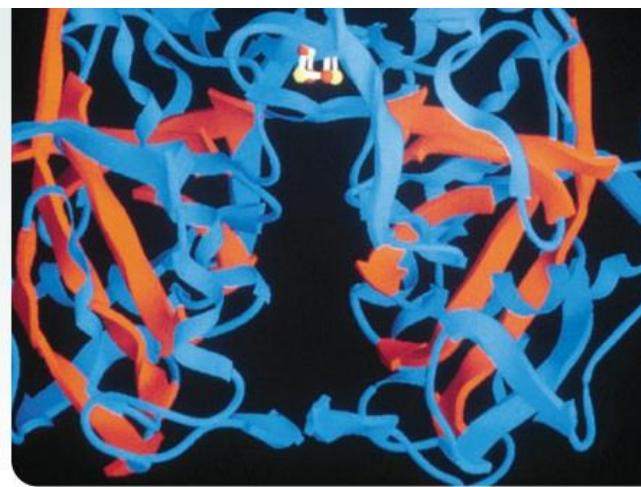


# 10

## Metabolism:

### The Use of Energy in Biosynthesis



The nitrogenase Fe protein's subunits are arranged like a pair of butterfly wings. Nitrogenase consists of the Fe protein and the MoFe protein; it catalyzes the reduction of atmospheric nitrogen during nitrogen fixation.

#### PREVIEW

- In anabolism, cells use free energy to construct more complex molecules and structures from smaller, simpler precursors.
- Biosynthetic pathways are organized to optimize efficiency by conserving biosynthetic raw materials and energy. This is accomplished in a number of ways, including the use of amphibolic pathways that function in both catabolic and anabolic directions. Some key reactions in amphibolic pathways require two enzymes: one for the catabolic reaction and another for the anabolic reaction.
- Precursor metabolites are carbon skeletons that serve as the starting substrates for biosynthetic pathways. They are intermediates of the central metabolic pathways.
- Four different pathways for CO<sub>2</sub> fixation have been identified in microorganisms. The most commonly used pathway is the Calvin cycle. All CO<sub>2</sub>-fixation pathways consume ATP and reducing power (e.g., NADPH).
- Gluconeogenesis is used to synthesize glucose from noncarbohydrate organic molecules. Glucose and other hexoses serve as precursor metabolites for the synthesis of other sugars and polysaccharides. The synthesis of the polysaccharide peptidoglycan is particularly complex, requiring many steps and occurring at several locations in the cell.
- Many of the precursor metabolites are used in amino acid biosynthetic pathways. The carbon skeletons are remodeled and amended by the addition of nitrogen and sometimes sulfur. Many amino acid biosynthetic pathways are branched. Thus a single precursor metabolite can produce a family of related amino acids. Anaplerotic reactions ensure that an adequate supply of precursor metabolites is available for amino acid biosynthesis.
- Certain amino acids and precursor metabolites contribute to the synthesis of nucleotides. Phosphorus assimilation is also required.
- The acetyl-CoA and malonyl-CoA pathways synthesize fatty acids. These pathways are not amphibolic, as they only function in the synthesis of lipids, not in their degradation.

*Biological structures are almost always constructed in a hierarchical manner, with subassemblies acting as important intermediates en route from simple starting molecules to the end products of organelles, cells, and organisms.*

