process that produces alcoholic beverages or acidic dairy products (general use)
- Any large-scale microbial process occurring with or without air (common definition used in industry)

Fermentation

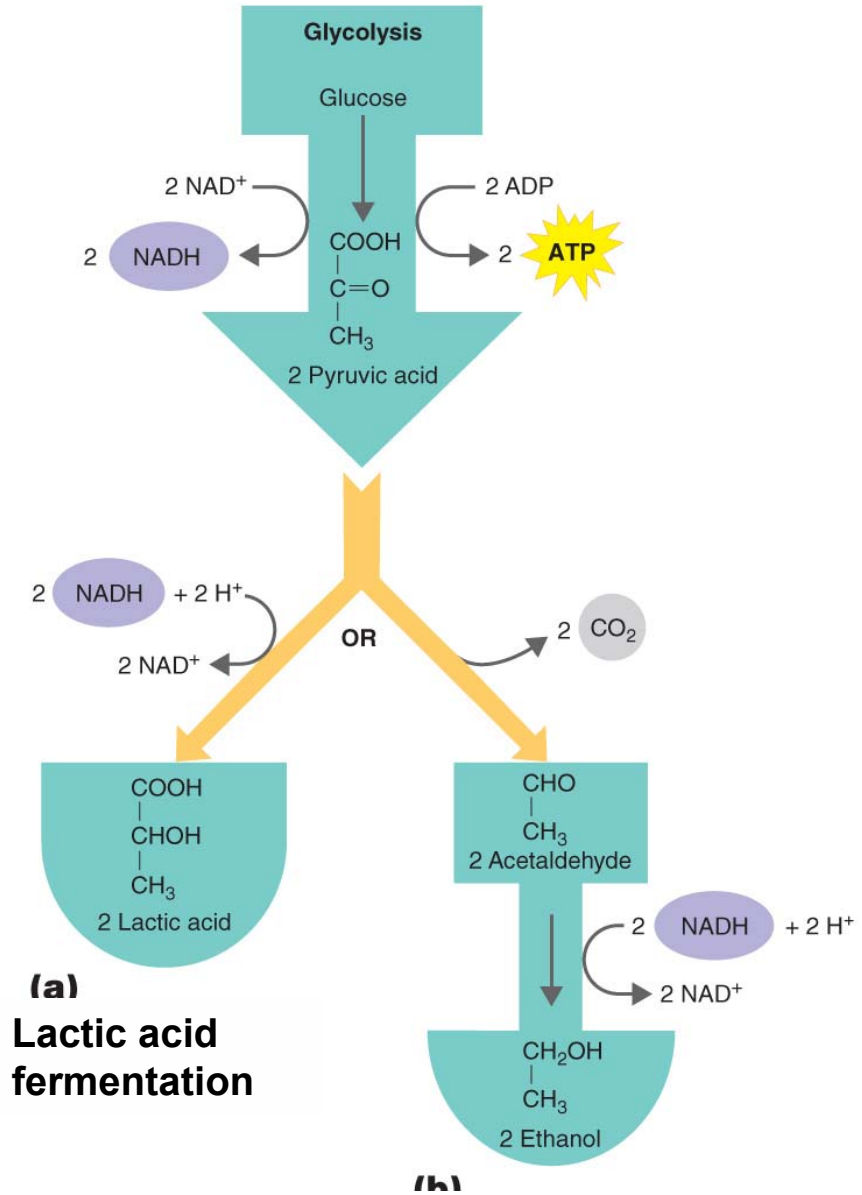
- **Alcohol fermentation**: produces ethanol + CO_2
- **Lactic acid fermentation**: produces lactic acid
  - Homolactic fermentation: produces lactic acid only
  - Heterolactic fermentation: produces lactic acid and other compounds

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



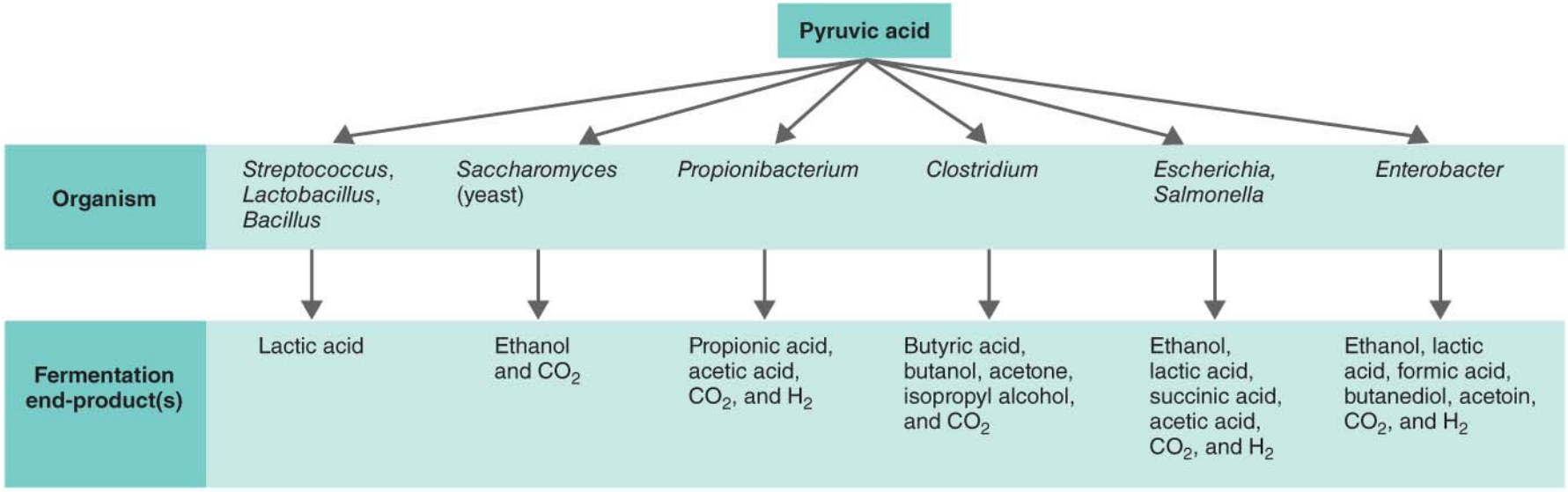
Types of Fermentation



Lactic acid fermentation

Alcohol fermentation

Fermentation.



(b)

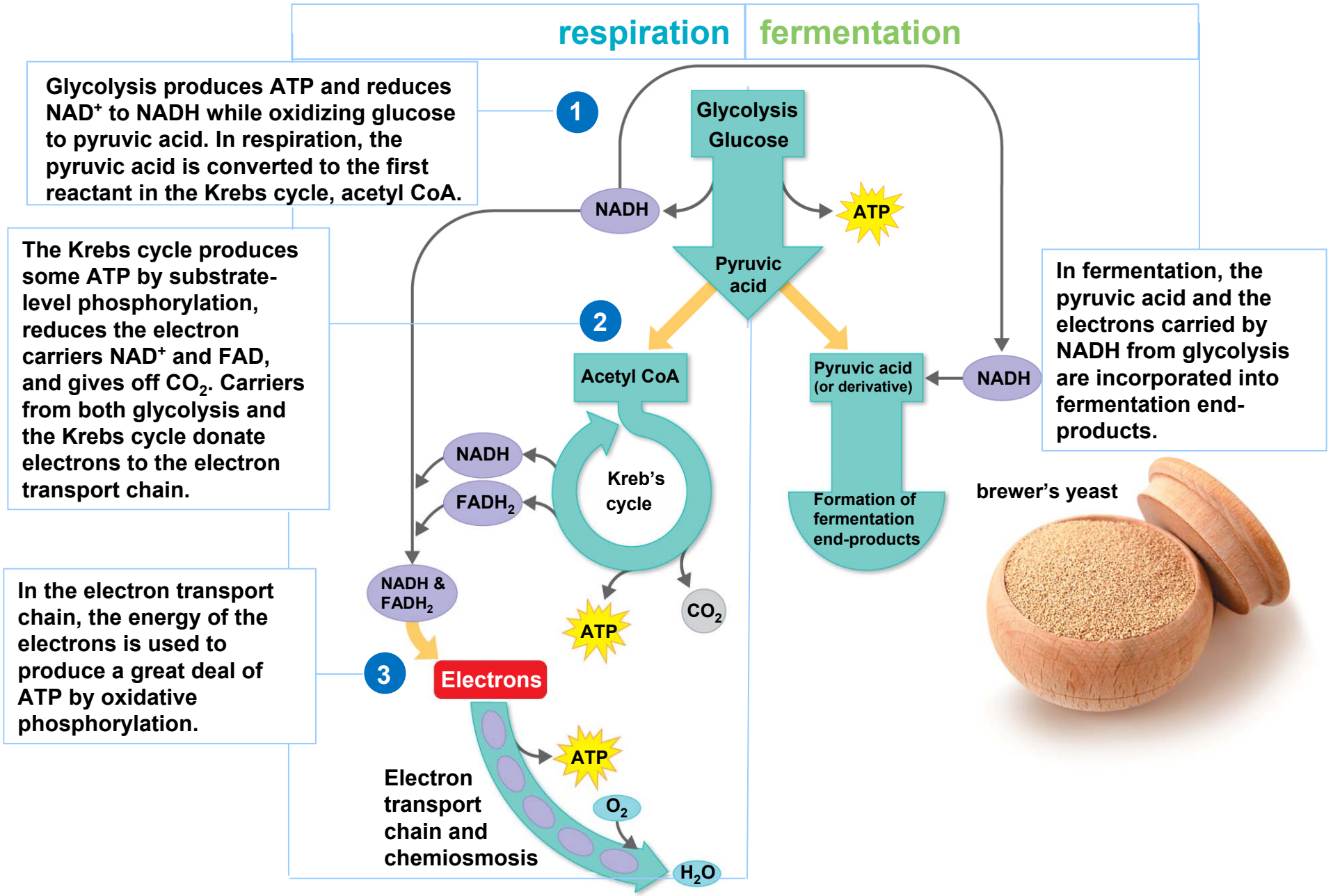
Some Industrial Uses for Different Types of Fermentations*

