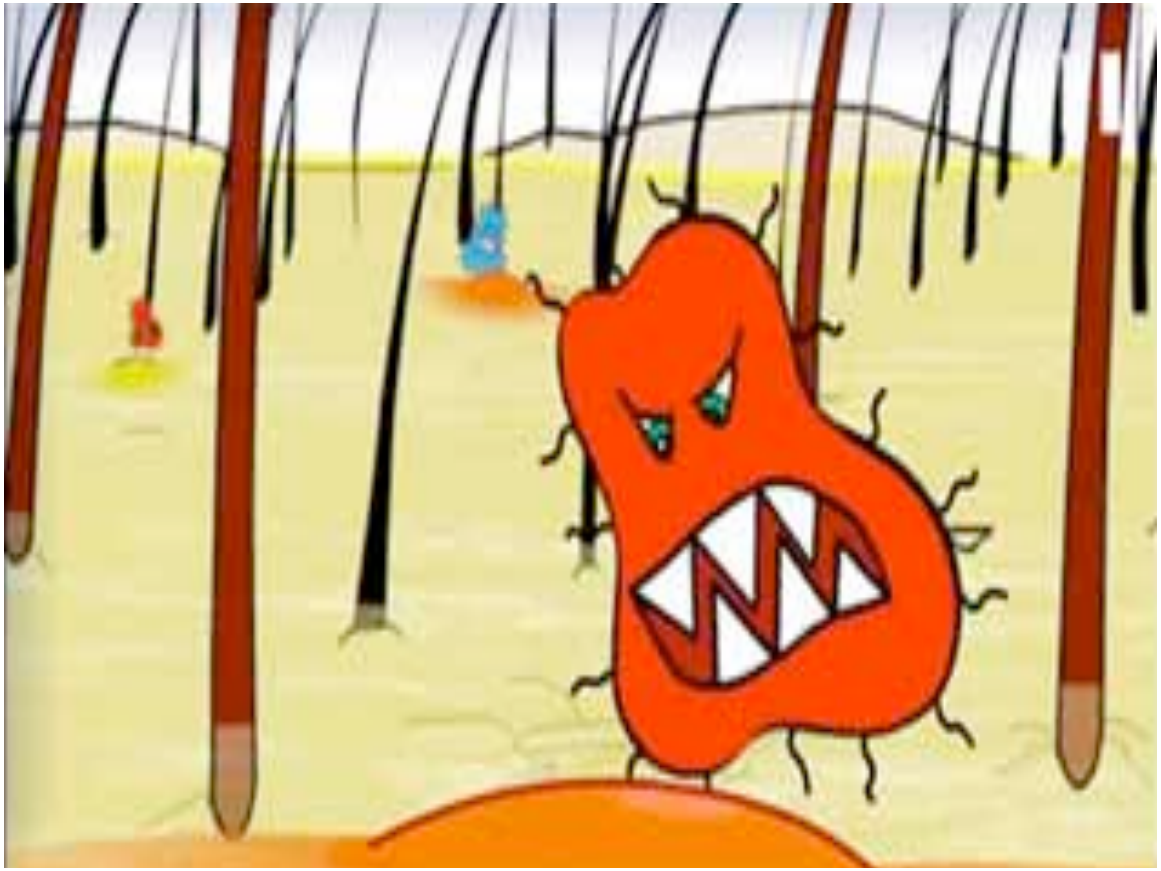
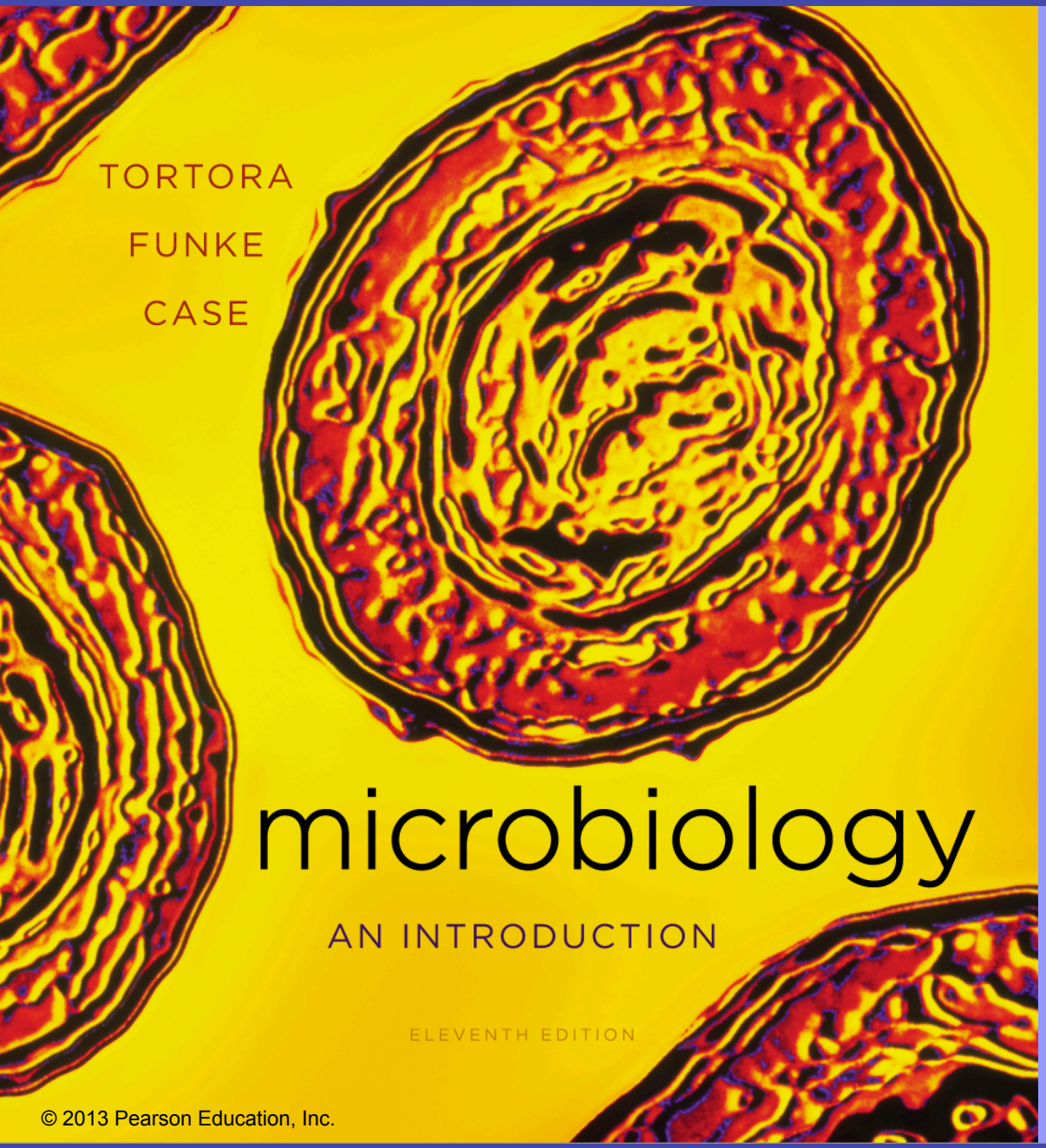


Module 3

Microbial Metabolism



Chapter 5

Microbial Metabolism

Lectures prepared by Helmut Kae

Catabolic and Anabolic Reactions

- **Metabolism**: the sum of the chemical reactions in an organism
- Two general types of metabolic reactions
  - **Catabolism**: provides energy and building blocks for anabolism
  - **Anabolism**: uses energy and building blocks to build large molecules
- Recall from Chapter 2
  - Energy can be stored when covalent bonds form
  - Energy can be released when covalent bonds broken

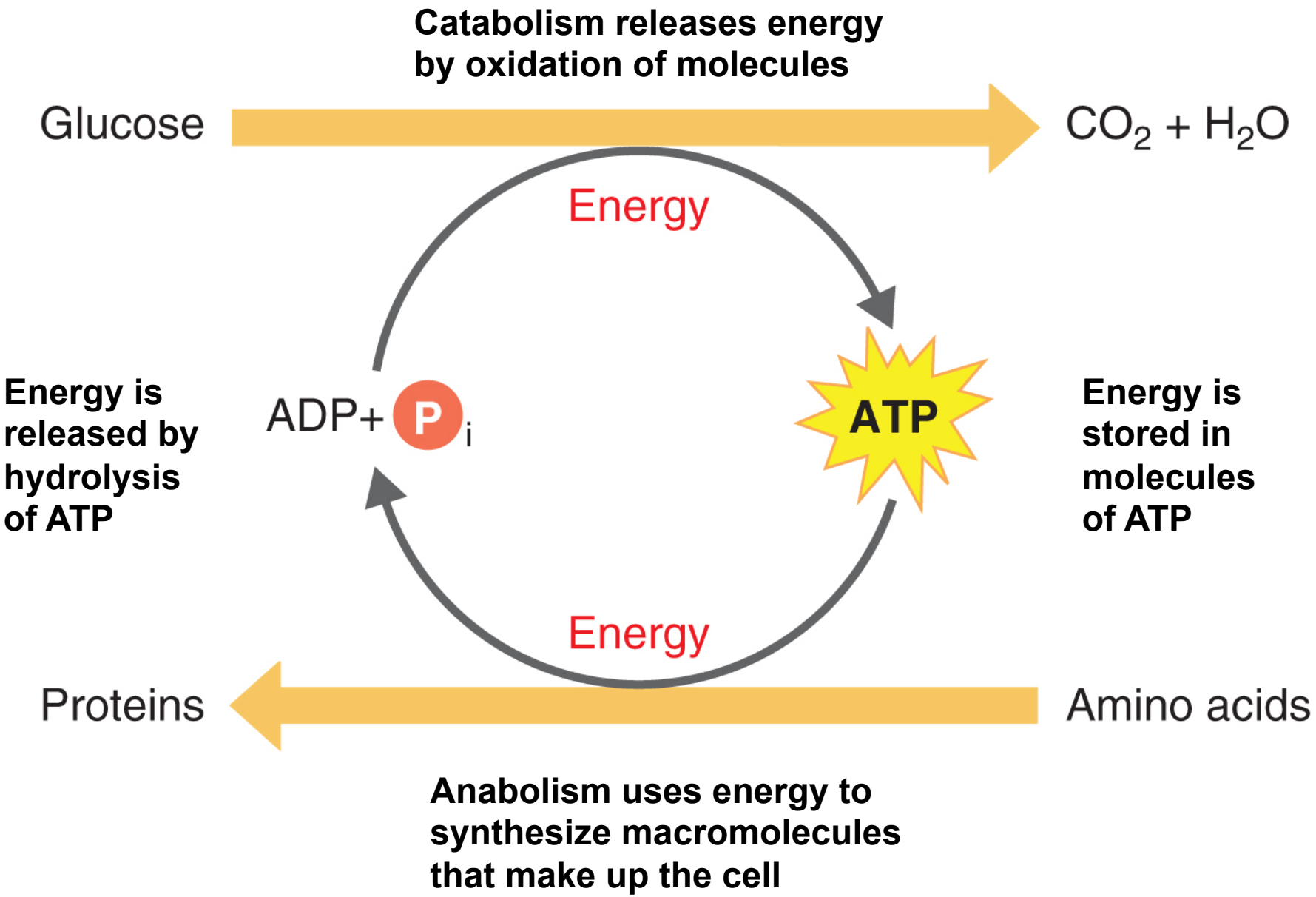
Catabolic Reactions

- Breakdown of complex substances to small molecules
  - **Degradative** reactions
  - Breaking of covalent bonds
  - What type of reaction used to break covalent bonds?
- Purpose is to generate energy, make building blocks
  - Releases energy stored in bonds
  - Breakdown products used to build new molecules
- Eg, breakdown of glucose for energy

Anabolic Reactions

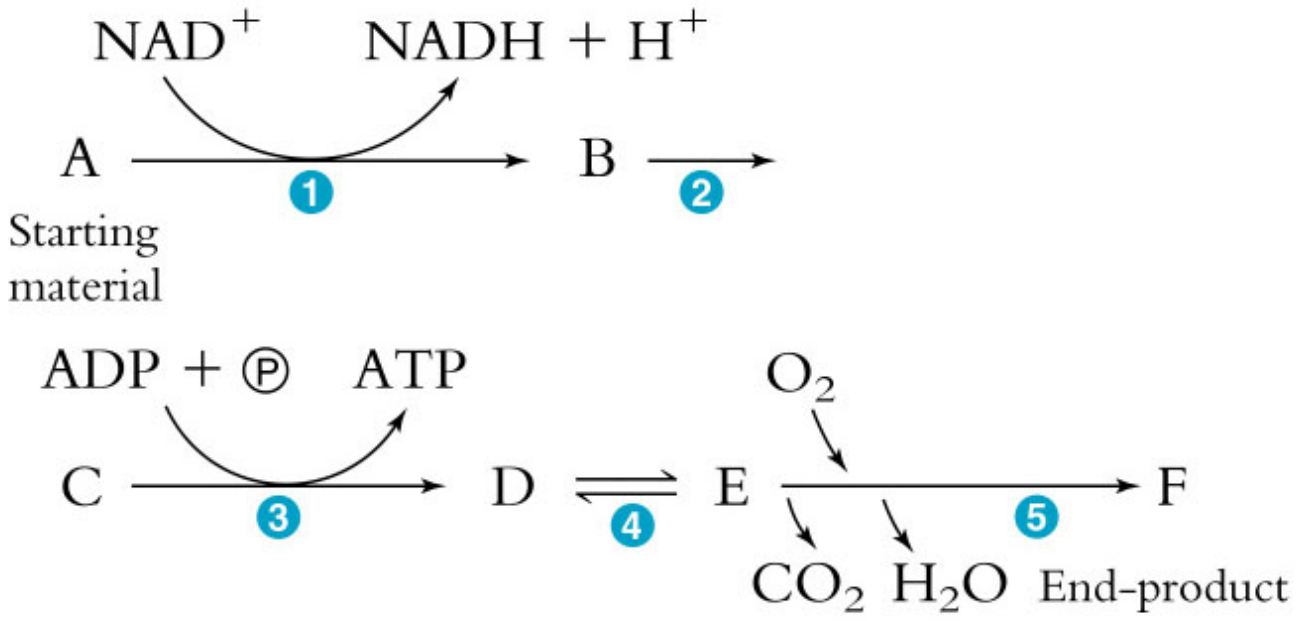
- Building macromolecules from small molecules
  - **Biosynthetic** reactions
  - Forming of covalent bonds
  - What type of reaction used to form covalent bonds?
- Requires energy to form bonds
- Purpose is to generate materials for cell growth
- Eg, making proteins from amino acids

Figure 5.1 The role of ATP in coupling anabolic and catabolic reactions.