—W. M. Becker and D. W. Deamer

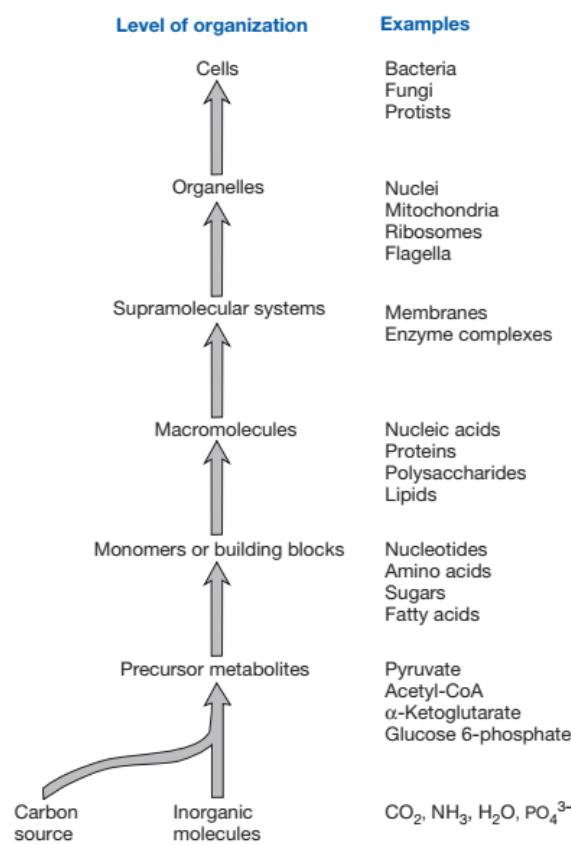
**A**s chapter 9 makes clear, microorganisms can obtain energy in many ways. Much of this energy is used in anabolism. During anabolism, a microorganism begins with simple inorganic molecules and a carbon source and constructs ever more complex molecules until new organelles and cells arise (**figure 10.1**). A microbial cell must manufacture many different kinds of molecules; here, we discuss the synthesis of only the most important types of cell constituents.

In this chapter we begin with a general introduction to anabolism and the role played by the precursor metabolites in biosynthetic pathways. We then focus on CO<sub>2</sub> fixation and the synthesis of carbohydrates, amino acids, purines and pyrimidines, and lipids. Because protein and nucleic acid synthesis is so significant and complex, the polymerization reactions that yield these macromolecules is described separately in chapter 11.

Anabolism is the creation of order. Because a cell is highly ordered and immensely complex, a lot of energy is required for biosynthesis. This is readily apparent from estimates of the biosynthetic capacity of rapidly growing *Escherichia coli* (**table 10.1**). Although most ATP dedicated to biosynthesis is employed in protein synthesis, ATP is also used to make other cell constituents.

It is intuitively obvious why rapidly growing cells need a large supply of ATP. But even nongrowing cells need energy for the biosynthetic processes they carry out. This is because nongrowing cells continuously degrade and resynthesize cellular molecules during a process known as **turnover**. Thus cells are never the same from one instant to the next. In addition, many nongrowing cells use energy to synthesize enzymes and other substances for release into

their surroundings. Clearly, metabolism must be carefully regulated if the rate of turnover is to be balanced by the rate of biosynthesis. It must also be regulated in response to a microbe's environment. Some of the mechanisms of metabolic regulation have already been introduced in chapter 8; others are discussed in chapter 12.



**Figure 10.1 The Construction of Cells.** The biosynthesis of prokaryotic and eukaryotic cell constituents. Biosynthesis is organized in levels of ever greater complexity.

## 10.1 PRINCIPLES GOVERNING BIOSYNTHESIS

Biosynthetic metabolism generally follows certain patterns and is shaped by a few basic principles. Six of these are now briefly discussed.

1. The construction of large **macromolecules** (complex molecules) from a few simple structural units (**monomers**) saves much genetic storage capacity, biosynthetic raw material, and energy. A consideration of protein synthesis clarifies this. Proteins—whatever size, shape, or function—are made of only 20 common amino acids joined by peptide bonds. Different proteins simply have different amino acid sequences but not new and dissimilar amino acids. Suppose that proteins were composed of 40 different amino acids instead of 20. The cell would then need the enzymes to manufacture twice as many amino acids (or would have to obtain the extra amino acids in its diet). Genes would be required for the extra enzymes, and the cell would have to invest raw materials and energy in the synthesis of these additional genes, enzymes, and amino acids. Clearly the use of a few monomers linked together by a single type of covalent bond makes the synthesis of macromolecules a highly efficient process. [Proteins and amino acids \(appendix I\)](#)
2. The use of many of the same enzymes for both catabolic and anabolic processes saves additional materials and energy. For example, most glycolytic enzymes are involved in both the synthesis and the degradation of glucose.
3. The use of separate enzymes to catalyze the two directions of a single step in an amphibolic pathway allows independent regulation of catabolism and anabolism (**figure 10.2**). Thus catabolic and anabolic pathways are never identical although many enzymes are shared. Although this is discussed in more detail in sections 8.8 through 8.10, note that the regulation of anabolism is somewhat different from that of catabolism. Both types of pathways can be regulated by their end products as well as by the concentrations of ATP, ADP, AMP, and  $\text{NAD}^+$ . Nevertheless, end product regulation generally assumes more importance in anabolic pathways.
4. To synthesize molecules efficiently, anabolic pathways must operate irreversibly in the direction of biosynthesis. Cells can