TABLE 5.4 Some Industrial Uses for Different Types of Fermentations*

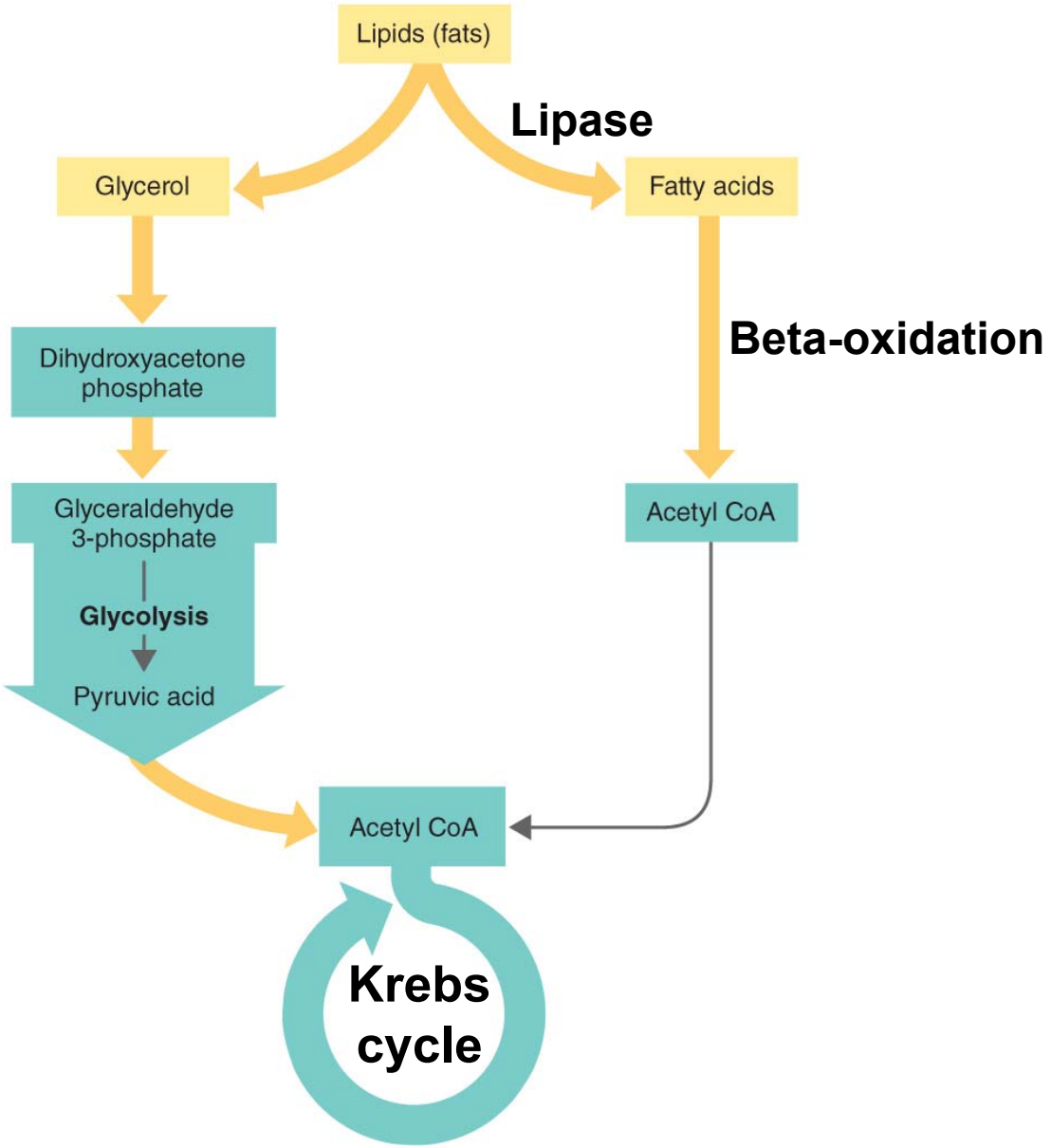
Fermentation End-Product(s)	Industrial or Commercial Use	Starting Material	Microorganism
Ethanol	Beer, wine	Starch, sugar	*Saccharomyces cerevisiae* (yeast, a fungus)
	Fuel	Agricultural wastes	*Saccharomyces cerevisiae* (yeast)
Acetic Acid	Vinegar	Ethanol	*Acetobacter*
Lactic Acid	Cheese, yogurt	Milk	*Lactobacillus, Streptococcus*
	Rye bread	Grain, sugar	*Lactobacillus delbrueckii*
	Sauerkraut	Cabbage	*Lactobacillus plantarum*
	Summer sausage	Meat	*Pediococcus*
Propionic Acid and Carbon Dioxide	Swiss cheese	Lactic acid	*Propionibacterium freudenreichii*
Acetone and Butanol	Pharmaceutical, industrial uses	Molasses	*Clostridium acetobutylicum*
Citric Acid	Flavoring	Molasses	*Aspergillus* (fungus)
Methane	Fuel	Acetic acid	*Methanosarcina*
Sorbose	Vitamin C (ascorbic acid)	Sorbitol	*Gluconobacter*

*Unless otherwise noted, the microorganisms listed are bacteria.

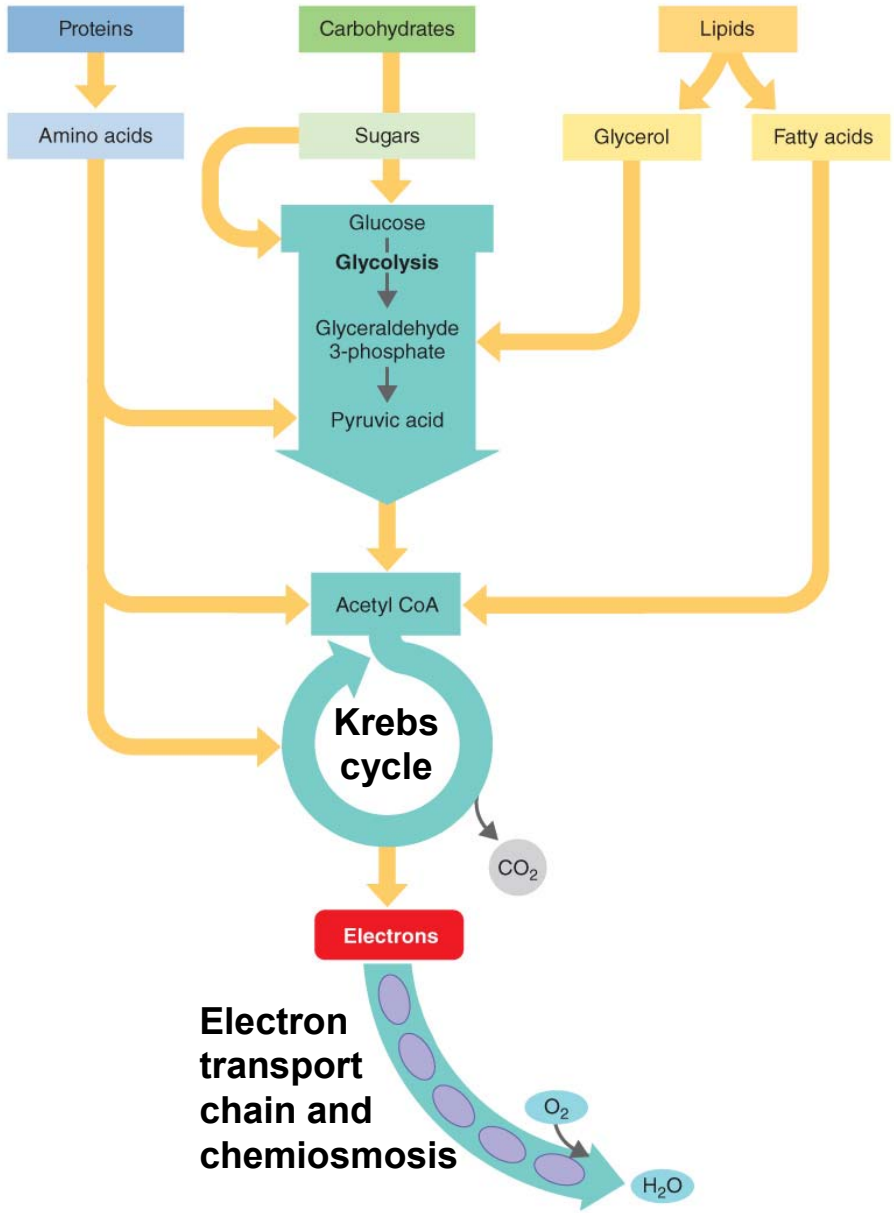
An Overview of Respiration and Fermentation.



