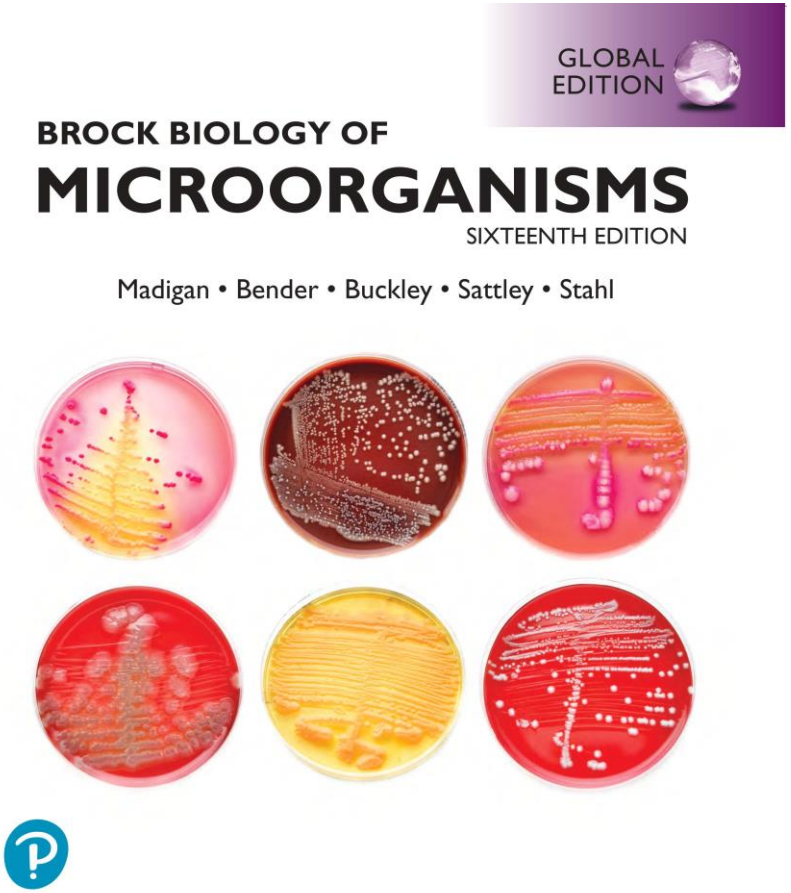
Brock Biology of Microorganisms

Sixteenth Edition, Global Edition



Chapter 3

Microbial Metabolism

I. Fundamentals of Metabolism

3.1 Defining the Requirements for Life

3.2 Electron Transfer Reactions

3.3 Calculating Changes in Free Energy

3.4 Cellular Energy Conservation

3.5 Catalysis and Enzymes

3.1 Defining the Requirements for Life (1 of 9)

- Metabolism: All biochemical reactions needed for life
  - Includes catabolism (to obtain energy) and anabolism (to make cellular material) (Figure 3.1)
  - Relies on **electron donors** directing electrons to **electron acceptors**
- Energy is neither created nor destroyed
  - Cells conserve energy by conversion into a form that can do work
  - Generate **adenosine triphosphate** (ATP) to store energy and fuel processes

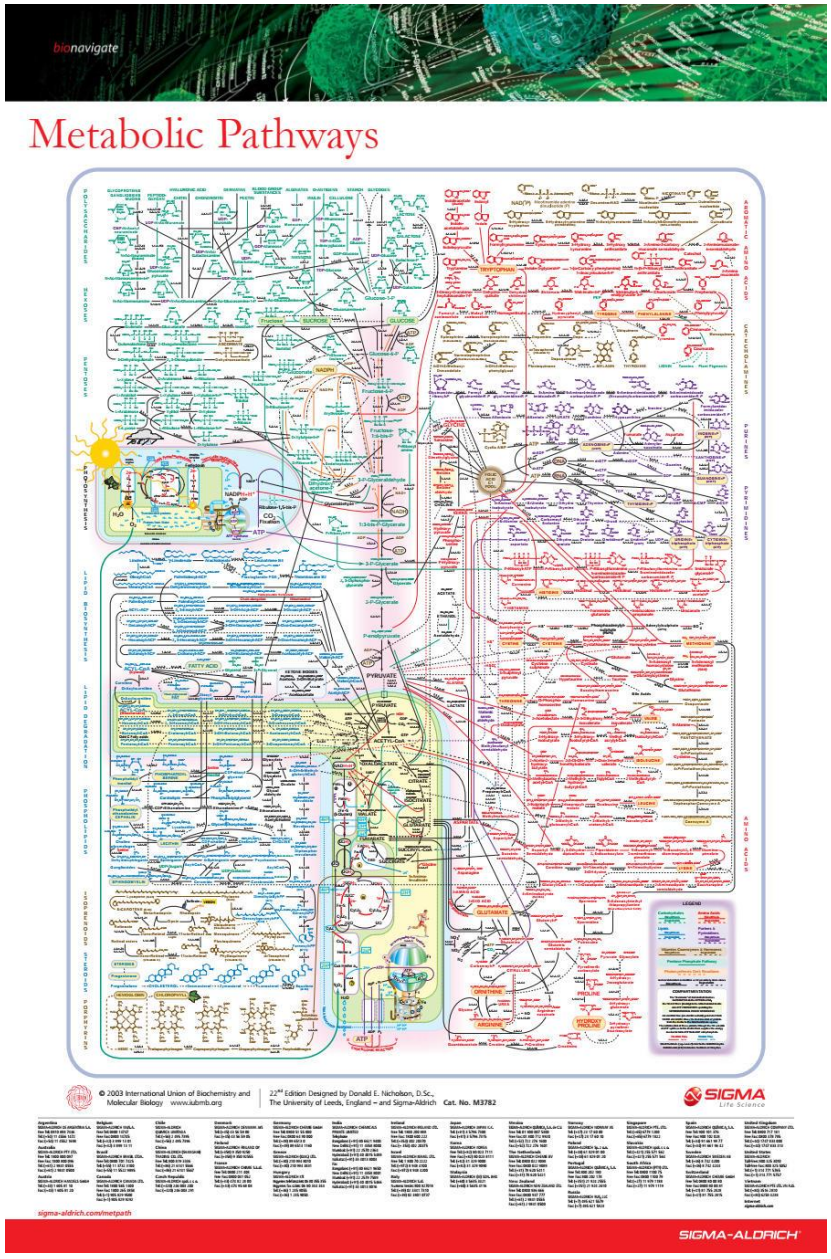
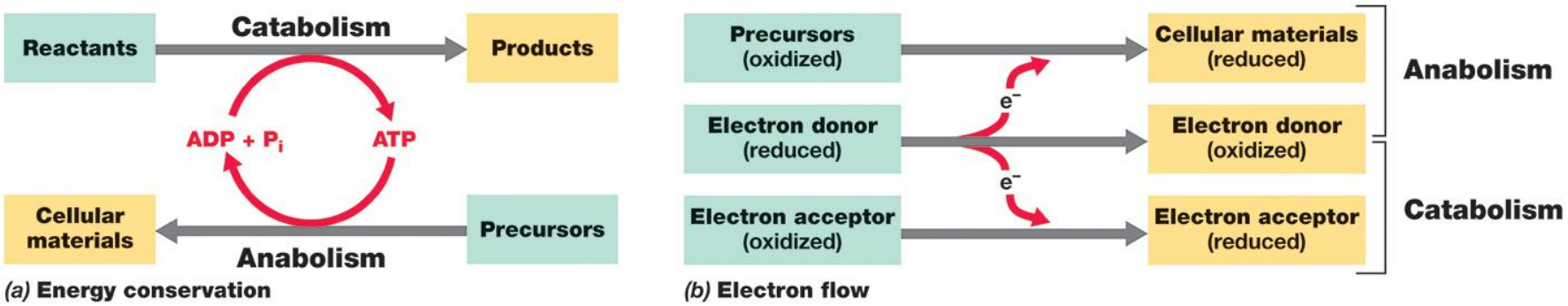


Figure 3.1 Metabolic Coupling with Respect to Energy Conservation and Electron Flow



3.1 Defining the Requirements for Life (2 of 9)

- All cells have certain fundamental metabolic requirements
  - Water
  - Carbon and other nutrients
  - **Free energy**: energy available to do work
  - **Reducing power**: source of electrons

3.1 Defining the Requirements for Life (3 of 9)

- Free Energy
  - Some reactions release energy, others require energy
  - The change in free energy during a reaction is referred to as $\Delta G^{0\prime}$ ("change in" and **standard conditions**: pH 7, 25°C, 1 atmosphere, all reactants and products = 1 M).
  - Measured in kJ/mol (heat energy)
  - **Exergonic:** Reactions with $-\Delta G^{0\prime}$ release free energy.
  - **Endergonic:** Reactions with $+\Delta G^{0\prime}$ require energy.

3.1 Defining the Requirements for Life (4 of 9)

- Free Energy
  - **Catabolic pathways**: exergonic cellular processes that generate free energy
    - Free energy produced is conserved by synthesizing energy-rich molecules, **e.g.,** ATP
    - ATP formation requires $\Delta G^{0\prime} = -31.8$ kJ/mol
    - Aerobic respiration of 1 mole glucose could produce 91 moles ATP under standard conditions, though 38 moles actually produced; some energy lost as heat

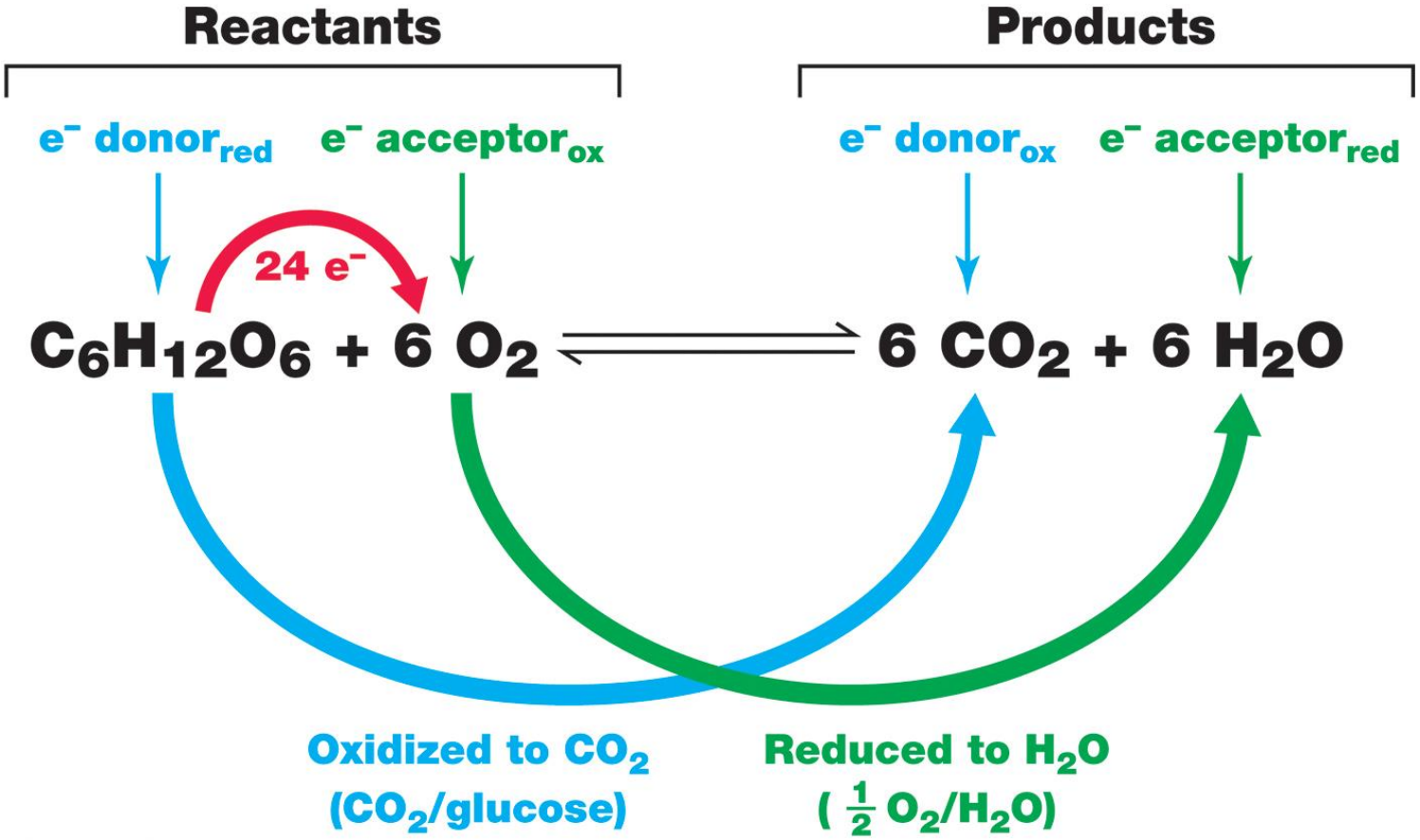
3.1 Defining the Requirements for Life (5 of 9)

- Free Energy
  - **Anabolic pathways**: endergonic cellular processes in which cellular synthesis requires energy
    - Energy comes from hydrolysis of ATP
  - Catabolic and anabolic reactions are fundamentally linked

3.1 Defining the Requirements for Life (6 of 9)

- Reducing Power
  - **Reducing power**: ability to donate electrons during electron transfer reactions (**redox reactions**)
  - Redox reactions include two **half reactions**.
    - **Electron donor:** transfers electrons (**oxidized**)
    - **Electron acceptor:** adds electrons (**reduced***)*
    - **e.g.,** Aerobic respiration of glucose (Figure 3.2)
      - Electron donor: glucose, electron acceptor: O_2

Figure 3.2 Example of an Oxidation–Reduction Reaction



3.1 Defining the Requirements for Life (7 of 9)

- Reducing Power
  - Also required by anabolic reactions
    - Biosynthesis requires free energy (ATP) and reducing power (electron carriers)

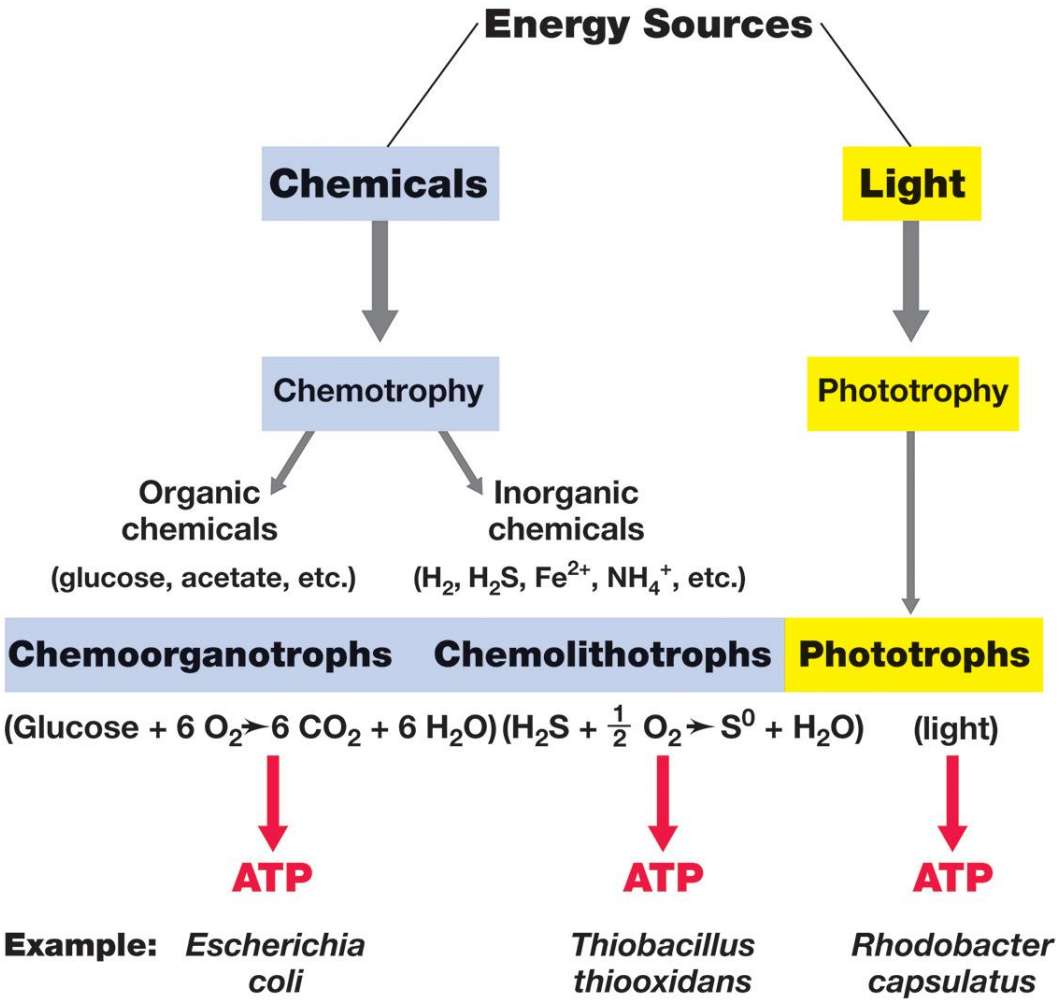
3.1 Defining the Requirements for Life (8 of 9)

- Metabolic Classes of Microorganisms (Figure 3.3)
  - **Phototrophs** obtain energy from light
    - Do not require chemicals as a source of energy
    - **Oxygenic** (O_2 produced) and **anoxygenic** (no O_2 produced) **photosynthesis**
  - **Chemotrophs** obtain energy from chemical reactions
    - **Aerobic** reactions require O_2 as electron acceptor
    - **Anaerobic** reactions use anything other than O_2 as electron acceptor
    - **Respiration** or **fermentation**
    - Energy source can be **organic** (containing carbon with some exceptions) or **inorganic**

3.1 Defining the Requirements for Life (9 of 9)

- Metabolic Classes of Microorganisms (Figure 3.3)
  - **Chemoorganotrophs** obtain energy and reducing power from organics
  - **Chemolithotrophs** obtain energy and reducing power from inorganics
  - **Heterotrophs** obtain carbon from organics
  - **Autotrophs** obtain carbon from CO_2
    - Also called **primary producers**: synthesize organic matter from inorganic carbon

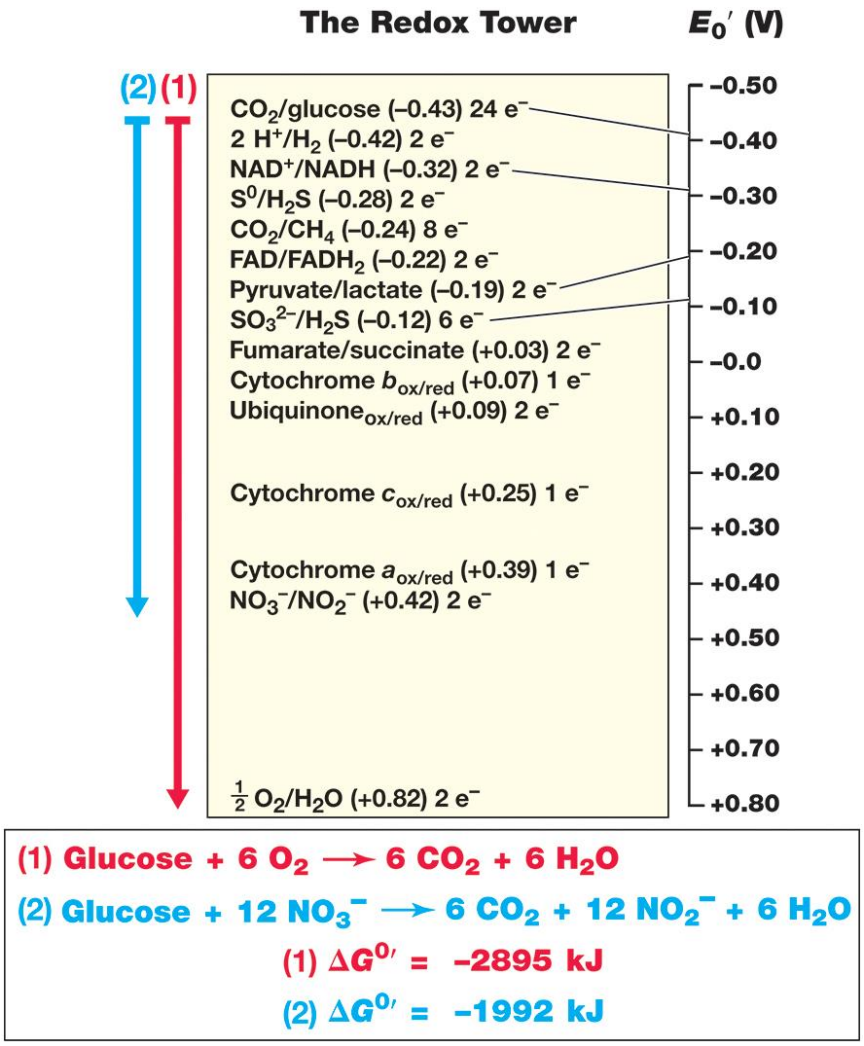
Figure 3.3 Classification of Metabolic Types Based on Energy Sources



3.2 Electron Transfer Reactions (1 of 5)

- Catabolism depends on electron flow from **electron donor** to **electron acceptor**
- **Reduction potential** (Figure 3.4): affinity of substance for electrons
- Redox Reactions and Reduction Potentials
  - Electrons cannot exist in solution
  - Must be transferred directly in redox reactions
  - Redox reactions occur in pairs (**half reactions** or **redox couple**)
  - **Oxidized** form on left, **reduced** form on right (**e.g.,** NO_3^-/NO_2^-)

Figure 3.4 The Redox Tower



3.2 Electron Transfer Reactions (2 of 5)

- Redox Reactions and Reduction Potentials
  - First half reaction produces electrons consumed by second half reaction
  - Oxidized reactant (**electron donor**) donates electrons, reduced reactant (**electron acceptor**) accepts electrons
  - Many redox couples in nature (Figure 3.4 and Table 3.1)
  - **Reduction potential (E_0')***:* tendency to donate electrons
    - expressed as volts (V) compared with reference (typically H_2)
    - Reduced substance of a redox couple has strong tendency to donate when couple has negative reduction potential (**e.g.,** glucose in CO_2/glucose, −0.43 V)
    - Oxidized substance will accept electrons when redox couple has positive reduction potential (**e.g.,** O_2 in ½ O_2/H_2O, +0.82 V)

Table 3.1 Reduction Potentials, E_0' (Volts), of Some Redox Half Reactions Commonly Encountered in Microbiology[a]

Pyruvate/glucose (−0.70) 4 e^-	$Flavodoxin_{ox/red}$ (−0.12) 2 e^-	NO_3^-/NH_4^+ (+0.34) 8 e^-
Acetate/pyruvate (−0.68) 4 e^-	SO_3^{2-}/H_2S (−0.12) 6 e^-	NO_2^-/NO (+0.36) 2 e^-
Acetate/acetaldehyde (−0.60) 2 e^-	$Menaquinone_{ox/red}$ (−0.07) 2 e^-	SeO_4^{2-}/SeO_3^{2-} (+0.48) 2 e^-
SO_4^{2-}/HSO_3^- (−0.52) 2 e^-	APS/AMP + HSO_3^- (−0.06) 2 e^-	Tetrachloroethene/trichloroethylene + Cl^- (+0.58) 2 e^-
$Ferredoxin_{ox/red}$ (−0.42) 2 e^-	Rubredoxin ox/red (−0.06) 1 e^-	$NO_3^-/\frac{1}{2}N_2$ (+0.74) 5 e^-
CO_2/methanol (−0.38) 6 e^-	Acrylyl-CoA/proprionyl-CoA (−0.02) 2 e^-	Fe^{3+}/Fe^{2+} (+0.77) 1 e^- (at pH 2)
$Coenzyme\ F420_{ox/red}$ (−0.36) 2 e^-	Fe^{3+}/Fe^{2+} (+0.20) 1 e^- (at pH 7)	Mn^{4+}/Mn^{2+} (+0.80) 2 e^-
CO_2/acetate (−0.28) 8 e^-	TMAO/TMA (+0.13) 2 e^-	ClO_3^-/Cl^- (+1.03) 4 e^-
$Methanophenazine_{ox/red}$ (−0.26) 2 e^-	AsO_4^{3-}/AsO_3^{3-} (+0.14) 2 e^-	NO/N_2O (+1.18) 1 e^-
FMN/$FMNH_2$ (−0.22) 2 e^-	DMSO/DMS (+0.23) 2 e^-	N_2O/N_2 (+1.36) 1 e^-
Acetaldehyde/ethanol (−0.20) 2 e^-	Chlorobenzoate/benzoate + Cl^- (+0.30) 2 e^-	

[a]Entries are read as: oxidized form/reduced form (E_0' in volts) and number of e^- transferred per half reaction. Values are organized from lowest (most electronegative) to highest (most electropositive) reduction potential.