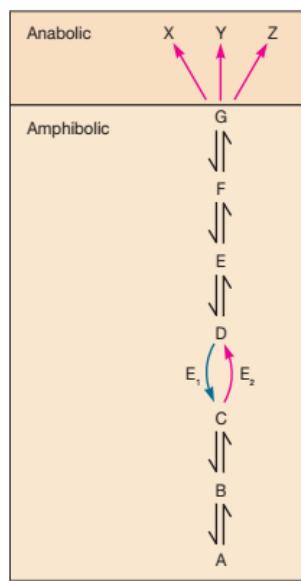
**Table 10.1 Biosynthesis in *Escherichia coli***

Cell Constituent	Number of Molecules per Cell <sup>a</sup>	Molecules Synthesized per Second	Molecules of ATP Required per Second for Synthesis
DNA	1 <sup>b</sup>	0.00083	60,000
RNA	15,000	12.5	75,000
Polysaccharides	39,000	32.5	65,000
Lipids	15,000,000	12,500.0	87,000
Proteins	1,700,000	1,400.0	2,120,000

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<sup>a</sup>Estimates for a cell with a volume of  $2.25 \mu\text{m}^3$ , a total weight of  $1 \times 10^{-12}\text{g}$ , a dry weight of  $2.5 \times 10^{-13}\text{g}$ , and a 20 minute cell division cycle.

<sup>b</sup>It should be noted that bacteria can contain multiple copies of their genomic DNA.



**Figure 10.2 A Hypothetical Biosynthetic Pathway.** The routes connecting G with X, Y, and Z are purely anabolic because they are used only for synthesis of the end products. The pathway from A to G is amphibolic—that is, it has both catabolic and anabolic functions. Most reactions are used in both roles; however, the interconversion of C and D is catalyzed by two separate enzymes,  $E_1$  (catabolic) and  $E_2$  (anabolic).

achieve this by connecting some biosynthetic reactions to the breakdown of ATP and other nucleoside triphosphates. When these two processes are coupled, the free energy made available during nucleoside triphosphate breakdown drives the biosynthetic reaction to completion. [The role of ATP in metabolism \(section 8.5\)](#)

- 5.** Compartmentation in eucaryotic cells—that is, localization of biosynthetic pathways into certain cellular compartments and catabolic pathways into others—makes it easier for catabolic and anabolic pathways to operate simultaneously yet independently. For example, fatty acid biosynthesis occurs in the cytoplasmic matrix, whereas fatty acid oxidation takes place within the mitochondrion.

**6.** Finally, anabolic and catabolic pathways often use different cofactors. Usually catabolic oxidations produce NADH, a substrate for electron transport. In contrast, when an electron donor is needed during biosynthesis, NADPH rather than NADH normally serves as the donor. Fatty acid metabolism provides a second example. Fatty acyl-CoA molecules are oxidized to generate energy, whereas fatty acid synthesis involves acyl carrier protein thioesters (see p. 242).

After macromolecules have been constructed from simpler precursors, they are assembled into larger, more complex structures such as supramolecular systems and organelles (figure 10.1). Macromolecules normally contain the necessary information to

form supramolecular systems spontaneously in a process known as **self-assembly**. For example, ribosomes are large assemblages of many proteins and ribonucleic acid molecules, yet they arise by the self-assembly of their components without the involvement of extra factors.

1. Define anabolism, turnover, and self-assembly.
  2. Summarize the six principles by which biosynthetic pathways are organized.