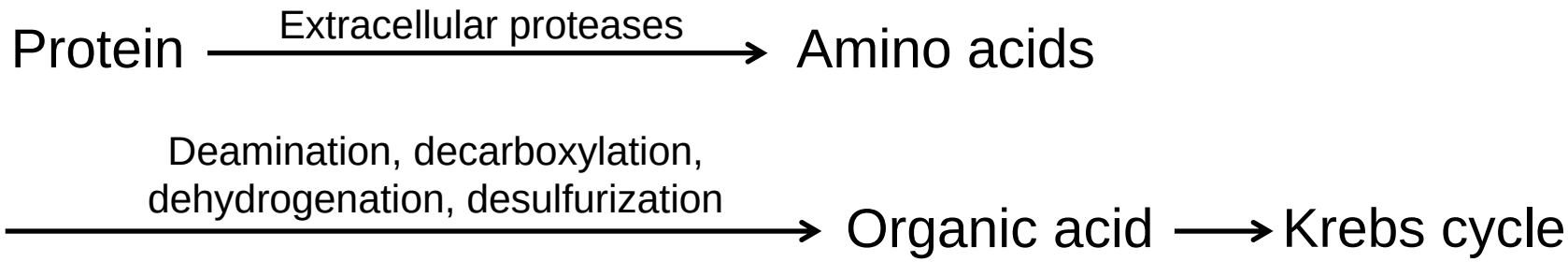
Lipid catabolism.



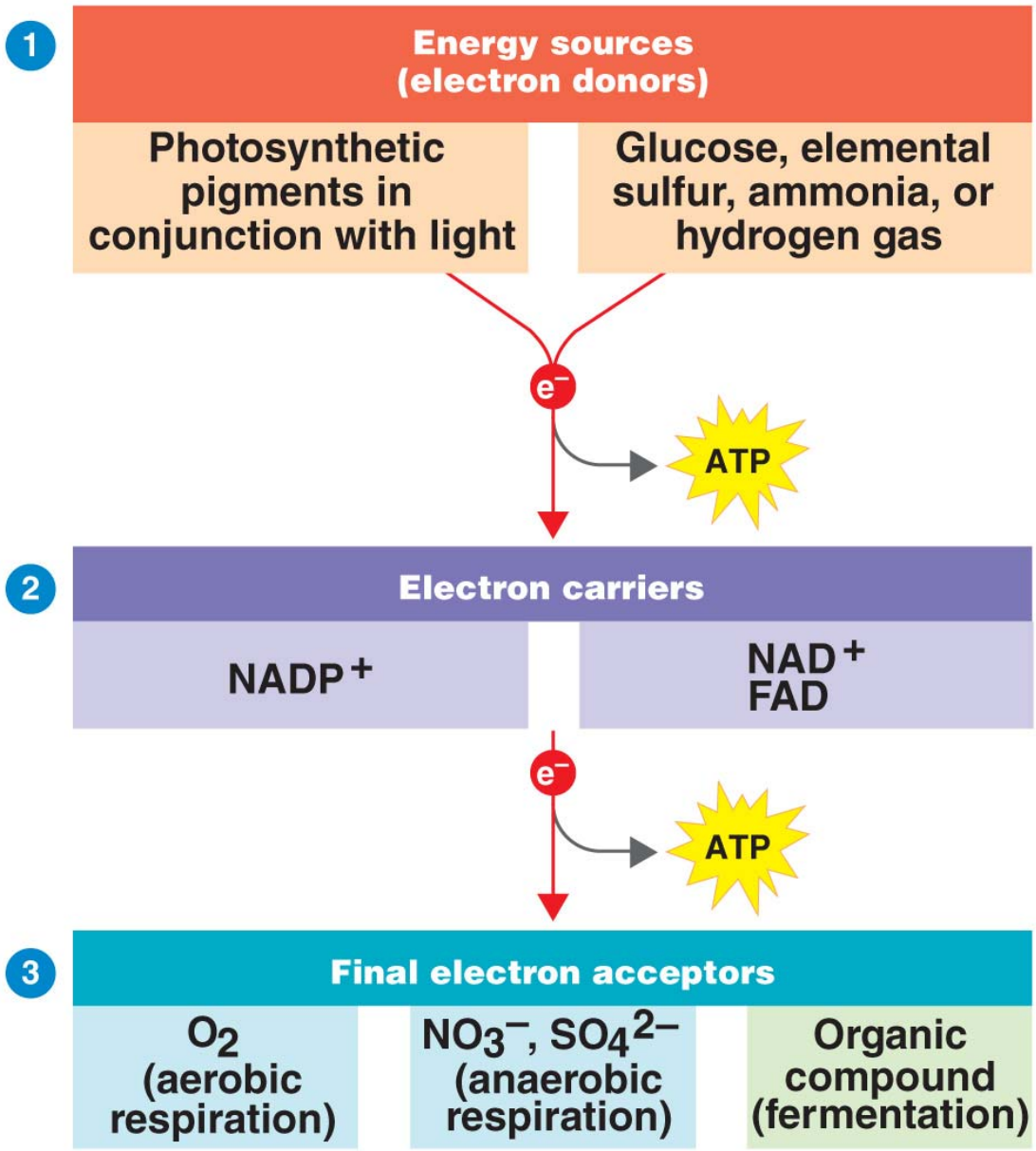
Catabolism of various organic food molecules.



Protein Catabolism



Requirements of ATP production.