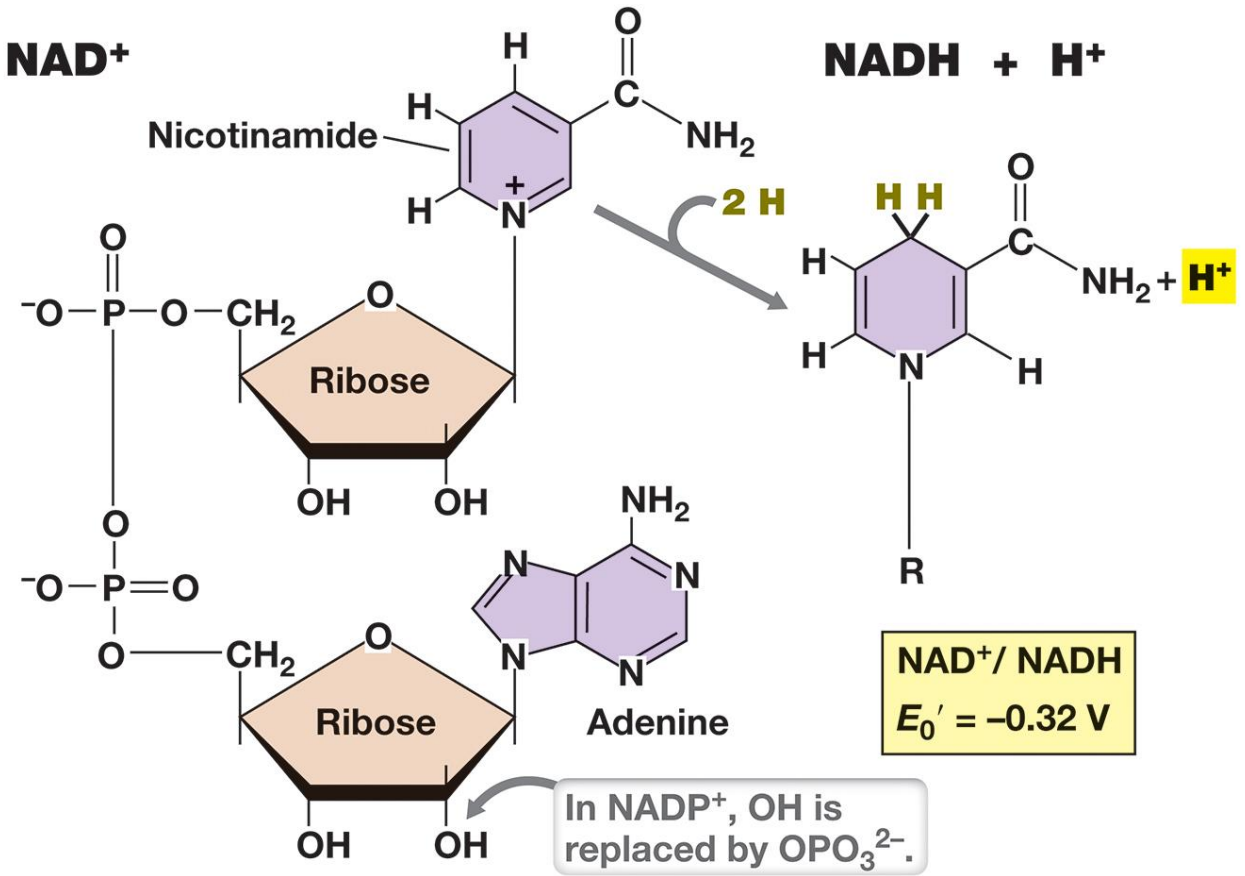
3.2 Electron Transfer Reactions (3 of 5)

- Redox Reactions and Reduction Potentials
  - Half reactions written as reductions
  - When two half reactions are combined, the donor half reaction is an oxidation; orientation is written in reverse overall
  - Reduction potentials can be used to determine whether any given substance will serve as donor or acceptor
  - The greater the difference in reduction potentials of two half reactions, the more energy will be available

3.2 Electron Transfer Reactions (4 of 5)

- Electron Carriers and NAD^+/NADH Cycling
  - Typically, electron movement proceeds through consecutive reactions
  - Soluble electron carriers such as nicotinamide adenine dinucleotide (NAD^+/NADH) needed to carry electrons (Figure 3.5)
    - NAD^+/NADH redox couple = −0.32 V; NADH good electron donor, NAD^+ weak electron acceptor
    - Reduction requires 2 e^- and 1 H^+
    - Reduction of NAD^+ results in NADH + H^+

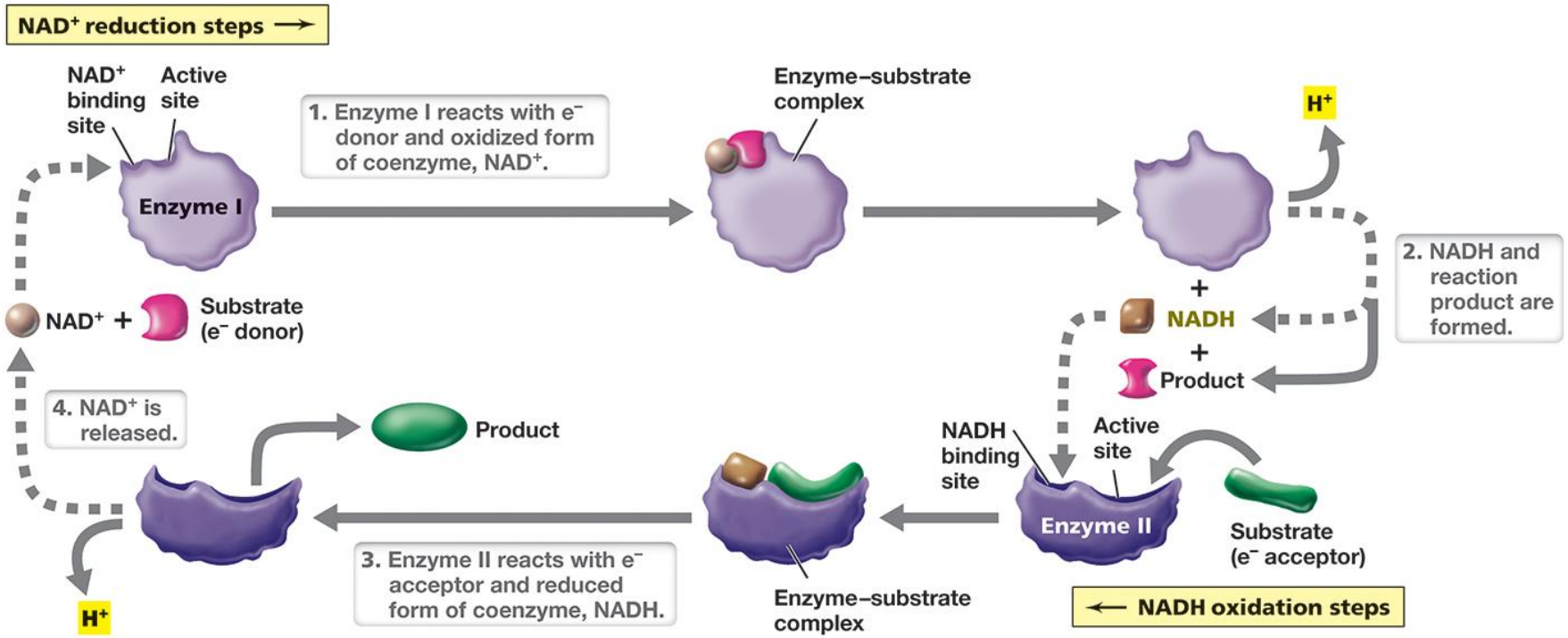
Figure 3.5 The Redox Coenzymes Nicotinamide Adenine Dinucleotide (NAD^+) and $NADP^+$



3.2 Electron Transfer Reactions (5 of 5)

- Electron Carriers and NAD^+/NADH Cycling
  - NAD^+/NADH are **coenzymes**
    - Allow many different electron donors and acceptors to interact (Figure 3.6)
  - Many other molecules are electron shuttles, **e.g.,** nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH) in anabolic biosynthetic reactions

Figure 3.6 NAD^+/NADH Cycling



3.3 Calculating Changes in Free Energy (1 of 4)

- The Redox Tower and Its Relationship to $\Delta G^{0\prime}$
  - The **redox tower** represents the range of possible reduction potentials with the most negative potential at top, most positive potential at bottom (Figure 3.4)
  - The further the electrons "drop," the greater the amount of energy released ($\Delta E_0'$ = change in reduction potential) (Table 3.2)
    - $\Delta G^0 = -nF\Delta E_0'$
    - n = # electrons transferred, F Faraday constant

Table 3.2 Example of Free-Energy-Change Calculations Using Electrochemical Potentials or G_f^0 Values

For the reaction in which acetate is oxidized completely to CO_2:[a]

$$CH_3COO^- + H^+ + 2\,O_2 \rightarrow 2\,CO_2 + 2\,H_2O$$

1. Calculation from E_0' values:[b]

$$\begin{aligned}\Delta G^{0\prime} &= -nF\Delta E_0' \\ &= [-8(96.5)(1.1)] \\ &= -849 \text{ kJ/reaction}\end{aligned}$$

2. Calculation from G_f^0 values:

$$\begin{aligned}\Delta G^{0\prime} &= [G_f^0 \text{(products)} - G_f^0 \text{(reactants)}] \\ &= [G_f^0 (2\,CO_2 + 2\,H_2O) - G_f^0 (CH_3COO^- + H^+ + 2\,O_2)] \\ &= -852 \text{ kJ/reaction}\end{aligned}$$

[a]The reaction is balanced and is an 8-electron oxidation (n = 8 in equation 2). G_f^0 values were taken from Table 3.3.

[b]F is the Faraday constant (96.5 kj/V) and $\Delta E_0'$ is calculated from the E_0' values in Figure 3.4 and Table 3.1.

3.3 Calculating Changes in Free Energy (2 of 4)

- Calculating $\Delta G^{0\prime}$ from the Free Energy of Formation
  - Can calculate $\Delta G^{0\prime}$ if **free energy of formation** (G_f^0; energy released or required during formation of a given molecule from its elements) of reactants and products known (Table 3.3)

Table 3.3 Free Energy of Formation (G_f^0, kJ/Mol) for Some Common Substances[a]

Sugars	Organic and fatty acids		Amino acids and alcohols	Gases and inorganic compounds
Fructose (−951.4)	Acetate (−369.4)	Ketoglutarate (−797.5)	Alanine (−371.5)	O_2, N_2, H_2, S^0, Fe^0 (0)
Glucose (−917.2)	Benzoate (−245.6)	Lactate (−517.8)	Aspartate (−700.4)	CH_4 (−50.8)
Lactose (−1515.2)	Butyrate (−352.6)	Malate (−845.1)	*n*-Butanol (−171.8)	CO_2 (−394.4); CO (−137.4)
Ribose (−369.4)	Caproate (−335.9)	Propionate (−361.1)	Ethanol (−181.7)	H_2O (−237.2); H^+ (−39.8); OH^- (−198.7)
Sucrose (−757.3)	Citrate (−1168.3)	Pyruvate (−474.6)	Glutamate (−699.6)	N_2O (+104.2); NO (+86.6)
	Formate (−351.1)	Succinate (−690.2)	Glutamine (−529.7)	NO_2^- (−37.2); NO_3^- (−111.3)
	Fumarate (−604.2)	Valerate (−344.3)	Glycerol (−488.5)	NH_3 (−26.57); NH_4^+ (−79.4)
	Glyoxylate (−468.6)		Mannitol (−942.6)	H_2S (−27.87); HS^- (+12.1)
			Methanol (−175.4)	SO_4^{2-} (−744.6); $S_2O_3^{2-}$ (−513.4)
			n-Propanol (−175.8)	Fe^{2+} (−78.8); Fe^{3+} (−4.6); FeS (−100.4)

[a]Values for free energy of formation taken from Speight, J. 2005. **Lange's Handbook of Chemistry**, 16th edition, and Thauer, R.K., K. Jungermann, and H. Decker. 1977. Energy conservation in anaerobic chemotrophic bacteria. **Bacteriol. Rev. 41:** 100–180.

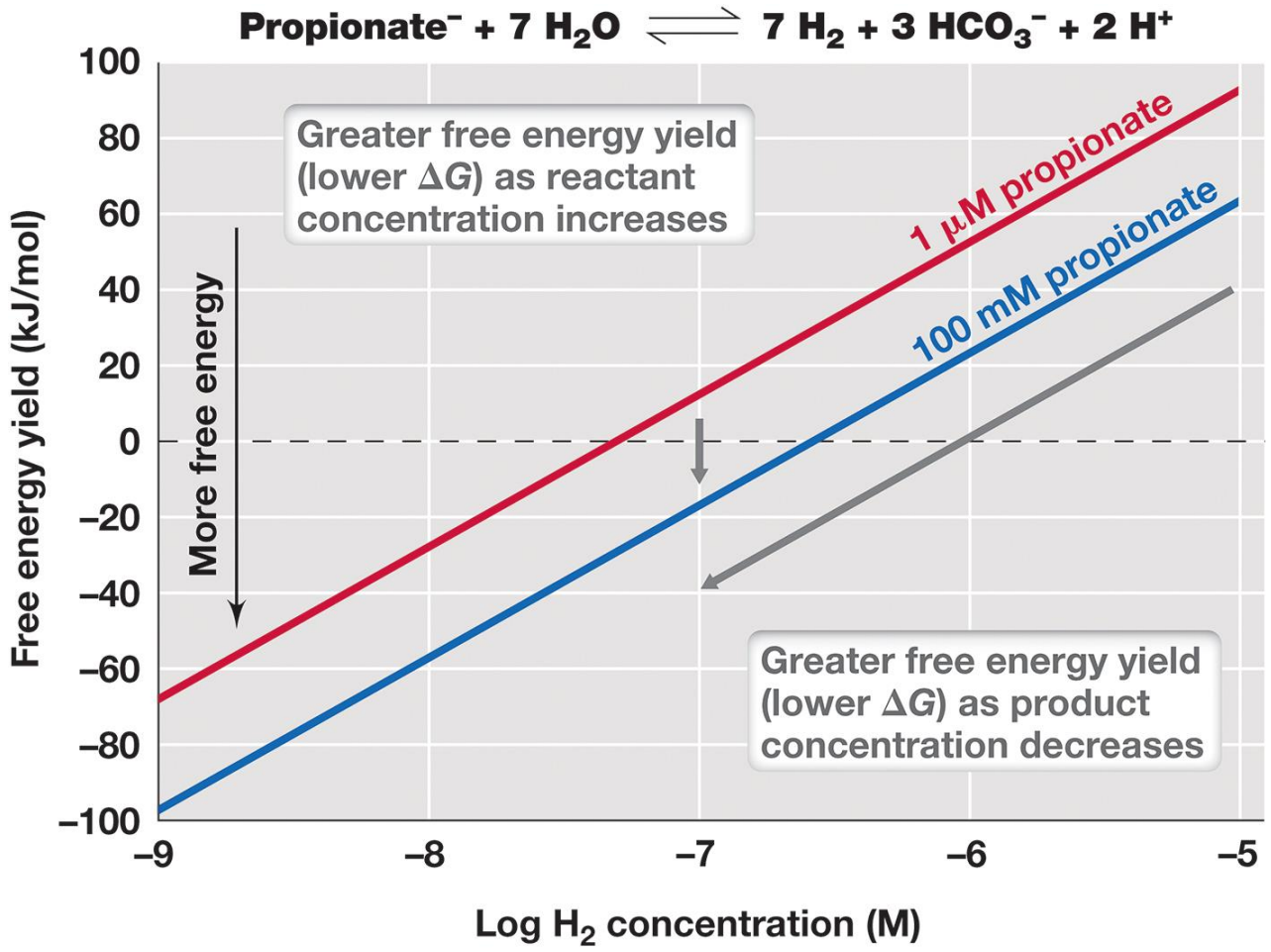
3.3 Calculating Changes in Free Energy (3 of 4)

- Calculating $\Delta G^{0\prime}$ from the Free Energy of Formation
  - For the reaction $A + B \leftrightarrow C + D$,
    - $\Delta G^{0\prime} = G_f^{\,0}[C + D] - G_f^{\,0}[A + B]$
  - $\Delta G^{0\prime}$ tells whether reaction is exergonic or endergonic

3.3 Calculating Changes in Free Energy (4 of 4)

- Calculating Free-Energy Change in Natural Condtions
  - $\Delta G^{0\prime}$ is not always a good estimate of actual free energy changes (Figure 3.7)
    - Actual concentrations of products and reactants are almost never at molar concentrations
    - $\Delta G^{0\prime}$ may misrepresent actual available energy in cells
  - More relevant ΔG: free energy under actual conditions
    - $\Delta G = \Delta G^{0\prime} + RT \ln K_{eq}$
    - R and T are physical constants and K_{eq} is the equilibrium constant for the reaction
  - Key: Only exergonic reactions yield energy that can be conserved by the cell.

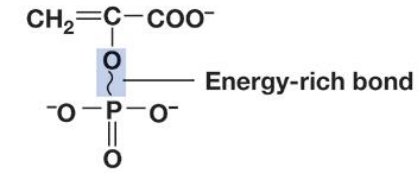
Figure 3.7 Calculating Free Energy Under Natural Conditions (ΔG)



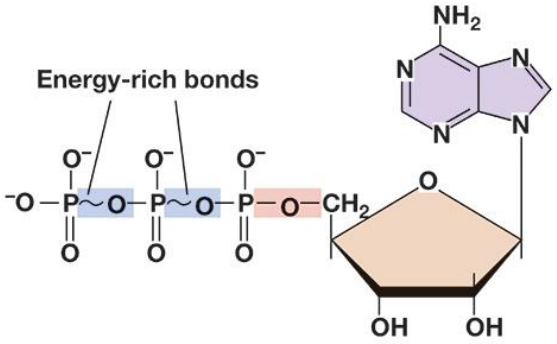
3.4 Cellular Energy Conservation (1 of 3)

- Adenosine Triphosphate (ATP)
  - Most important energy-rich phosphate compound (Figure 3.8)
  - Two high energy phosphate bonds

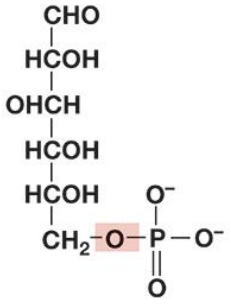
Figure 3.8 Energy-Rich Bonds in Compounds That Conserve Energy in Microbial Metabolism



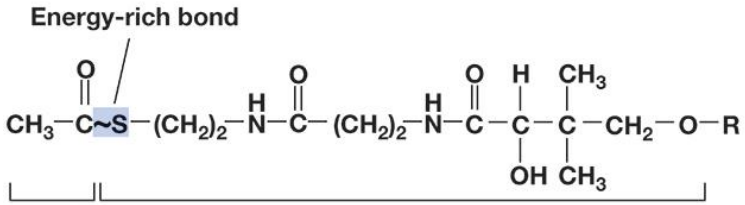
Phosphoenolpyruvate



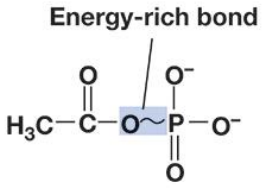
Adenosine triphosphate (ATP)



Glucose 6-phosphate



Acetyl-CoA



Acetyl phosphate

Compound	$G^{0'}$ kJ/mol
$\Delta G^{0'}$ > 30 kJ	
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Acetyl-CoA	−35.7
$\Delta G^{0'}$ < 30 kJ	
AMP	−14.2
Glucose 6-phosphate	−13.8

Pearson

3.4 Cellular Energy Conservation (2 of 3)

- Energy-Rich Compounds
  - Several others have energy-rich phosphate or sulfur bonds
    - Not all phosphate bonds are energy-rich
    - Cells need compounds where $\Delta G^{0\prime\prime} < -31.8$ kJ/mol to synthesize ATP
  - **Coenzyme A** derivatives have energy-rich thioester bonds

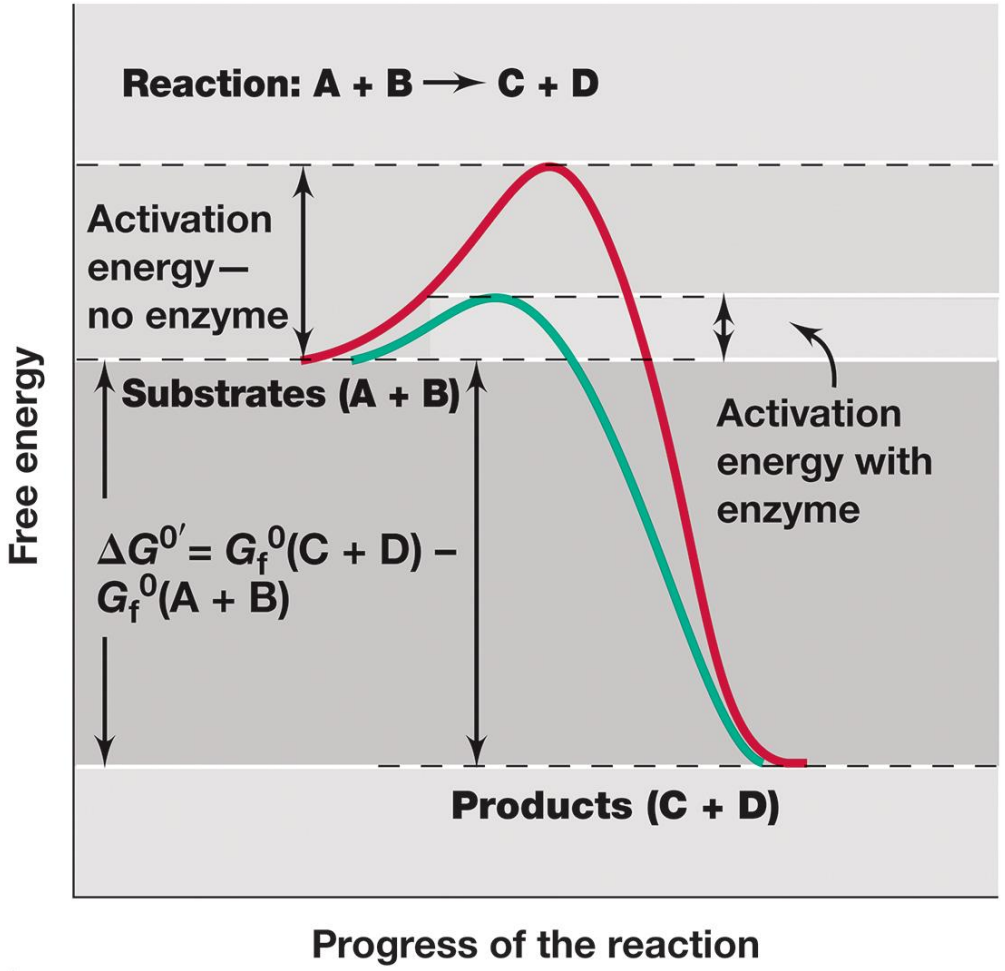
3.4 Cellular Energy Conservation (3 of 3)

- Mechanisms of Energy Conservation
  - ATP generated through 1 of 3 mechanisms
    - **Substrate-level phosphorylation**: energy-rich substrate bond hydrolyzed directly to drive ATP formation (**e.g.,** hydrolysis of phosphoenolpyruvate)
    - **Oxidative phosphorylation**: Movement of electrons generates **proton motive force** (electrochemical gradient) used to synthesize ATP
    - **Photophosphorylation**: light used to form proton motive force

3.5 Catalysis and Enzymes (1 of 4)

- Free energy calculations do not provide information on reaction rates.
- **Activation energy***:* minimum energy required for chemical reaction to begin (Figure 3.9)
  - A **catalyst** (facilitates reaction without being consumed) is usually required to overcome activation energy barrier.