## 10.2 THE PRECURSOR METABOLITES

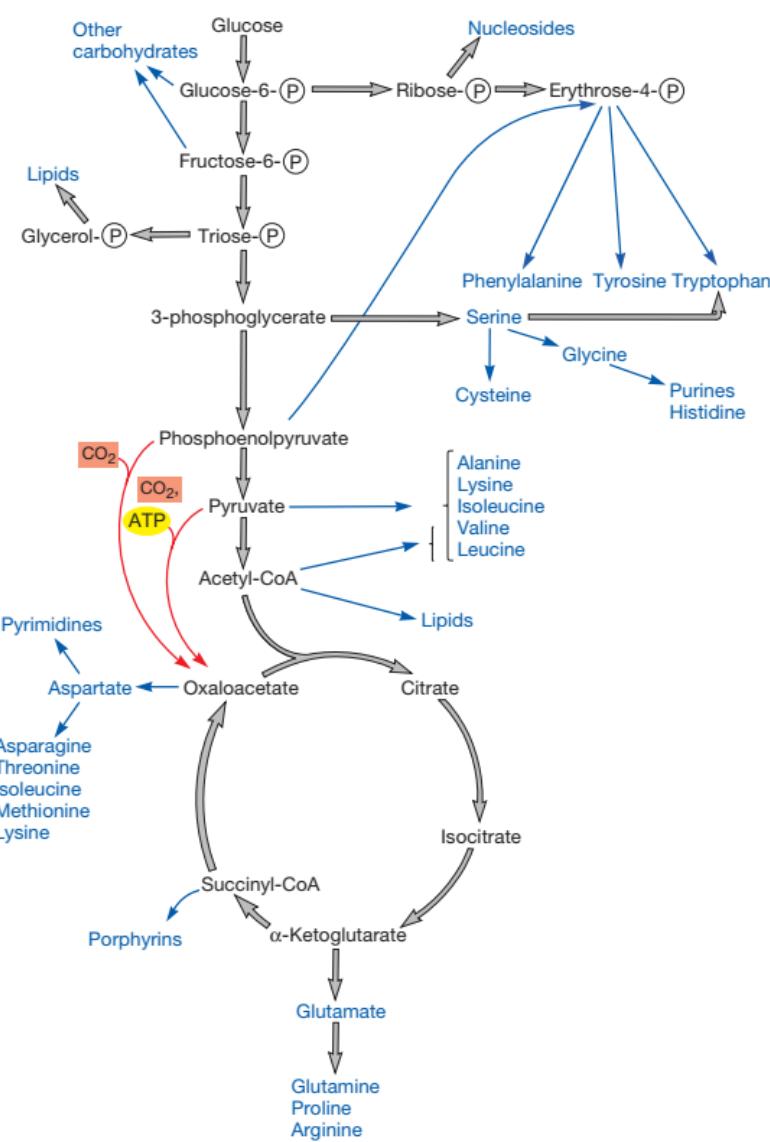
The generation of the **precursor metabolites** is a critical step in anabolism. Precursor metabolites are **carbon skeletons** (i.e., carbon chains) used as the starting substrates for the synthesis of monomers and other building blocks needed for the synthesis of macromolecules. Precursor metabolites are referred to as carbon skeletons because they are molecules that lack functional moieties such as amino and sulfhydryl groups; these are added during the biosynthetic process. The precursor metabolites and their use in biosynthesis are shown in **figure 10.3**. Several things should be noted in this figure. First, all the precursor metabolites are intermediates of the glycolytic pathways (Embden-Meyerhof pathway or the Entner-Doudoroff pathway, and the pentose phosphate pathway) and the tricarboxylic acid (TCA) cycle. Therefore, these pathways play a central role in metabolism and are often referred to as the **central metabolic pathways**. Note, too, that most of the precursor metabolites are used for synthesis of amino acids and nucleotides.