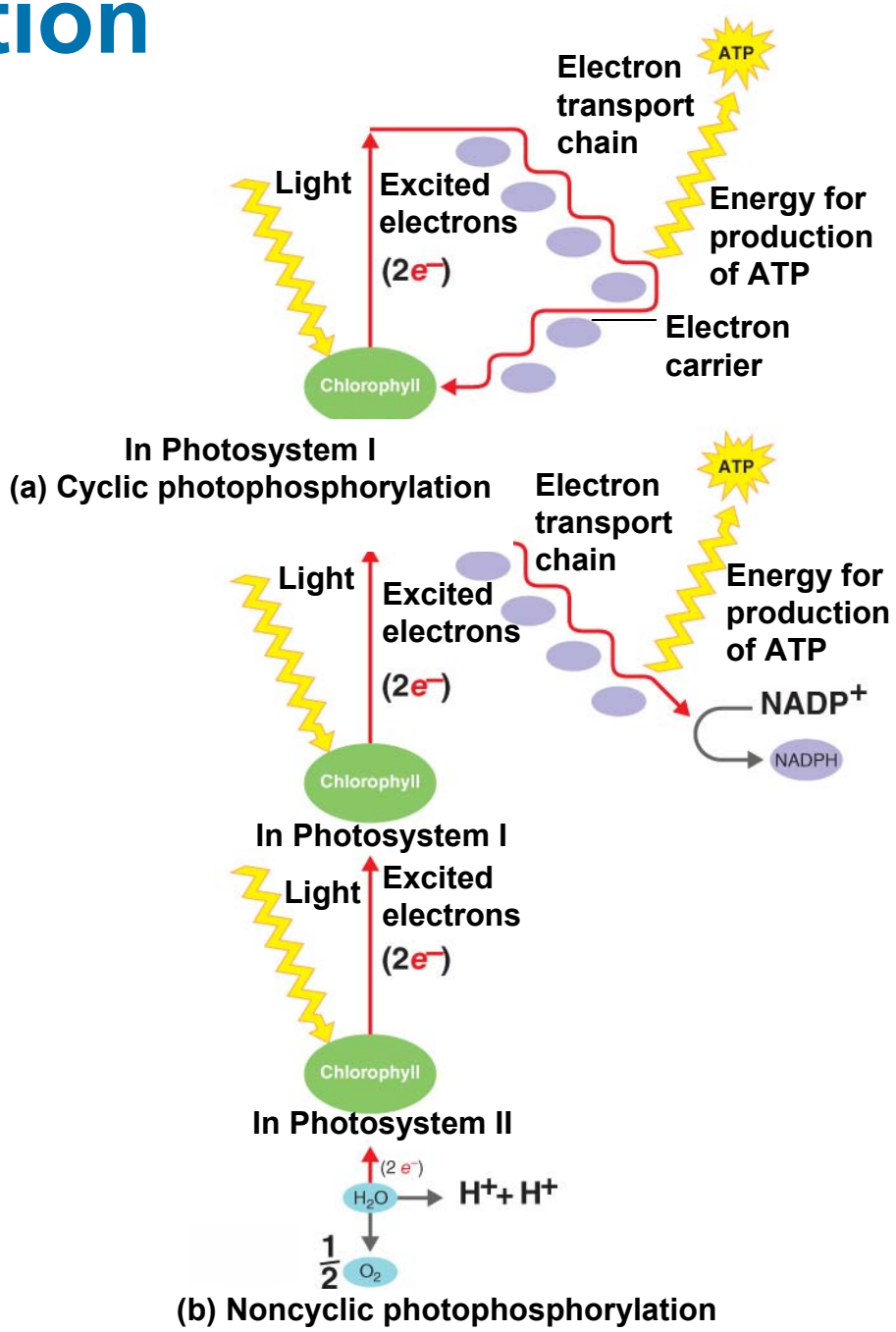


Photosynthesis

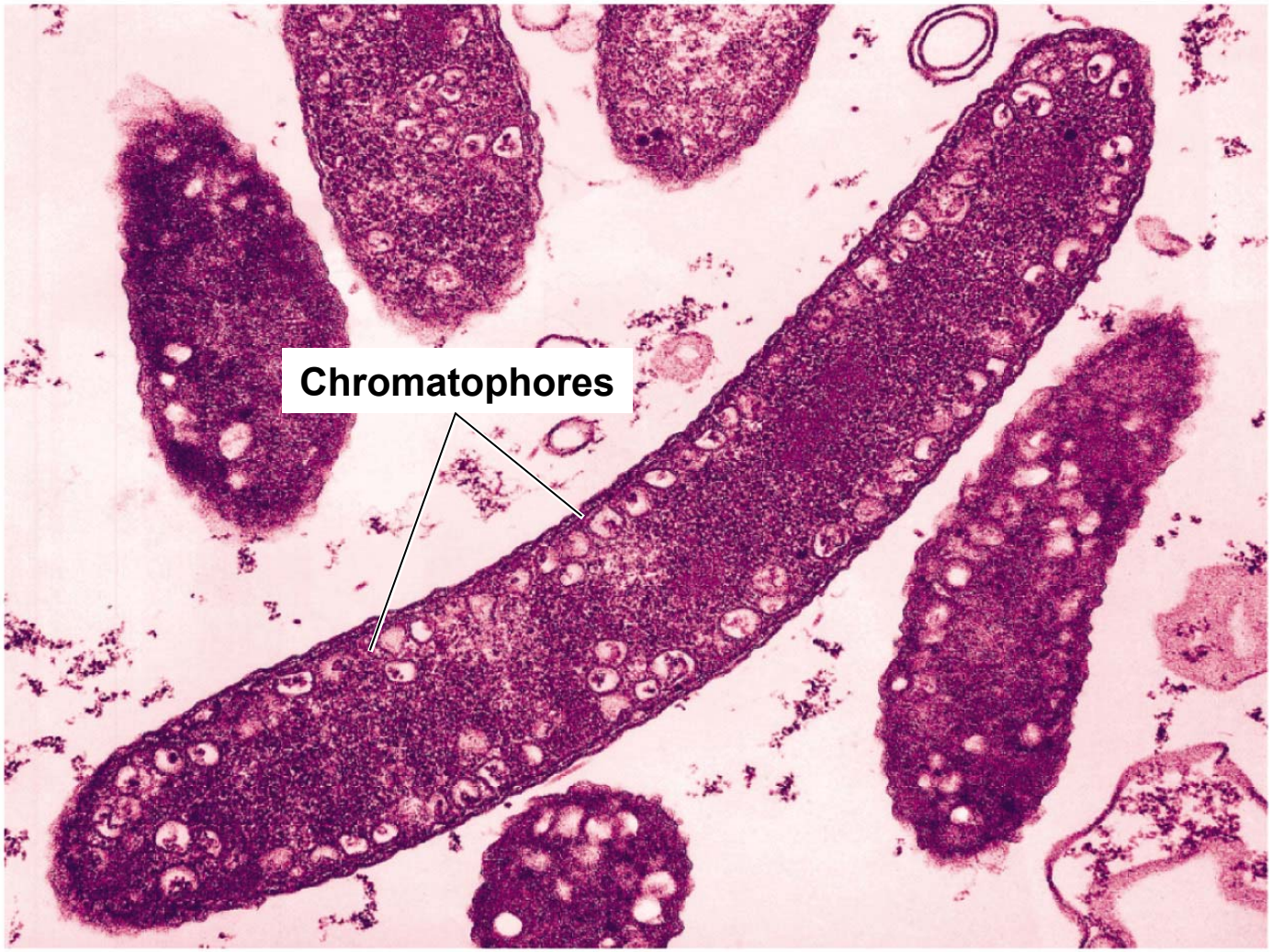
- Photo: conversion of light energy into chemical energy (ATP) /// Light-dependent (light) reactions
- Synthesis = Carbon fixation: fixing carbon dioxide into organic molecules // Light-independent (dark) reaction = Calvin-Benson Cycle
- In bacteria photosynthesis occurs in chromatophores

Photophosphorylation

- Light causes chlorophyll to give up electrons
- Energy released from transfer of electrons (oxidation) from chlorophyll through a system of carrier molecules are used to generate ATP
- During “dark reaction” this energy is used to fix carbon dioxide // make glucose



Chromatophores.



Photosynthesis / Two “Types”

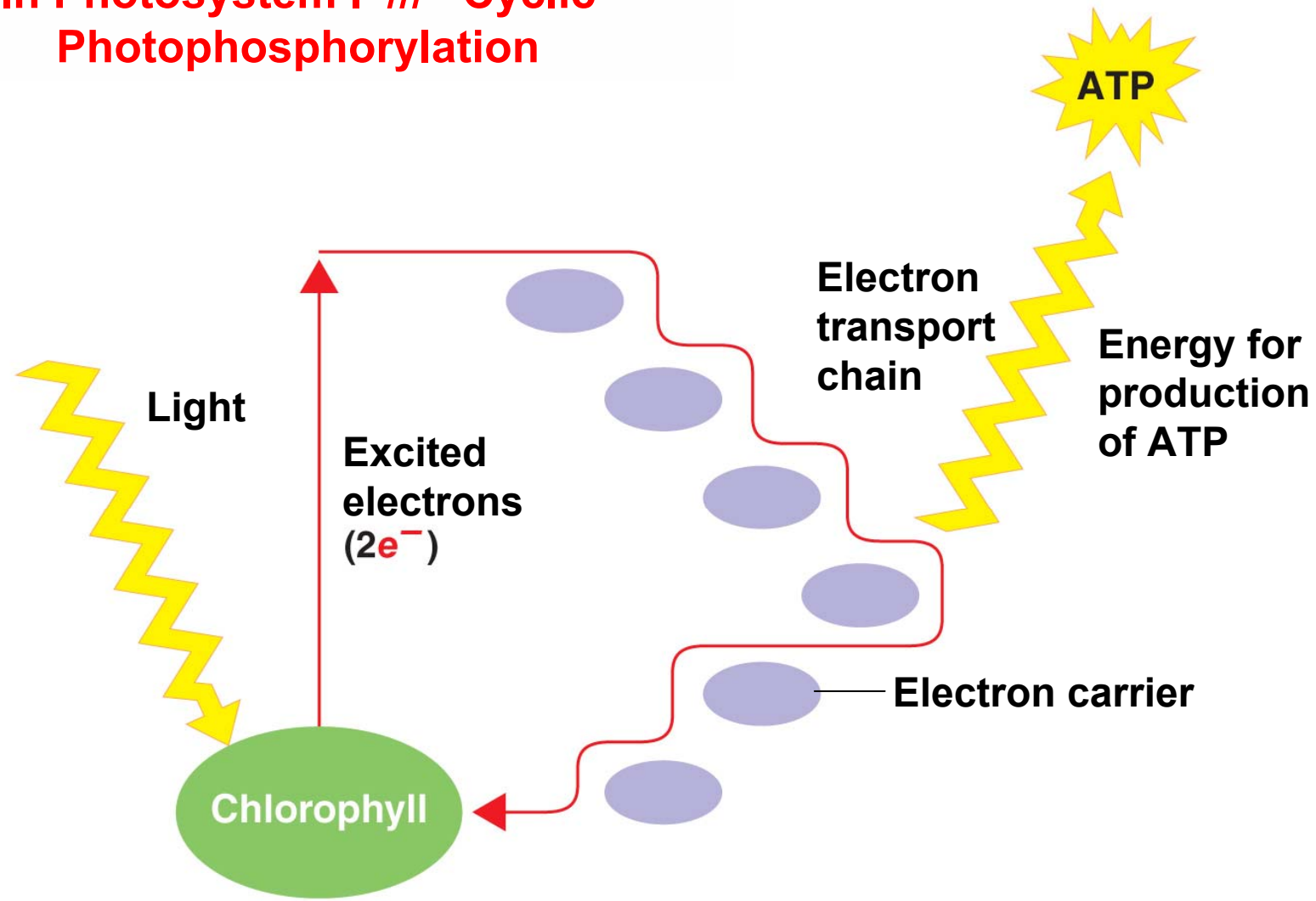
- Oxygenic:

$$6\ CO_2 + 12\ H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\ H_2O + 6\ O_2$$

- Anoxygenic:

$$6\ CO_2 + 12\ H_2S + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\ H_2O + 12\ S$$