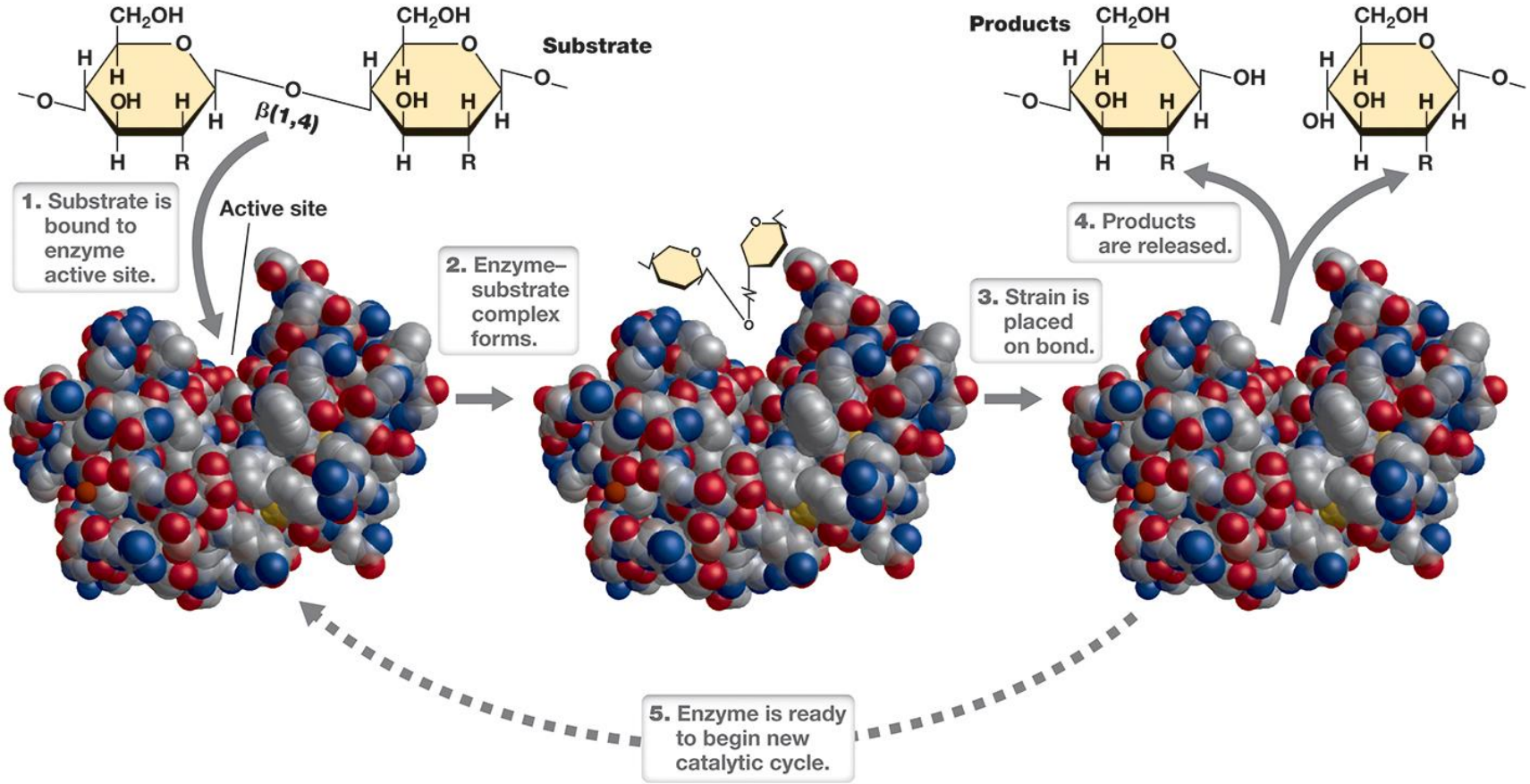
Figure 3.9 Activation Energy and Catalysis



3.5 Catalysis and Enzymes (2 of 4)

- Enzymes
  - Catalysts lower activation energy, increasing reaction rate
  - **Enzymes**: major cellular catalysts
    - typically proteins (some RNAs)
    - highly specific as a result of structure
    - in reaction, enzyme combines with reactant (**substrate**), forming **enzyme-substrate complex**, releasing **product** and enzyme (Figure 3.10)
    - active site: region of enzyme that binds substrate

Figure 3.10 The Catalytic Cycle of an Enzyme



3.5 Catalysis and Enzymes (3 of 4)

- Enzymes
  - Many contain small nonprotein, nonsubstrate molecules that participate in catalysis.
    - **Prosthetic groups**
      - tightly bound, usually covalently and permanently (**e.g.,** heme in cytochromes)
    - **Coenzymes**
      - Loosely, transiently bound
      - Most are derivatives of vitamins. (Table 3.1)

3.5 Catalysis and Enzymes (4 of 4)

- Enzyme Catalysis
  - Binding and proper positioning of substrate needed for catalysis
  - Enzyme-substrate complex aligns reactive groups and strains specific bonds, reducing activation energy
  - To catalyze endergonic reactions, **coupling** used between endergonic and exergonic reactions
    - Overall reaction has negative or zero free-energy change
  - All enzymes theoretically reversible, but highly exergonic or endergonic reaction catalysis usually goes in one direction
    - Another enzyme needed for reverse reaction

II. Catabolism: Chemoorganotrophs

3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle

3.7 Principles of Fermentation

3.8 Principles of Respiration: Electron Carriers

3.9 Principles of Respiration: Generating a Proton Motive Force

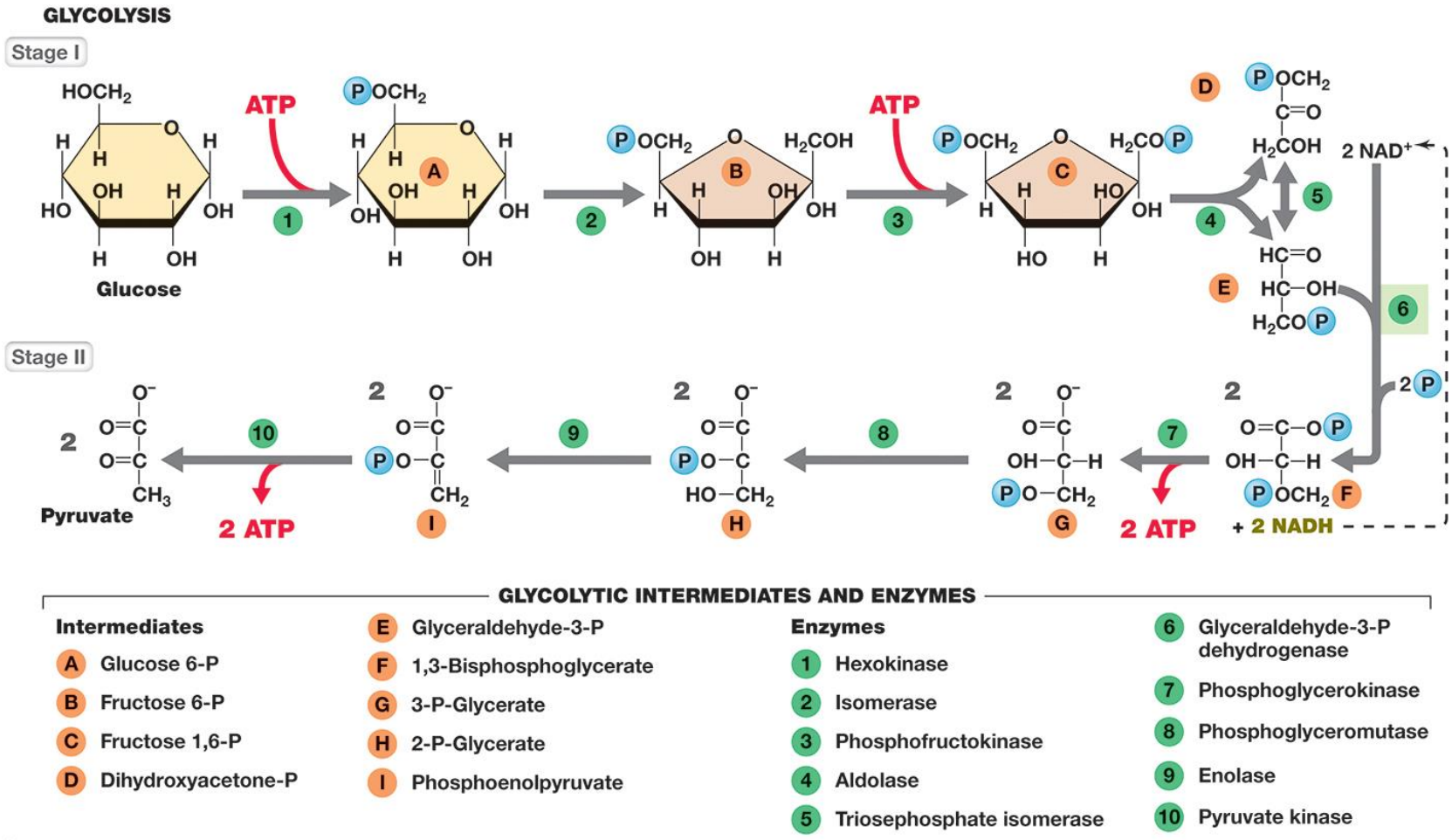
3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle (1 of 8)

- Glycolysis **(Embden–Meyerhof–Parnas pathway)**
  - Nearly universal pathway for glucose catabolism that oxidizes glucose to pyruvate
  - Can participate in multiple forms of catabolism (fermentation, aerobic respiration, anaerobic respiration)

3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle (2 of 8)

- Glycolysis
  - Two stages
    - Stage I: “preparatory,” form key intermediate
    - Stage II: redox, energy conserved, 2 pyruvate formed (Figure 3.11)

Figure 3.11 Embden–Meyerhof–Parnas Pathway (Glycolysis)



3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle (3 of 8)

- Glycolysis
  - Glucose phosphorylated to glucose-6-phosphate
  - Glucose-6-phosphate isomerized to fructose-6-phosphate
  - Phosphorylation produces fructose-1,6-bisphosphate
  - Aldolase splits fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate
  - Dihydroxyacetone phosphate isomerized to second glyceraldehyde-3-phosphate

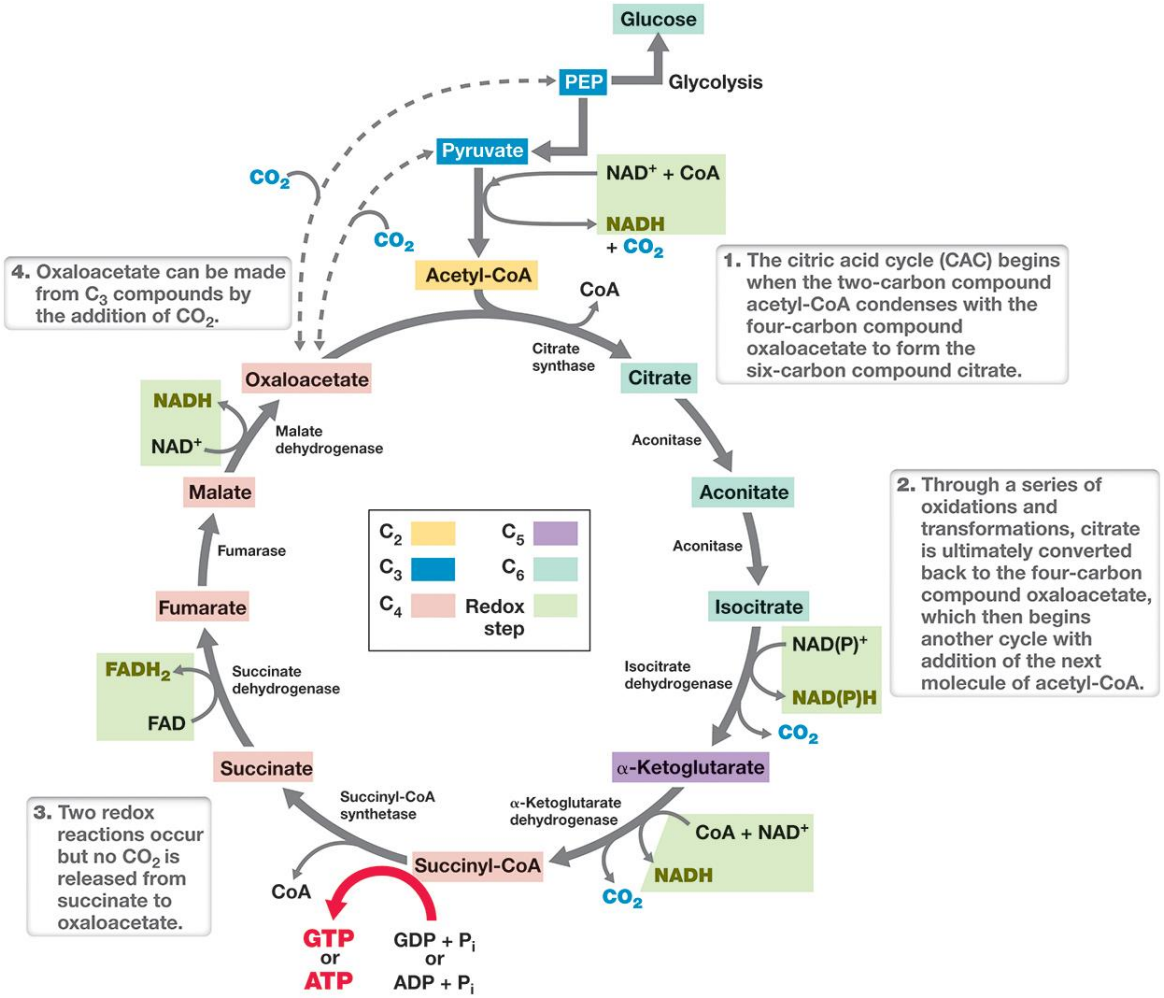
3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle (4 of 8)

- Glycolysis
  - Glyceraldehyde-3-phosphate oxidized to 1,3-bisphosphoglycerate (2x), reducing NAD^+ to NADH
  - ATP synthesized by substrate-level phosphorylation when 1,3-bisphosphoglycerate converted to 3-phosphoglycerate (2x)
  - Later, phosphoenolpyruvate converted to pyruvate (x2)
  - Glycolysis uses 2 ATP in stage I and produces 4 in stage II; net is 2 ATP, 2 NADH, 2 pyruvate per glucose
  - Redox balance requires pyruvate reduction or respiration

3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle (5 of 8)

- The Citric Acid Cycle (CAC)
  - Pathway by which pyruvate is oxidized to CO_2 (Figure 3.12)
  - Pyruvate from glycolysis is decarboxylated, producing CO_2, NADH, energy rich **acetyl-coA** (which enters CAC)
  - Acetyl-CoA + intermediate oxaloacetate forms citric acid.
  - 2 CO_2, 3 NADH, 1 $FADH_2$, 1 ATP formed per oxidized pyruvate
  - oxaloacetate regenerated
  - Redox balance achieved through respiration

Figure 3.12 The Citric Acid Cycle



3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle (6 of 8)

- Biosynthesis and the Citric Acid Cycle
  - α-Ketoglutarate **and** oxaloacetate*:* precursors of several amino acids; OAA also converted if necessary to phosphoenolpyruvate, a precursor of glucose
  - succinyl-CoA*:* required for synthesis of cytochromes, chlorophyll, and related molecules
  - acetate*:* necessary for fatty acid biosynthesis
  - Like glycolysis, CAC oxidizes organics and biosynthesizes key intermediates

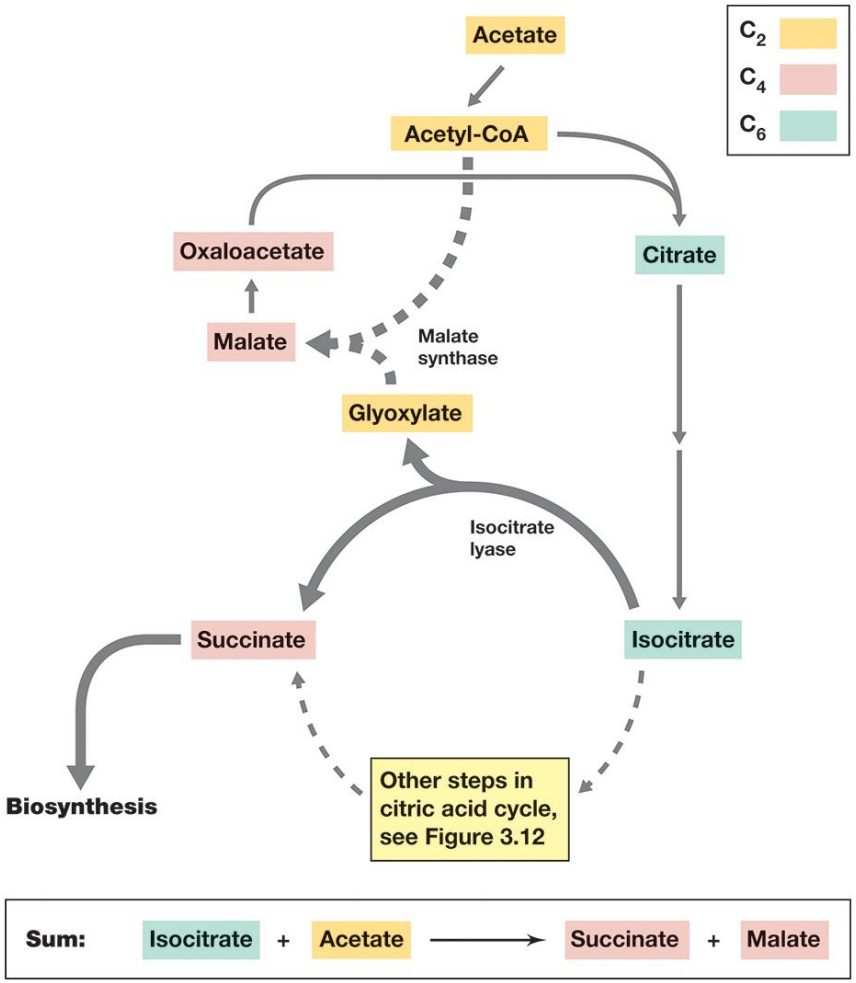
3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle (7 of 8)

- Other Pathways for Chemoorganotrophy
  - Glycolysis and the CAC can oxidize several C_4-C_6 compounds (**e.g.,** glucose, citrate, malate, fumarate, succinate)
  - Unrelated catabolic pathways can be linked for oxidation (**e.g.,** isomerization)

3.6 Glycolysis, the Citric Acid Cycle, and the Glyoxylate Cycle (8 of 8)

- Other Pathways for Chemoorganotrophy
  - Some C_2 (**e.g.,** acetate) compounds catabolized through **glyoxylate cycle** (Figure 3.13)
    - Includes several CAC enzymes + isocitrate lyase and malate synthase
  - C_3 compounds are carboxylated by pyruvate carboxylase or phosphoenolpyruvate carboxylase; glyoxylate cycle unnecessary

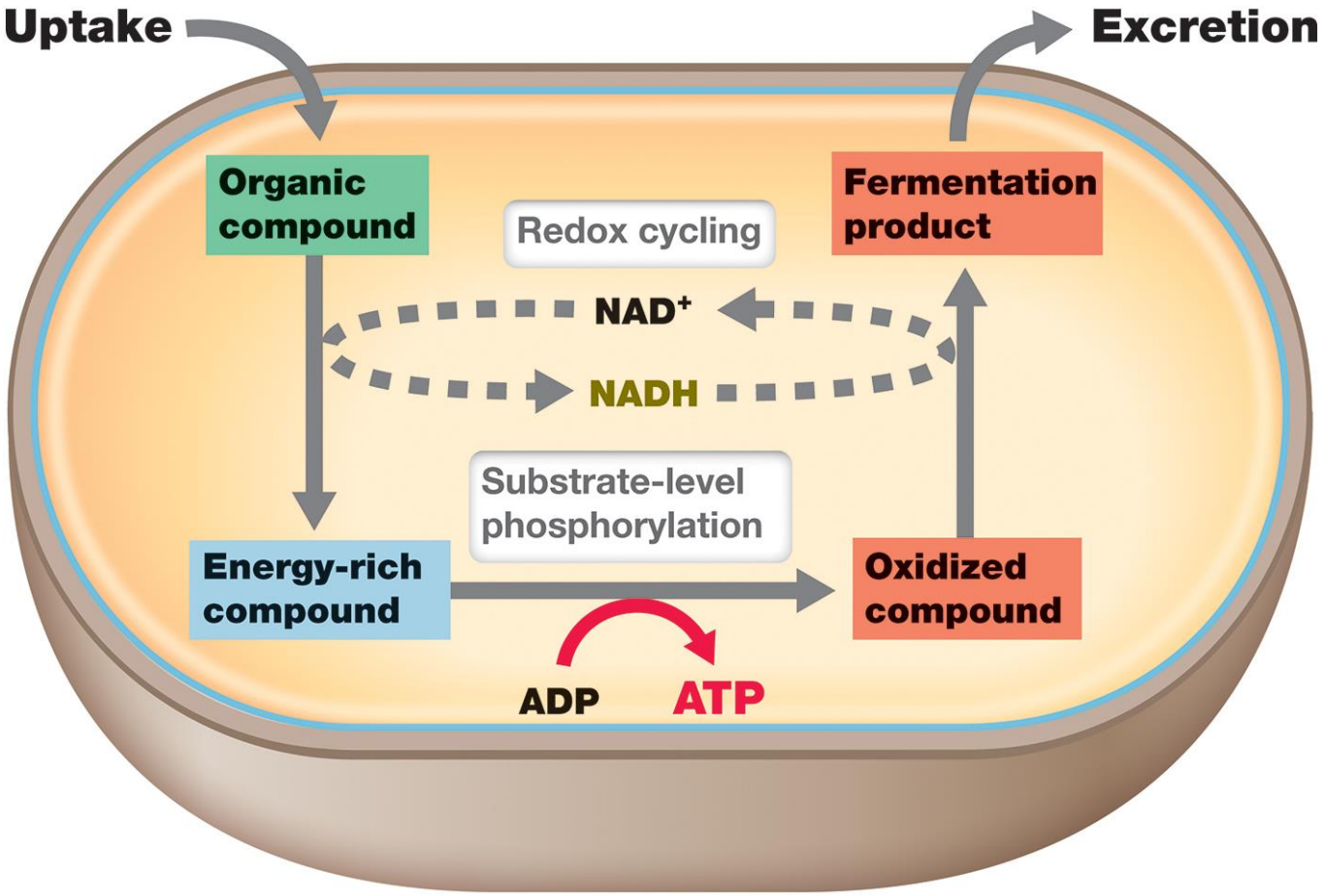
Figure 3.13 The Glyoxylate Cycle



3.7 Principles of Fermentation (1 of 4)

- Fermentation of glucose involves substrate-level phosphorylation and redox balance via pyruvate reduction + excretion as waste (Figure 3.14)
- Many fermentation products are useful for humans (**e.g.,** beer and wine, yogurt, cheese, effect of microbiome on health)

Figure 3.14 The Essentials of Fermentation



3.7 Principles of Fermentation (2 of 4)

- Energy Conservation and Redox Balance in Fermentation
  - All fermentations must do two things:
    - Conserve energy
    - Redox balance
  - Need to produce compounds containing high-energy bonds for ATP synthesis
  - Must oxidize NADH back to NAD^+
    - Donate electrons back to an electron acceptor derived from original organic donor

3.7 Principles of Fermentation (3 of 4)

- Energy Conservation and Redox Balance in Fermentation
  - **e.g.,** Yeast ferments glucose to 2 ethanol + 2 CO_2
    - ATP from glycolysis
    - NAD^+ regenerated by donating electrons from NADH to pyruvate (electron acceptor), producing ethanol and CO_2
  - **e.g.,** Lactic acid bacteria ferment glucose to 2 lactic acid
    - Different enzymes reduce pyruvate to lactic acid
    - Important in fermenting food and human health

3.7 Principles of Fermentation (4 of 4)

- Fermentative Diversity
  - Tremendous diversity: sugars, amino acids, fatty acids, purines, pyrimidines, aromatics
  - Acetate, other volatile fatty acids are products
    - Allow additional ATP synthesis from substrate-level phosphorylation
    - Involves coenzyme-A derivatives (**e.g., Clostridium butyricum**)
  - Redox balance can be improved by producing H_2 (low reduction potential)
    - Associated with ferredoxin and hydrogenase

3.8 Principles of Respiration: Electron Carriers (1 of 6)

- **Respiration**: electrons transferred from reduced electron donors to external electron acceptors (**e.g.,** O_2)
- NADH and $FADH_2$ produced in glycolysis and citric acid cycle must be reoxidized for redox balance
  - In respiration, reoxidation occurs during **electron transport**
    - Occurs in cytoplasmic membrane
    - Forms electrochemical gradient (usually protons) that conserves energy through ATP synthesis