Catabolic and Anabolic Reactions

- A **metabolic pathway** is a sequence of chemical reactions in a cell
- Metabolic pathways are controlled by enzymes



Enzymes

- Enzymes are biological **catalysts**
  - "Allow" reactions to occur
  - Not used up in that reaction
- Specific for a single chemical reaction
- Specificity is due to structure of enzyme
  - Proteins (enzymes) have characteristic 3D structure
  - Structure is essential for function
- If enzyme loses shape, enzyme doesn't work
  - Denaturation (Denatured)

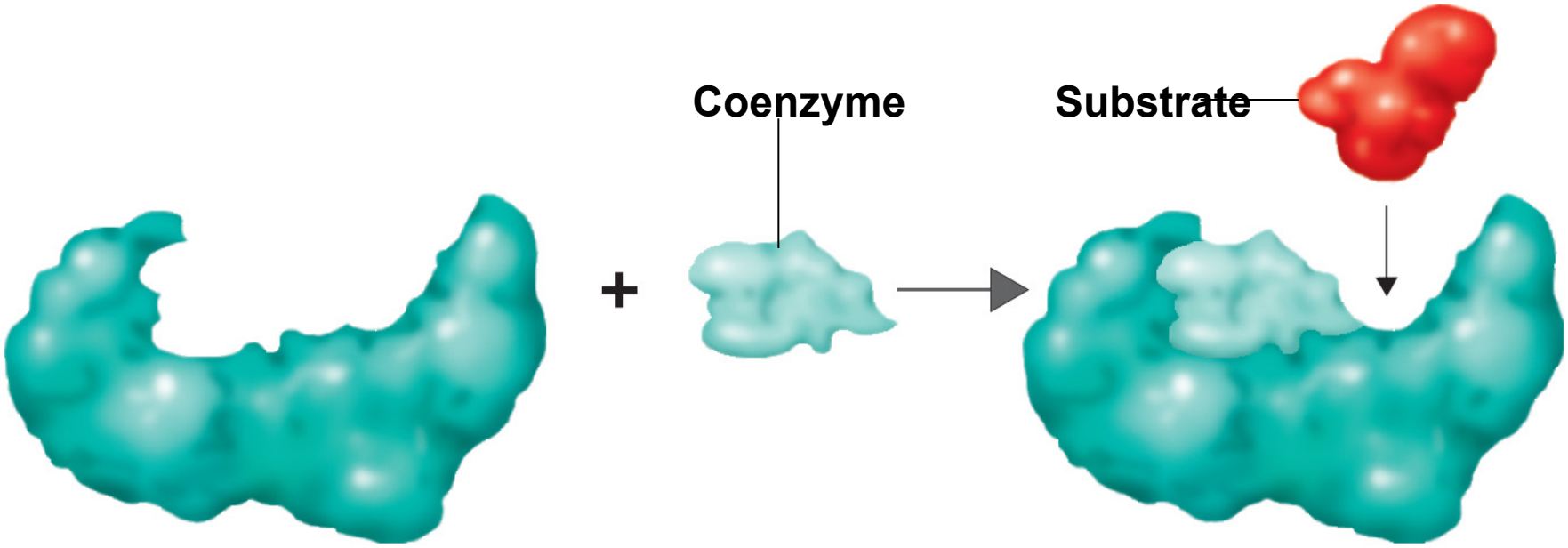
Enzymes

- Enzymes act on one or more "**substrates**"
  - Substrates bind at **active site**
- Each enzyme is specific:
  - For one (set of) substrate(s)
  - For one reaction
- Names of enzymes end in "-ase"
  - Sucr*ase*, Catal*ase*, DNA polymer*ase*
- Eg, the enzyme *sucrase* catalyses the hydrolysis of sucrose into glucose and fructose
  - That's it, no other reactions

Enzyme Components

- Many enzymes are made entirely of proteins
- Some consist of 2 components:
  - **Apoenzyme**: protein
  - **Cofactor**: nonprotein component
    - **Coenzyme**: organic cofactor
- **Holoenzyme**: apoenzyme plus cofactor
  - Apoenzyme alone doesn't function

Enzyme Components



Apoenzyme (protein portion), inactive

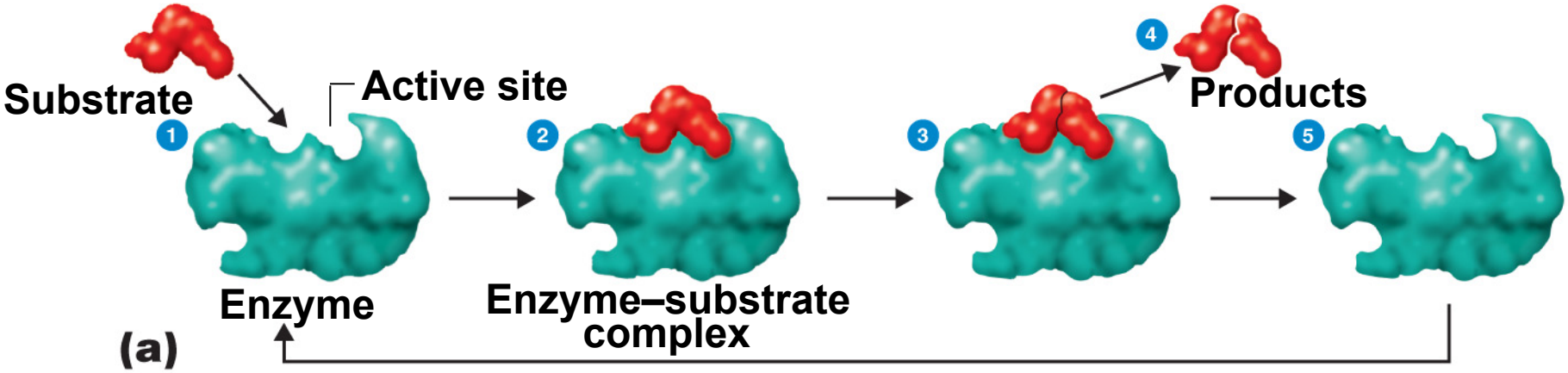
Cofactor (nonprotein portion), activator

Holoenzyme (whole enzyme), active

Enzyme Mechanism

- General sequence of events in an enzymatic reaction
  - Substrate(s) bind to active site
    - **Enzyme-substrate complex**
  - The substrate(s) is transformed
  - Transformed molecule(s), product(s), released
    - No longer fits active site
    - Enzyme only binds to substrate
  - Enzyme is free to bind to new substrate(s)
- Sequence continues until enzyme is shut off, or enzyme breaks down

Enzyme Mechanism

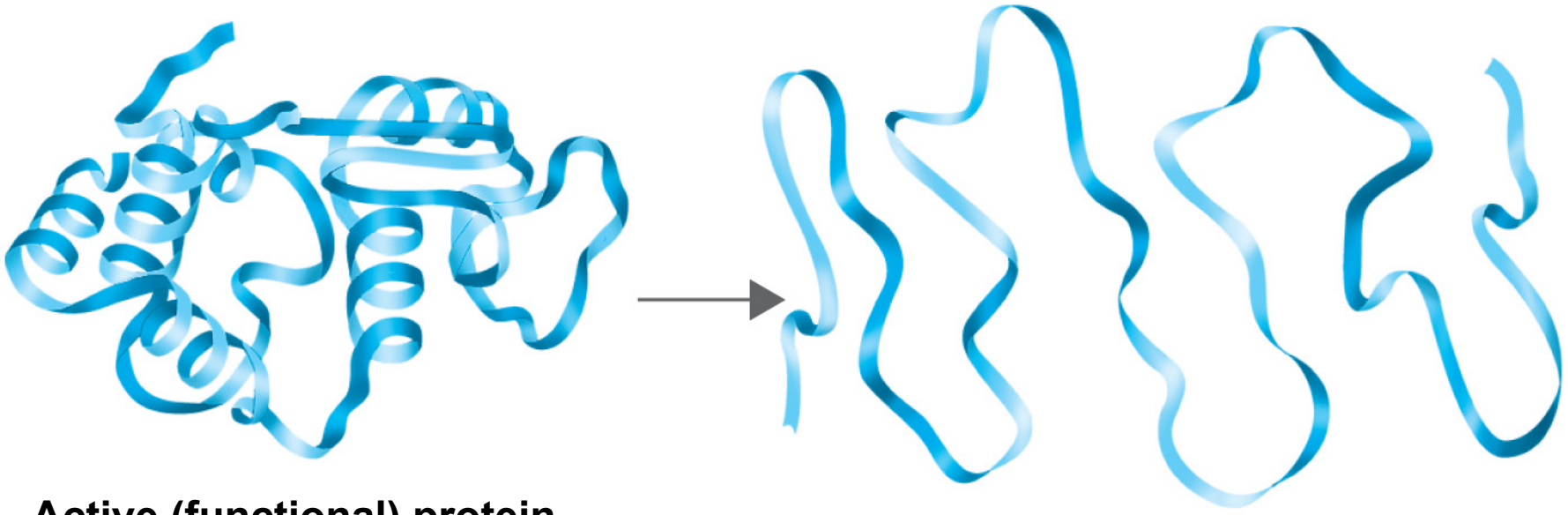


Factors Influencing Enzyme Activity

- Temperature
- pH
- Inhibitors

Factors Influencing Enzyme Activity

- Enzymes require specific conditions to function
- Hostile environments can cause proteins to denature



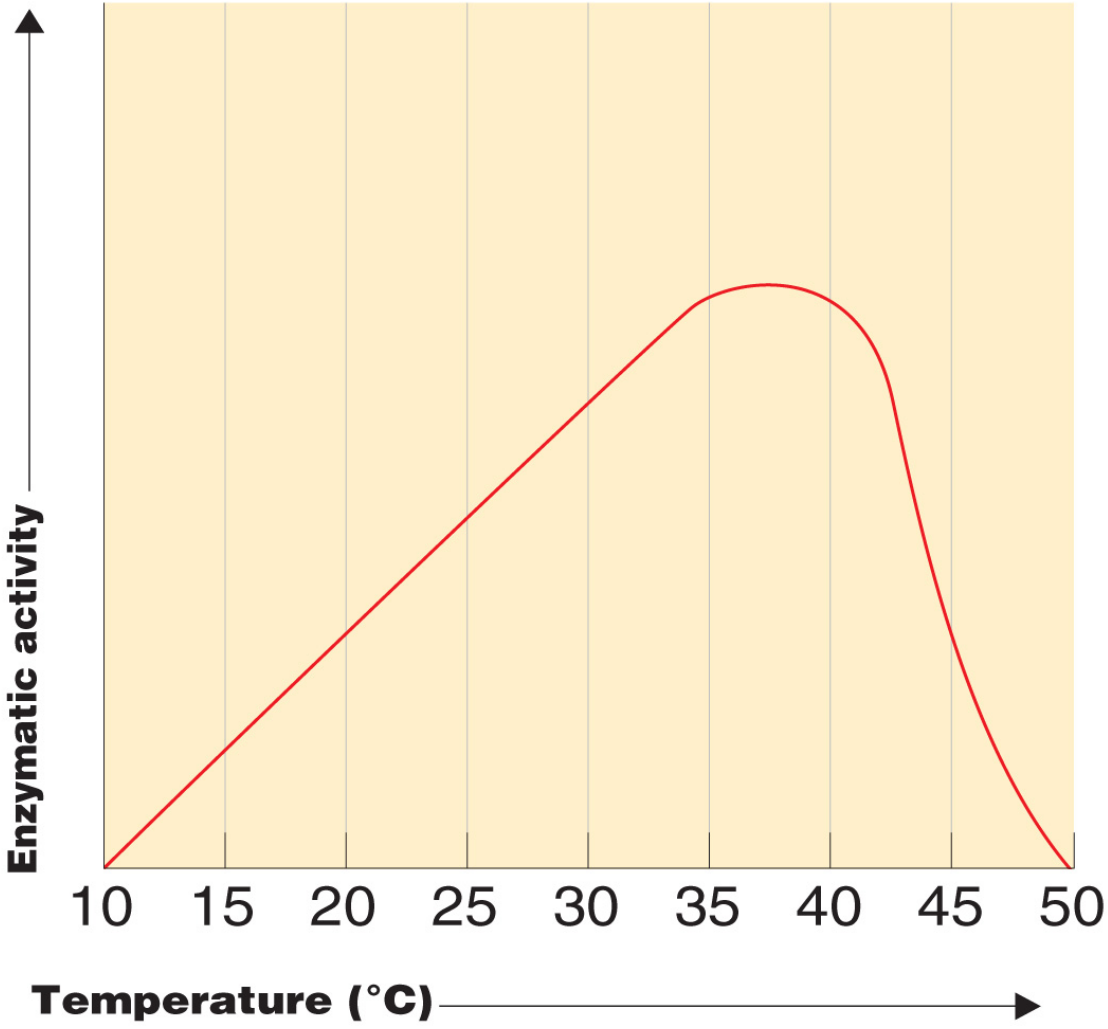
Active (functional) protein

Denatured protein

Factors Influencing Enzyme Activity

- Temperature
  - In general, chemical reactions **speed up** as temperature increases **slightly**
  - For enzymes, **too high** temperature increases cause **denaturation**

Figure 5.5a Factors that influence enzymatic activity, plotted for a hypothetical enzyme.

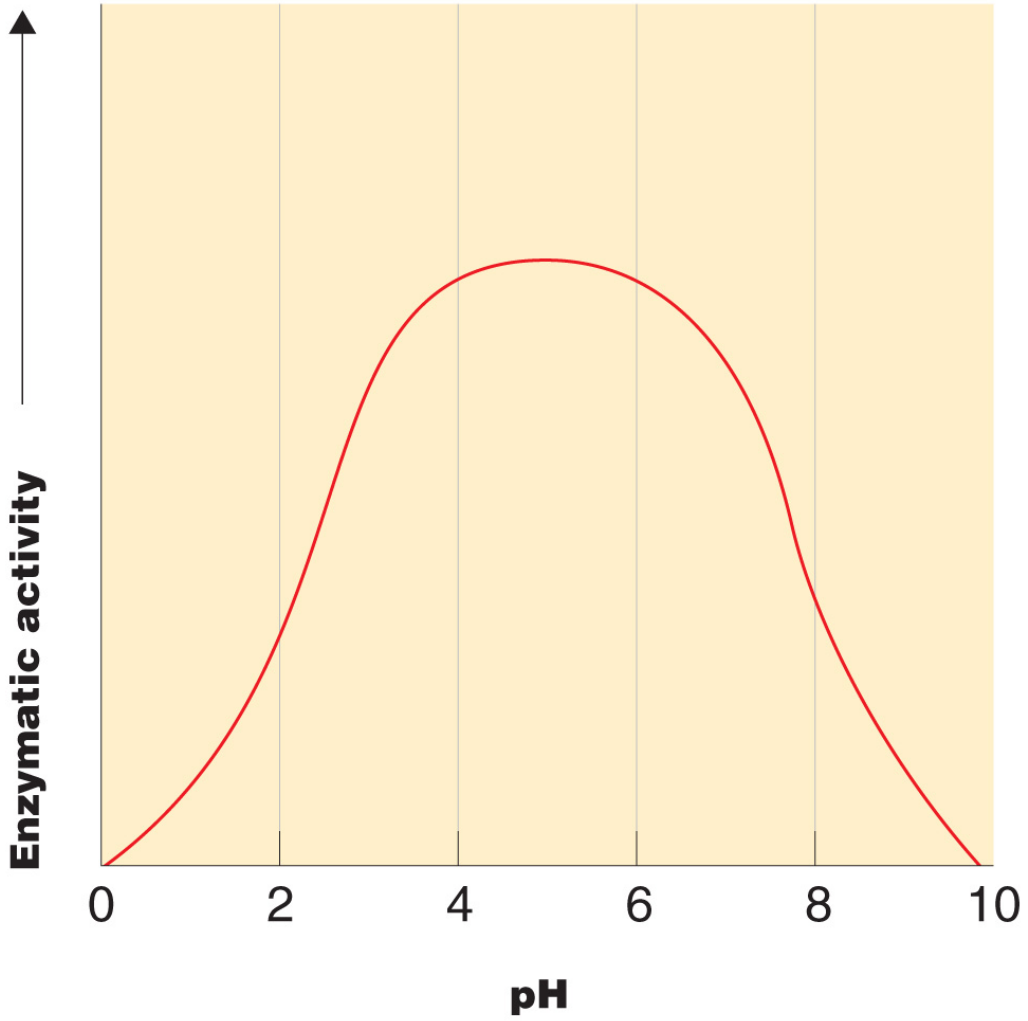


(a) Temperature. The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.

Factors Influencing Enzyme Activity

- pH
  - pH is a measure of hydrogen ions (H^+) in a solution
  - All enzymes have **preferred** pH
  - Shift from preferred pH will denature enzymes

Figure 5.5b Factors that influence enzymatic activity, plotted for a hypothetical enzyme.