From careful examination of figure 10.3, it should be clear that if an organism is a chemoorganotroph using glucose as its energy, electron, and carbon source, it generates the precursor metabolites as it generates ATP and reducing power. But what if the chemoorganotroph is using an amino acid as its sole source of carbon, electrons, and energy? And what about autotrophs? How do they generate precursor metabolites from  $\text{CO}_2$ , their carbon source? Heterotrophs growing on something other than glucose degrade that carbon and energy source into one or more intermediates of the central metabolic pathways. From there, they can generate the remaining precursor metabolites. Autotrophs must first convert  $\text{CO}_2$  into organic carbon from which they can generate the precursor metabolites. Many of the reactions that autotrophs use to generate the precursor metabolites are reactions of the central metabolic pathways, operating either in the catabolic direction or in the anabolic direction. Thus the central metabolic pathways are important to the anabolism of both heterotrophs and autotrophs.

We begin our discussion of anabolism by first considering CO<sub>2</sub> fixation by autotrophs. Once CO<sub>2</sub> is converted to organic carbon, the synthesis of other precursor metabolites, amino acids, nucleotides, and additional building blocks is essentially the same in both autotrophs and heterotrophs. Recall that the precursor metabolites provide the carbon skeletons for the synthesis of other important organic molecules. In the process of transforming a precursor metabolite into an amino acid or a nucleotide, the carbon skeleton is modified in a number of ways, including the

**Figure 10.3** The Organization of Anabolism.

Biosynthetic products (in blue) are derived from precursor metabolites, which are intermediates of amphibolic pathways. Two major anaplerotic reactions are shown in red. These reactions ensure an adequate supply of TCA cycle-derived precursor metabolites and are especially important to fermentative organisms, in which only certain TCA cycle reactions operate.



addition of nitrogen, phosphorus, and sulfur. Thus as we discuss the synthesis of monomers from precursor metabolites, we will also address the assimilation of nitrogen, sulfur, and phosphorus.

### 10.3 THE FIXATION OF CO<sub>2</sub> BY AUTOTROPHS

Autotrophs use CO<sub>2</sub> as their sole or principal carbon source and the reduction and incorporation of CO<sub>2</sub> requires much energy. Many autotrophs obtain energy by trapping light during photosynthesis, but some derive energy from the oxidation of reduced inorganic electron donors. Autotrophic CO<sub>2</sub> fixation is crucial to life on

Earth because it provides the organic matter on which heterotrophs depend. **Chemolithotrophy** (section 9.11); **Phototrophy** (section 9.12)

Four different CO<sub>2</sub>-fixation pathways have been identified in microorganisms. Most autotrophs use the **Calvin cycle**, which is also called the Calvin-Benson cycle or the reductive pentose phosphate cycle. The Calvin cycle is found in photosynthetic eukaryotes and most photosynthetic bacteria. It is absent in some obligatory anaerobic and microaerophilic bacteria. Autotrophic archaea also use an alternative pathway for CO<sub>2</sub> fixation. We consider the Calvin cycle first, and then briefly introduce the three other CO<sub>2</sub>-fixation pathways.

### The Calvin Cycle

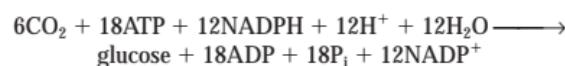
The Calvin cycle is also called the reductive pentose phosphate cycle because it is essentially the reverse of the pentose phosphate pathway. Thus many of the reactions are similar, in particular the sugar transformations. The reactions of the Calvin cycle occur in the chloroplast stroma of eucaryotic microbial autotrophs. In cyanobacteria, some nitrifying bacteria, and thiobacilli (sulfur-oxidizing chemolithotrophs), the Calvin cycle is associated with inclusion bodies called **carboxysomes**. These are polyhedral structures that contain the enzyme critical to the Calvin cycle and may be the site of CO<sub>2</sub> fixation. [The breakdown of glucose to pyruvate: The pentose-phosphate pathway \(section 9.3\)](#)