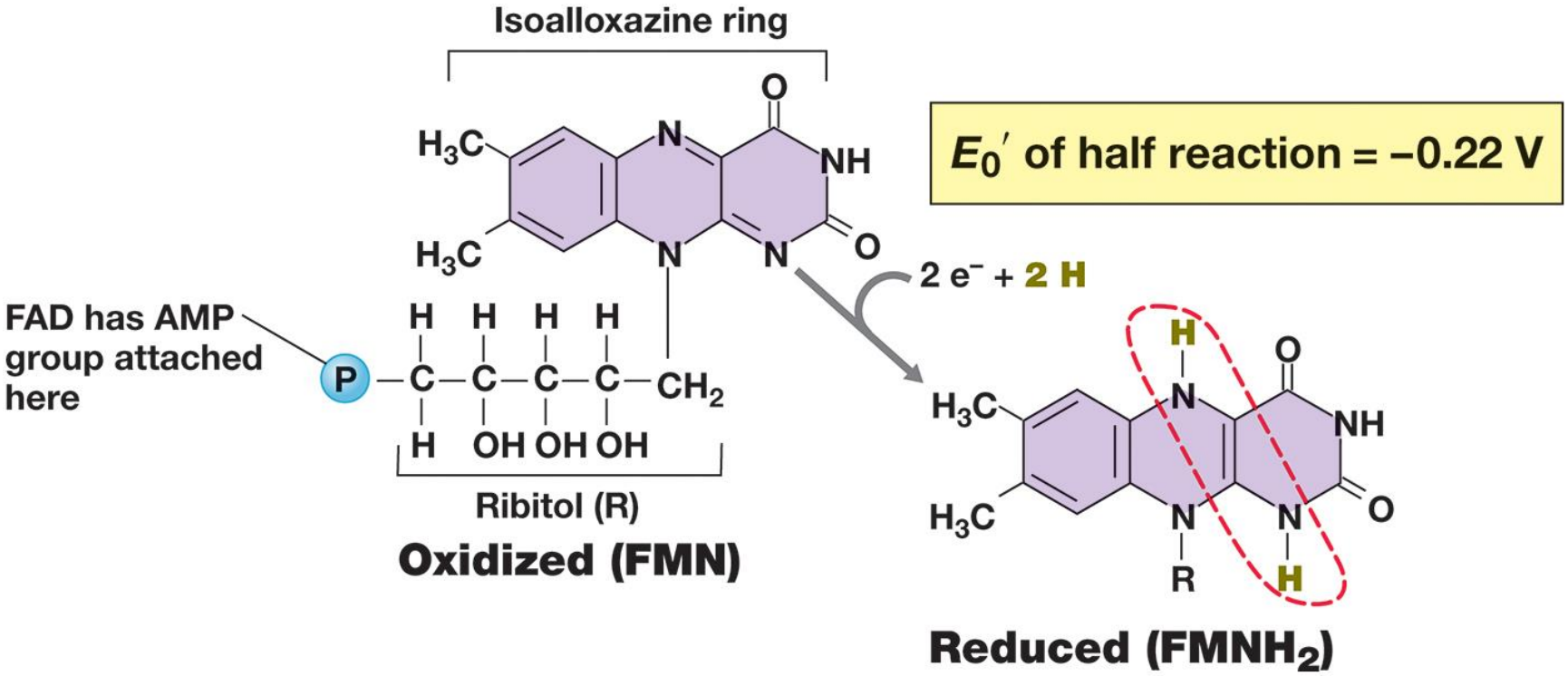
3.8 Principles of Respiration: Electron Carriers (2 of 6)

- NADH Dehydrogenases and Flavoproteins
  - Electron transport occurs in membranes
  - Many oxidation–reduction enzymes (**e.g.,** NADH dehydrogenases, flavoproteins, iron–sulfur proteins, cytochromes)
  - Also **quinones***:* nonprotein electron carriers
  - Arranged with **increasingly more positive** reduction potential
    - NADH dehydrogenase 1st, cytochromes last

3.8 Principles of Respiration: Electron Carriers (3 of 6)

- NADH Dehydrogenases and Flavoproteins
  - **NADH dehydrogenases:** active site binds NADH, accept two electrons and two protons that are transferred to flavoproteins, regenerating NAD^+
  - **Flavoproteins:** contain deriviative of riboflavin (Figure 3.15) as prosthetic group (**e.g.,** FMN, FAD) that accepts two electrons and two protons but only donate electrons

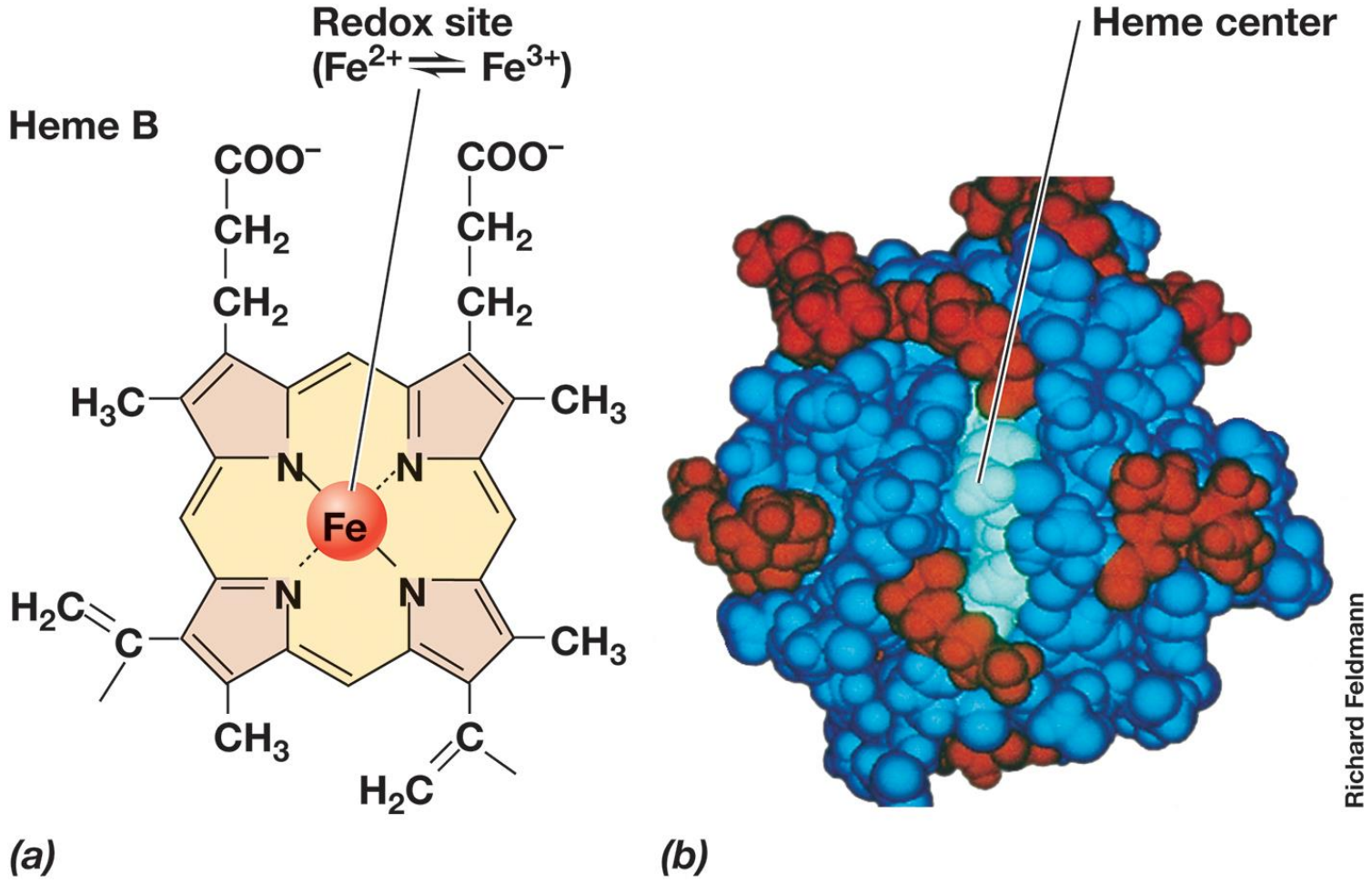
Figure 3.15 Flavin Mononucleotide (FMN), a Hydrogen Atom Carrier



3.8 Principles of Respiration: Electron Carriers (4 of 6)

- Cytochromes, Other Iron Proteins, and Quinones
  - Cytochromes: proteins that contain heme prosthetic groups (Figure 3.16)
    - Oxidized/reduced by 1 electron via the iron atom (Fe^{2+} or Fe^{3+})
    - Several classes, differ widely in reduction potentials, designated by letters based on heme
    - sometimes form complexes (**e.g.,** cytochrome bc_1)

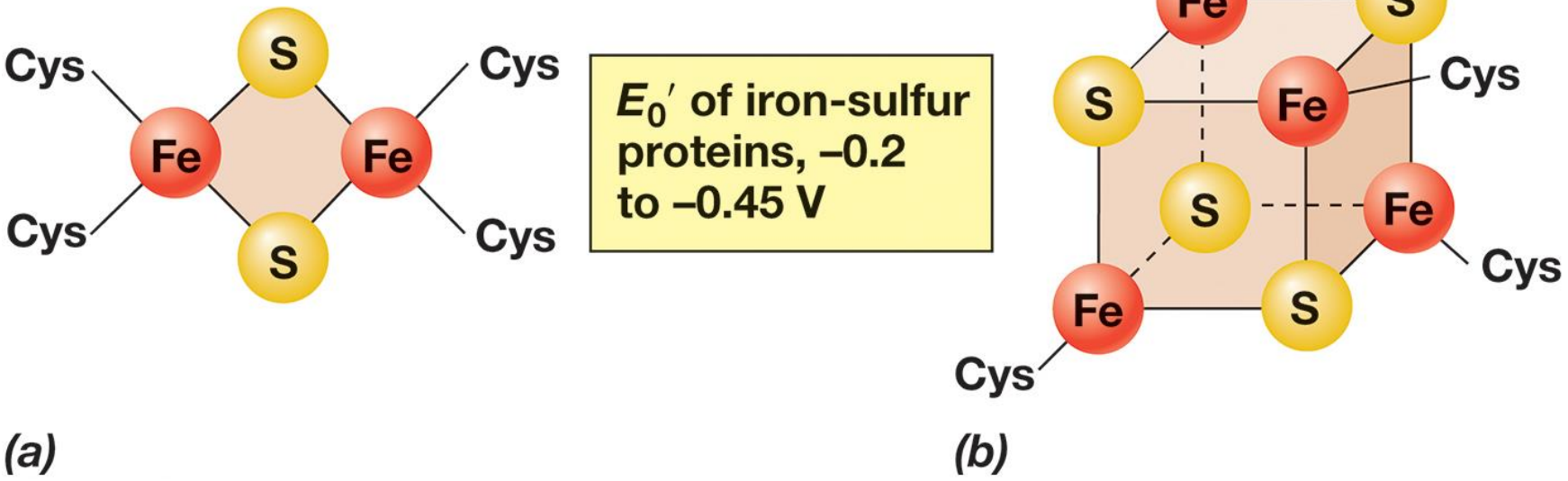
Figure 3.16 Cytochrome and Its Structure



3.8 Principles of Respiration: Electron Carriers (5 of 6)

- Cytochromes, Other Iron Proteins, and Quinones
  - Nonheme iron proteins
    - contain clusters of iron and sulfur (Fe_2S_2 and Fe_4S_4; Figure 3.17)
    - **e.g.,** ferredoxin: low reduction potential, important in H_2 production
    - Reduction potentials vary.
    - only carry electrons

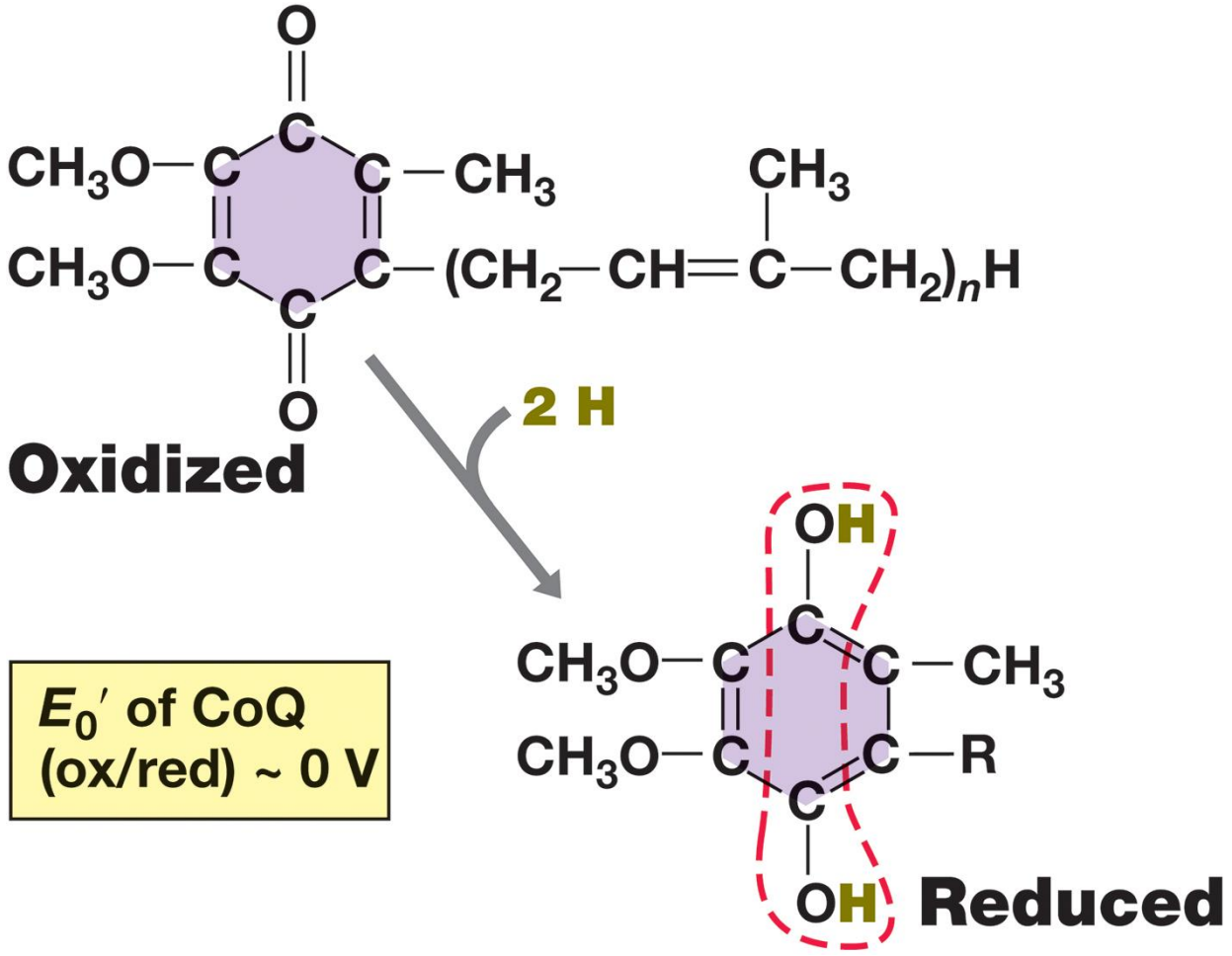
Figure 3.17 Arrangement of the Iron–Sulfur Centers of Nonheme Iron–Sulfur Proteins



3.8 Principles of Respiration: Electron Carriers (6 of 6)

- Cytochromes, Other Iron Proteins, and Quinones
  - Quinones: small hydrophobic nonprotein redox molecules (Figure 3.18)
    - can move within membrane
    - Accept two electrons and two protons but transfer electrons only
    - typically link iron–sulfur proteins and cytochromes
    - ubiquinone (coenzyme Q) and menaquinone most common

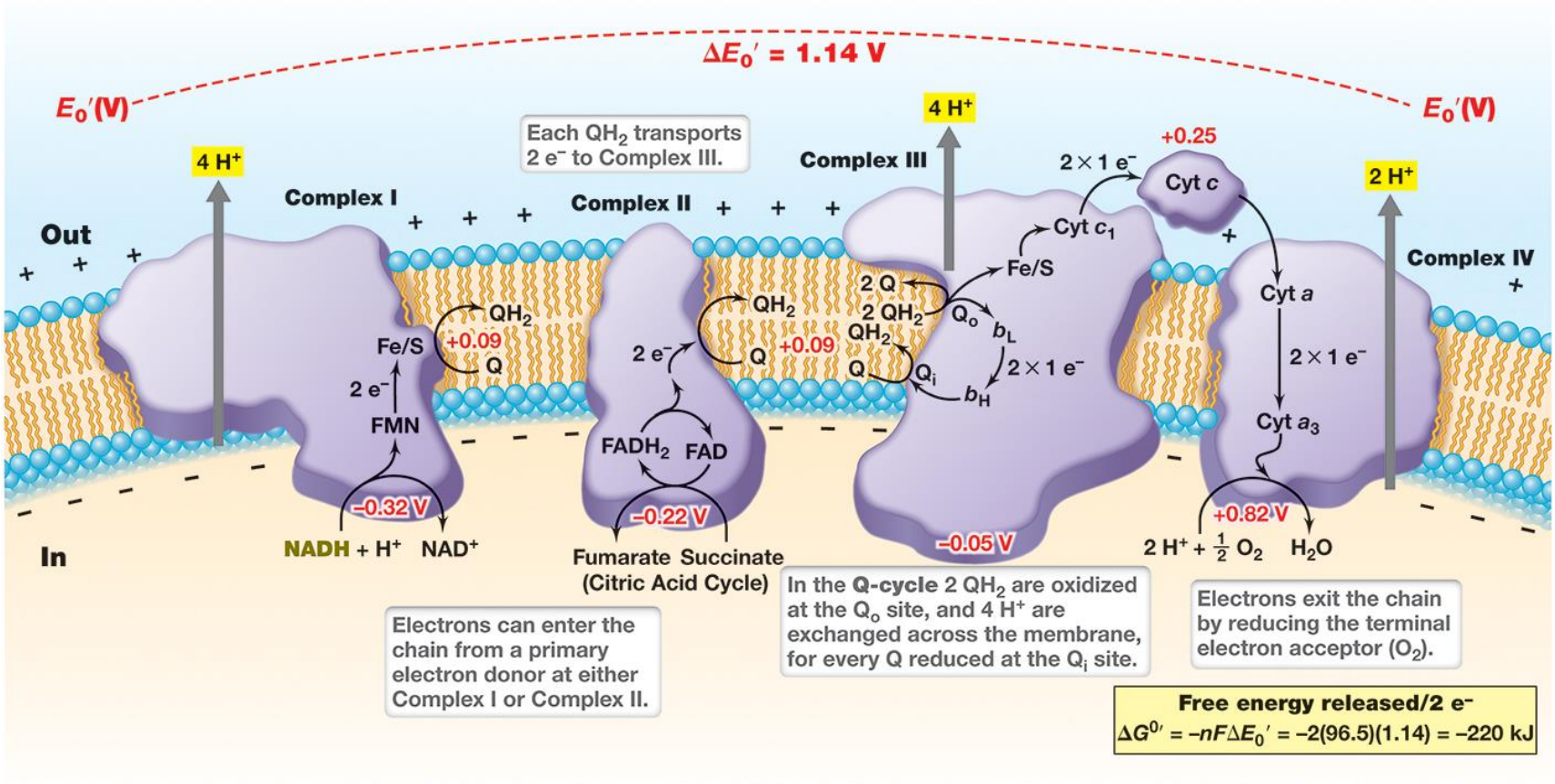
Figure 3.18 Structure of Oxidized and Reduced Forms of Ubiquinone (Coenzyme Q, or CoQ)



3.9 Principles of Respiration: Generating a Proton Motive Force (1 of 11)

- Electron Transport
  - Organized in cytoplasmic membrane (Figure 3.19)
    - Composition varies
    - Multiple chains can sometimes function
  - Starts when electron donor adds electrons
    - In chemoorganotrophs, from NADH or $FADH_2$
    - In chemolithotrophs, many different inorganics
  - Electron carriers embedded in order of increasingly positive reduction potentials
  - Passed until they reduce a terminal electron acceptor (**e.g.,** O_2)

Figure 3.19 Generation of the Proton Motive Force During Aerobic Respiration



3.9 Principles of Respiration: Generating a Proton Motive Force (2 of 11)

- Electron Transport
  - Electron movements are exergonic, providing free energy to pump protons to outer surface of membrane
    - Generates proton motive force
    - H^+ (charged and polar) cannot diffuse across membrane
    - Separation of H^+ and OH^- creates pH difference and electrochemical potential across membrane
  - **e.g., Paracoccus denitrificans** (Figure 3.19)

3.9 Principles of Respiration: Generating a Proton Motive Force (3 of 11)

- Generation of the Proton Motive Force: Complexes I and II
  - **Complex I (NADH: quinone oxidoreductase, NADH dehydrogenase)**
    - Begins electron transport
    - Composed of many proteins that function as a unit
    - NADH oxidized to NAD^+, quinone reduced and diffuses to Complex III, four H^+ released

3.9 Principles of Respiration: Generating a Proton Motive Force (4 of 11)

- Generation of the Proton Motive Force: Complexes I and II
  - **Complex II (succinate dehydrogenase complex)**
    - Alternative entry point
    - 2 e^- from $FADH_2$ and 2 H^+ from cytoplasm transferred to ubiquinone (Q) to make ubiquinol
    - Less energy conserved due to lack of H^+ translocation

3.9 Principles of Respiration: Generating a Proton Motive Force (5 of 11)

- Complexes III and IV: *bc*1 and *c*- and *a*-Type **Cytochromes**
  - **Complex III (cytochrome bc_1 complex)**
    - transfers e^- from QH_2 ubiquinol (reduced quinone) to cytochrome *c*
    - pumps 2 H^+ from QH_2 outside of cytoplasmic membrane
    - ***Q* cycle** (electron bifurcation) sends electrons to cytochrome *c* and subunit b_L; 4 H^+ transferred across membrane
    - Cytochrome *c* shuttles e^- to Complex IV.