In Photosystem I /// Cyclic Photophosphorylation

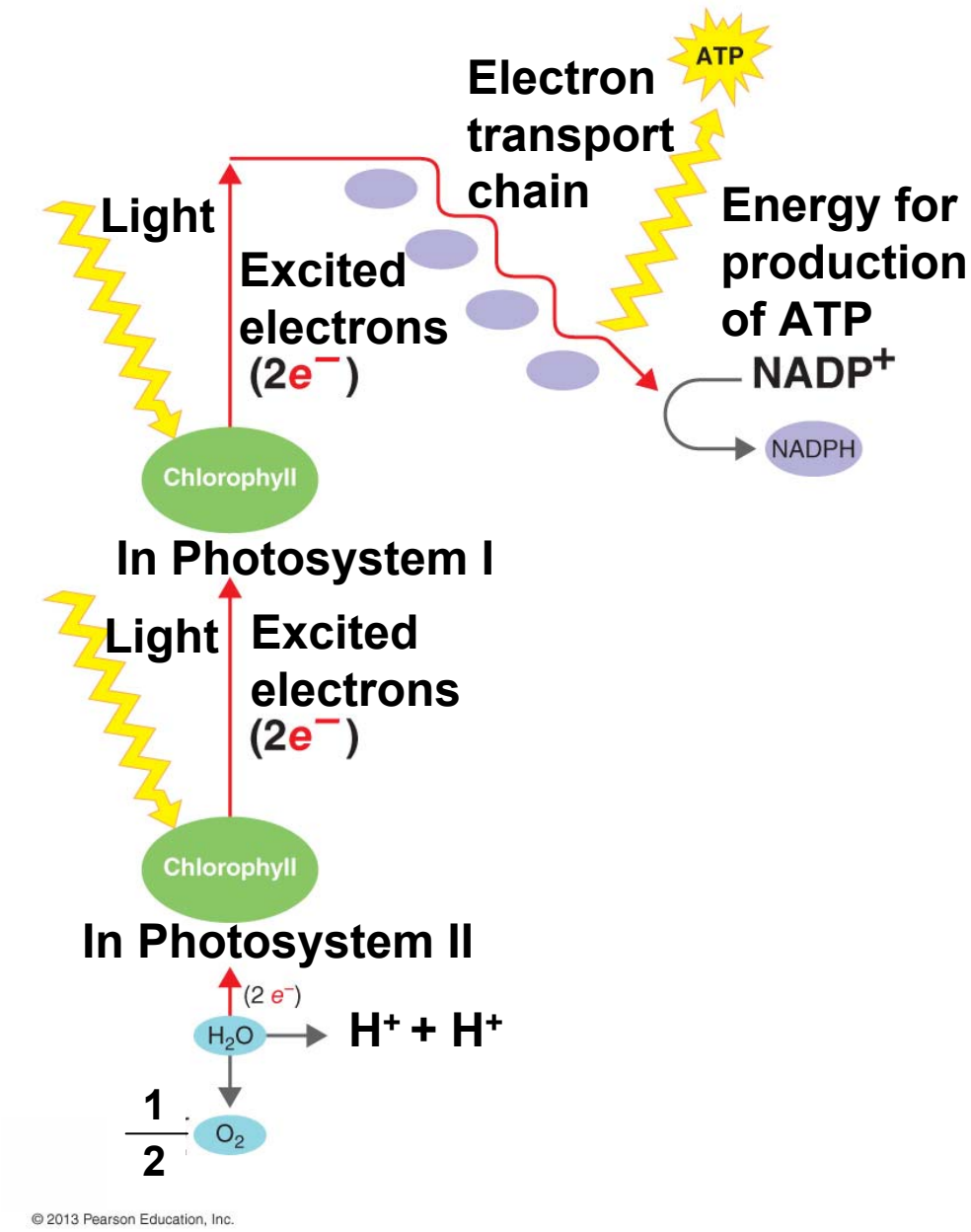


The light dependent reaction

Noncyclic photophosphorylation

Used in oxygenic organisms

The light dependent reaction



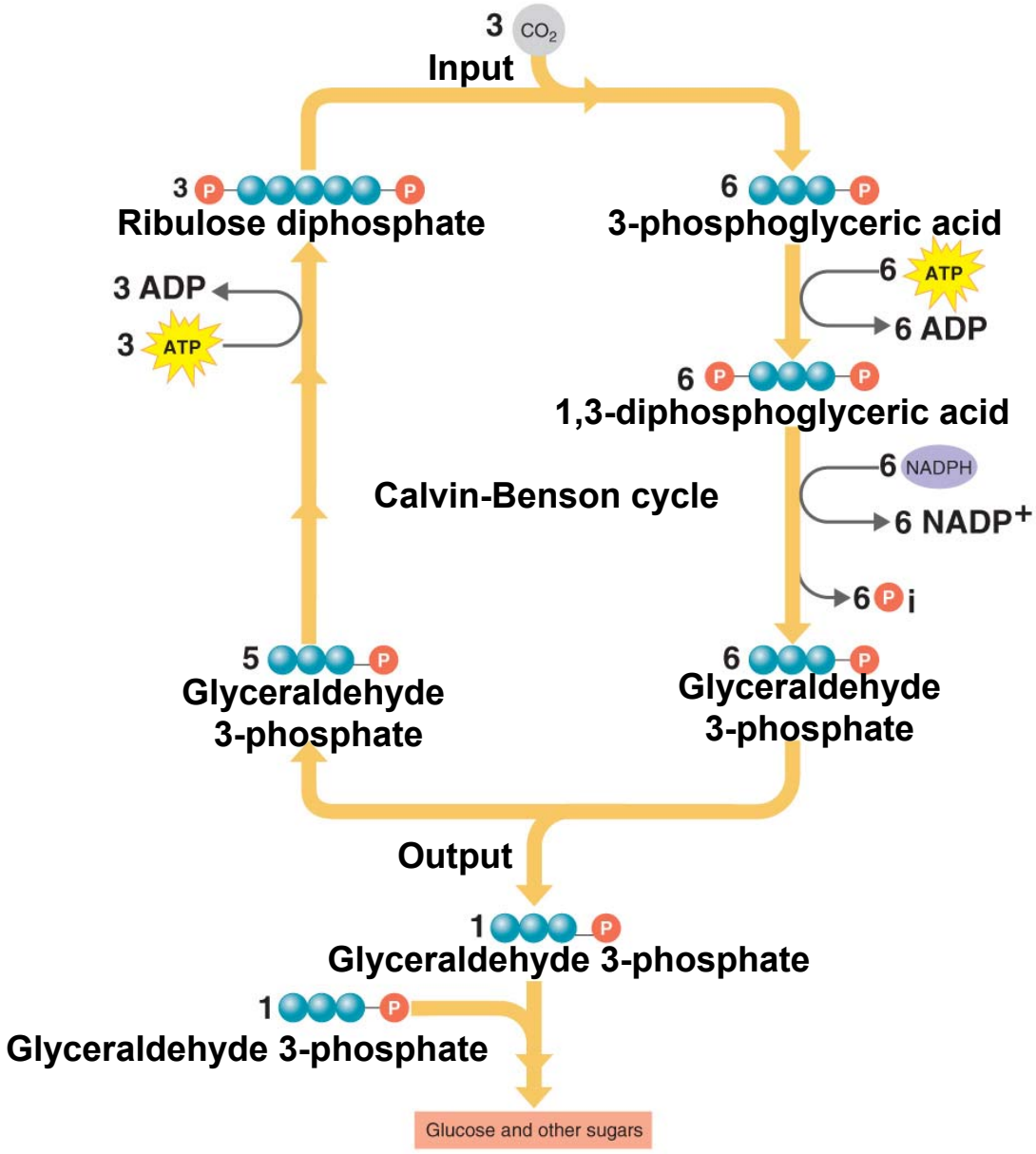
A simplified version of the Calvin-Benson cycle.

The light independent reaction

Cycle must turn 6X to make one glucose molecule

---

investment of 6C02 + 12 molecules of NADPH + 18 molecules of ATP



Photosynthesis Compared in Selected Eukaryotes and Prokaryotes

TABLE 5.6 Photosynthesis Compared in Selected Eukaryotes and Prokaryotes

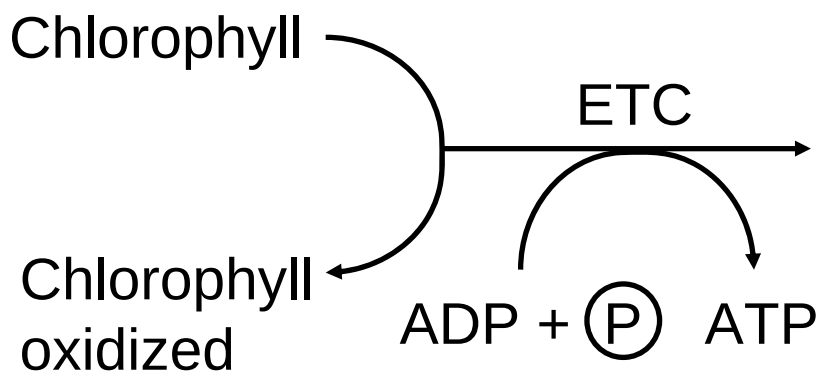
Characteristic	Eukaryotes	Prokaryotes		
	Algae, Plants	**Cyanobacteria**	**Green Bacteria**	**Purple Bacteria**
Substance That Reduces CO_2	H atoms of H_2O	H atoms of H_2O	Sulfur, sulfur compounds, H_2 gas	Sulfur, sulfur compounds, H_2 gas
Oxygen Production	Oxygenic	Oxygenic (and anoxygenic)	Anoxygenic	Anoxygenic
Type of Chlorophyll	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll *a*	Bacteriochlorophyll *a* or *b*
Site of Photosynthesis	Chloroplasts with thylakoids	Thylakoids	Chlorosomes	Chromatophores
Environment	Aerobic	Aerobic (and anaerobic)	Anaerobic	Anaerobic