The Calvin cycle is divided into three phases: carboxylation phase, reduction phase, and regeneration phase ([figure 10.4](#) and appendix II). During the carboxylation phase, the enzyme **ribulose-1,5-bisphosphate carboxylase**, also called ribulose bisphosphate carboxylase/oxygenase (**Rubisco**), catalyzes the addition of CO<sub>2</sub> to the 5-carbon molecule ribulose-1,5-bisphosphate (RuBP), forming a six-carbon intermediate that rapidly and spontaneously splits into two molecules of 3-phosphoglycerate (PGA) ([figure 10.5](#)). Note that PGA is an intermediate of the Embden-Meyerhof pathway (EMP), and in the reduction phase, PGA is reduced to glyceraldehyde 3-phosphate by two reactions that are essentially the reverse of two EMP reactions. The difference is that the Calvin cycle enzyme glyceraldehyde 3-phosphate dehydrogenase uses NADP<sup>+</sup> rather than NAD<sup>+</sup> (compare figures 10.4 and 9.5). Finally, in the regeneration phase, RuBP is regenerated, so that the cycle can repeat. In addition, this phase produces carbohydrates such as glyceraldehyde 3-phosphate, fructose 6-phosphate, and glucose 6-phosphate, all of which are precursor metabolites (figure 10.4). This portion of the cycle is similar to the pentose phosphate pathway and involves the transketolase and transaldolase reactions.

To synthesize fructose 6-phosphate or glucose 6-phosphate from CO<sub>2</sub>, the cycle must operate six times to yield the desired hexose and reform the six RuBP molecules.



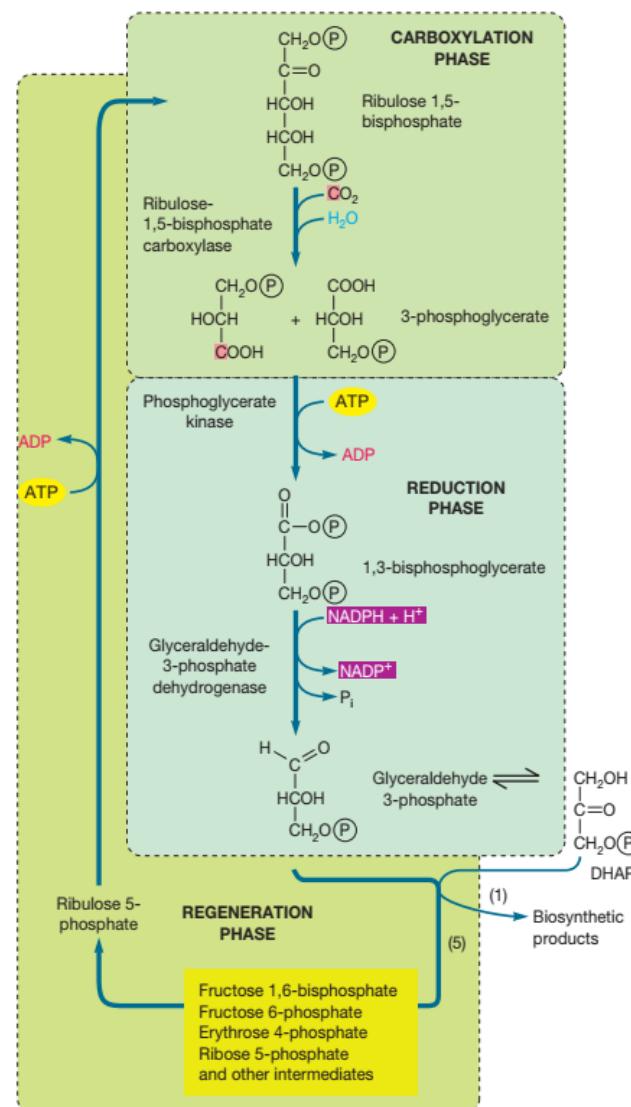
The incorporation of one CO<sub>2</sub> into organic material requires three ATPs and two NADPHs. The formation of glucose from CO<sub>2</sub> may be summarized by the following equation.



The precursor metabolites formed in the Calvin cycle can then be used to synthesize other precursor metabolites and essential molecules, as described in sections 10.4 through 10.7.