3.9 Principles of Respiration: Generating a Proton Motive Force (6 of 11)

- Complexes III and IV: *bc*1 and *c*- and *a*-Type **Cytochromes**
  - **Complex IV (cytochromes *a* and a_3)**
    - terminal oxidase; reduces O_2 to H_2O
    - Needs 4 e^- and 4 H^+ from cytoplasm
    - pumps 1 H^+ per electron

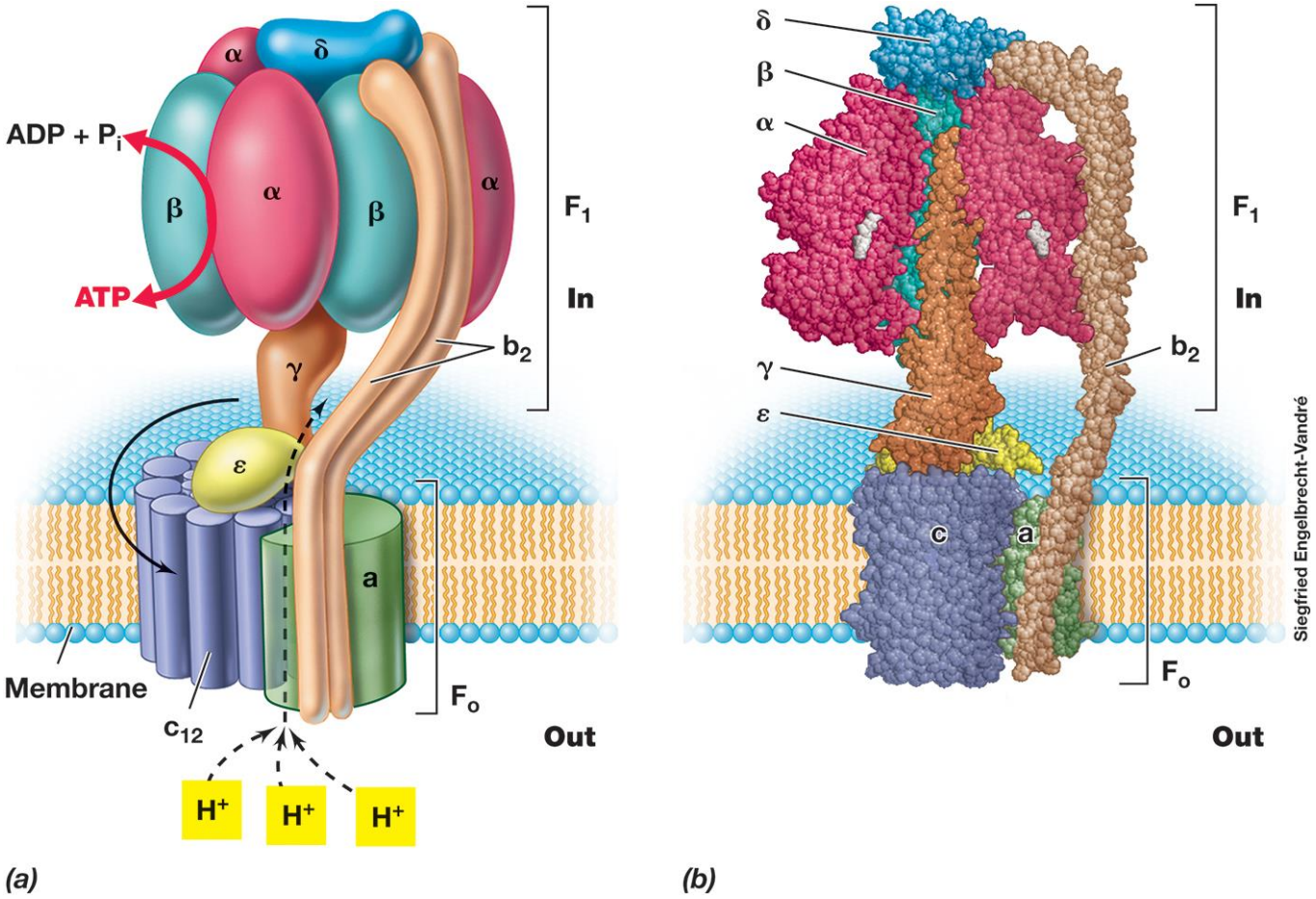
3.9 Principles of Respiration: Generating a Proton Motive Force (7 of 11)

- Complexes III and IV: *bc*1 and *c*- and *a*-Type **Cytochromes**
  - For every 2 e^- from NADH to O_2, 10 H^+ transferred outside membrane (4 at Complex 1, 4 at Complex III, 2 at Complex IV), 2 consumed in cytoplasm (H_2O)
  - For every 2 e^- from $FADH_2$ to O_2, 6 H^+ transferred outside membrane (4 at Complex III, 2 at Complex IV), 2 consumed in cytoplasm (H_2O)

3.9 Principles of Respiration: Generating a Proton Motive Force (8 of 11)

- ATP Synthase (ATPase)
  - Uses energy from proton motive force (pmf) to form ATP (Figure 3.20)
    - pmf generates torque; mechanical energy catalyzes ADP + P_i to ATP
    - **oxidative phosphorylation** from respiratory electrons
    - **photophosphorylation** from light energy

Figure 3.20 Structure and Function of the Reversible ATP Synthase (ATPase) in *Escherichia Coli*



3.9 Principles of Respiration: Generating a Proton Motive Force (9 of 11)

- ATP Synthase (ATPase)
  - Two components
    - F_1: multiprotein complex extending into cytoplasm that catalyzes ATP synthesis
    - F_o: membrane-integrated proton-translocating multiprotein complex
    - found in nearly all organisms, highly conserved
    - reversible reaction $(\text{ATP} \leftrightarrow \text{ADP} + P_i)$

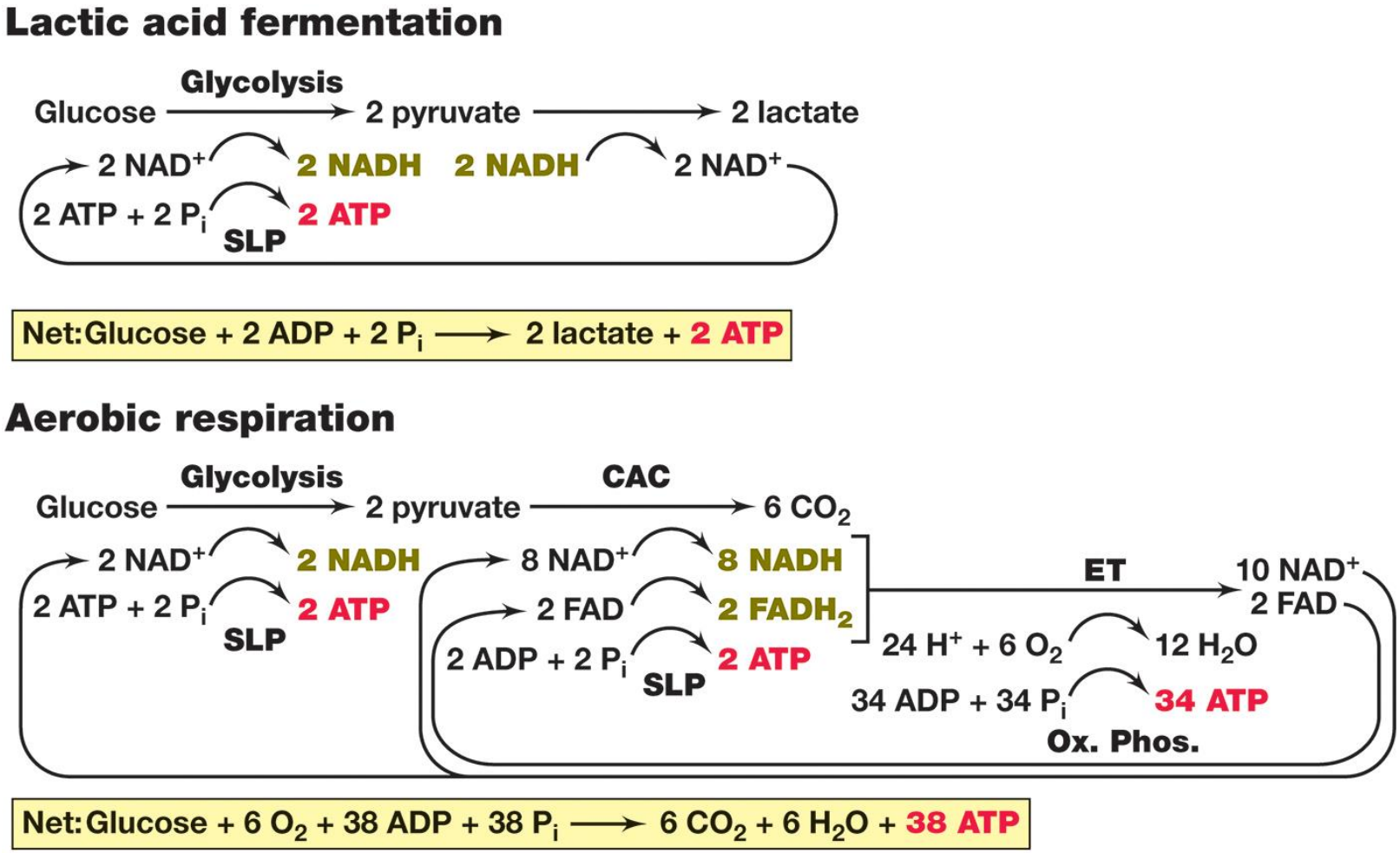
3.9 Principles of Respiration: Generating a Proton Motive Force (10 of 11)

- ATP synthase (ATPase)
  - For every full rotation of F_0 *c* ring, 3 ATP formed by F_1
    - In ***E. coli***, approximately 3.3 H^+ required per ATP
    - Number of c subunits varies between organisms, so # of H^+ needed also varies
  - Oxidative phosphorylation conserves much more energy because substrate is completely oxidized (Figure 3.21)
    - **e.g.,** 38 ATP in aerobic respiration, 2 ATP in lactic acid fermentation

3.9 Principles of Respiration: Generating a Proton Motive Force (11 of 11)

- ATP synthase (ATPase)
  - ATPases are reversible
    - ATP hydrolysis can reverse ATPase activity and transport protons out of cytoplasm, generating instead of dissipating pmf
    - ATPases in strict fermenters generate pmf for motility and transport by hydrolyzing ATP from substrate-level phosphorylation

Figure 3.21 Energetics in Fermentation and Aerobic Respiration



III. Catabolism: Electron Transport and Metabolic Diversity

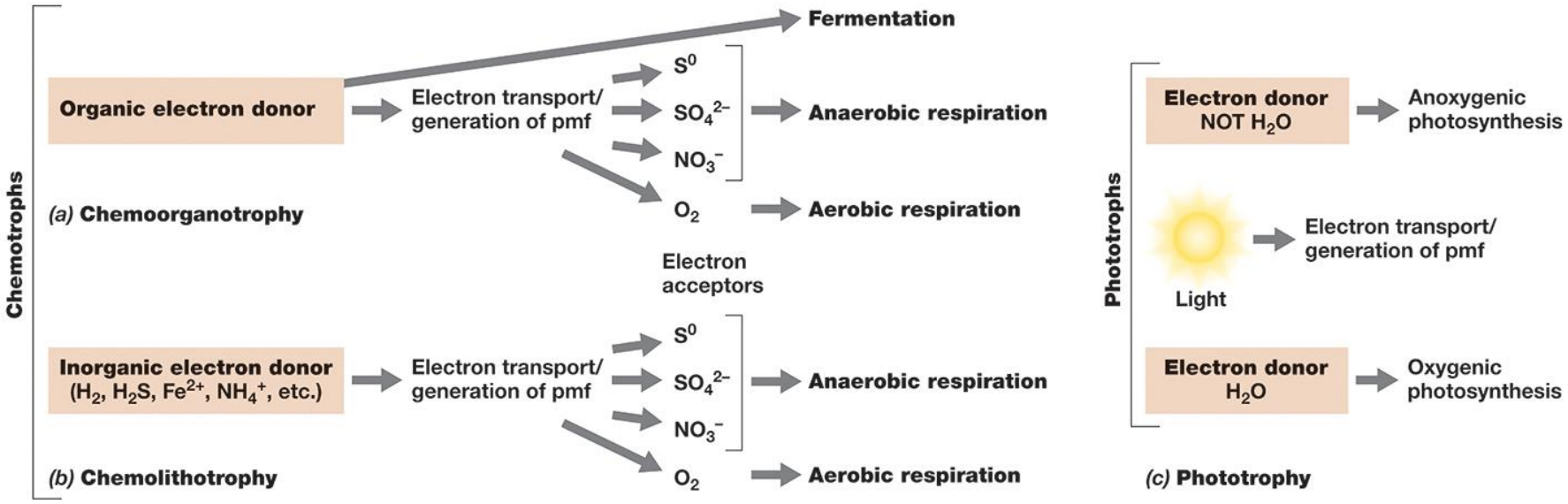
3.10 Anaerobic Respiration and Metabolic Modularity

3.11 Chemolithotrophy and Phototrophy

3.10 Anaerobic Respiration and Metabolic Modularity (1 of 7)

- Respiration can occur under both oxic (O_2) and anoxic (no O_2) conditions
  - Aerobic respiration requires O_2 as terminal electron acceptor
  - **Anaerobic respiration** uses other terminal electron acceptors (Figure 3.22)
- Originally no O_2 in atmosphere
  - Anaerobic microbes dominated much of history of life
  - Still occurs widely today

Figure 3.22 Metabolic Diversity and Its Relationship to Oxygen



3.10 Anaerobic Respiration and Metabolic Modularity (2 of 7)

- Fermentation and anaerobic respiration both occur in anaerobes
  - Respiration requires an external electron acceptor, generates ATP by oxidative phosphorylation
  - Fermentation does not require an external electron acceptor, generates ATP by substrate-level phosphorylation

3.10 Anaerobic Respiration and Metabolic Modularity (3 of 7)

- Respiration in ***Escherichia coli***
  - ***E. coli*** is a versatile chemoorganotroph with electron transport similar but not identical to **Paracoccus denitrificans**
  - Grows by aerobic respiration (O_2), fermentation (no external electron acceptors), anaerobic respiration (with nitrate)

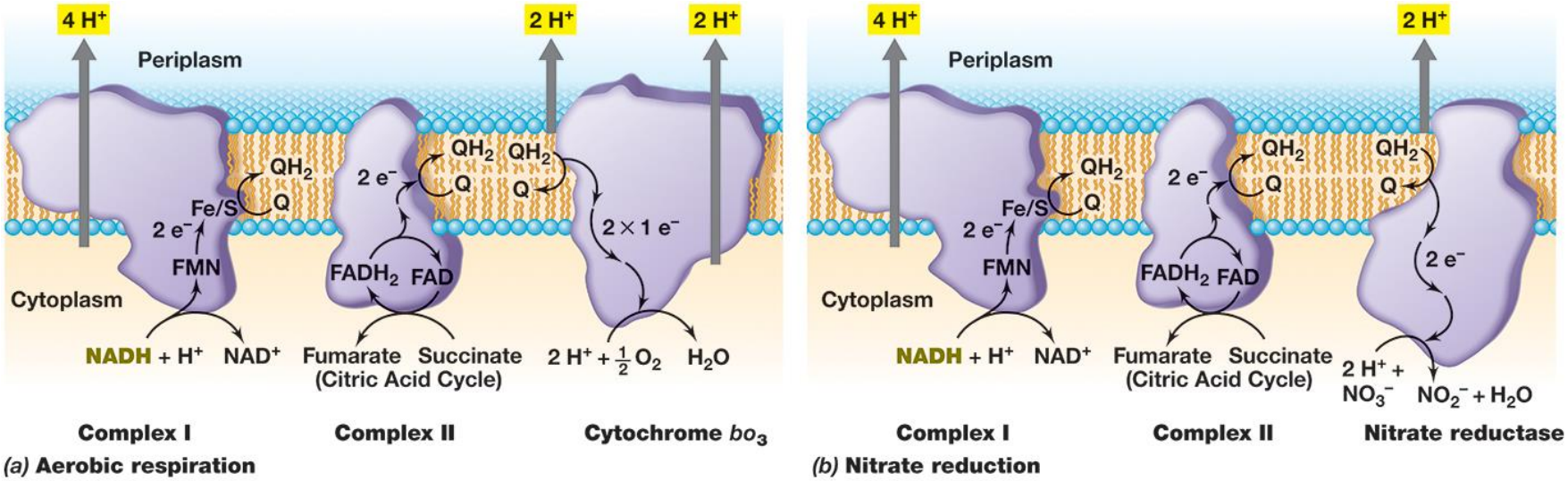
3.10 Anaerobic Respiration and Metabolic Modularity (4 of 7)

- Respiration in ***Escherichia coli***
  - Can optimize under multiple conditions
    - With organic carbon source, grows fastest by aerobic respiration
    - Grows faster with nitrate respiration than fermentation
    - Can insert many different proteins into electron transport chain

3.10 Anaerobic Respiration and Metabolic Modularity (5 of 7)

- Respiration in ***Escherichia coli***
  - Basic organization (Figure 3.23)
    - complex I, complex II, quinones, terminal reductase
  - Can swap components
    - alternative quinones
    - alternative dehydrogenases/terminal reductases (5+ sets each)

Figure 3.23 Respiration and Nitrate-Based Anaerobic Respiration in *Escherichia Coli*



3.10 Anaerobic Respiration and Metabolic Modularity (6 of 7)

- Respiration in ***Escherichia coli***
  - No complex III
    - Conserves less energy than ***P.denitrificans***
    - Exchanges only 8 H^+ instead of 10 for every 2 electrons
  - If no O_2 and nitrate present, ***E. coli*** uses **nitrate reductase** as terminal reductase
    - NO_3^-/NO_2^- couple is less electropositive
    - Provides less energy
    - Only 6 H^+ exchanged for every 2 electrons

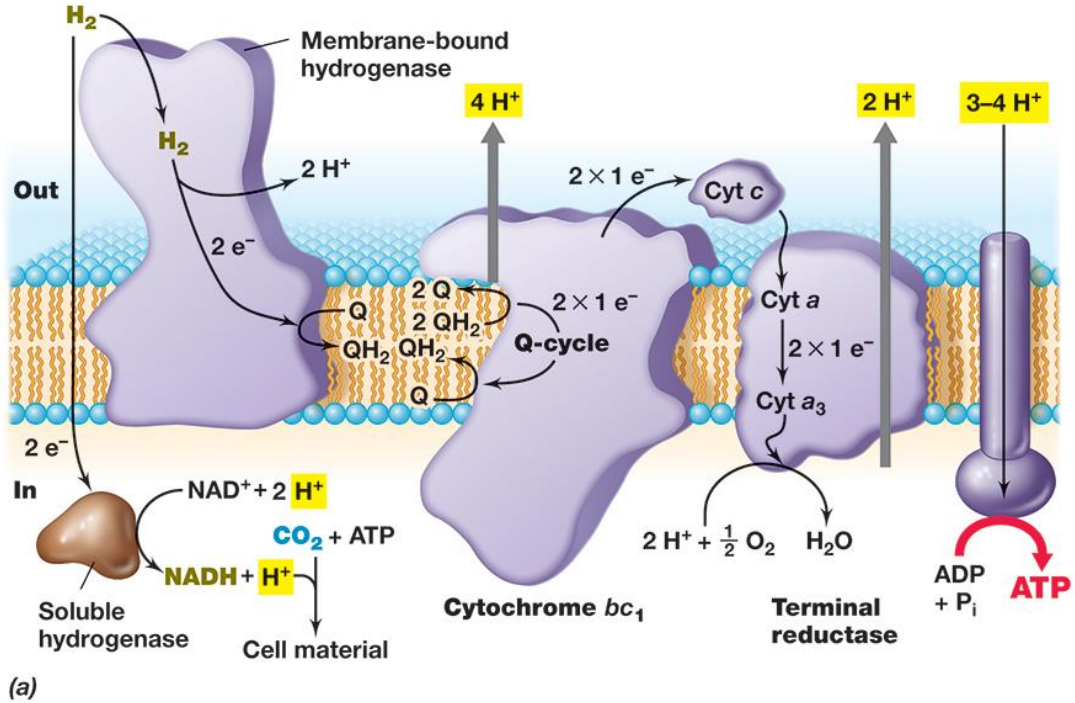
3.10 Anaerobic Respiration and Metabolic Modularity (7 of 7)

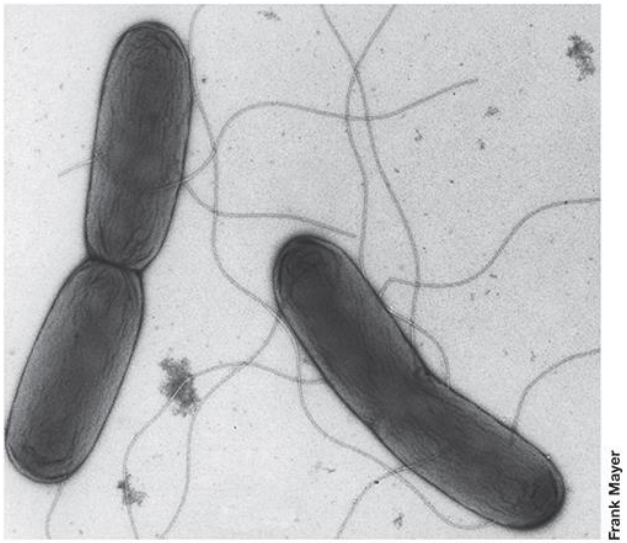
- Respiration in ***Escherichia coli***
  - For any electron donor, aerobic organisms always conserve more energy and will outcompete anaerobic organisms
  - O_2 consumed very quickly
  - Anoxic habitats and anaerobic microbes widespread

3.11 Chemolithotrophy and Phototrophy (1 of 6)

- **Chemolithotrophy**
  - Uses inorganic chemicals as electron donors
    - **e.g.,** hydrogen sulfide (H_2S), hydrogen gas (H_2), ferrous iron (Fe^{2+}), ammonium (NH_4^+)
  - Begins with oxidation of inorganic electron donor, electrons enter electron transport chain
    - **e.g., Ralstonia eutropha:** aerobic chemolithotroph with H_2 as electron donor ($2\ H^+/H_2$, −0.42 V) (Figure 3.24)

Figure 3.24 Energy Conservation in Ralstonia Eutropha, an Autotrophic Chemolithotroph That Oxidizes H_2





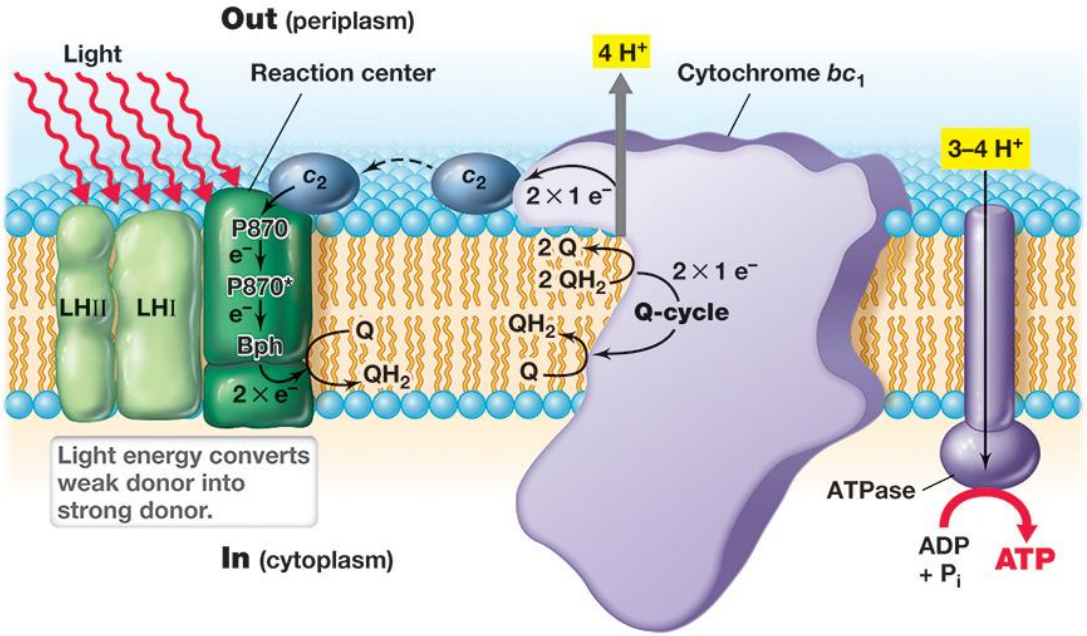
3.11 Chemolithotrophy and Phototrophy (2 of 6)

- Chemolithotrophy
  - Both chemoorganotrophs and chemolithotrophs depend on oxidative phosphorylation
  - Can be aerobic or anerobic
  - Major difference is source of cellular carbon
    - Chemoorganotrophs are heterotrophs, using organics as carbon sources
    - Chemolithotrophs typically use CO_2 as carbon source (autotrophs)
      - Use **reverse electron transport** to form reducing power

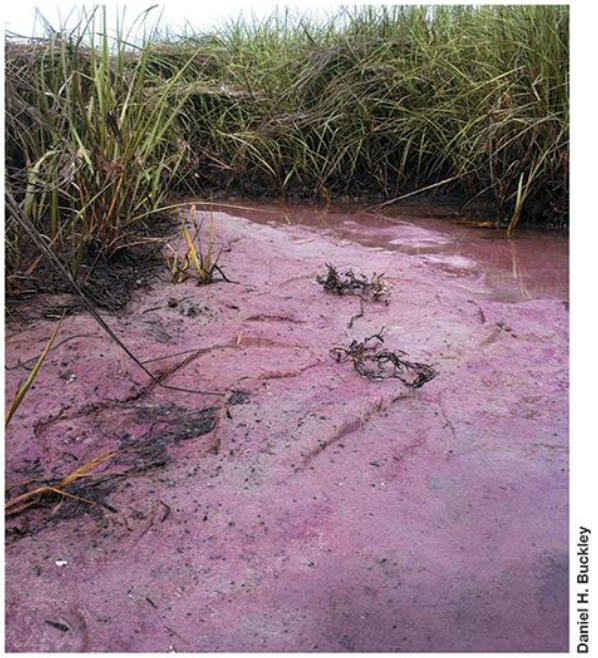
3.11 Chemolithotrophy and Phototrophy (3 of 6)

- Phototrophy
  - Uses light to generate proton motive force
  - ATP synthase makes ATP by photophosphorylation (Figure 3.25a)
    - **oxygenic** (**e.g.,** cyanobacteria, algae, plants), forming O_2 as waste product, **or anoxygenic** (many **Bacteria**)
    - anoxygenic phototrophs evolved first, more metabolic diversity

Figure 3.25 Electron Flow in Anoxygenic Photosynthesis in a Purple Bacterium



(a) **Electron transport in purple bacteria**



Daniel H. Buckley

(b) **Bloom of purple bacteria in a salt marsh**

3.11 Chemolithotrophy and Phototrophy (4 of 6)

- Phototrophy
  - Purple bacteria are anoxygenic prototrophs common in anoxic aquatic environments
    - produce **photosynthetic reaction center** that converts light into chemical energy
    - reaction centers contain photopigments (**e.g.,** chlorophylls, bacteriochlorophylls)
    - photopigments absorb light, transfer energy to photosynthetic reaction center, forms proton motive force that ATP synthase uses to make ATP

3.11 Chemolithotrophy and Phototrophy (5 of 6)

- Phototrophy
  - Major difference between respiratory and photosynthetic electron transport in purple bacteria is electrons are returned (**cyclic photophosphorylation**)