All Organisms Have Two Requirements

- Source of energy
- Source of carbon

Phototrophs

- Phototrophs are organisms that use sun light as a source of energy to make the chemical energy of ATP)

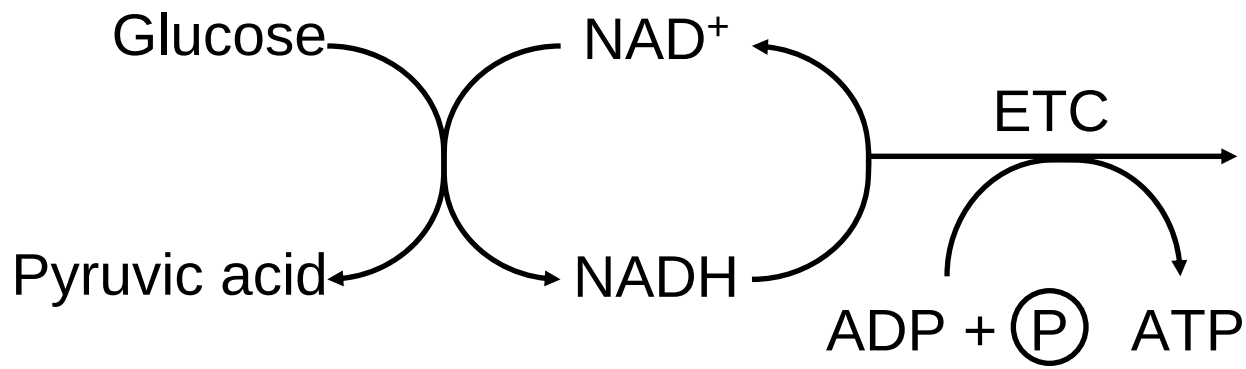


Phototrophs

- Phototrophs then must acquire carbon using one of these two mechanisms:
  - The Autotrophs VS Heterotrophs
  - **Photoautotrophs - use energy (ATP) in the Calvin-Benson cycle to fix CO_2 // convert CO2 into sugar – becomes organic source for organism's source of carbon**
  - **Photoheterotrophs - use light energy to make ATP but can not convert CO2 into sugar // therefore use organic compounds as source of carbon (e.g. alcohols, fatty acids, carbohydrates, etc.)**

Chemotrophs

- Chemotrophs // Produces energy from reduced compounds to make ATP



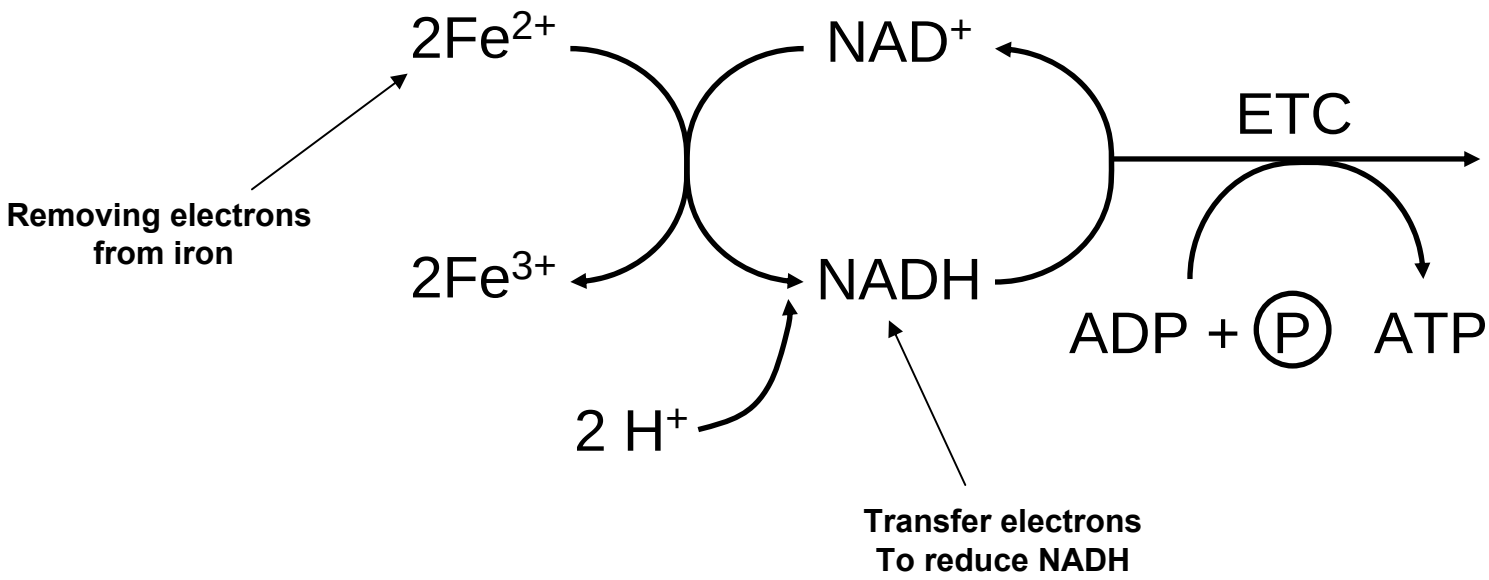
- The carbon will come from two mechanisms (e.g. chemoheterotrops or chemoautotrops)

Chemoheterotrophs

- Energy comes from reduced compounds
- Carbon comes from organic molecules
  - Energy is used in anabolism to build new macromolecules

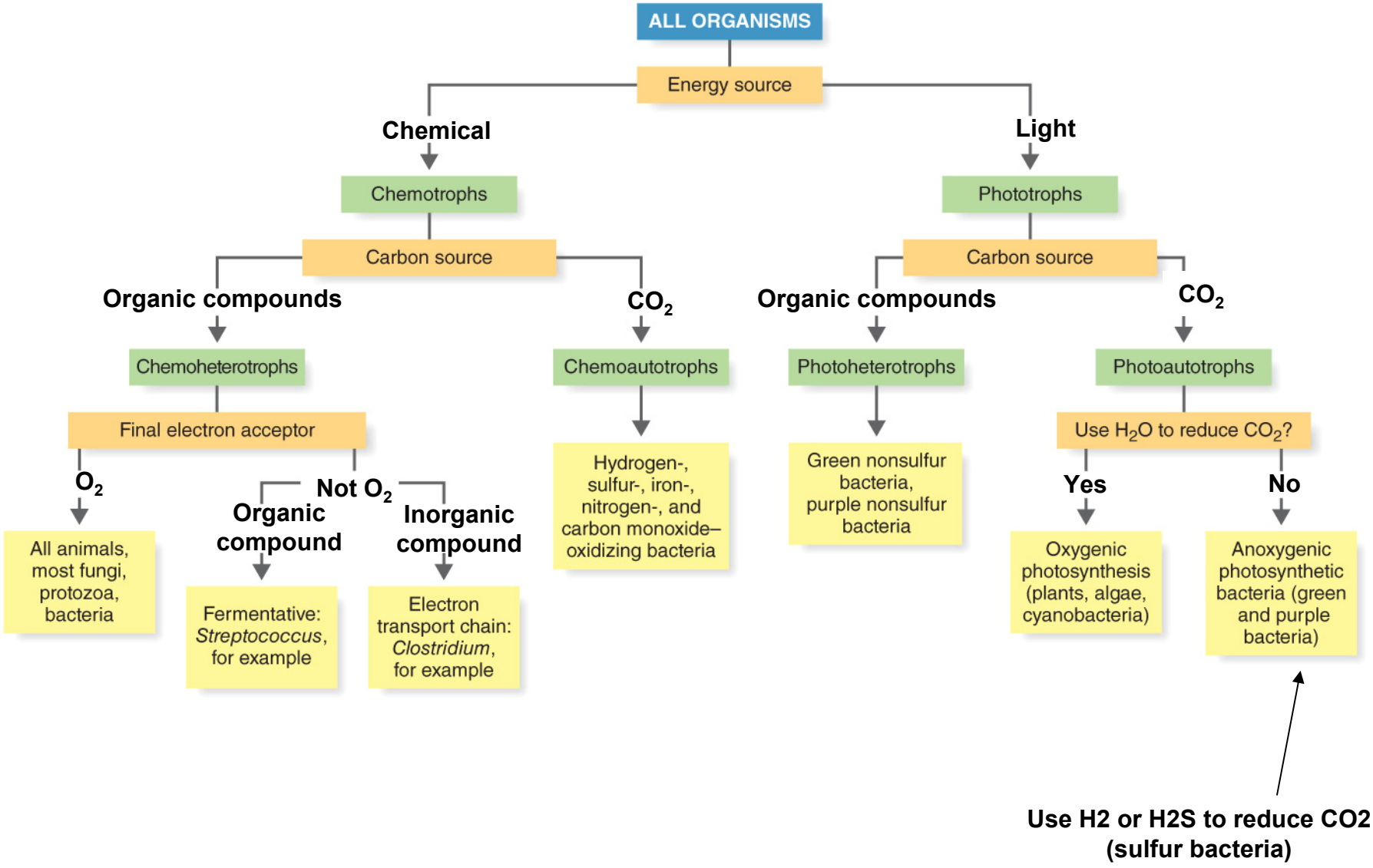
Chemoautotroph

- In this example -- “energy” comes from inorganic source // used by *Thiobacillus ferrooxidans*



- Energy is used in the Calvin-Benson cycle to fix CO_2

A nutritional classification of organisms.