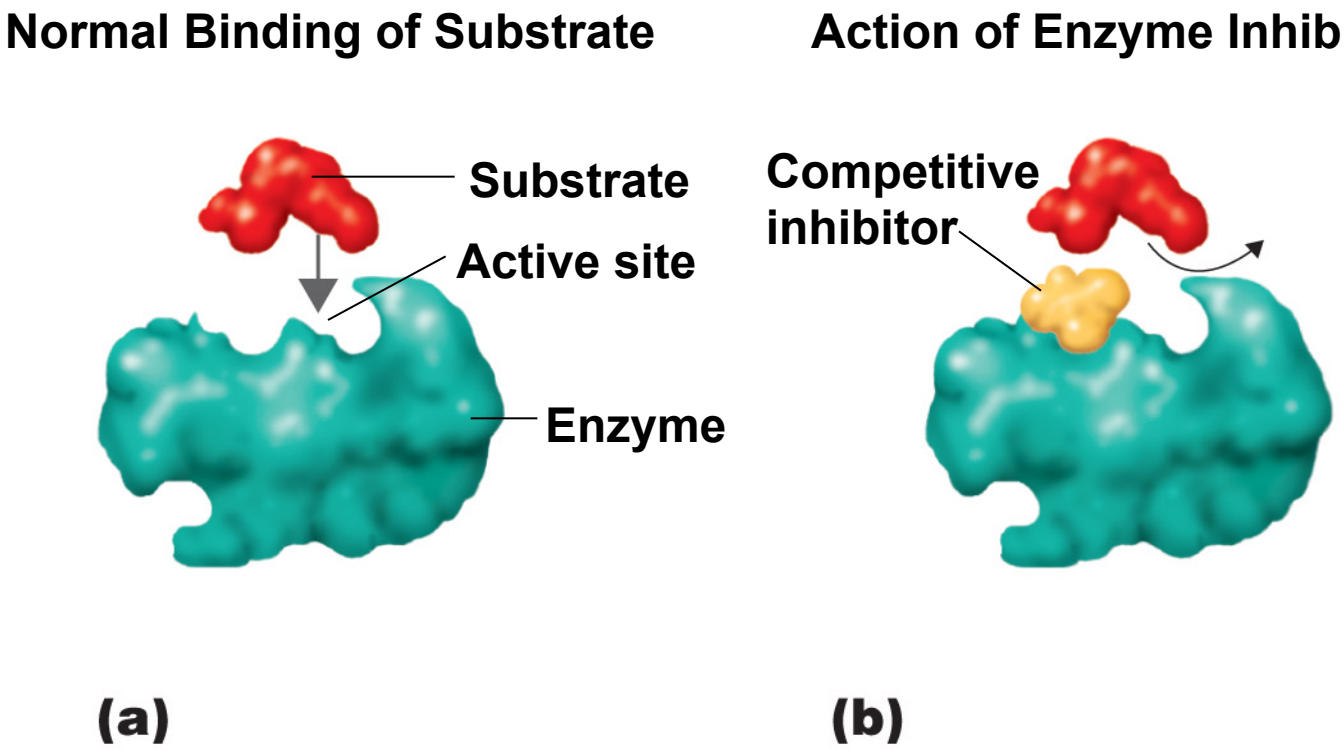
(b) pH. **The enzyme illustrated is most active at about pH 5.0.**

Factors Influencing Enzyme Activity

- Certain chemicals that bind to enzymes
  - Binding disrupts enzyme function
- Function as enzyme **inhibitors**
- Two classes of inhibitors
  - **Competitive** inhibitors
  - **Noncompetitive** inhibitors

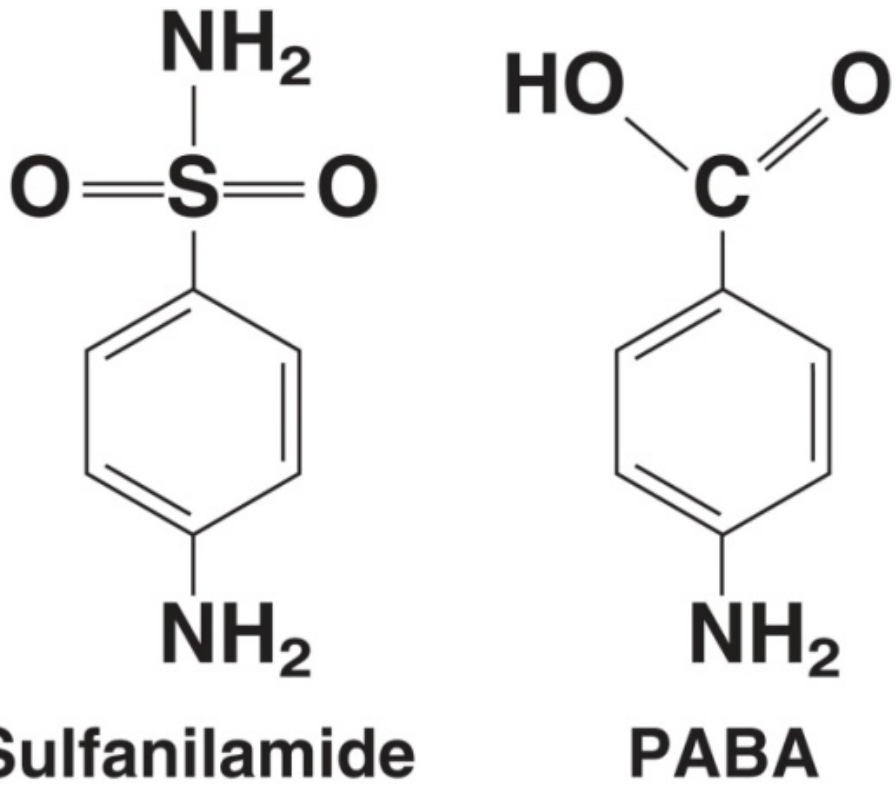
Competitive Inhibitors

- Bind at active site
- *Compete* with substrate for the active site of enzyme



The Sulfa Drugs

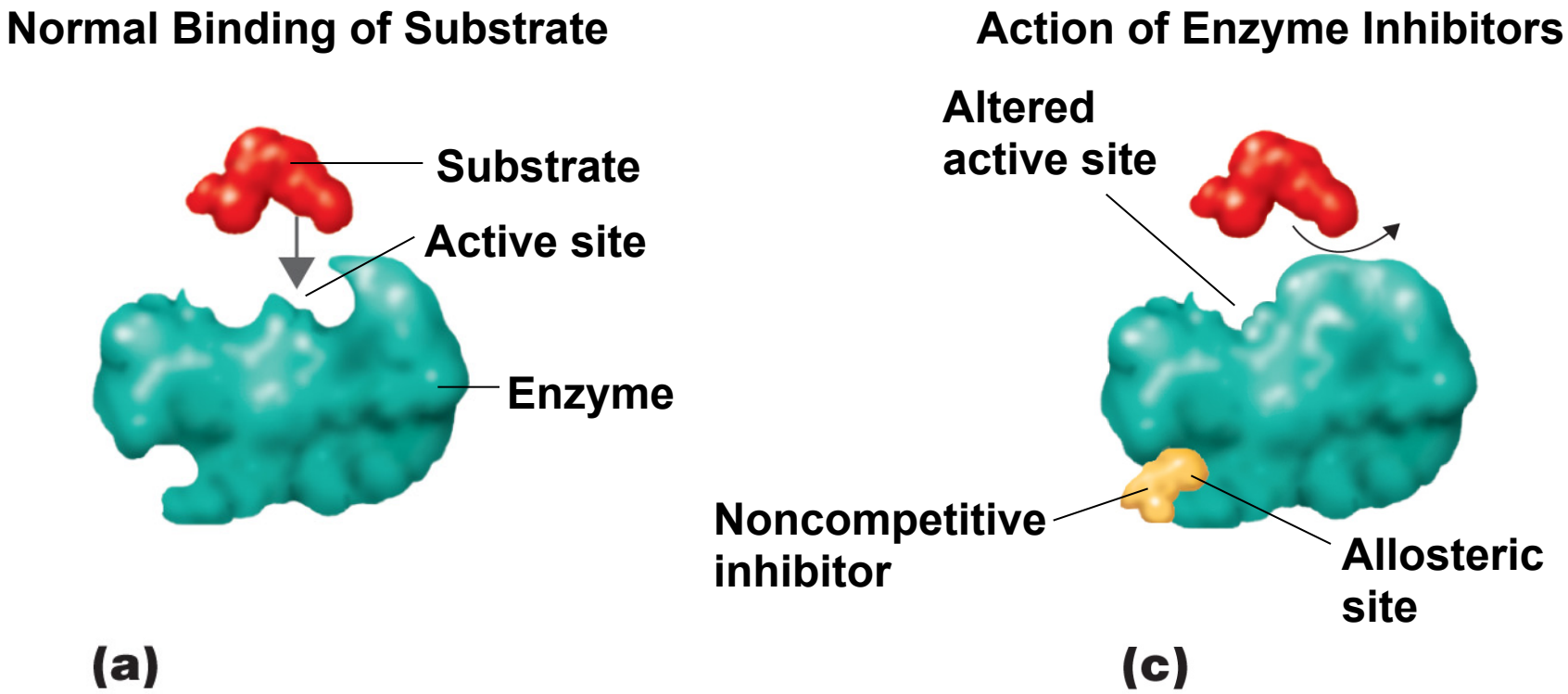
- **Sulfa drugs** inhibit Tryptophan (an essential amino acid) synthesis via competitive inhibition
- Sulfa drugs were the first effective antibacterial drugs



Sulfanilamide PABA

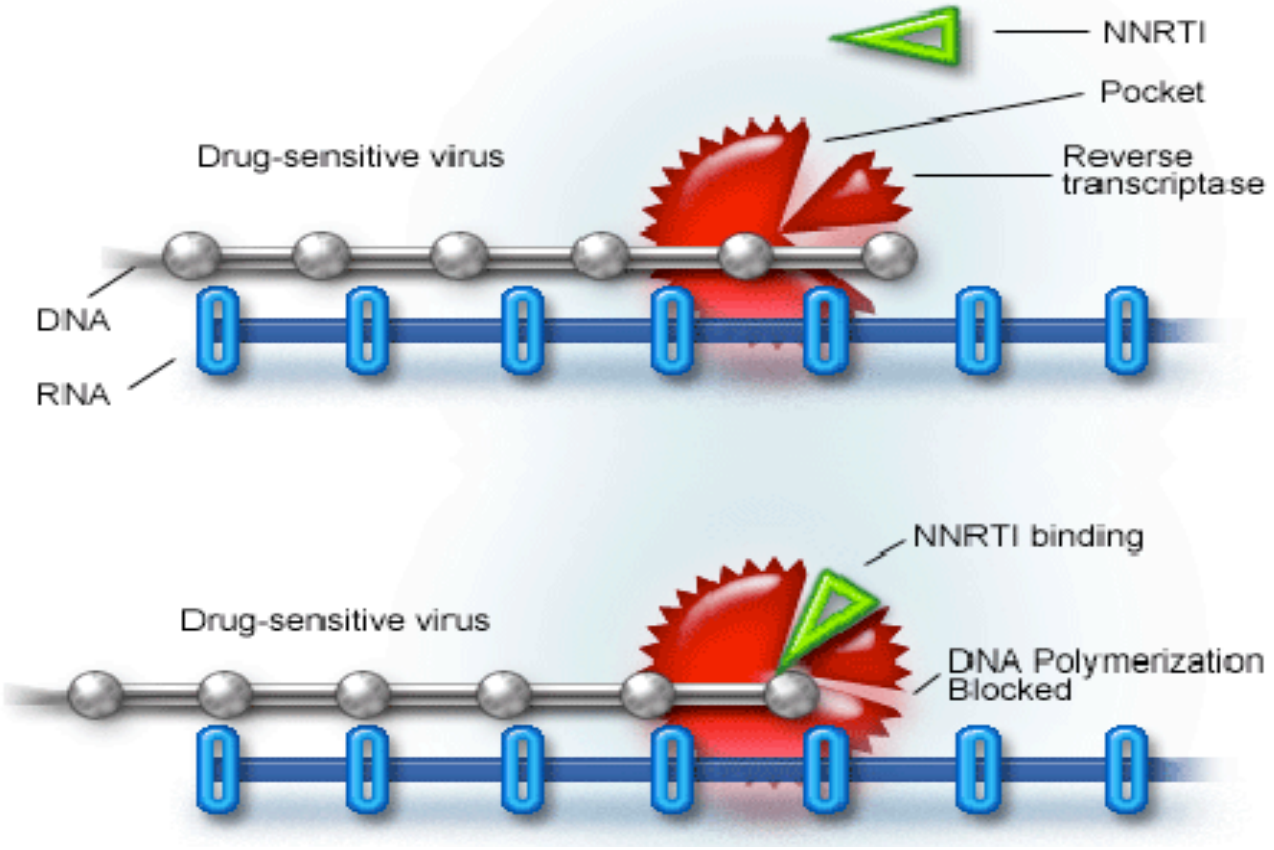
Noncompetitive Inhibition

- Bind away from active site
- Aka **allosteric** inhibition



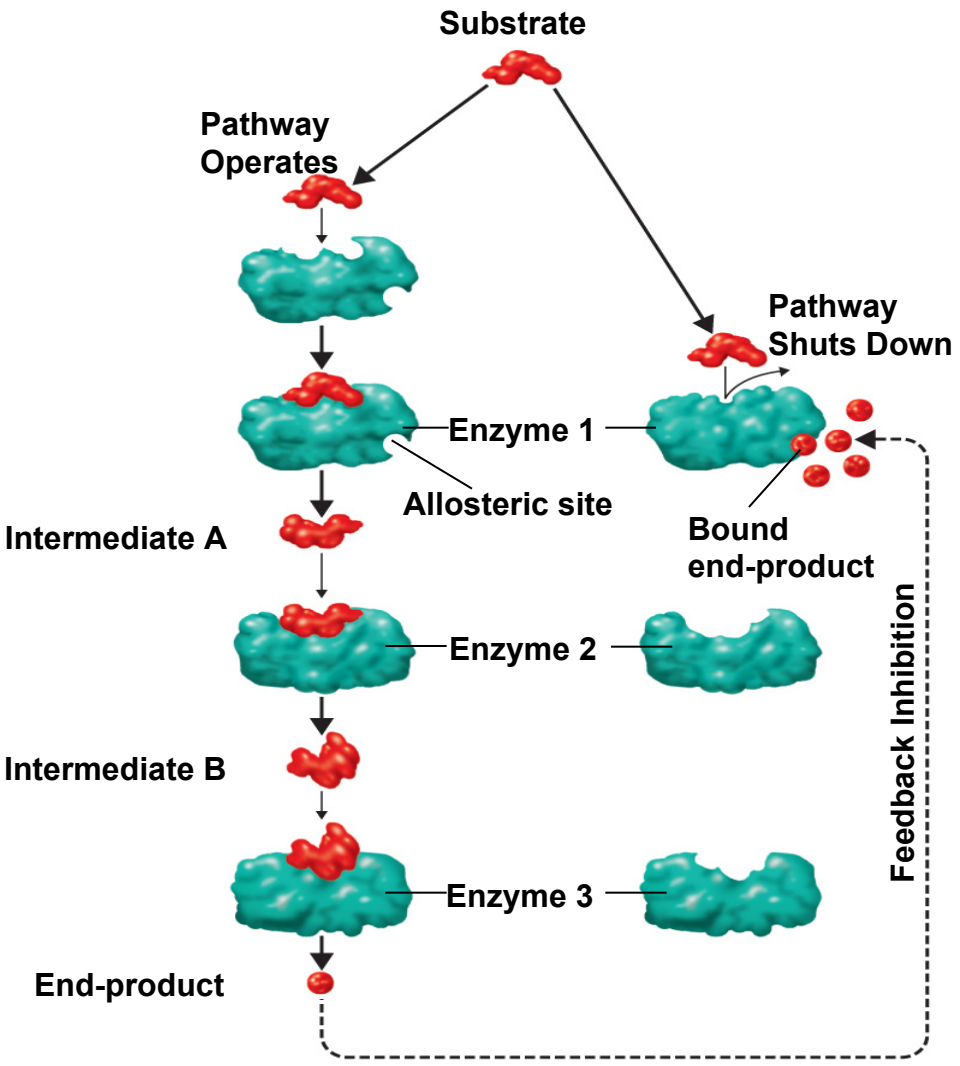
Noncompetitive Inhibition

- **Nevirapine** (NNRTI) prevents replication of HIV via non-competitive inhibition



Enzyme Inhibitors: Feedback Inhibition

- Control of enzymatic activity by use of allosteric inhibition
  - Mechanism to stop cell from wasting chemical resources



Energy Production

Learning Objectives

5-8 Explain the term *oxidation-reduction*.