3.11 Chemolithotrophy and Phototrophy (6 of 6)

- Generation of Reducing Power
  - Reducing power (NADH) necessary to produce cellular material
  - Can come from variety of electron donors (**e.g.,** H_2S)
  - Use **reverse electron transport** (pushing electrons from quinone pool backward to reduce NAD^+ to NADH)

IV. Biosynthesis

3.12 Autotrophy and Nitrogen Fixation

3.13 Sugars and Polysaccharides

3.14 Amino Acids and Nucleotides

3.15 Fatty Acids and Lipids

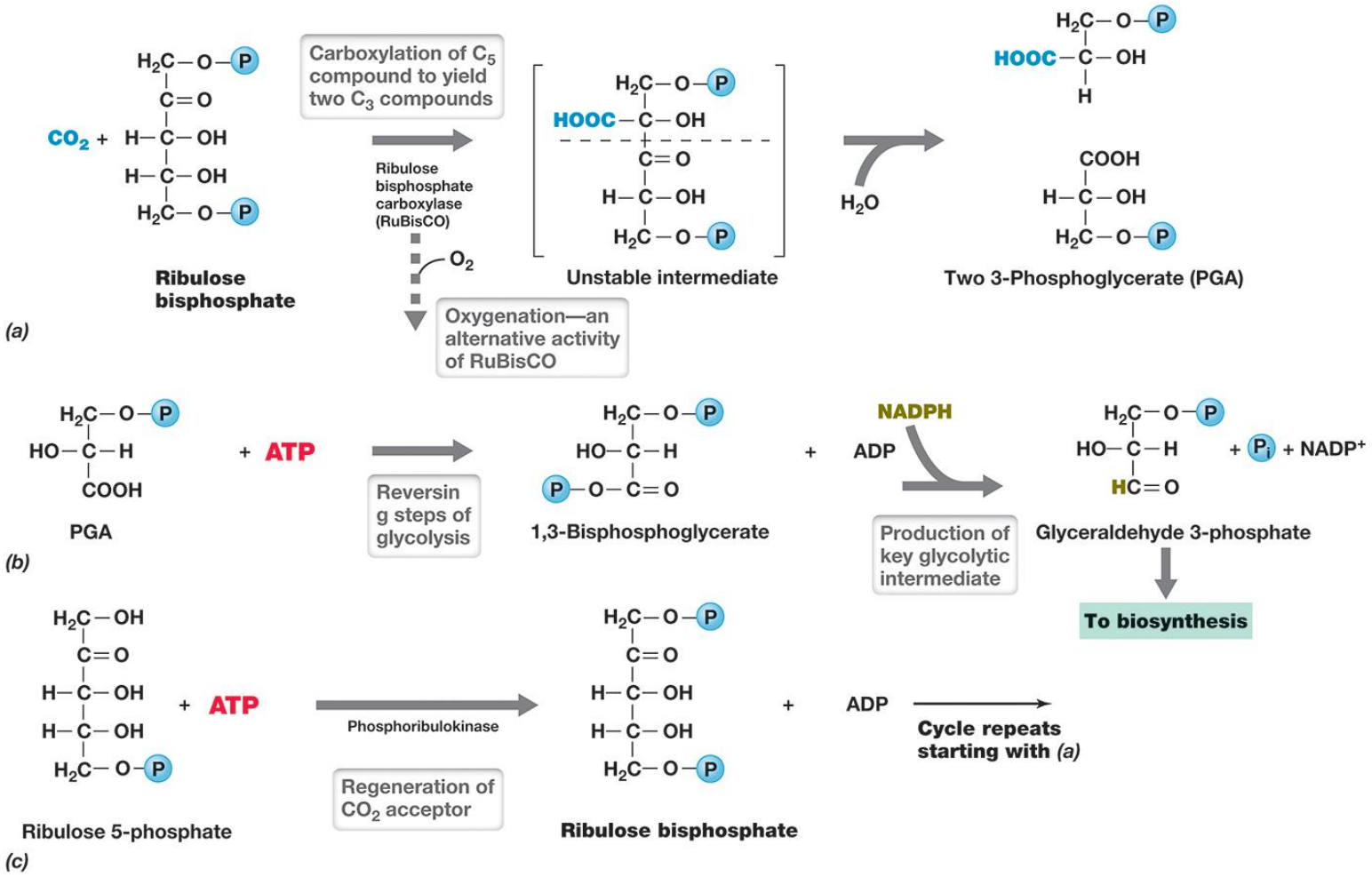
3.12 Autotrophy and Nitrogen Fixation (1 of 6)

- **Anaboliism**: Biosynthesis of cellular macromolecules
- Cells require carbon and nitrogen to perform biosynthesis
- Atmospheric sources (CO_2 and N_2) must be chemically reduced for assimilation (**CO_2 fixation** and **N_2 fixation**)
- Requires ATP and reducing power

3.12 Autotrophy and Nitrogen Fixation (2 of 6)

- The Calvin Cycle (Figure 3.26)
  - Used by many autotrophs, including all oxygenic phototrophs
  - Found in purple bacteria, cyanobacteria, algae, green plants, most chemolithotrophic **Bacteria**, few **Archaea**
  - Requires CO_2, a CO_2 acceptor, NADPH, ATP, **ribulose bisphophate carboxylase** (RubisCO), and **phosphoribulokinase**
  - First step catalyzed by RubisCO, forming two molecules of 3-phosphoglyceric acid (PGA) from ribulose bisphophate and CO_2
  - PGA then phosphorylated and reduced to glyceraldehyde-3-phosphate
  - glucose formed by reversal of glycolysis

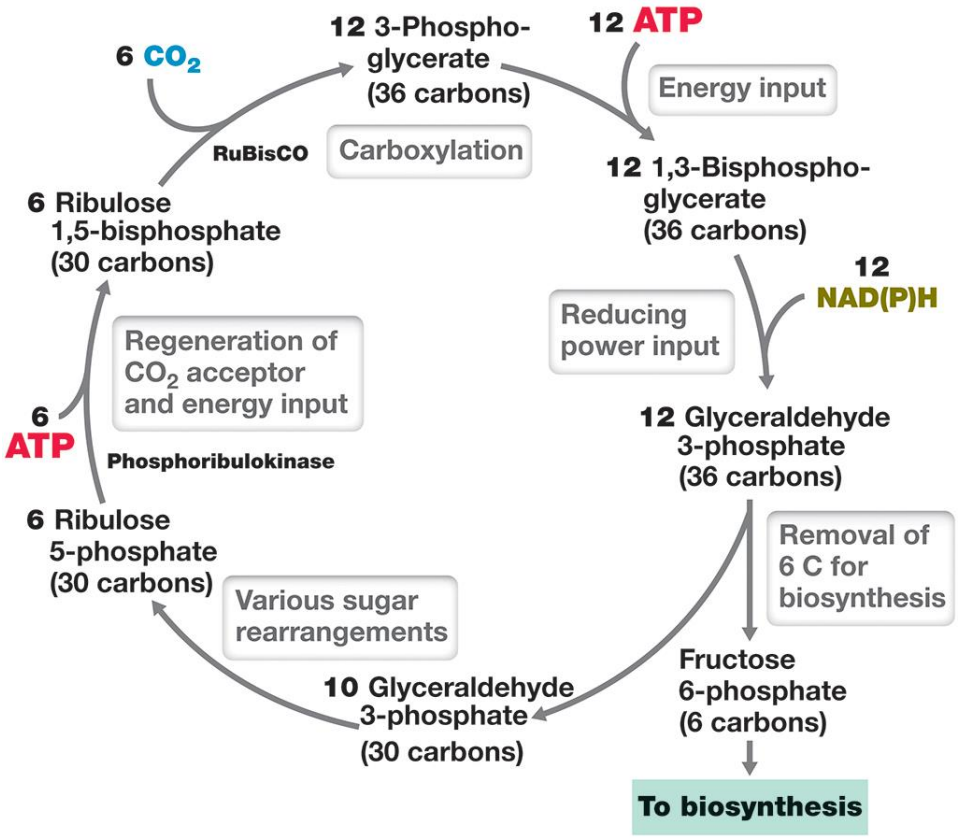
Figure 3.26 Key Reactions of the Calvin Cycle



3.12 Autotrophy and Nitrogen Fixation (3 of 6)

- The Calvin Cycle
  - Easiest to consider cycle as six molecules of CO_2 required to make one hexose ($C_6H_{12}O_6$)
  - 6 ribulose bisphosphate and 6 CO_2 required (Figure 3.27)
  - Results in 6 molecules of ribulose 5-phosphate (30 carbons) + one hexose (6 carbons) for biosynthesis
  - Phosphoribulokinase phosphorylates each ribulose 5-phosphate to regenerate ribulose bisphosphate
  - 12 NADPH and 18 ATP required to synthesize one glucose
  - Many alternative pathways for CO_2 fixation

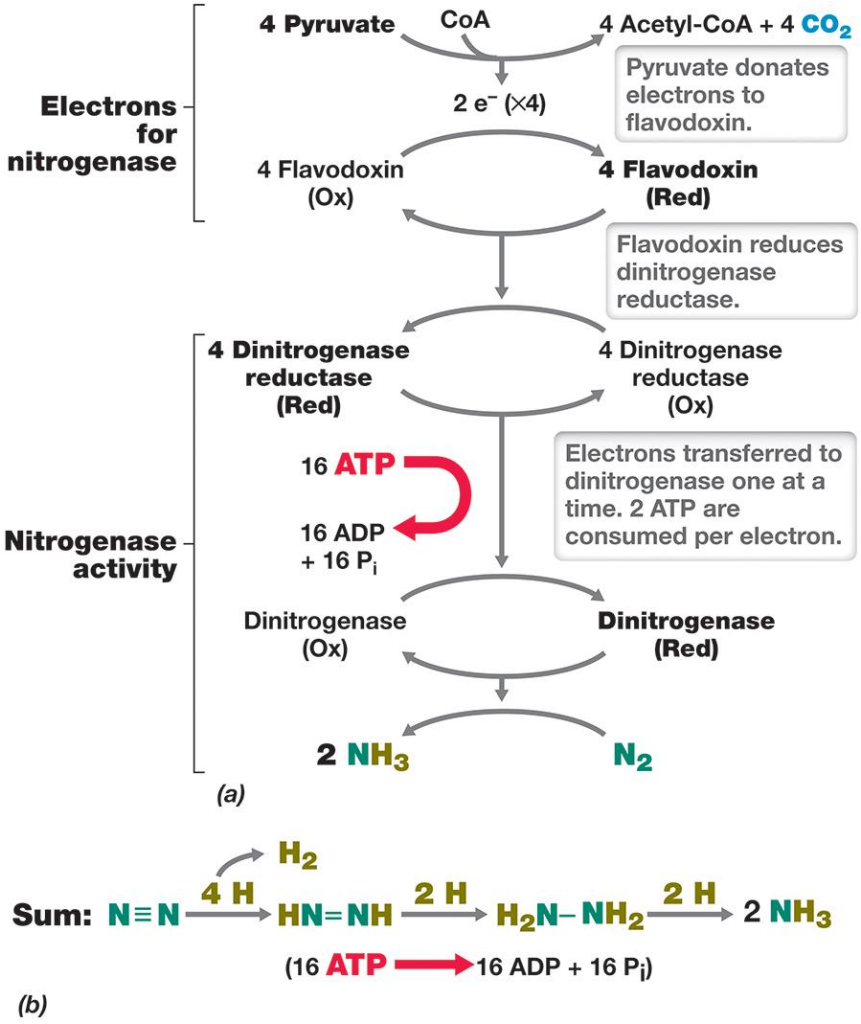
Figure 3.27 The Calvin Cycle



3.12 Autotrophy and Nitrogen Fixation (4 of 6)

- Nitrogen Fixation
  - Nitrogen needed for proteins, nucleic acids, other organics
  - Most microbes obtain this nitrogen from “fixed” nitrogen (ammonia, NH_3, or nitrate, NO_3^-)
  - Many prokaryotes can conduct nitrogen fixation: form ammonia (NH_3) from gaseous dinitrogen (N_2). (Figure 3.28)

Figure 3.28 Biological Nitrogen Fixation by Nitrogenase



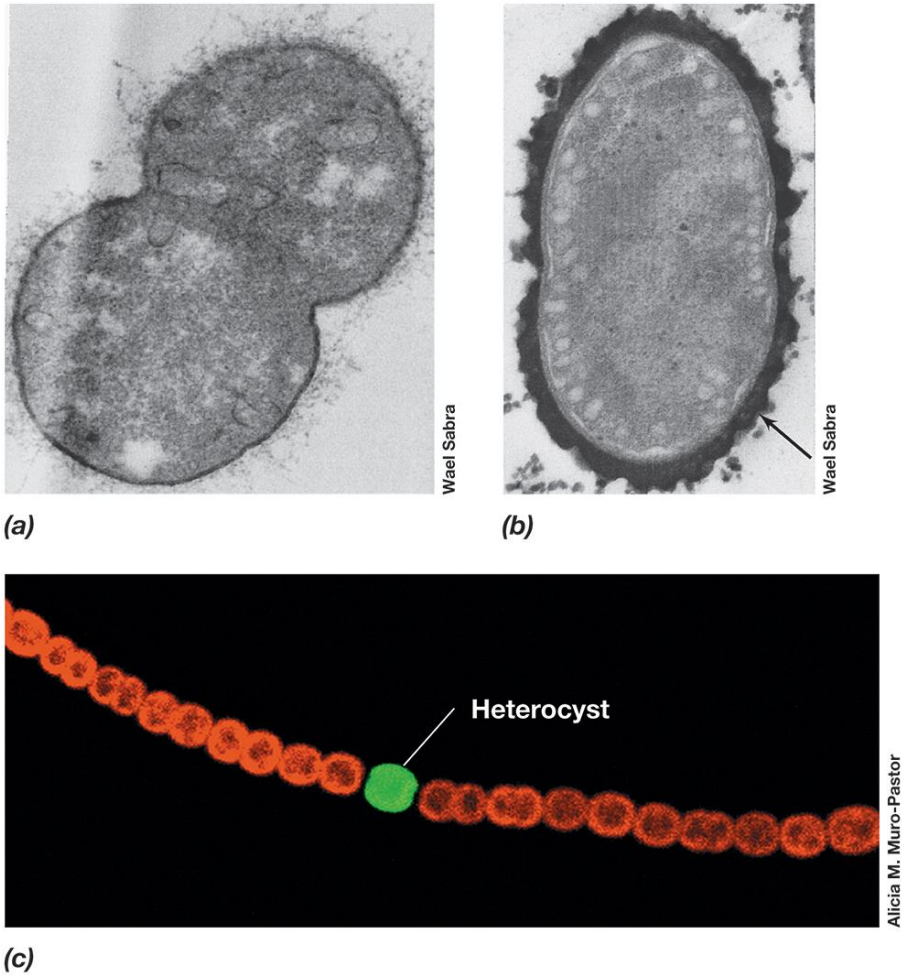
3.12 Autotrophy and Nitrogen Fixation (5 of 6)

- Nitrogen Fixation
  - Catalyzed by **nitrogenase** enzyme complex
    - Consists of **dinitrogenase** and **dinitrogenase reductase**
    - **Iron-molybdenum cofactor** (FeMo-co) of dinitrogenase is where N_2 reduction occurs
    - Triple bond stability makes activation and reduction very energy demanding
    - Electron donor $\rightarrow$ dinitrogenase reductase $\rightarrow$ dinitrogenase $\rightarrow N_2$
    - **6** electrons needed; **8** actually consumed because H_2 must be produced
    - 16 ATP required to lower protein's reduction potential, enabling dinitrogenase reductase to reduce dinitrogenase

3.12 Autotrophy and Nitrogen Fixation (6 of 6)

- Nitrogen Fixation
  - Catalyzed by **nitrogenase** enzyme complex
    - inhibited by oxygen
    - in obligate aerobes, nitrogenase is protected from oxygen by combination of removal by respiration, production of oxygen-retarding slime layers, localization of nitrogenase in differentiated **heterocyst** (Figure 3.29)

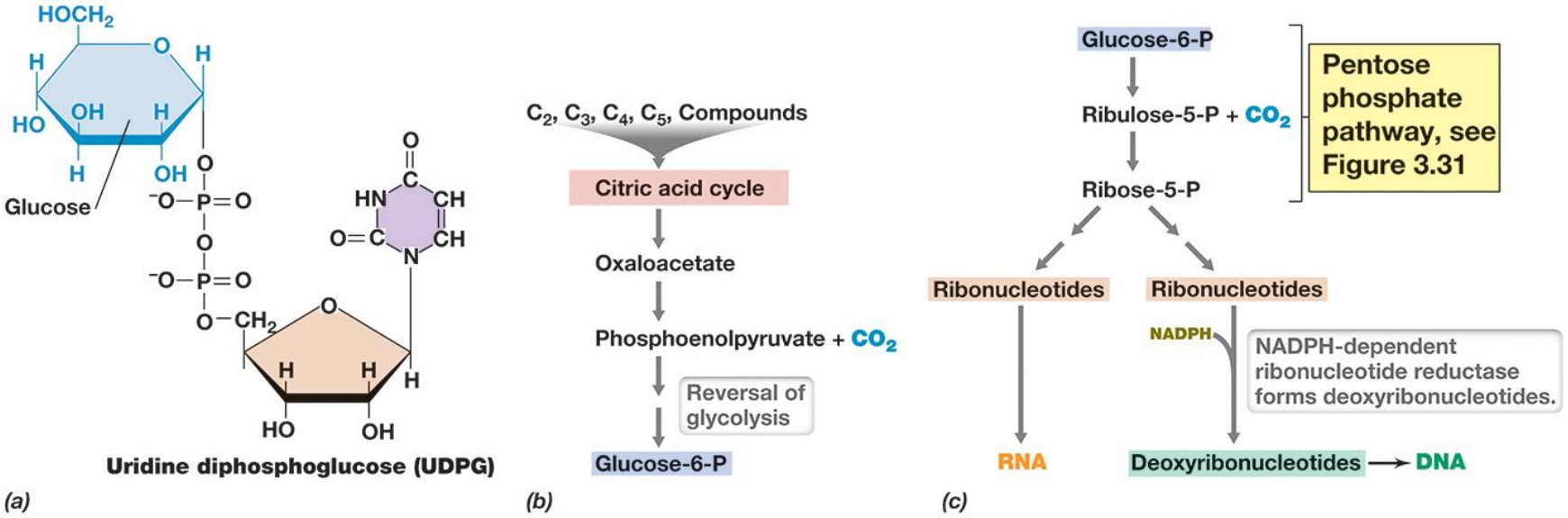
Figure 3.29 Two Ways of Protecting Nitrogenase from O_2



3.13 Sugars and Polysaccharides (1 of 3)

- Polysaccharide Biosyntheses and Gluconeogenesis
  - Polysaccharides synthesized from activated glucose (Figure 3.30a)
    - uridine diphosphoglucose (UDPG)
      - precursor of glucose derivatives for biosynthesis of structural polysaccharides (**e.g.,** *N*-acetylglucosamine, *N*-acetylmuramic acid, lipopolysaccharide)
    - adenosine diphosphoglucose *(***ADPG***)*
    - add activated glucose to polymer fragment
  - **Gluconeogenesis**: synthesis of glucose from phosphoenolpyruvate (Figure 3.30b)

Figure 3.30 Sugar Metabolism



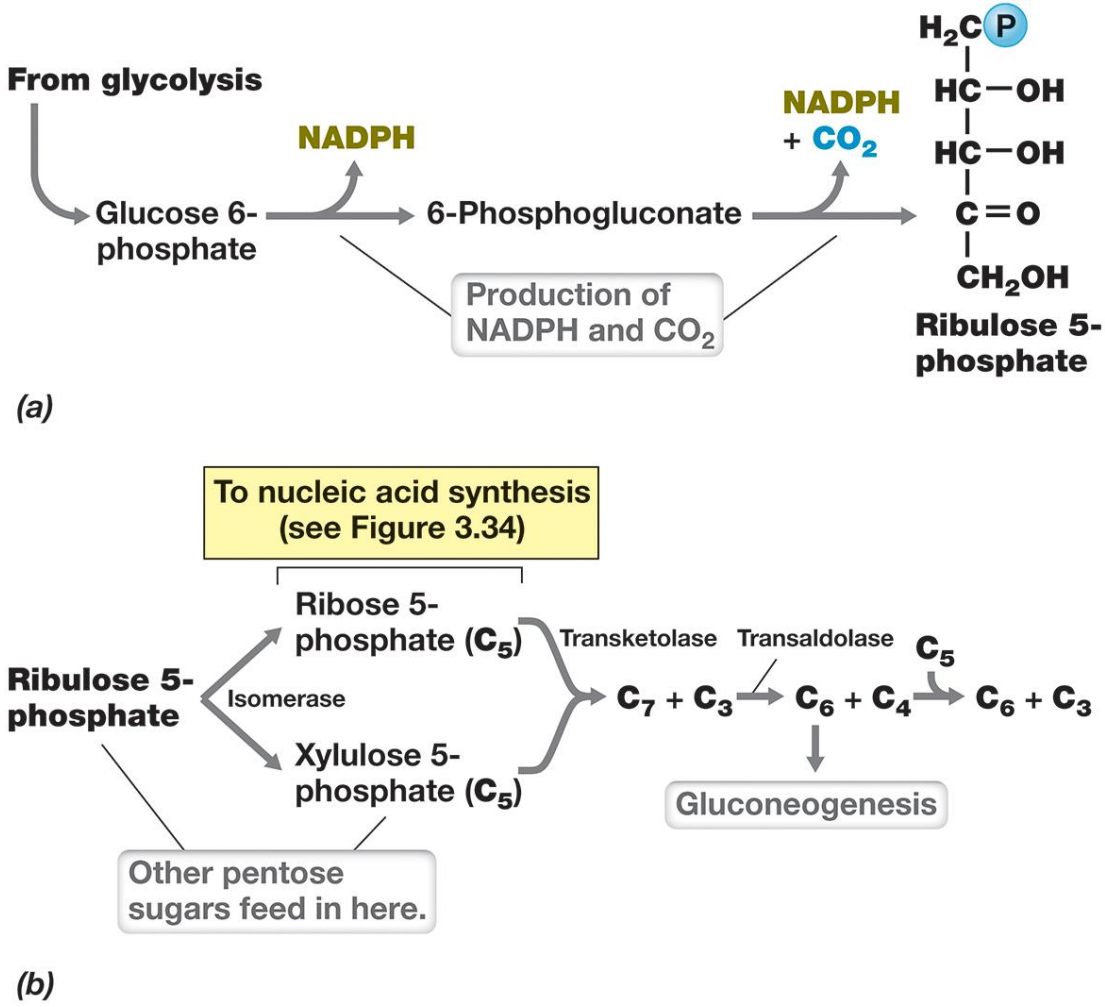
3.13 Sugars and Polysaccharides (2 of 3)

- Pentose Metabolism and the Pentose Phosphate Pathway
  - Pentoses (C_5 sugars)
    - formed by the removal of one carbon atom from a hexose (Figure 3.30c)
    - required for the synthesis of nucleic acids (**e.g.,** ribonucleotide reductase converts ribo- to deoxyribonucleotides)

3.13 Sugars and Polysaccharides (3 of 3)

- Pentose Metabolism and the Pentose Phosphate Pathway
  - Major pathway for pentose production is the **pentose phosphate pathway** (Figure 3.31)
    - glucose oxidized to CO_2, NADPH, ribulose-5-phosphate (which leads to several derivatives)
    - pentoses can be catabolized through this pathway
    - produces several 4-7 carbon-sugars
    - generates NADPH for deoxyribonucleotide and fatty acid biosynthesis