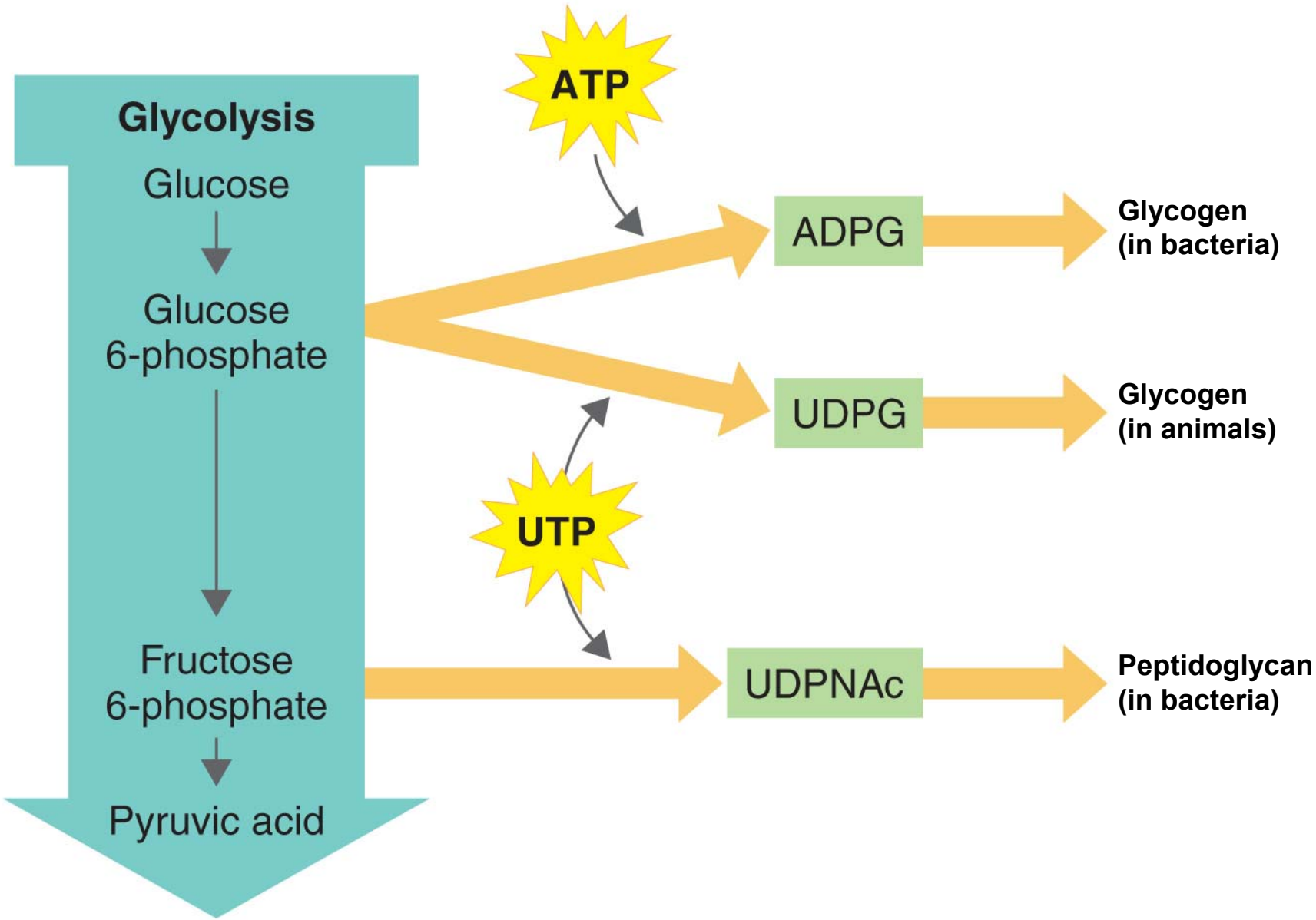
Metabolic Diversity among Organisms

Nutritional Type	Energy Source	Carbon Source	Example
Photoautotroph	Light	CO_2	Oxygenic: Cyanobacteria, plants Anoxygenic: Green bacteria, purple bacteria
Photoheterotroph	Light	Organic compounds	Green bacteria, purple nonsulfur bacteria
Chemoautotroph	Chemical	CO_2	Iron-oxidizing bacteria
Chemoheterotroph	Chemical	Organic compounds	Fermentative bacteria Animals, protozoa, fungi, bacteria

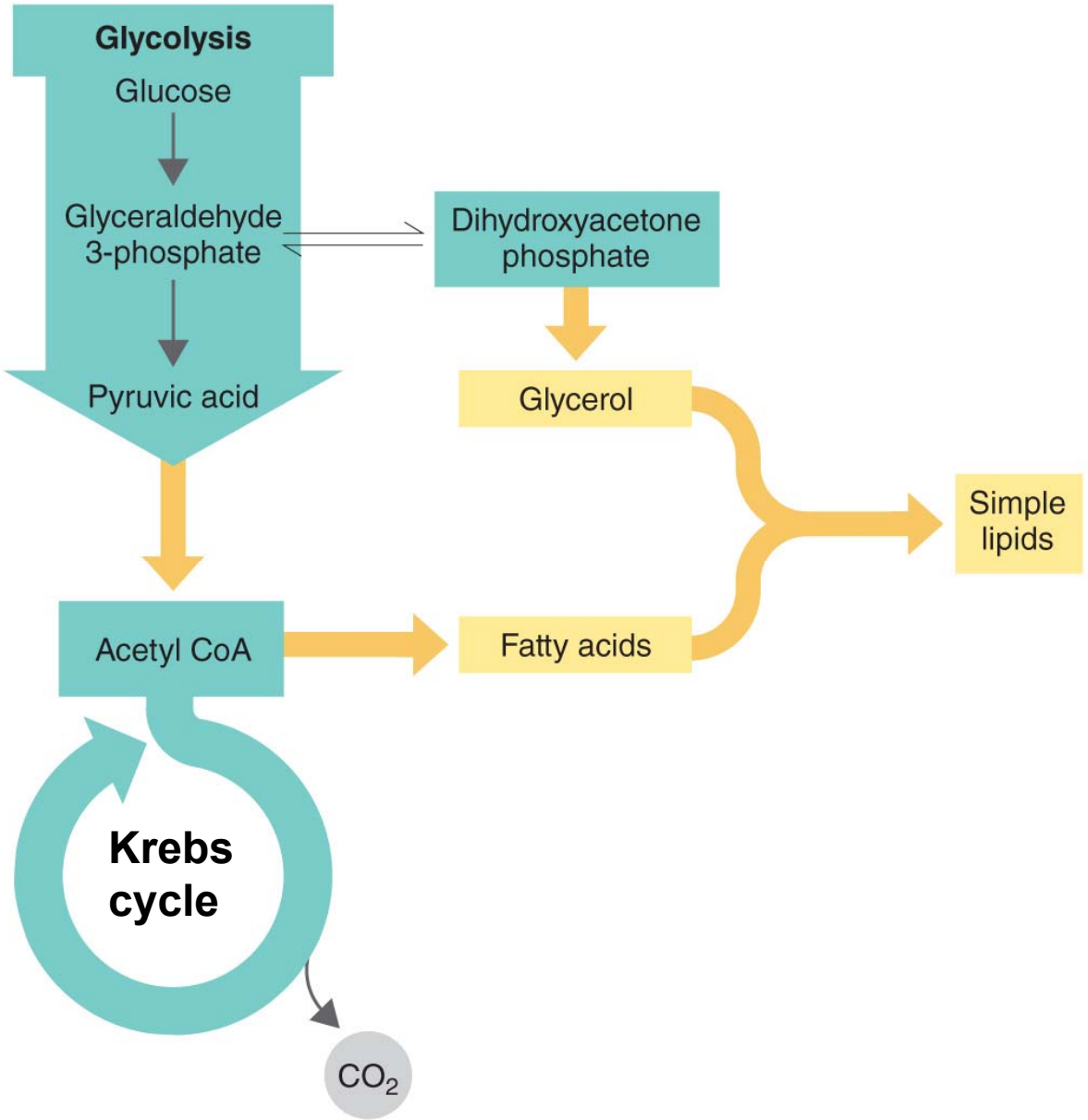
Four Major Classes of Macromolecules

- How do cells make:
  - Polysacharides
  - Amino acids
  - Lips
  - Nucleic Acids