5-9 List and provide examples of three types of phosphorylation reactions that generate ATP.

5-10 Explain the overall function of metabolic pathways.

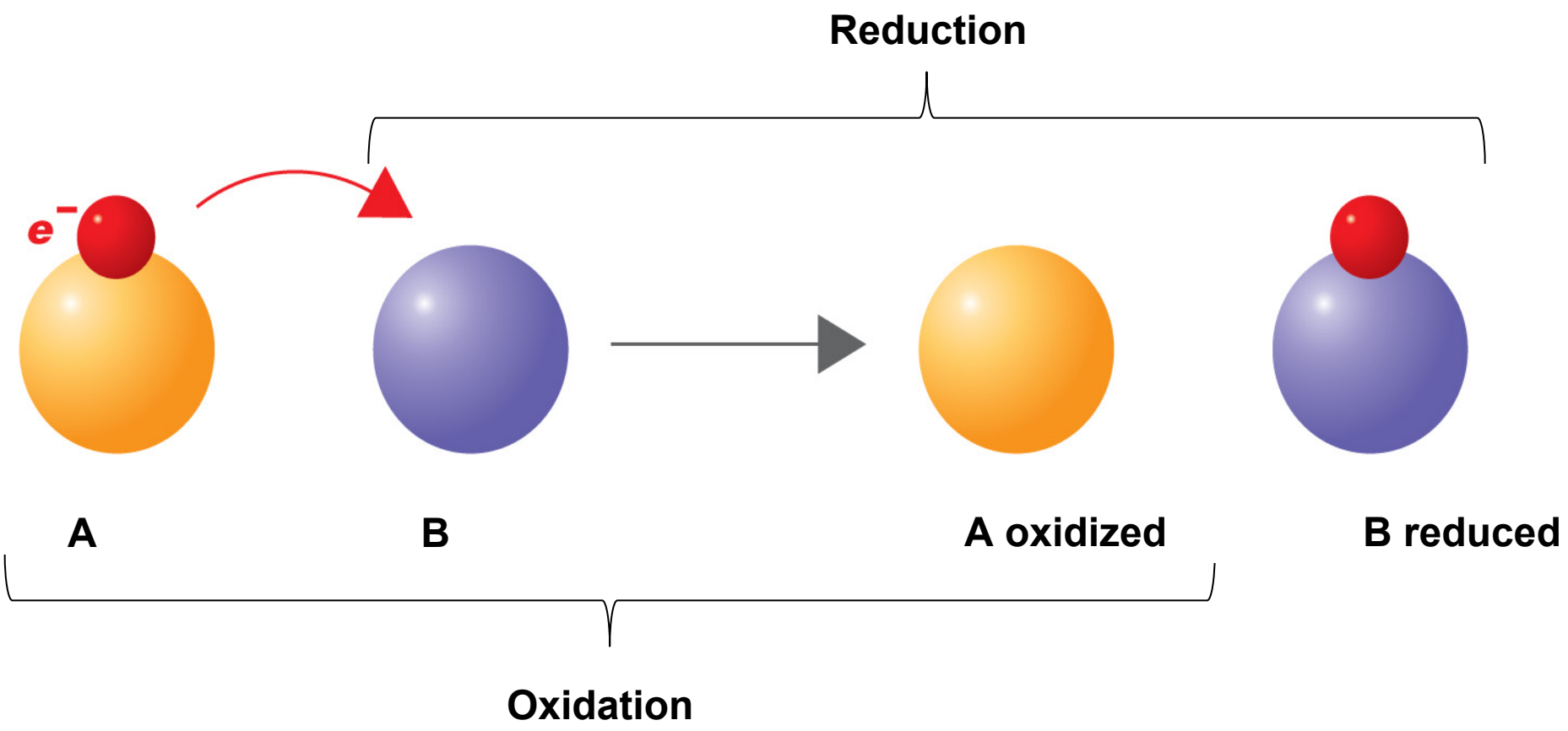
Energy Production

- Energy is stored in covalent bonds
  - Recall: covalent bonds form by sharing electrons
- Energy is stored in electrons of covalent bonds
- Catabolism involves stripping "**high energy**" electrons from molecules and concentrate them in the bonds of ATP
- Reactions that involve removing and adding electrons are called "**oxidation-reduction**" reactions

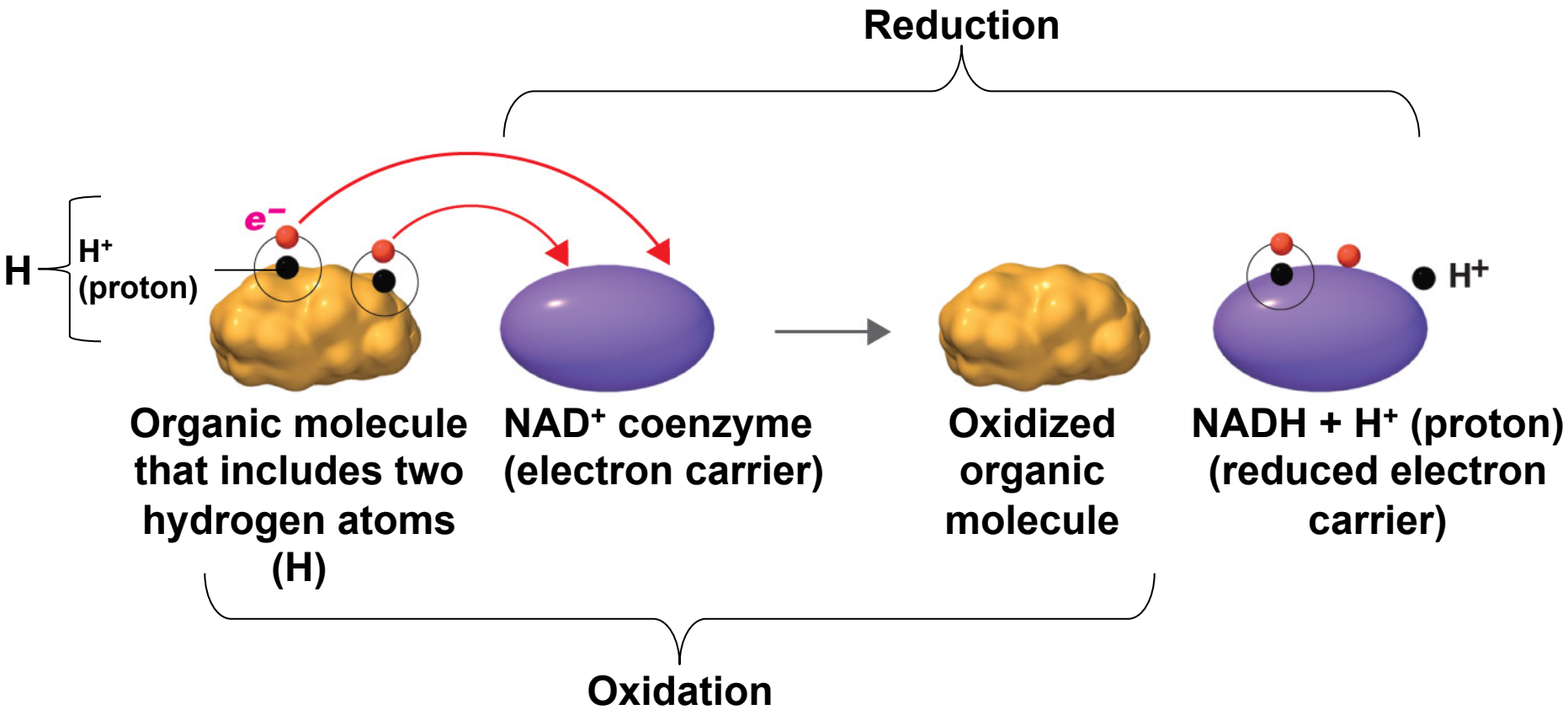
Oxidation-Reduction Reactions

- **Oxidation**: removal of electrons
- **Reduction**: gain of electrons
- **Redox reaction**: an oxidation reaction paired with a reduction reaction
- Catabolism is the oxidation of highly reduced molecules

A Redox Reduction

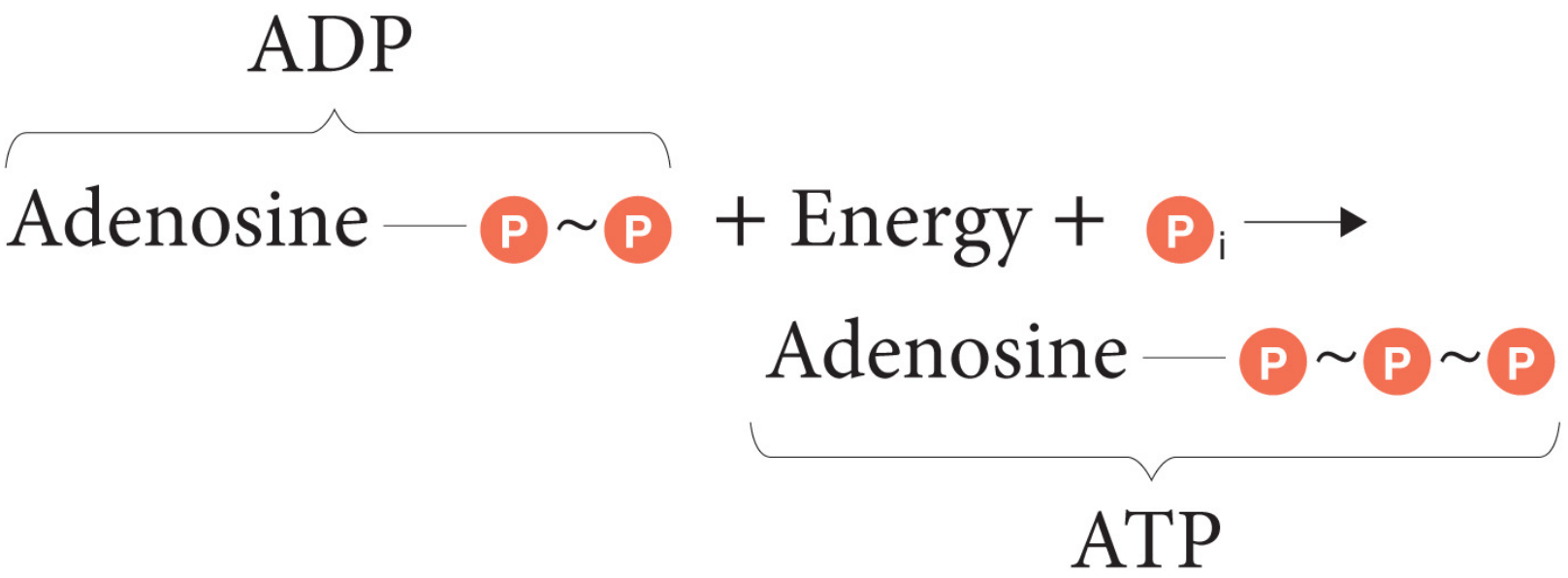


Biological Oxidation



The Generation of ATP

- Energy released by redox reactions “trapped” by ATP
- ATP is generated by the **phosphorylation** of ADP
  - Addition of a phosphate
  - Requires energy

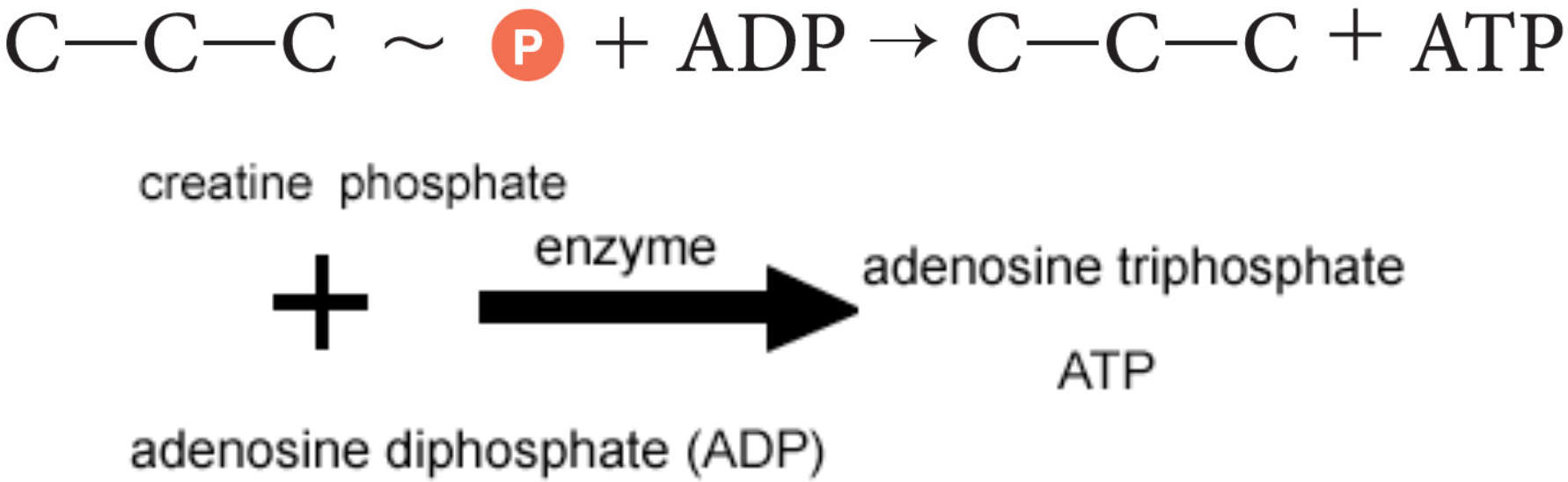


Generation of ATP

- 3 Types of phosphorylations to generate ATP
  - Substrate-level phosphorylation
  - Oxidative phosphorylation
  - Photophosphorylation

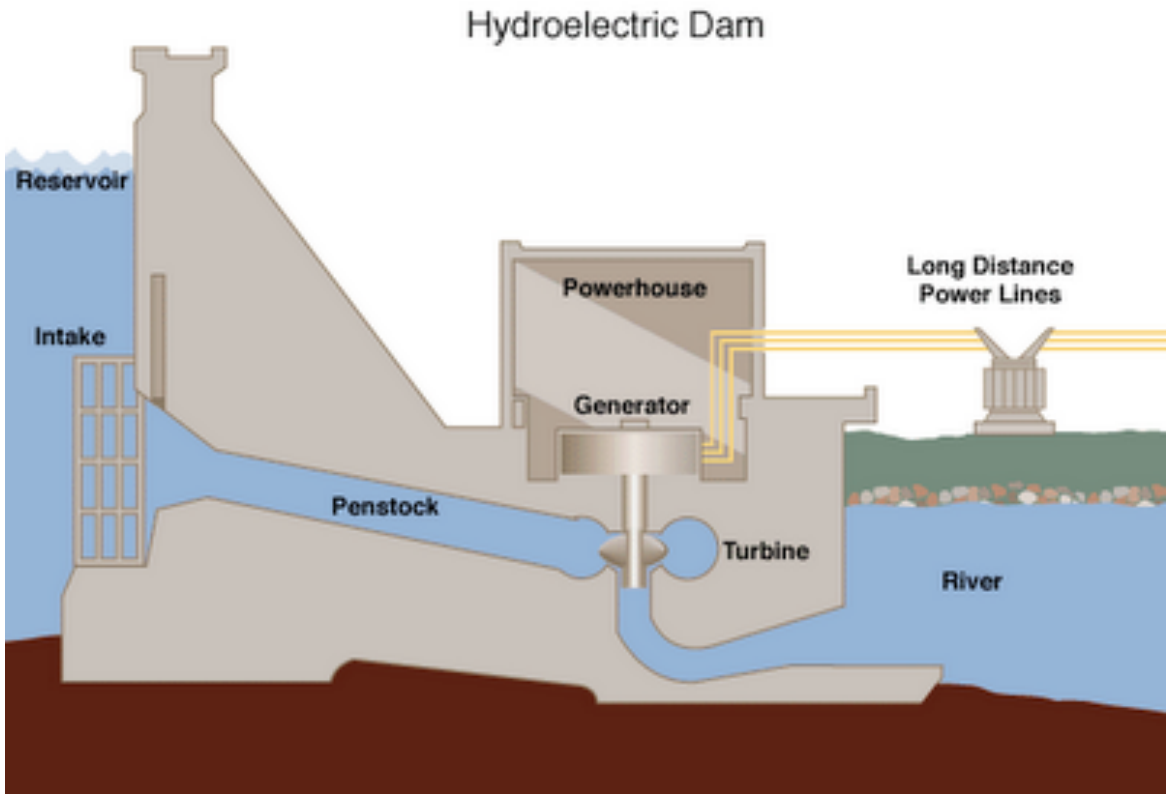
Substrate-Level Phosphorylation

- **Direct transfer** of a PO_4^- to ADP generates ATP
- Enzymatic process
- Quick and easy
- Not highly efficient