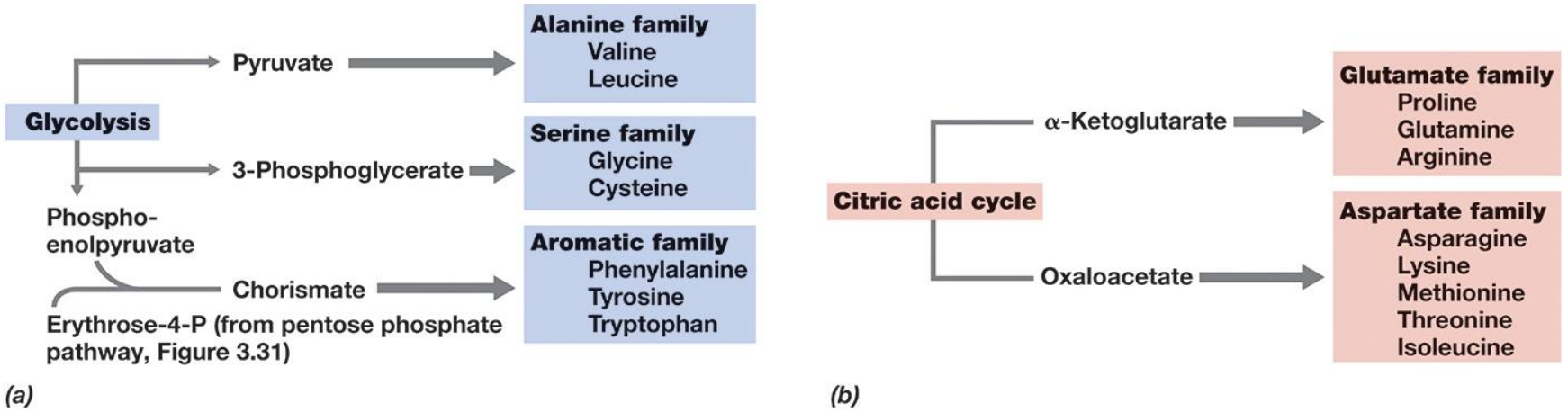
Figure 3.31 Pentose Phosphate Pathway



3.14 Amino Acids and Nucleotides (1 of 3)

- Amino acid and nucleotide biosynthesis typically use long, multistep pathways.
- Monomers of Proteins: Amino Acids
  - If amino acids are not obtained from environment, synthesized from glucose or other carbon sources
  - Grouped into structural **families** based on shared biosynthetic steps
  - Carbon skeletons come from intermediates of glycolysis or citric acid cycle (Figure 3.32)

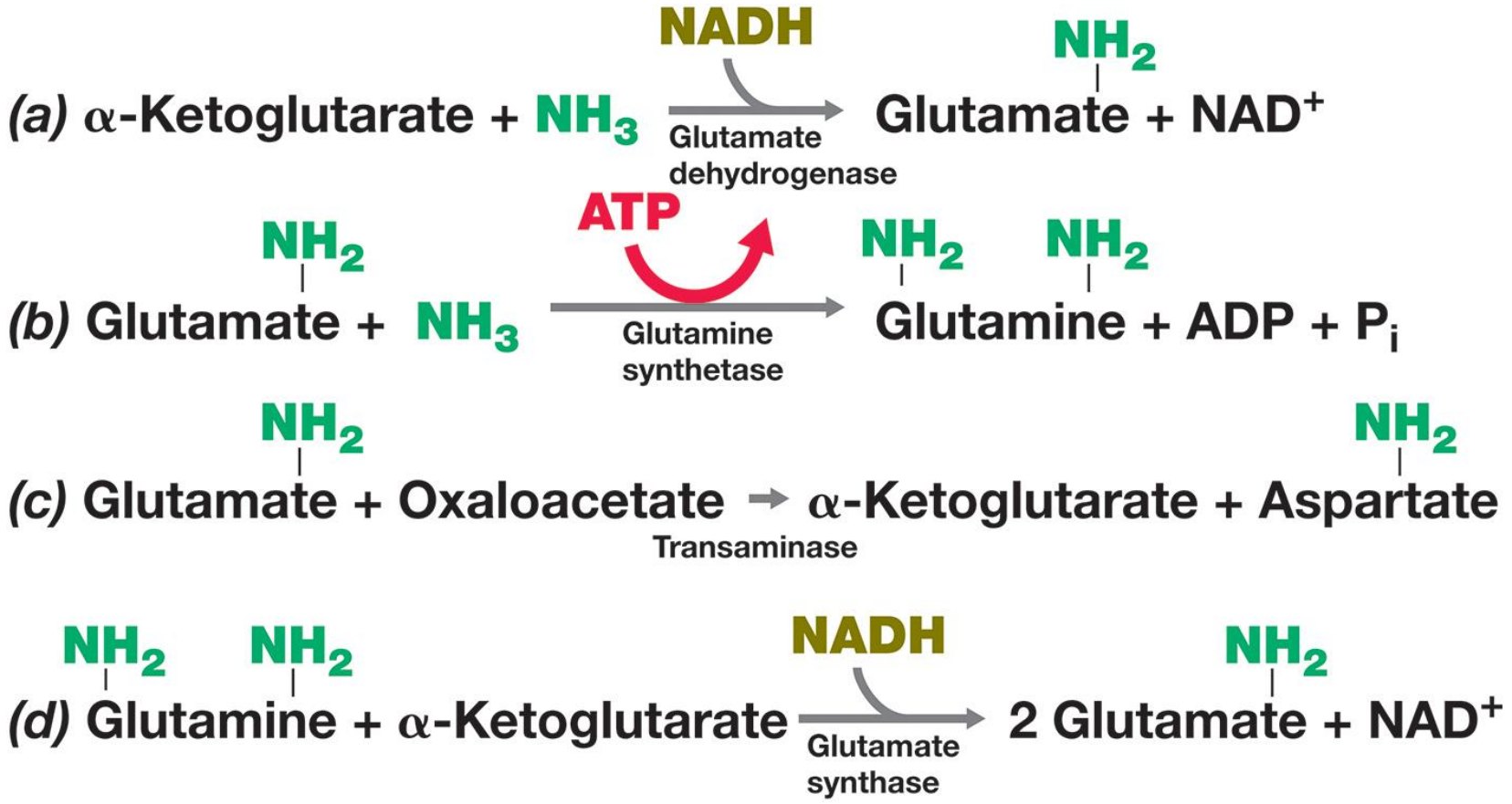
Figure 3.32 Amino Acid Families



3.14 Amino Acids and Nucleotides (2 of 3)

- Monomers of Proteins: Amino Acids
  - Amino group ($-NH_2$) typically from inorganic nitrogen source (**e.g.,** ammonia, NH_3)
  - Ammonia is incorporated by glutamine dehydrogenase or glutamine synthetase in biosynthesis of glutamate or glutamine (Figure 3.33)
  - Amino group from glutamate or glutamine can be transferred by transaminase/aminotransferase shuttles

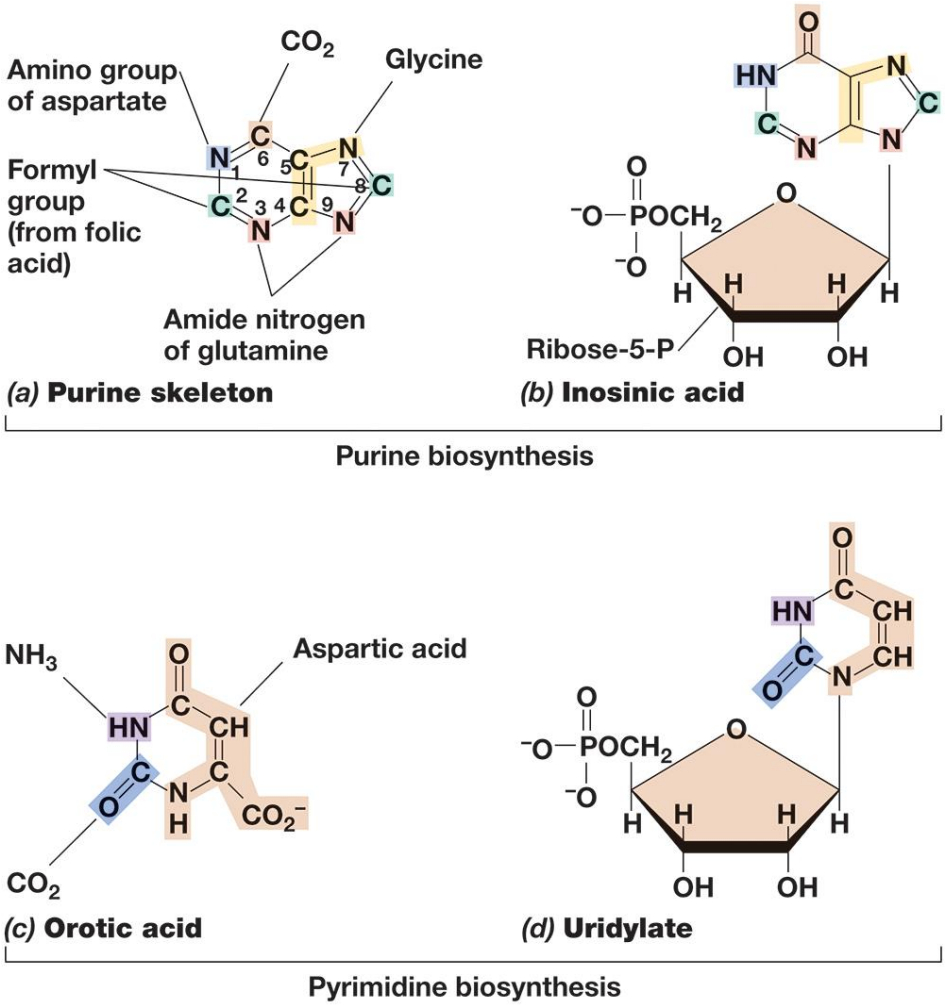
Figure 3.33 Ammonia Incorporation in Bacteria



3.14 Amino Acids and Nucleotides (3 of 3)

- Monomers of Nucleic Acids: Nucleotides
  - Purine and pyrimidine biosynthesis is complex (Figure 3.34)
    - Constructed from multiple carbon and nitrogen sources
    - Purines: Inosinic acid skeleton is precursor to adenine and guanine
    - Pyrimidines: Uridylate skeleton is precursor to thymine, cytosine, and uracil

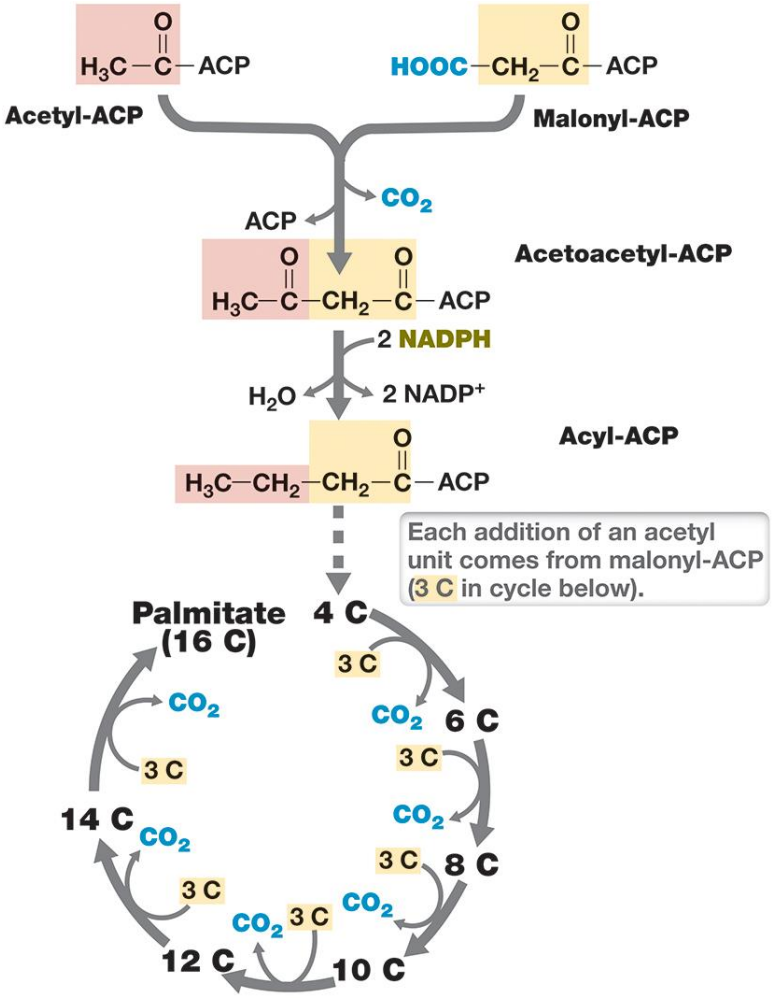
Figure 3.34 Biosynthesis of Purines and Pyrimidines



3.15 Fatty Acids and Lipids (1 of 4)

- Fatty Acids: Backbone of Microbial Lipids
  - **Archaea** do not contain fatty acids; have hydrophobic isoprenoids instead
- Fatty Acid Biosynthesis
  - Biosynthesized two carbons at a time (Figure 3.35)
    - **Acyl carrier protein (ACP)** holds the growing fatty acid during synthesis and releases it after final length
    - each C_2 acetyl actually originates from C_3 malonate; CO_2 released

Figure 3.35 The Biosynthesis of the C_{16} Fatty Acid Palmitate



3.15 Fatty Acids and Lipids (2 of 4)

- Fatty Acid Biosynthesis
  - Can be unsaturated, branched, or contain odd numbers of carbon atoms
  - Varies between species and at different temperatures
    - lower temps: shorter, more unsaturated
    - higher temps: longer, more saturated
    - **Bacteria** most commonly have C_{12}-C_{20} lipids

3.15 Fatty Acids and Lipids (3 of 4)

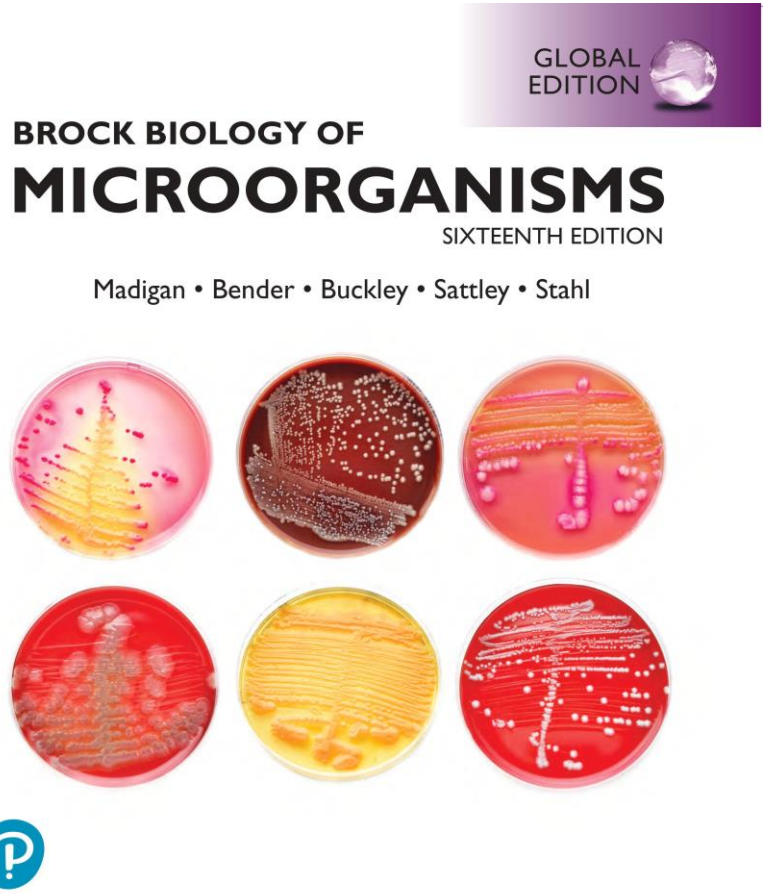
- Lipid Biosynthesis
  - In **Bacteria** and **Eukarya**, assembly of lipids first involves addition of fatty acids to glycerol.
    - Simple triglycerides have glycerol esterified to 3 fatty acids
    - In complex lipids, one carbon is modified with a polar group (**e.g.,** phosphate, ethanolamine, sugar)

3.15 Fatty Acids and Lipids (4 of 4)

- Lipid Biosynthesis
  - In **Archaea**, lipids are made from isoprene, but glycerol contains a polar group (like **Bacteria** and **Eukarya**)
    - Polar groups necessary for canonical membrane architecture (hydrophobic interior, hydrophilic surfaces)

Brock Biology of Microorganisms

Sixteenth Edition, Global Edition



Chapter 4

Microbial Growth and Its Control

I. Culturing Microbes and Measuring Their Growth

4.1 Feeding the Microbe: Cell Nutrition

4.2 Growth Media and Laboratory Culture

4.3 Microscopic Counts of Microbial Cell Numbers

4.4 Viable Counting of Microbial Cell Numbers

4.5 Turbidimetric Measures of Microbial Cell Numbers

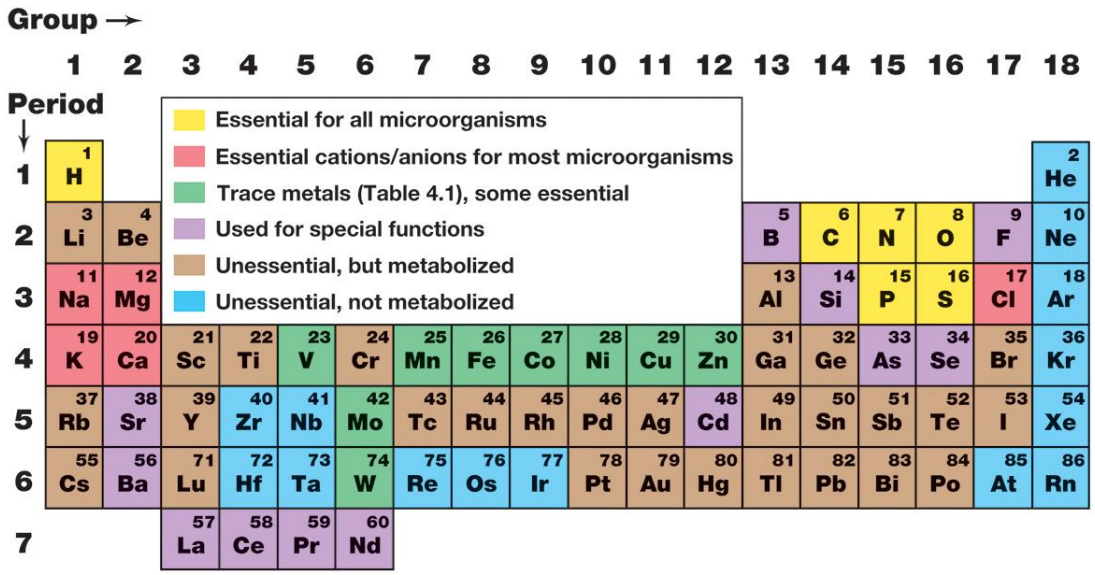
4.1 Feeding the Microbe: Cell Nutrition (1 of 8)

- **Nutrients**
  - Supply of elements required by cells for growth
- **Macronutrients**
  - Nutrients required in large amounts
- **Micronutrients**
  - Nutrients required in minute amounts
  - Trace metals and growth factors

4.1 Feeding the Microbe: Cell Nutrition (2 of 8)

- Chemical Makeup of a Cell
  - Handful of elements dominate living systems
  - C, O, N, H, P, S are ~96% of dry weight of bacterial cell (Figure 4.1) and required by all life
  - K, Na, Ca, Mg, Cl, Fe ~3.7% of dry weight
  - 62 total elements can be metabolized
  - Macromolecules (proteins, lipids, polysaccharides, lipopolysaccharides, nucleic acids) ~96% of dry weight
    - Mostly proteins and RNA, not DNA

Figure 4.1 Elemental and Macromolecular Composition of a Bacterial Cell

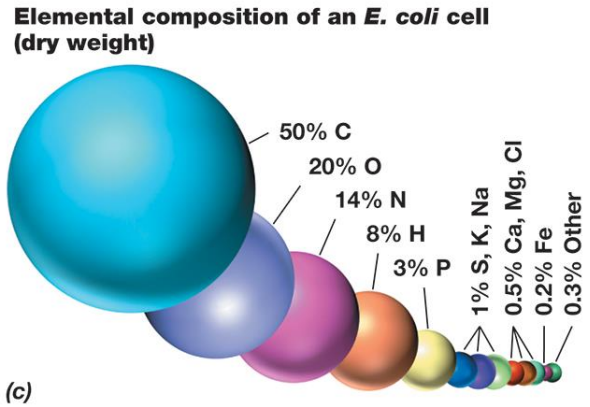


(a)

Macromolecular composition of a cell

Macromolecule	Percent of dry weight
Protein	55
Lipid	9.1
Polysaccharide	5.0
Lipopolysaccharide	3.4
DNA	3.1
RNA	20.5

(b)



(c)

Pearson

4.1 Feeding the Microbe: Cell Nutrition (3 of 8)

- Carbon, Nitrogen, and Other Macronutrients
  - Heterotrophs (require organic carbon (C)) obtain C from breakdown of organic polymers or uptake of monomers (amino acids, fatty acids, organic acids, sugars, nitrogen bases, other organics)
  - Autotrophs synthesize organics from carbon dioxide (CO_2)

4.1 Feeding the Microbe: Cell Nutrition (4 of 8)

- Carbon, Nitrogen, and Other Macronutrients
  - Nitrogen (N): mostly proteins, ammonia (NH_3), nitrate (NO_3^-), or nitrogen gas (N_2).
    - Nearly all microbes can use NH_3.
    - many use nitrate (NO_3^-)
    - some use organics (**e.g.**, amino acids) or N_2 (nitrogen-fixing)
  - Oxygen (O) and hydrogen (H): from water

4.1 Feeding the Microbe: Cell Nutrition (5 of 8)

- Carbon, Nitrogen, and Other Macronutrients
  - Phosphorus (P)
    - nucleic acids and phospholipids
    - usually inorganic phosphate (PO_4^{3-})
  - Sulfur (S)
    - sulfur-containing amino acids (cysteine and methionine)
    - vitamins (**e.g.**, thiamine, biotin, lipoic acid)
    - microbes assimilate sulfate (SO_4^{-2}), sulfide (H_2S), or organics

4.1 Feeding the Microbe: Cell Nutrition (6 of 8)

- Carbon, Nitrogen, and Other Macronutrients
  - Potassium (K)
    - required by several enzymes
  - Magnesium (Mg)
    - stabilizes ribosomes, membranes, and nucleic acids
    - also required by many enzymes
  - Calcium (Ca) and sodium (Na)
    - required by some microbes (**e.g.**, marine microbes)

4.1 Feeding the Microbe: Cell Nutrition (7 of 8)

- Micronutrients: Trace Metals and Growth Factors
  - Many enzymes require metal ion or small organic as a cofactor for catalysis
  - Iron (Fe)
    - cellular respiration, related oxidation-reduction reactions
  - **Trace metals** (Table 3.1): required in small amounts

Table 4.1 Micronutrients Needed by Microorganisms[a] (1 of 2)

I. Trace elements

Element	Function
Boron (B)	Autoinducer for quorum sensing in bacteria; also found in some polyketide antibiotics
Cobalt (Co)	Vitamin B_{12}; transcarboxylase (only in propionic acid bacteria)
Copper (Cu)	In respiration, cytochrome *c* oxidase; in photosynthesis, plastocyanin, some superoxide dismutases
Iron (Fe)[b]	Cytochromes; catalases; peroxidases; iron–sulfur proteins; oxygenases; all nitrogenases
Manganese (Mn)	Activator of many enzymes; component of certain superoxide dismutases and of the water-splitting enzyme in oxygenic phototrophs (photosystem II)
Molybdenum (Mo)	Certain flavin-containing enzymes; some nitrogenases, nitrate reductases, sulfite oxidases, DMSO–TMAO reductases; some formate dehydrogenases
Nickel (Ni)	Most hydrogenases; coenzyme F_{430} of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Carbonic anhydrase; nucleic acid polymerases; many DNA-binding proteins

Table 4.1 Micronutrients Needed by Microorganisms[a] (2 of 2)

II. Growth factors

Growth factor	Function
PABA (*p*-aminobenzoic acid)	Precursor of folic acid
Folic acid	One-carbon metabolism; methyl transfers
Biotin	Fatty acid biosynthesis; some CO_2 fixation reactions
B_{12} (Cobalamin)	One-carbon metabolism; synthesis of deoxyribose
B_1 (Thiamine)	Decarboxylation reactions
B_6 (Pyridoxine)	Amino acid/keto acid transformations
Nicotinic acid (Niacin)	Precursor of NAD^+
Riboflavin	Precursor of FMN, FAD
Pantothenic acid	Precursor of coenzyme A
Lipoic acid	Decarboxylation of pyruvate and α-ketoglutarate
Vitamin K	Electron transport
Coenzymes M and B	Methanogenesis[c]
F_{420} and F_{430}	Methanogenesis[c]

[a]Not all trace elements or growth factors are needed by all organisms, and many growth factors are biosynthesized and not required from the environment.
[b]Iron is typically needed in larger amounts than the other trace metals shown.
[c]The production of methane (CH4) by methanogens (**Archaea**).