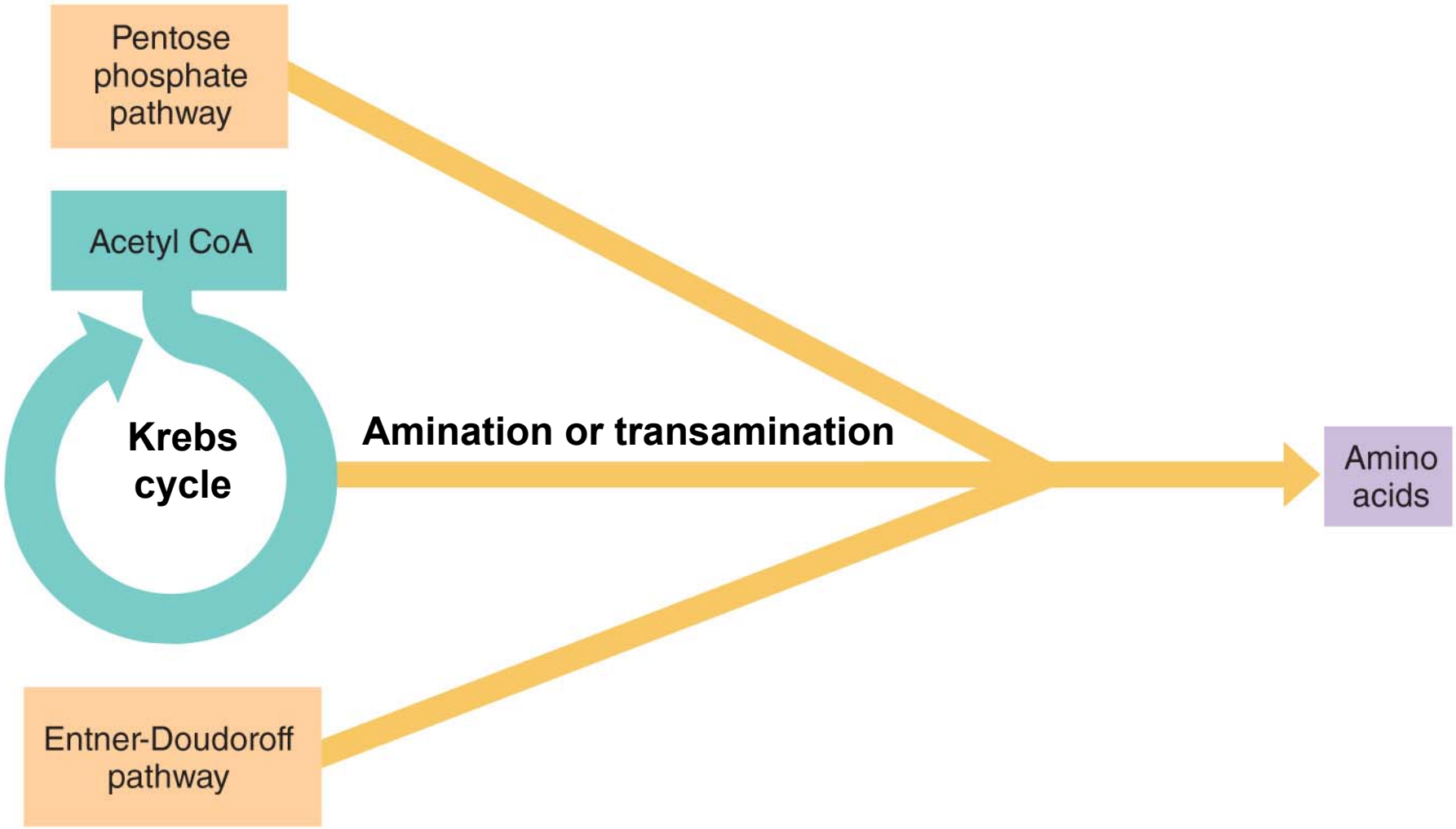
The biosynthesis of polysaccharides.



The biosynthesis of simple lipids.

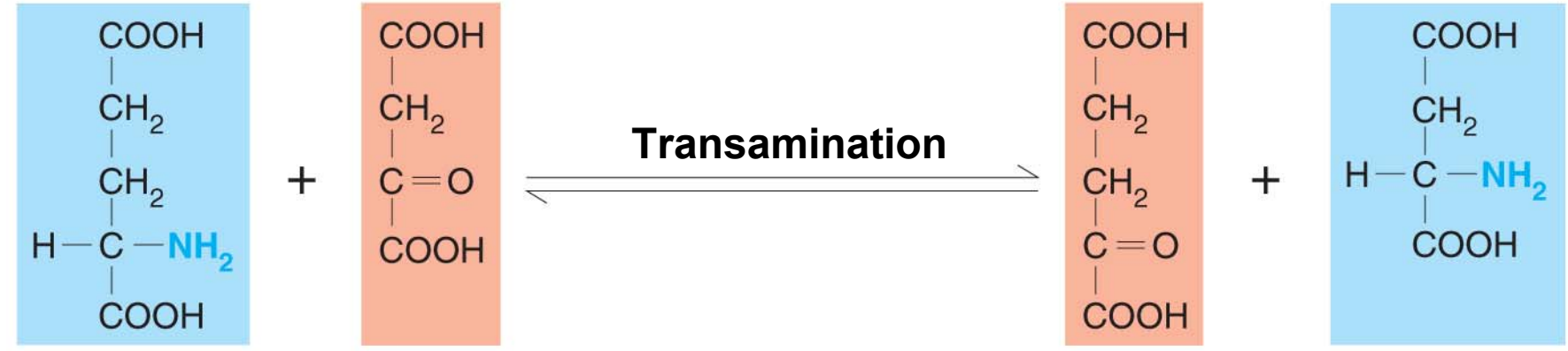


The biosynthesis of amino acids.



(a) Amino acid biosynthesis

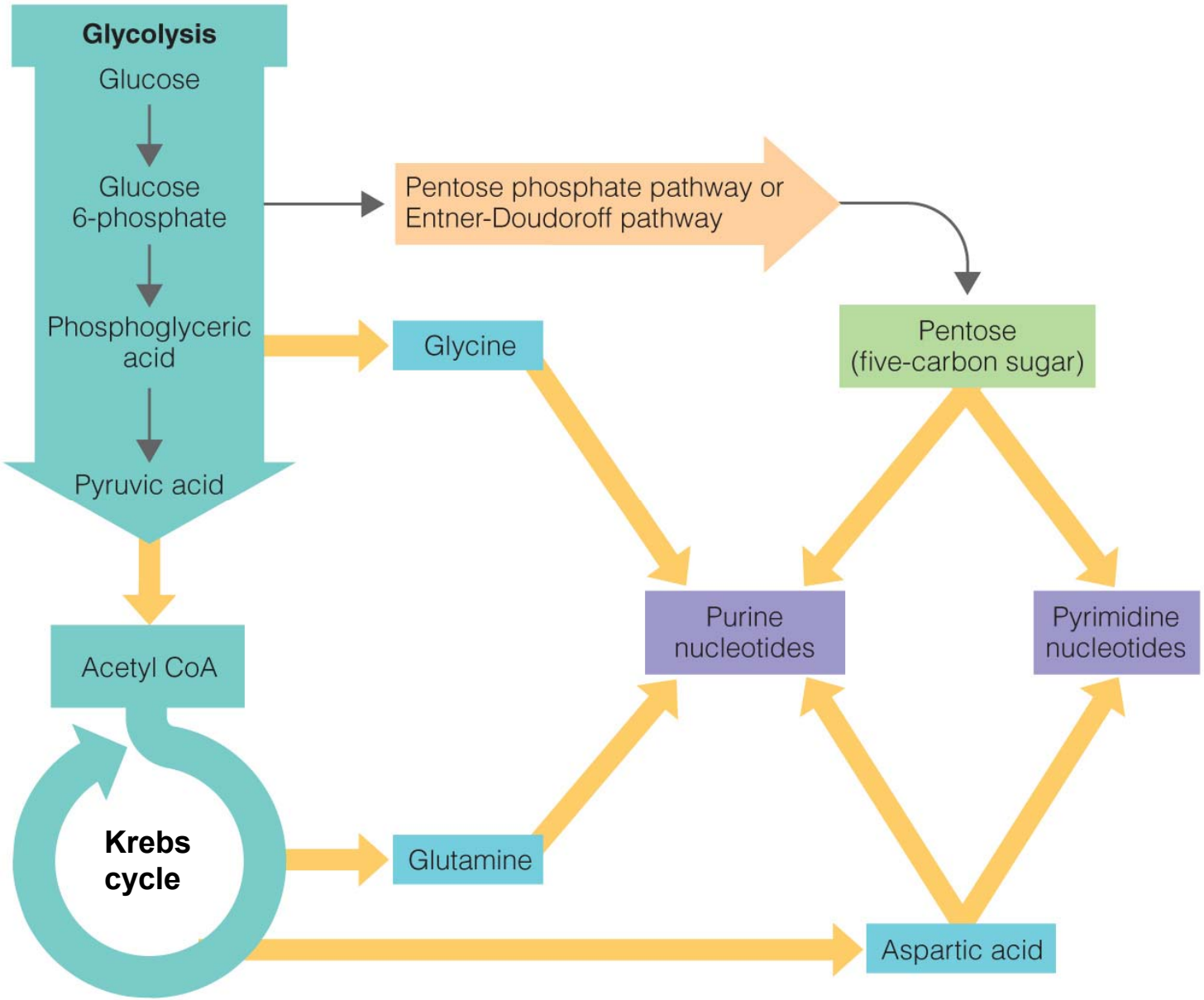
The biosynthesis of amino acids.



Glutamic acid **Oxaloacetic acid** **α-Ketoglutaric acid** **Aspartic acid**

(b) Process of transamination

The biosynthesis of purine and pyrimidine nucleotides.



The Integration of Metabolism

- **Amphibolic pathways**:
  - Metabolic pathways that have both catabolic and anabolic functions
  - A *bridge between two phases* of metabolism

Amphibolic Pathways

The integration of metabolism.