Oxidative Phosphorylation

- "High energy" electrons from organic molecule (food) used to generate a **chemiosmotic** (proton) gradient
  - Gradient used to drive ATP production



Photophosphorylation

- Occurs only in photosynthetic cells
- Light transfers energy to “low-energy” electrons
  - Electrons get “excited”
- Excited electrons used to generate chemiosmotic gradient, drive ATP production
  - Similar to oxidative phosphorylation, using light instead of food to “build dam”

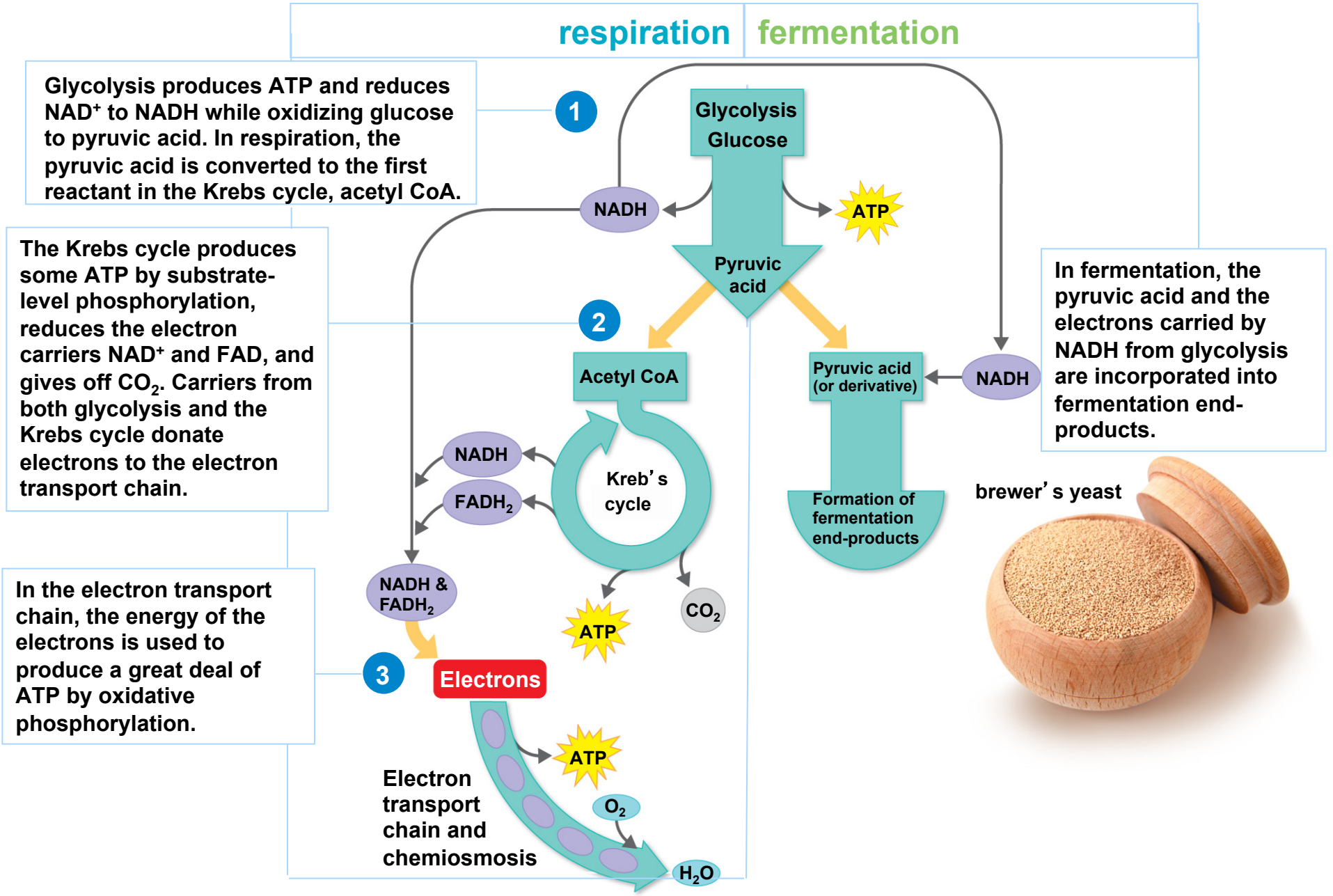
Metabolic Pathways of Energy Production

- Catabolism involves series of controlled reactions
  - Releasing energy in one reaction generates too much heat, can't be harnessed efficiently
- Catabolism is a series of redox reactions
  - Electrons extracted to generate ATP
  - Sequence of reactions called a **pathway**
- Every reaction in a pathway performed by one enzyme

Carbohydrate Catabolism

- Carbohydrates are primary source of energy
  - **Glucose** is most common energy source
- Glucose is broken down via two general processes
  - **Cellular respiration**
    - Glucose + O_2 → CO_2 + H_2O + energy
  - **Fermentation**
    - Glucose → Energy + Organic end-product, i.e. ethanol, lactic acid

Figure 5.11 An Overview of Respiration and Fermentation.

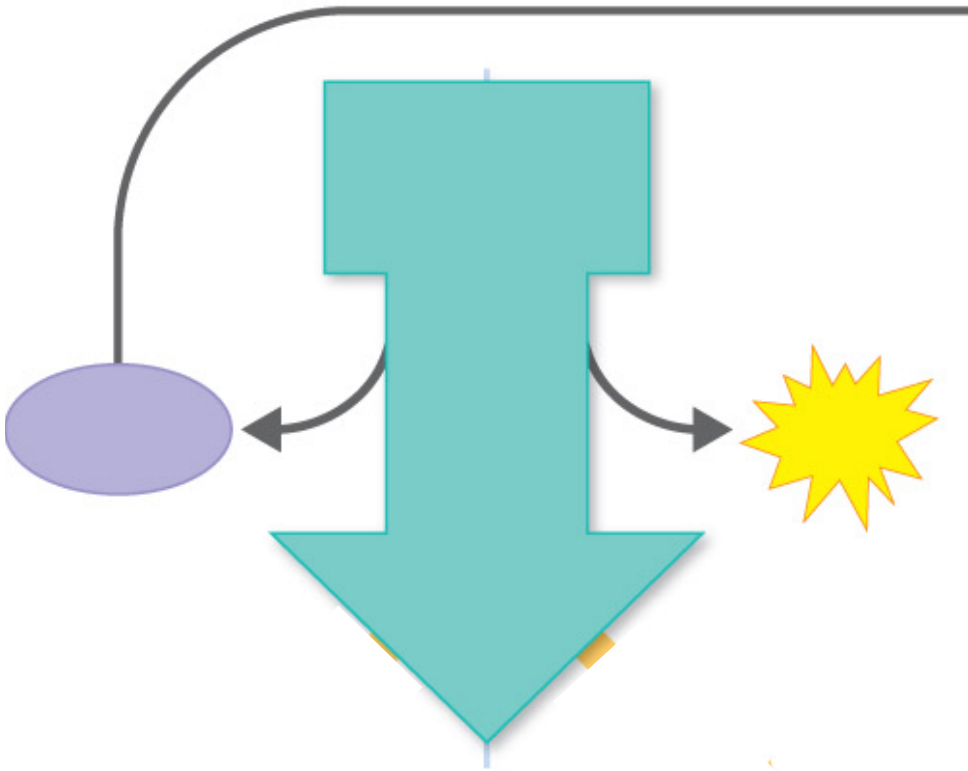


Cellular Respiration

- **Complete** oxidation (catabolism) of glucose
  - Waste products are inorganic
- Most of the energy is produced via oxidative phosphorylation
- Two types of respiration
  - **Aerobic** Respiration – with oxygen
  - **Anaerobic** Respiration – without oxygen
- Multiple pathways involved
  - **Glycolysis**
  - **Krebs Cycle**
  - **Electron transport Chain**

Glycolysis

- The oxidation of glucose (6 Carbon) to 2 pyruvic acid (3 Carbon)
- Main end products
  - 2 pyruvic acids (aka pyruvate)
  - 2 NADH – an electron carrier
  - 2 ATP – via SLP



A Word About Electron Carriers

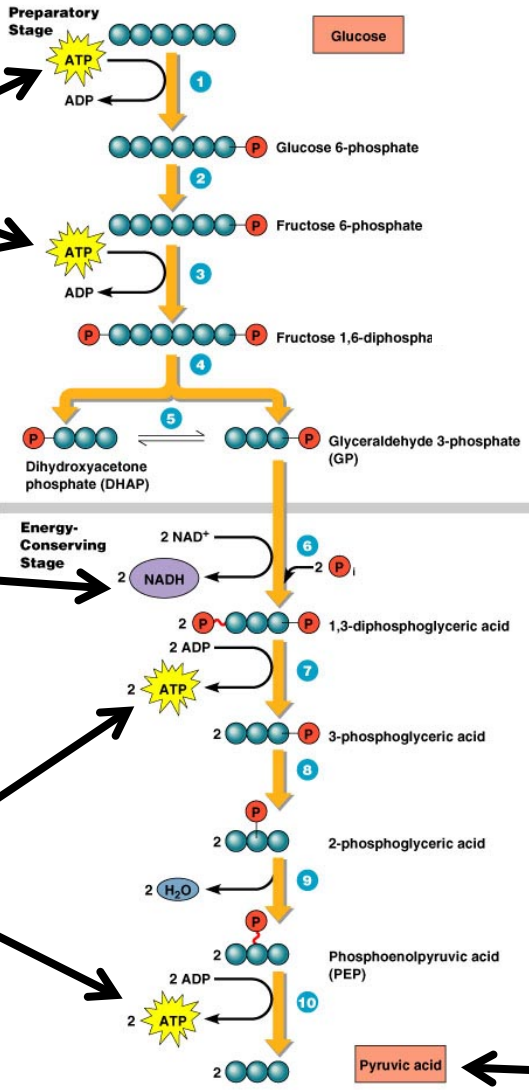
- NAD+ (empty electron carrier) removes electrons from organic molecule (food → glucose)
  - Becomes NADH (full electron carrier)
- NADH takes electrons to their final destination
  - Stay tuned ….

Glycolysis

1 - Requires 2 ATP to "get started"

2 – NAD+ is reduced to NADH
2 NADH produced

3 – 4 ATP produced
Overall, 2 ATP produced by glycolysis



Glycolysis DOES NOT require oxygen

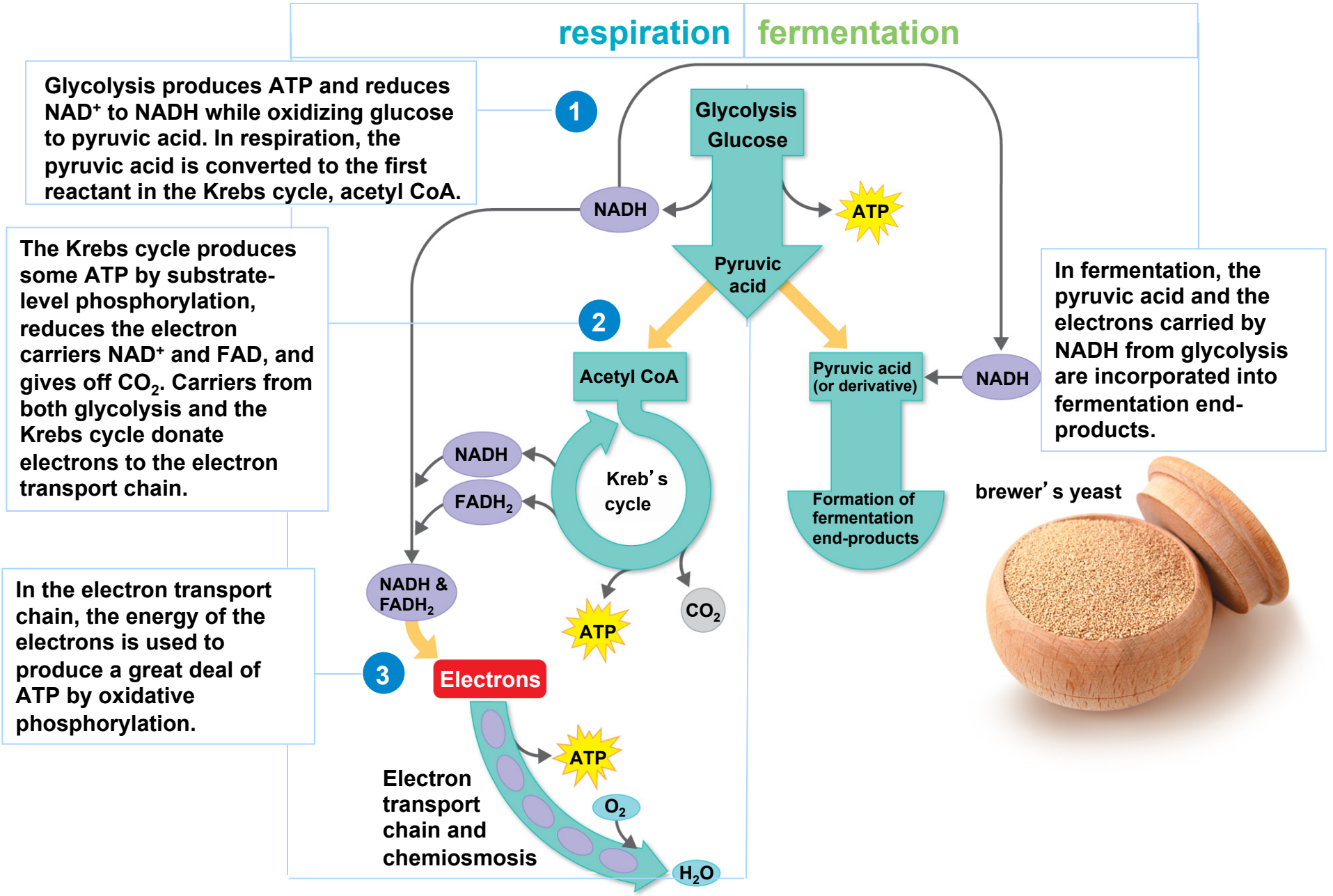
This part of glycolysis occurs twice per glucose molecule