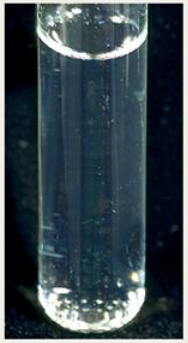
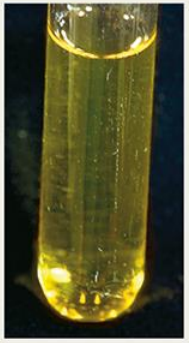
4.1 Feeding the Microbe: Cell Nutrition (8 of 8)

- Micronutrients: Trace Metals and Growth Factors
  - **Growth factors**: organic micronutrients
    - vitamins (Table 4.1)
      - Most function as coenzymes.
      - Most frequently required growth factors
    - others: amino acids, purines, pyrimidines, other organics

4.2 Growth Media and Laboratory Culture (1 of 5)

- Culture media (Table 4.2)
  - Nutrient solutions used to grow microbes in the laboratory
  - Typically sterilized in an **autoclave**

Table 4.2 Examples of Culture Media for Microorganisms with Simple and Demanding Nutritional Requirements[a]

Defined culture medium for *Escherichia coli*	Defined culture medium for *Leuconostoc mesenteroides*	Complex culture medium for either *E. coli* or *L. mesenteroides*	Defined culture medium for *Thiobacillus thioparus*
K_2HPO_4 7 g	K_2HPO_4 0.6 g	Glucose 15 g	KH_2PO_4 0.5 g
KH_2PO_4 2 g	KH_2PO_4 0.6 g	Yeast extract 5 g	NH_4Cl 0.5 g
$(NH_4)_2SO_4$ 1 g	NH_4Cl 3 g	Peptone 5 g	$MgSO_4$ 0.1 g
$MgSO_4$ 0.1 g	$MgSO_4$ 0.1 g	KH_2PO_4 2 g	$CaCl_2$ 0.05 g
$CaCl_2$ 0.02 g	Glucose 25 g	Distilled water 1000 ml	KCl 0.5 g
Glucose 4–10 g	Sodium acetate 25 g	pH 7	$Na_2S_2O_3$ 2 g
Trace elements (Fe, Co, Mn, Zn, Cu, Ni, Mo) 2–10 μg each	Amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine) 100–200 mg of each		Trace elements (as in first column) 2–10 μg each
Distilled water 1000 ml	Purines and pyrimidines (adenine, guanine, uracil, xanthine) 10 mg of each		Distilled water 1000 ml
pH 7	Vitamins (biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, *p*-aminobenzoic acid) 0.01–1 mg of each		pH 7
	Trace elements (as in first column) 2–10 μg each		Carbon source: CO_2 from air
(a)	Distilled water 1000 ml	*(b)*	
	pH 7		

[a]The photos are tubes of *(a)* the defined medium for **Escherichia coli**, and *(b)* the complex medium described. Note how the complex medium is colored from the various organic extracts and digests that it contains. Photo credits: Cheryl L. Broadie and John Vercillo, Southern Illinois University at Carbondale.

4.2 Growth Media and Laboratory Culture (2 of 5)

- Classes of Culture Media
  - **Defined media**: exact chemical composition known
  - **Complex media**: composed of digests of microbial, animal, or plant products (**e.g.**, yeast and meat extracts)
  - **Selective medium**
    - contains compounds that selectively inhibit growth of some microbes but not others
  - **Differential medium**
    - contains an indicator, usually a dye, that detects particular metabolic reactions during growth

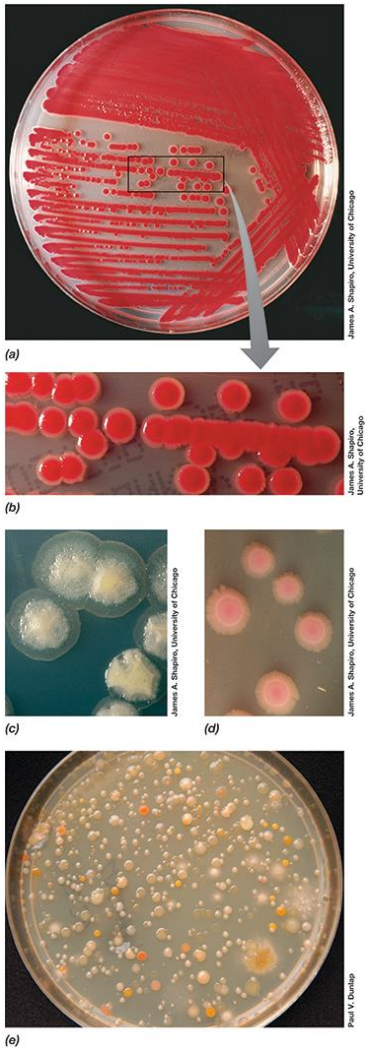
4.2 Growth Media and Laboratory Culture (3 of 5)

- Nutritional Requirements and Biosynthetic Capacity
  - Different microorganisms may have vastly different nutritional requirements
  - Necessary to understand physiology & nutritional requirements and supply with nutrients in proper form and amount

4.2 Growth Media and Laboratory Culture (4 of 5)

- Laboratory Culture
  - Either liquid or solid (Figure 4.2)
    - Solid media are prepared by addition of the gelling agent **agar** to liquid media.
    - When grown on solid media, cells form isolated masses (**colonies**).
    - **Colony morphology** (visible characteristics)
      - Sometimes used to identify microorganisms
      - Routinely used to determine if a culture is pure (one microbe), contaminated, or mixed

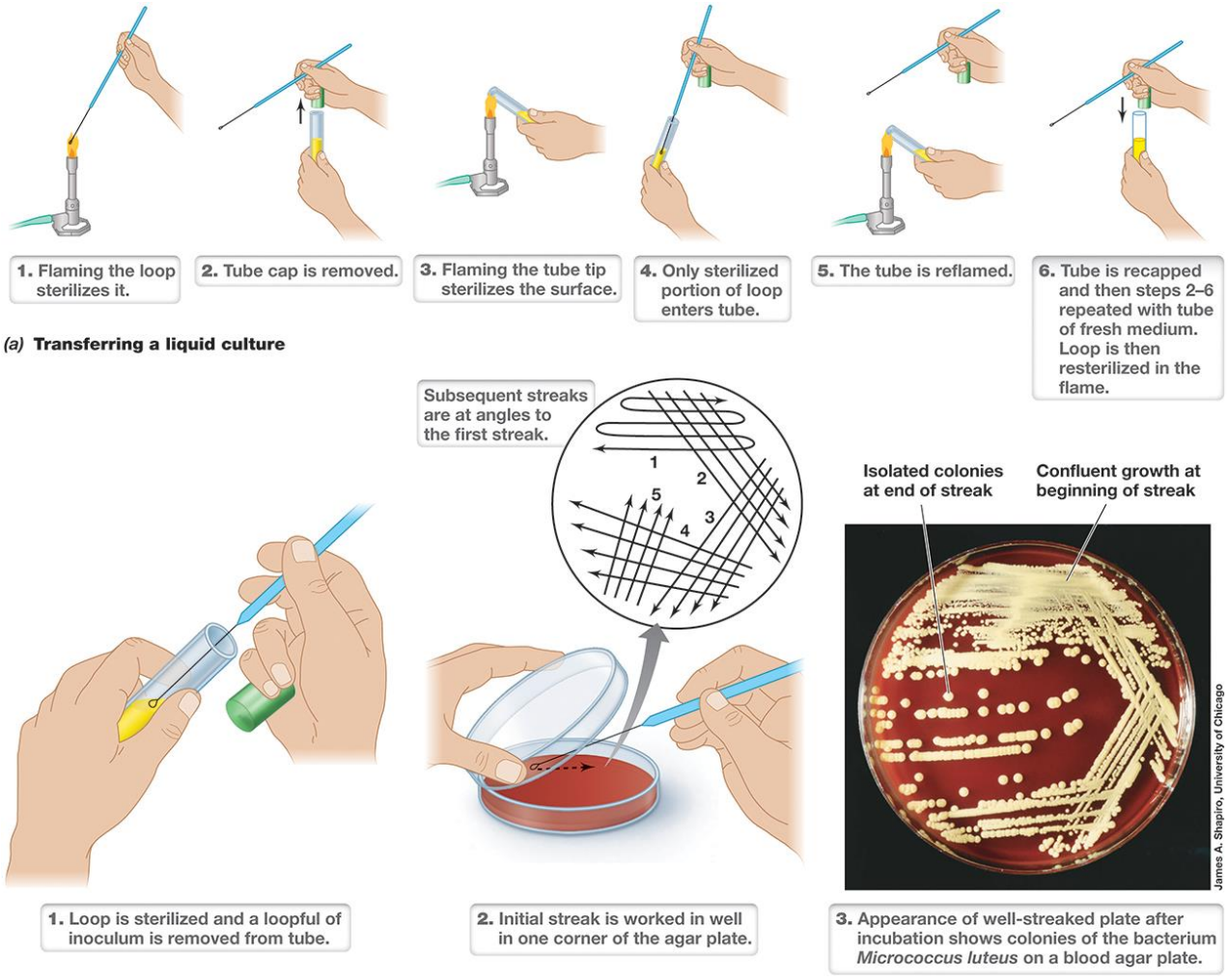
Figure 4.2 Bacterial Colonies



4.2 Growth Media and Laboratory Culture (5 of 5)

- Laboratory Culture
  - **Aseptic technique** (transfer without contamination) (Figure 4.3)
  - Airborne contaminants everywhere
  - Pure cultures containing a single microbe usually require **streak plate** technique with **inoculating loop**

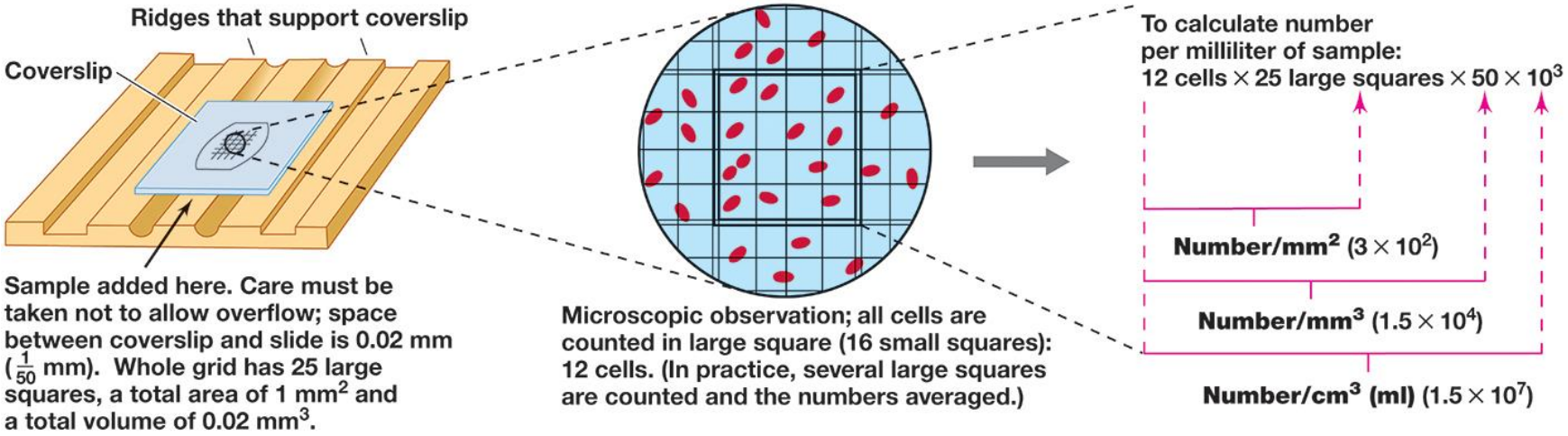
Figure 4.3 Aseptic Technique



4.3 Microscopic Counts of Microbial Cell Numbers (1 of 2)

- Total Cell Count
  - **Microscopic cell count:** observing and enumerating cells present
  - Dried on slides or on liquid samples
  - Counting chambers with squares etched on a slide used for liquid samples (Figure 4.4)
  - Several limitations

Figure 4.4 Direct Microscopic Counting Procedure Using the Petroff–Hausser Counting Chamber



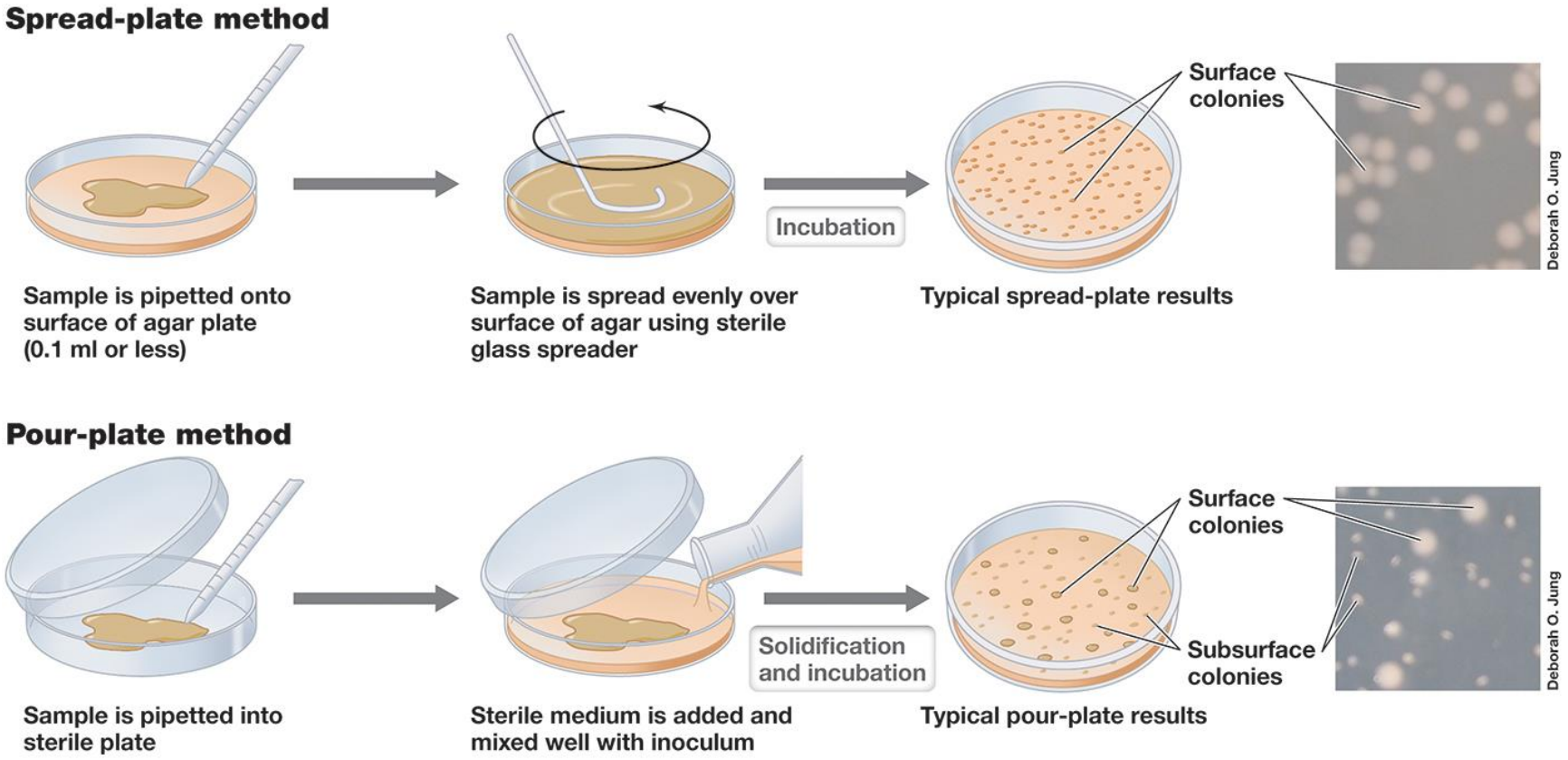
4.3 Microscopic Counts of Microbial Cell Numbers (2 of 2)

- Microscopic cell counts in microbial ecology
  - Often used on natural samples
  - Use stains to visualize and provide phylogenetic or metabolic properties
  - **e.g.**, DAPI binds DNA.
  - Other fluorescent stains differentiate live and dead cells.
  - **Phylogenetic stains** can determine proportions of **Bacteria** and **Archaea** in a sample.

4.4 Viable Counting of Microbial Cell Numbers (1 of 4)

- Viable (alive/living) counts: measurement of living, reproducing population
  - Two main ways to perform viable (plate) counts (Figure 4.5):
    - **spread-plate** method
    - **pour-plate** method
  - Count colonies on plates with 30–300 colonies
  - Reporting in **colony-forming units** instead of number of viable cells accounts for clumps.

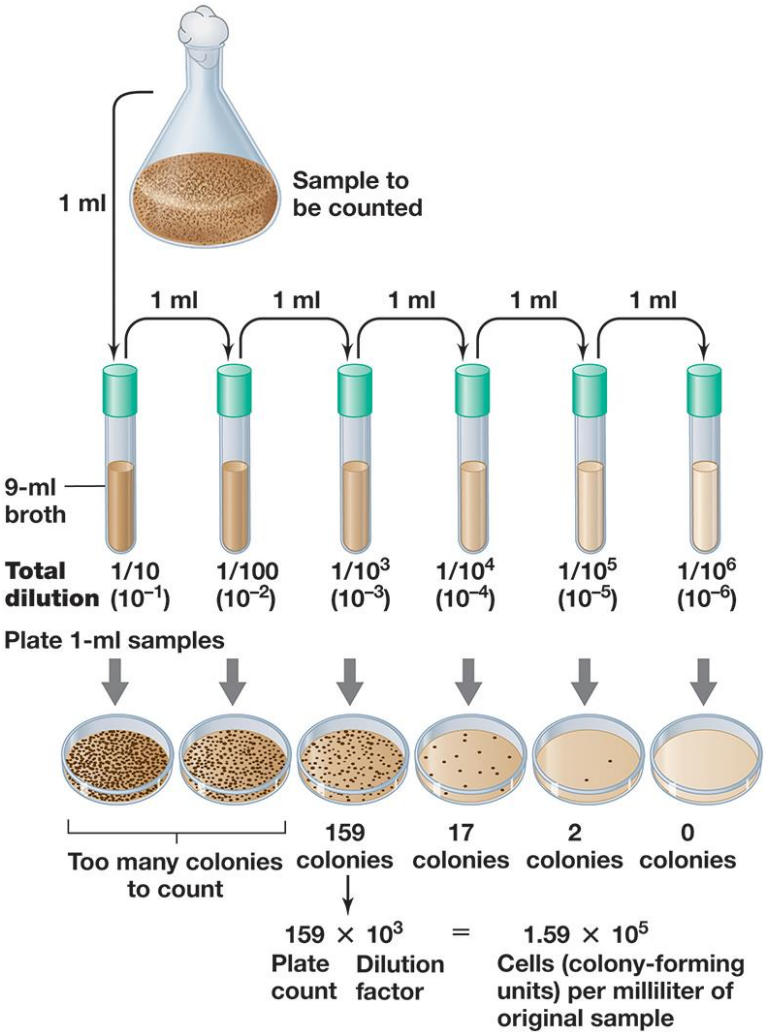
Figure 4.5 Two Methods for the Viable Count



4.4 Viable Counting of Microbial Cell Numbers (2 of 4)

- Diluting a Sample
  - Samples often contain thousands, millions, billions of viable cells
  - Ten-fold dilutions commonly used (Figure 4.6)
  - **Serial** (successive) dilutions needed for dense cultures

Figure 4.6 Procedure for Viable Counting Using Serial Dilutions of the Sample and the Pour-Plate Method



4.4 Viable Counting of Microbial Cell Numbers (3 of 4)

- Applications of the Plate Count
  - quick and easy
  - used in food, dairy, medical, and aquatic microbiology
  - high sensitivity
  - can target particular species in mixed samples (**e.g.**, **Staphylococcus**)
  - common in wastewater and other water analyses

4.4 Viable Counting of Microbial Cell Numbers (4 of 4)

- "The great plate count anomaly": Direct microscopic counts of natural samples reveal far more organisms than those recoverable on plates.
- Why is this?
  - Different organisms may have vastly different requirements for growth.
  - May underestimate actual by one to several orders of magnitude

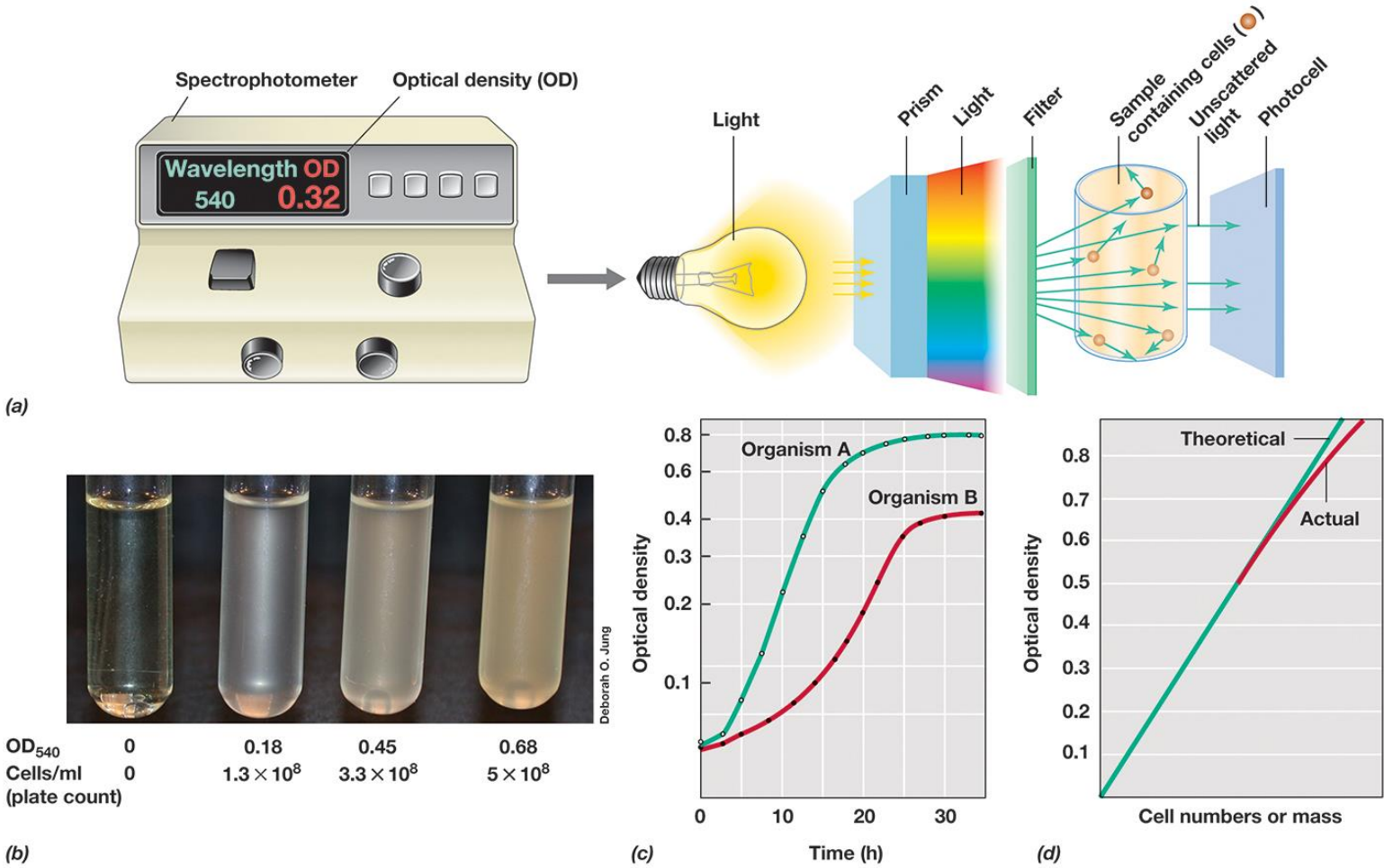
4.5 Turbidimetric Measures of Microbial Cell Numbers (1 of 3)

- Cell suspensions are **turbid** (cloudy) because cells scatter light.
- More cells, more light scattered, more turbid suspension
- **Turbidity measurements** are rapid, widely used for estimates

4.5 Turbidimetric Measures of Microbial Cell Numbers (2 of 3)

- Optical Density and Its Relationship to Cell Numbers
  - Measured with a spectrophotometer (Figure 4.7)
  - Unit is **optical density** (**OD**) at specified wavelength (**e.g.**, OD_{540} for measurements at 540 nm [green light]).
  - For unicellular organisms, OD is proportional to cell number within limits.
  - To relate a direct cell count to a turbidity value, a standard curve must first be established.

Figure 4.7 Turbidity Measurements of Microbial Growth



4.5 Turbidimetric Measures of Microbial Cell Numbers (3 of 3)

- Other Issues with Turbidimetric Growth Estimates
  - quick and easy to perform
  - typically do not require destruction or significant disturbance of sample
  - Same sample can be checked repeatedly.
  - sometimes problematic (**e.g.**, microbes that form clumps or biofilms in liquid medium)