4 – 2 pyruvic acid produced

Alternatives to Glycolysis

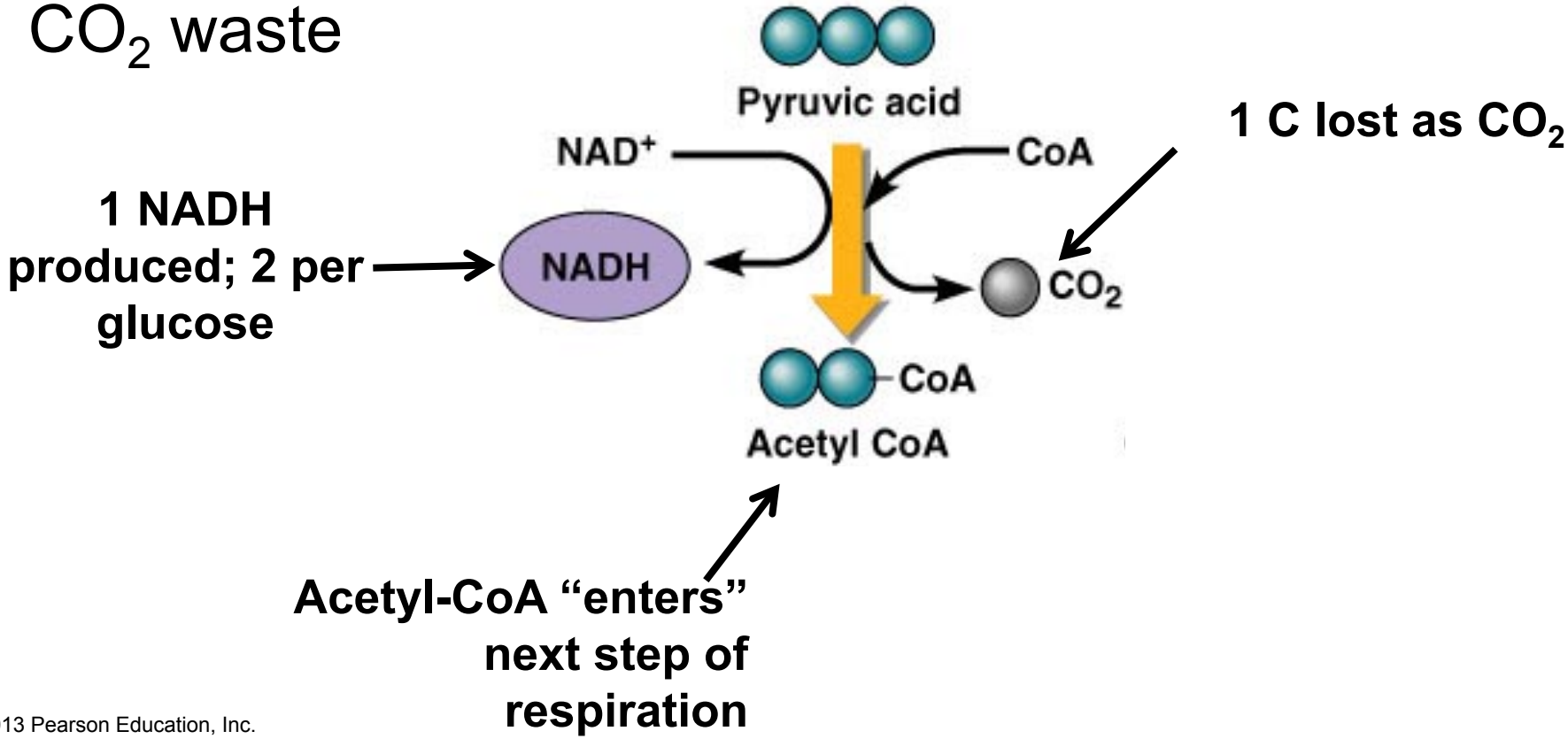
- **Pentose phosphate pathway**
  - Uses pentoses and NADPH (electron carrier for anabolism)
  - Operates with glycolysis
- **Entner-Doudoroff pathway**
  - Produces NADPH and ATP
  - Does not involve glycolysis

Figure 5.11 An Overview of Respiration and Fermentation.



Intermediate Step

- Pyruvic acid (from glycolysis) is oxidized and decarboxylated
- NADH is produced
- CO_2 waste



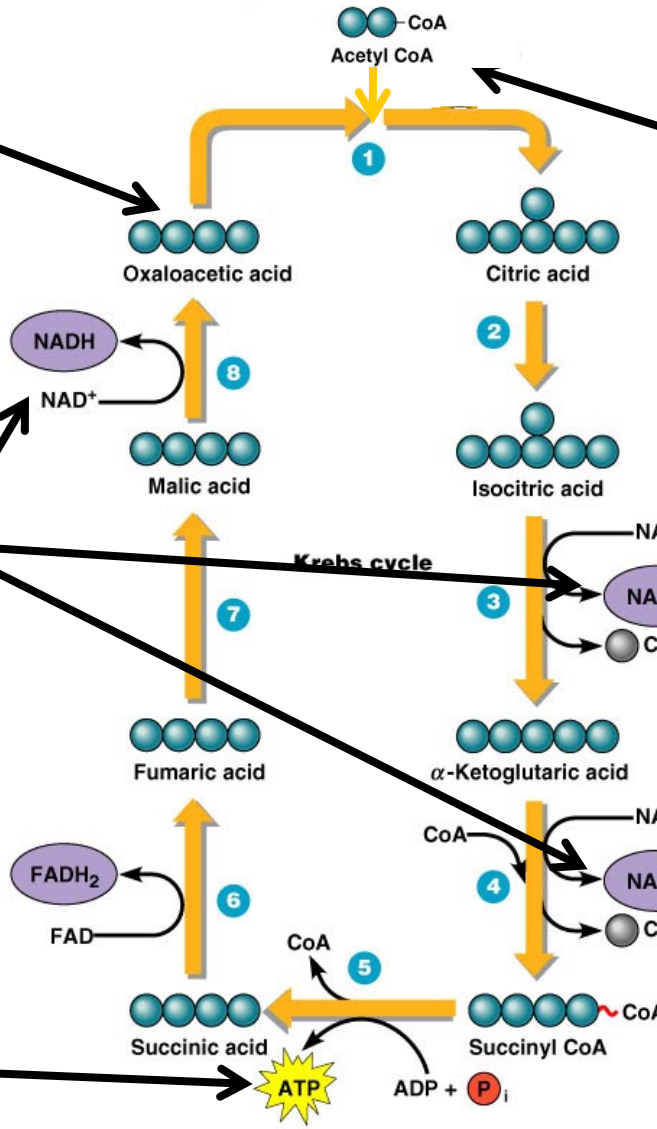
The Krebs Cycle

- Aka tricarboxylic acid cycle (TCA); citric acid cycle
- Series of 8 reactions
- Completely catabolizes organic molecule to CO_2
  - Released as waste
- Main products are NADH, $FADH_2$, 1 ATP
  - Electron carriers
- Final organic end-product is same as starting reactant
  - A “cycle”

The Krebs Cycle

Cyclical pathway, last product is same as starting reactant

1 – Acetyl-CoA enters Krebs cycle

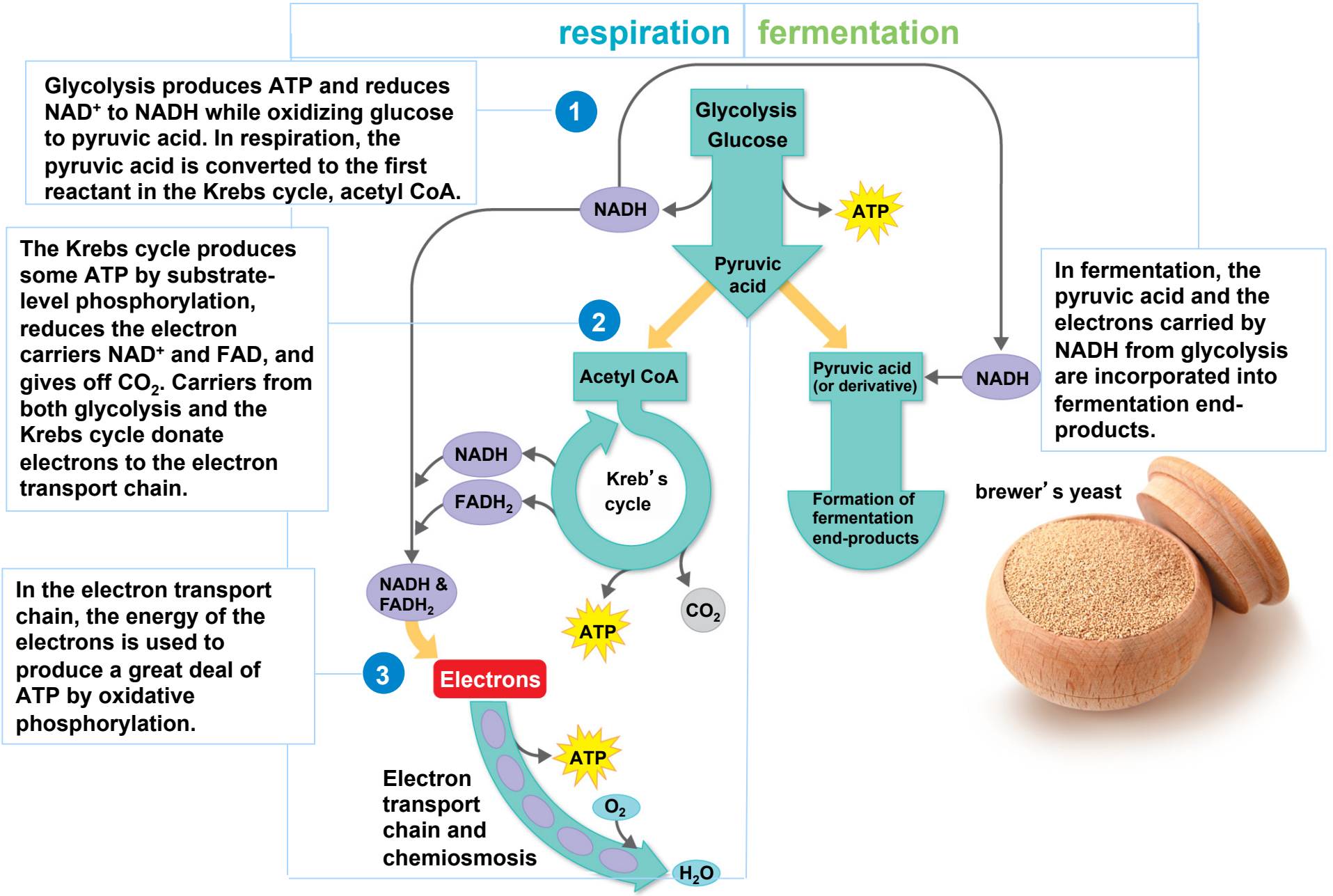


Generates NADH, $FADH_2$

CO_2 generated as waste

1 ATP produced

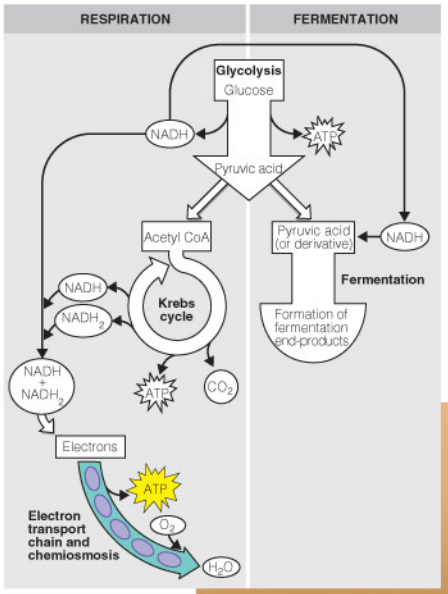
Figure 5.11 An Overview of Respiration and Fermentation.

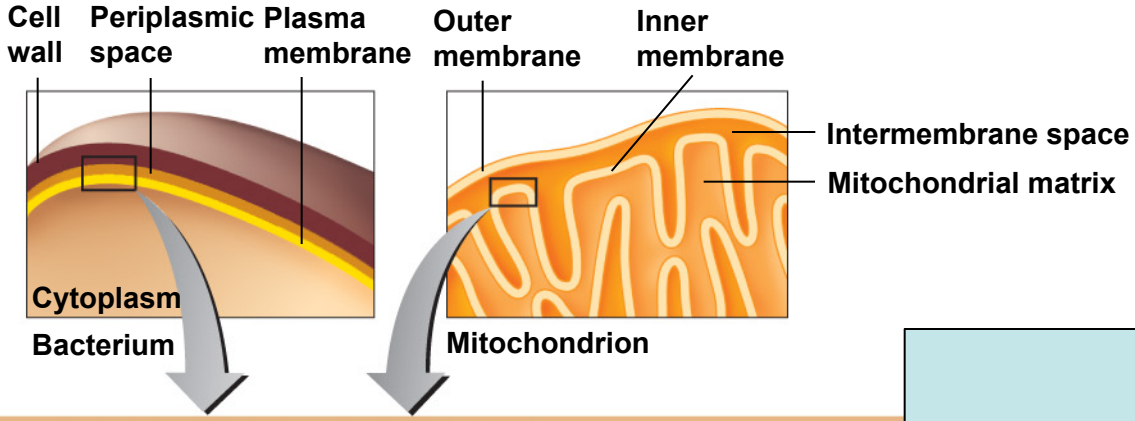


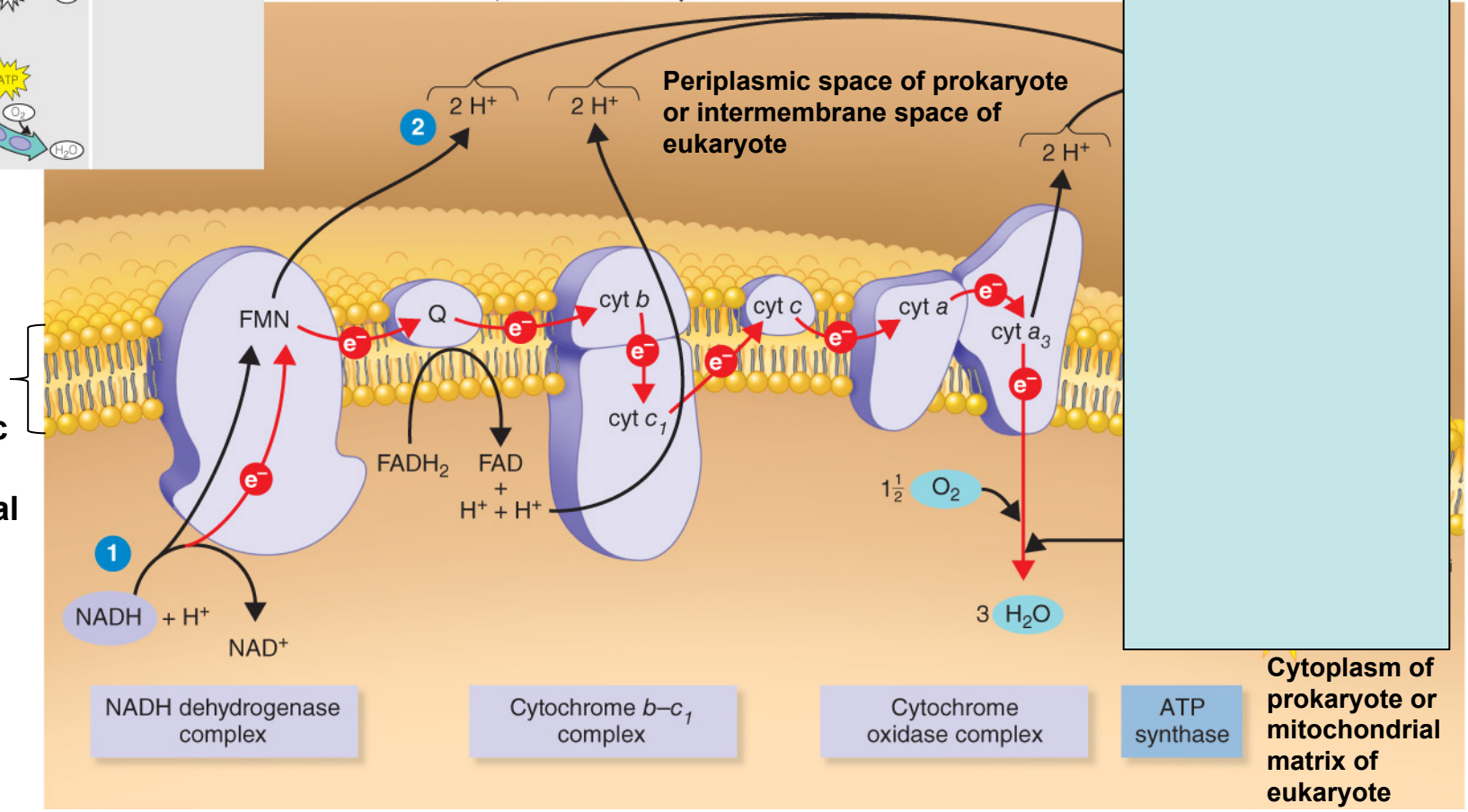
The Electron Transport Chain

- A series of electron carrier enzymes in membrane
- NADH, $FADH_2$ pass electrons to ETC
  - Become NAD+, FAD
  - Return to glycolysis, Krebs cycle
- Energy released from electrons used to drive H^+ from inside cell to outside cell
  - Produces H^+ concentration gradient – **chemiosmotic** gradient
- Electrons end up on "**final electron carrier**" – waste

Figure 5.16 Electron transport and the chemiosmotic generation of ATP.







Prokaryotic plasma membrane or eukaryotic inner mitochondrial membrane

Oxidative Phosphorylation

- ETC generates chemiosmotic gradient
- H^+ gradient favors diffusion into cell
  - H^+ cannot diffuse across membrane freely – Why?
- H^+ re-enters cell via **facilitated diffusion**
  - Through transporter called **ATP synthase**
- ATP synthase captures energy in gradient
  - Similar to water driving dam turbine
  - **Produces ATP → Oxidative phosphorylation**

Figure 5.15 Chemiosmosis.

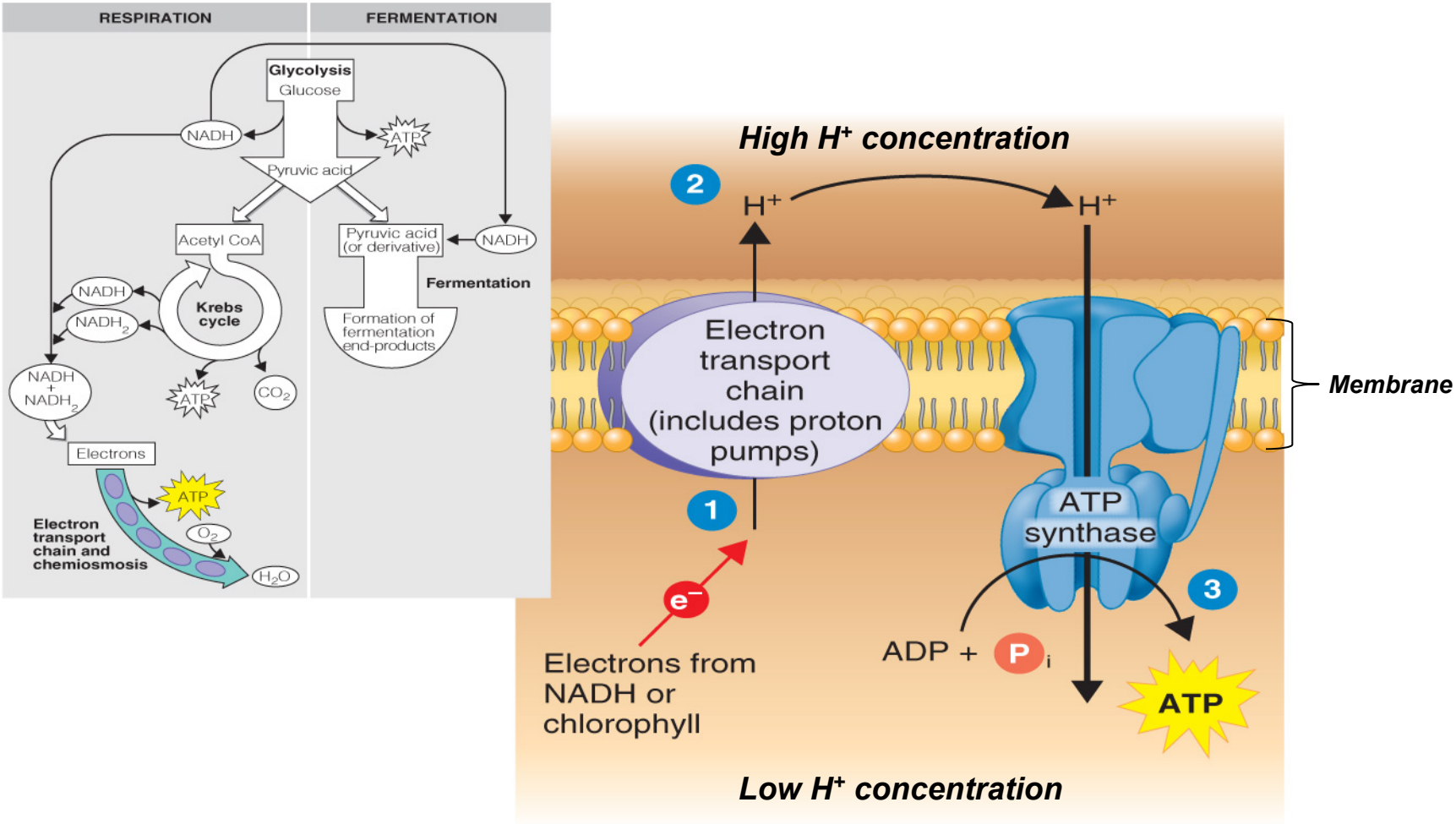
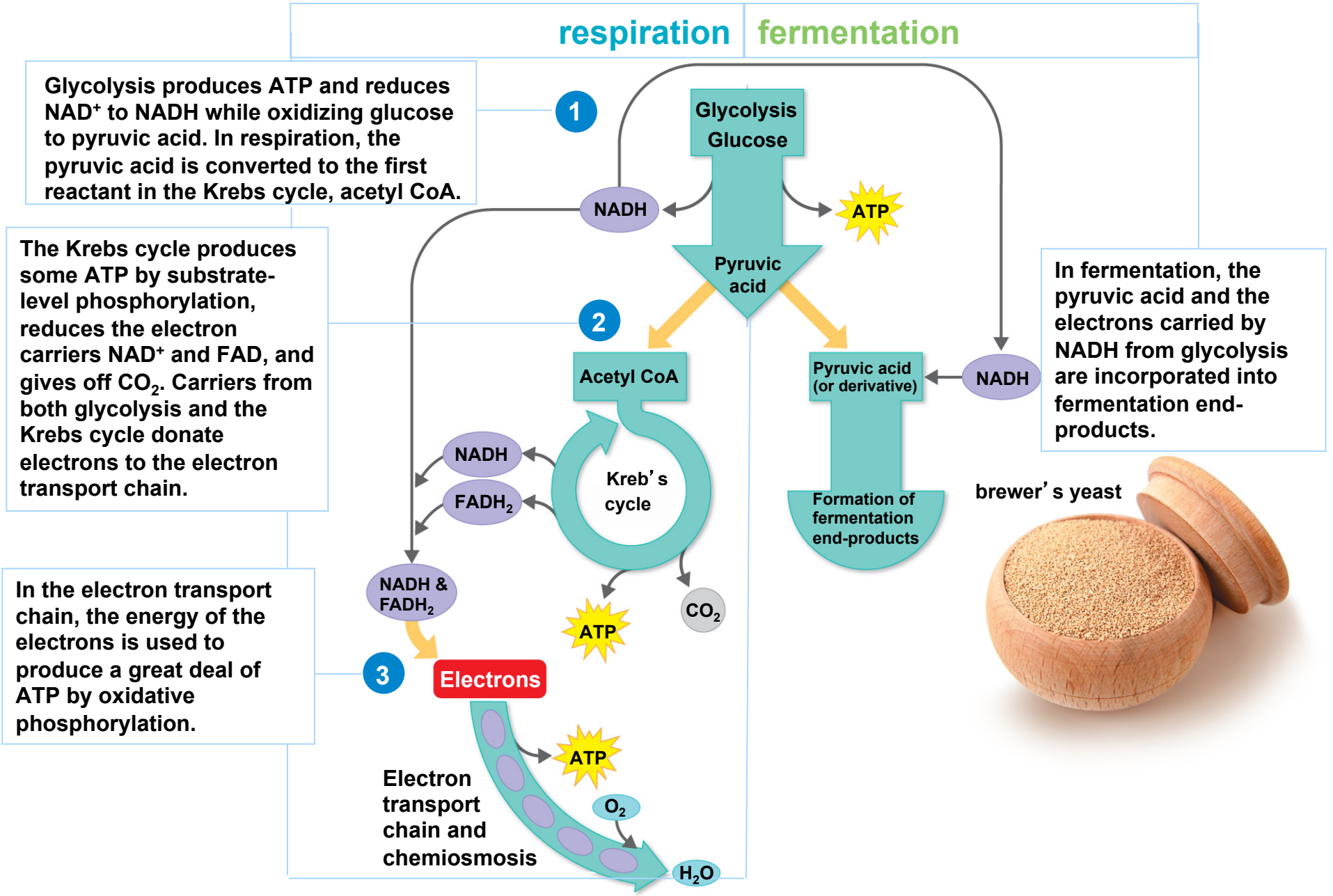


Figure 5.11 An Overview of Respiration and Fermentation.



A Summary of Respiration

- **Aerobic respiration**: the final electron acceptor in the electron transport chain is molecular oxygen (O_2)
  - Organism is an aerobe
  - Oxygen is converted to **water** → waste
- **Anaerobic respiration**: the final electron acceptor in the electron transport chain is NOT O_2
  - Usually inorganic ion
  - Organism is an anaerobe
  - Yields less energy than aerobic respiration because only part of the Krebs cycle operates 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-}	$CH_4 + H_2O$

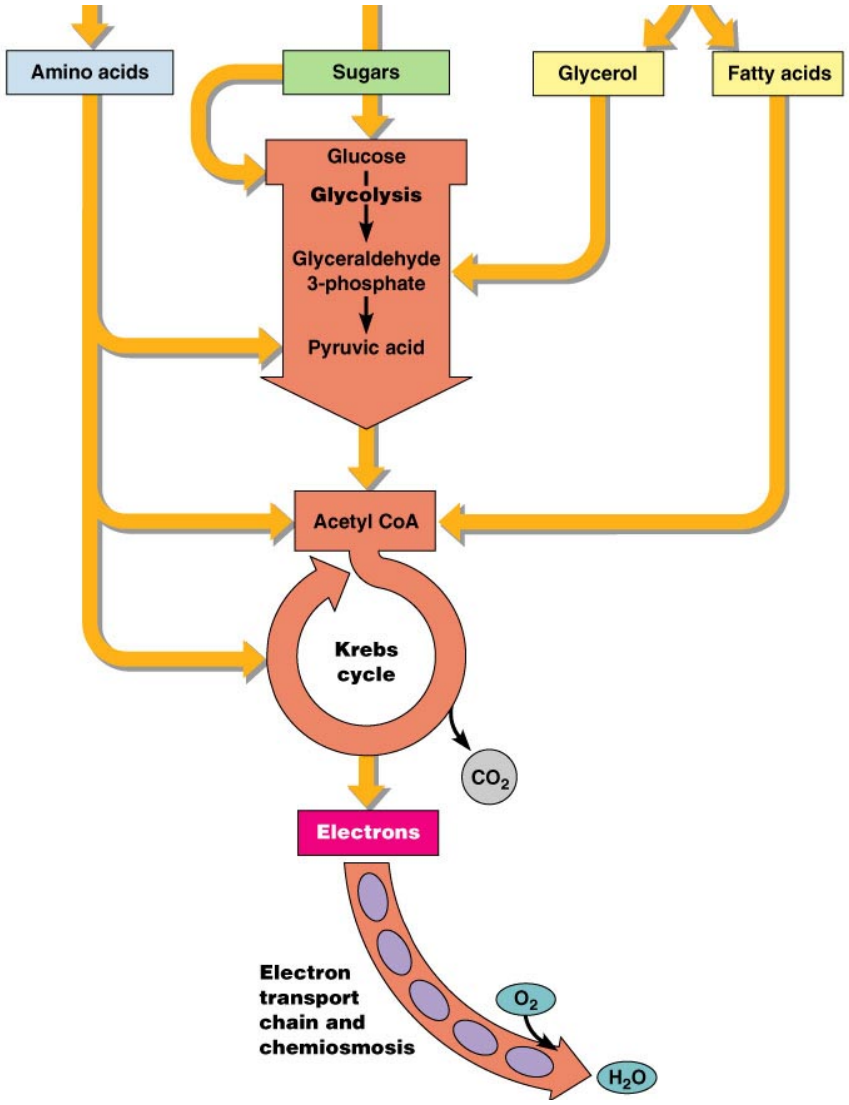
Carbohydrate Catabolism

- 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**

Cellular Respiration

- Other energy sources can be used
  - Eg, can oxidize lipids, proteins
- Polymers broken down by enzymes
- Smaller subunits enter catabolism at various points of glycolysis, Krebs cycle



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)

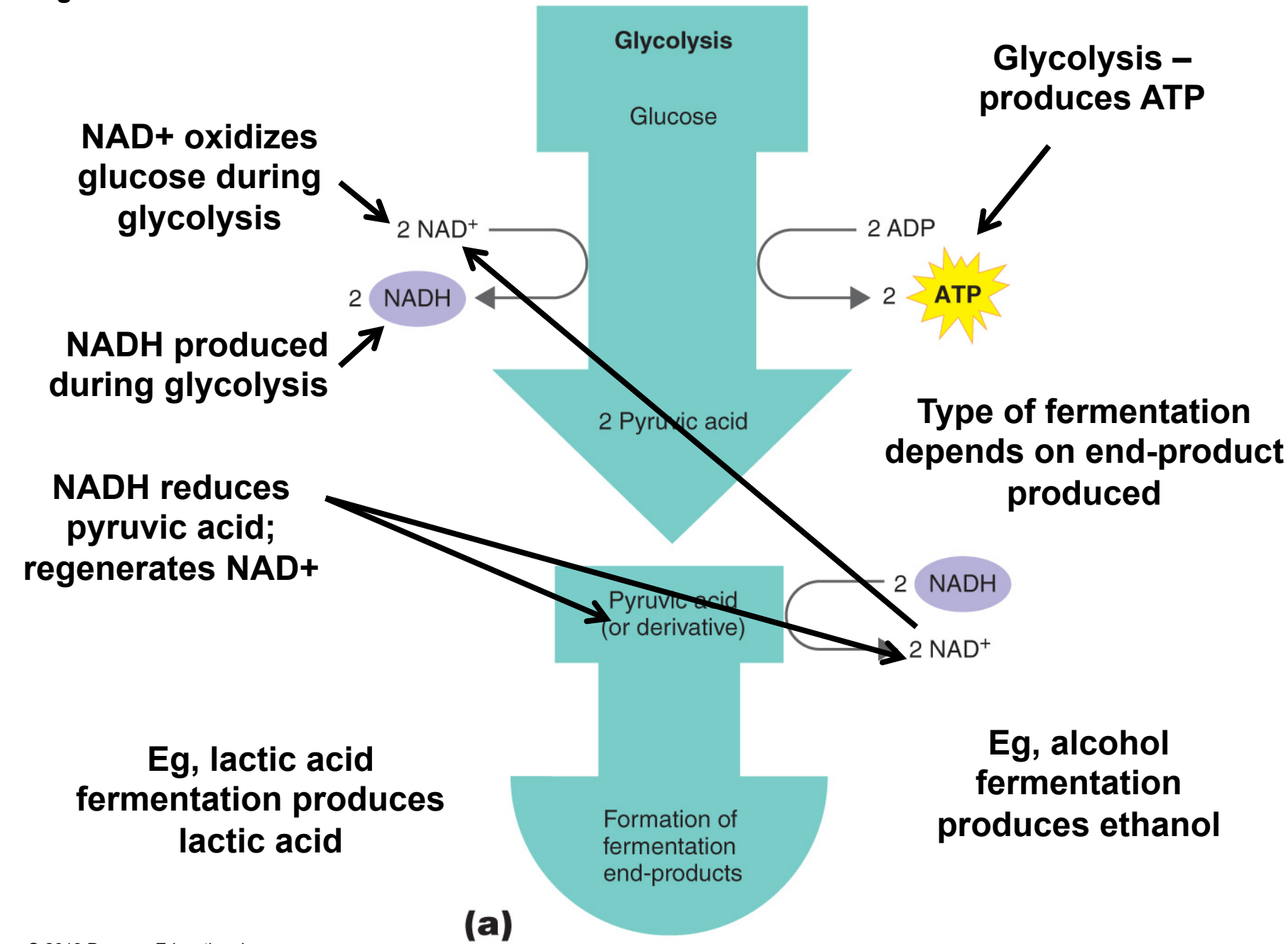
Fermentation

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

Fermentation

- ATP generated only via **glycolysis**
- Pyruvic acid converted into organic molecule end-product → waste
  - Main purpose is to regenerate NAD+ (from NADH) for glycolysis
  - NAD+, can participate in glycolysis again
- Produces only small amount of energy - energy still left in end-product

Figure 5.18a Fermentation.



NAD+ oxidizes glucose during glycolysis

Glycolysis – produces ATP

NADH produced during glycolysis

Type of fermentation depends on end-product produced

NADH reduces pyruvic acid; regenerates NAD+

Eg, lactic acid fermentation produces lactic acid

Eg, alcohol fermentation produces ethanol

Table 5.4 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.

Chemotherapy

- Cyanides → highly toxic
  - Inhibit Cytochrome c Oxidase, enzyme in ETC
- Disrupts ETC
  - Cell cannot use oxygen
  - Cell cannot produce ATP
  - Cell dies
- Strict fermenters not affected by cyanide

Catabolism and You

- Muscle cells have huge ATP demand
  - Only enough for about 3 seconds of activity
- 3 ways muscles create ATP
  - Phosphagen system
  - Glycogen-lactic acid system
  - Aerobic respiration

Phosphagen System

- **Creatine phosphate**
  - Phosphate containing molecule
- **Creatine kinase** can transfer P from Creatine phosphate to ADP → ATP
  - Substrate level phosphorylation
- Fast and easy
- Not efficient
  - About 10 seconds worth
- Useful for sprinters or low repetition/high weight exercise

Glycogen-Lactic Acid System

- Glycogen
  - Glucose polymer → stored in muscle cells
- When necessary, cell hydrolyses glycogen
  - Produces ATP via lactic acid fermentation
- Slower than phosphagen system, but produces more ATP
  - Produces enough ATP for about 90 sec
- Lactic acid build-up causes muscles to burn
  - Have to switch off eventually
- Useful for swimmers, medium distance sprints

Aerobic respiration

- Muscles can use oxygen to burn glucose from:
  - Remaining glycogen in muscles
  - Glucose present in bloodstream
  - Fatty acid reserves
  - Proteins (only in extreme conditions, like starvation)
- Much slower than phosphagen and Glycogen systems
  - But can supply ATP for hours as long as there is glucose and oxygen
- Marathon, endurance activities

Photosynthesis

- Photo: conversion of light energy into chemical energy (ATP)
  - **Light-dependent (light) reactions**
- Synthesis:
  - **Carbon fixation**: fixing carbon into organic molecules
  - **Light-independent (dark) reaction**: Calvin-Benson cycle

Light-dependent (Light) Reactions

- Light energy is absorbed by **chlorophyll**
  - In thylakoids of chloroplasts in eukaryotes
  - In thylakoids of prokaryotes
- Light energizes or "**excites**" electrons in chlorophyll
- Excited electrons are passed on to ETC
  - ATP is generated
- Occurs in two ways:
  - Cyclic (anoxygenic)
  - Noncyclic (oxygenic)

Figure 5.25a Photophosphorylation.

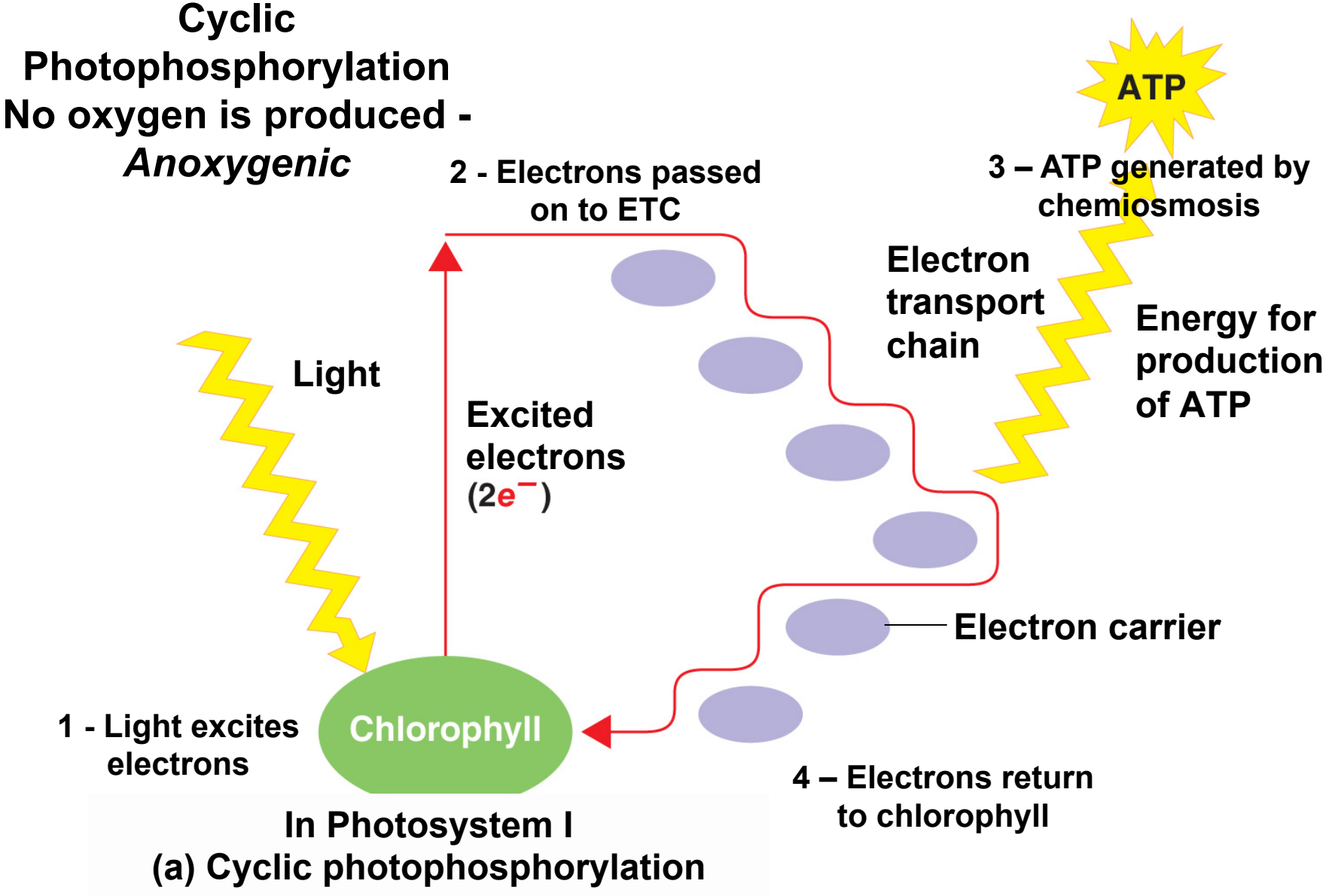
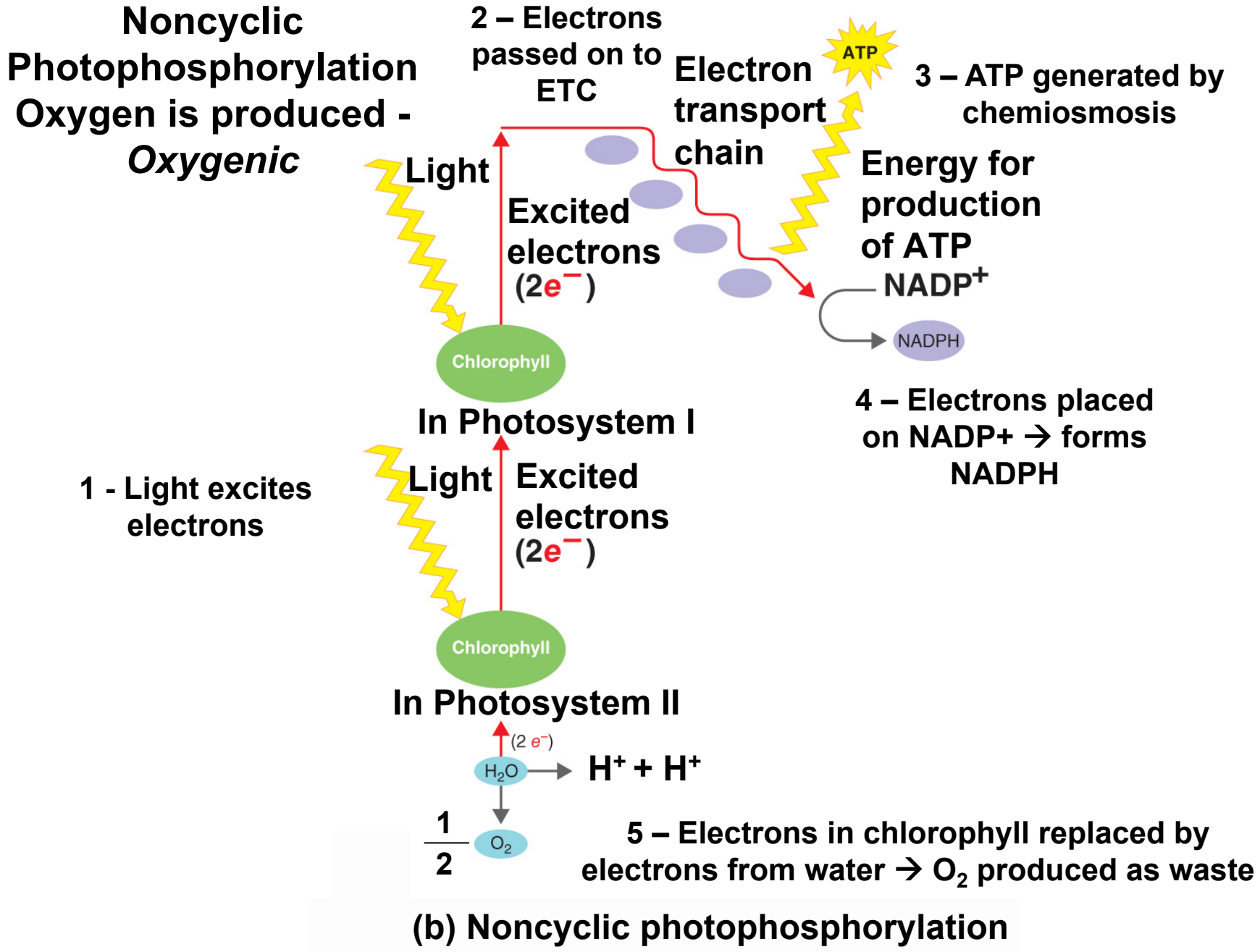


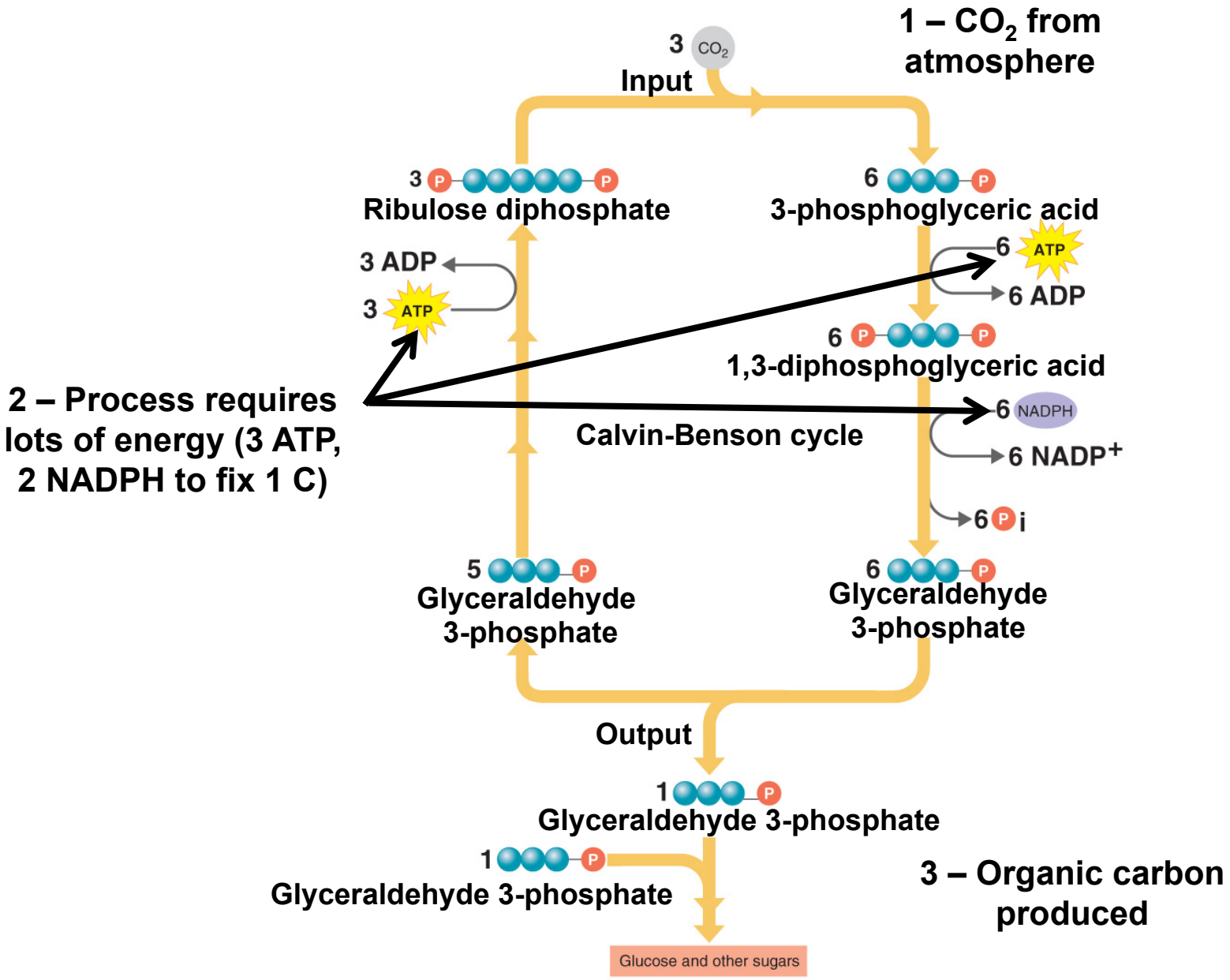
Figure 5.25b Photophosphorylation.



Light-independent (Dark) Reactions

- Aka Calvin-Benson Cycle
- Uses ATP, NADPH produced by noncyclic photophosphorylation to “fix” CO_2

Figure 5.26 A simplified version of the Calvin-Benson cycle.



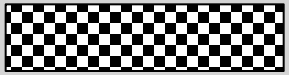
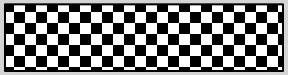
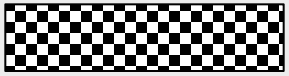
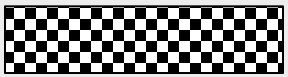
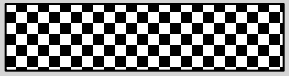
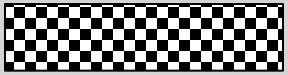
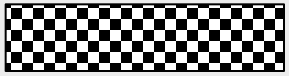
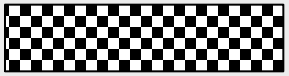
Metabolic Diversity Among Organisms

- Organisms classified according to metabolism
  - Based upon “nutritional pattern”
- Specifically, look at basic requirements to sustain life
  - Source of energy
  - Source of carbon

Metabolic Diversity Among Organisms

- Two classifications based on energy sources
  - Phototrophs – use light as primary source of energy
  - Chemotrophs – use inorganic and organic molecules (chemicals) for energy
- Two classifications based on carbon sources
  - Autotrophs – use carbon dioxide (inorganic)
    - Aka “lithotrophs” (litho = rock)
  - Heterotrophs – use organic carbon source
    - Aka organotroph

Metabolic Diversity Among Organisms

Nutritional Type	Energy Source	Carbon Source
Photoautotroph		
Photoheterotroph		
Chemoautotroph		
Chemoheterotroph		

Chemoheterotrophs

- Heterotrophs classified according to their source of organic molecule
  - Saprophytes – live on dead organic matter
  - Parasites – get nutrients from living hosts
- Metabolic diversity of great interest
  - Can cause problems, provide potential solutions
    - *Rhodococcus erythropolis* can cause disease in humans, animals
    - BUT, can remove sulfur from petroleum