### Other CO<sub>2</sub>-Fixation Pathways

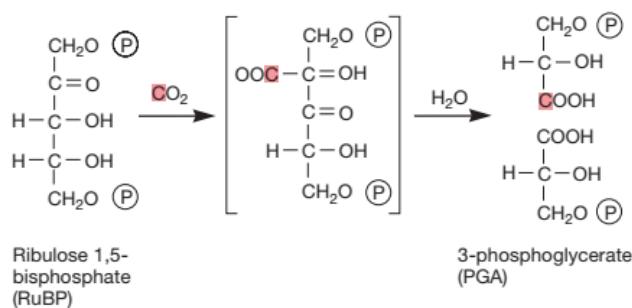
Certain bacteria and archaea fix CO<sub>2</sub> using the reductive TCA cycle, the 3-hydroxypropionate cycle, or the acetyl-CoA pathway. The **reductive TCA cycle** ([figure 10.6](#)) is used by some chemolithoautotrophs (e.g., *Thermoproteus* and *Sulfolobus*, two archaeal genera, and the bacterial genus *Aquifex*) and anoxygenic phototrophs such as *Chlorobium*, a green sulfur bacterium. The reductive TCA cycle



**Figure 10.4** The Calvin Cycle. This is an overview of the cycle with only the carboxylation and reduction phases in detail. Three ribulose 1,5-bisphosphates are carboxylated to give six 3-phosphoglycerates in the carboxylation phase. These are converted to six glyceraldehyde 3-phosphates, which can be converted to dihydroxyacetone phosphate (DHAP). Five of the six trioses (glyceraldehyde phosphate and dihydroxyacetone phosphate) are used to reform three ribulose 1,5-bisphosphates in the regeneration phase. The remaining triose is used in biosynthesis. The numbers in parentheses at the lower right indicate this carbon flow.

is so named because it runs in the reverse direction of the normal, oxidative TCA cycle (compare figures 10.6 and 9.9). A few archaeal genera and the green nonsulfur bacteria (another group of anoxygenic phototrophs) use the **3-hydroxypropionate cycle** to fix CO<sub>2</sub>.

**Figure 10.7** shows the cycle as it is thought to function in the green nonsulfur bacterium *Chloroflexus aurantiacus*. How its product, glyoxylate, is assimilated is unclear. Methanogens use portions of the **acetyl-CoA pathway** for carbon fixation; the pathway as it is used by *Methanobacterium thermoautotrophicum* is illustrated in **figure 10.8**. Both the acetyl-CoA pathway and methanogenesis involve the activity of a number of unusual enzymes and coenzymes. These are described in more detail in chapter 20. *Phylum Crenarchaeota* (section 20.2); *Aquificae* and *Thermotogae* (section 21.1); Photosynthetic bacteria (section 21.3)

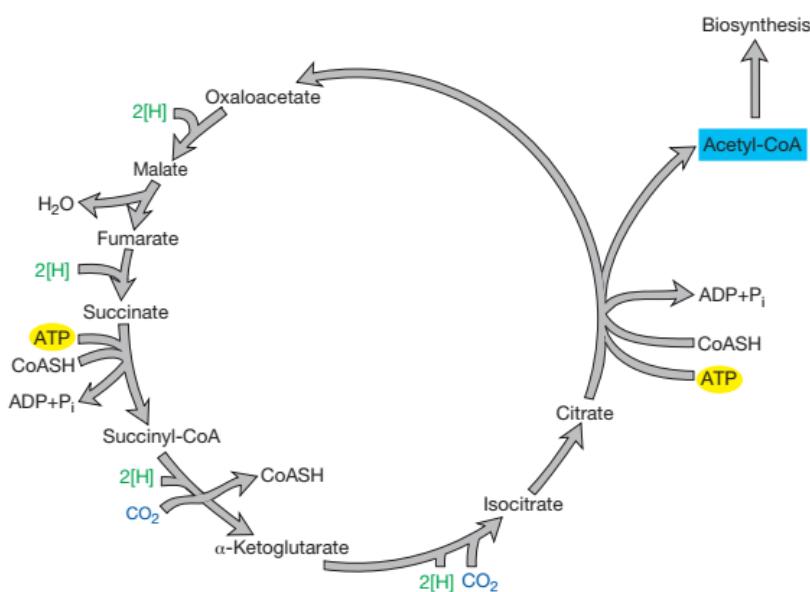


**Figure 10.5 The Ribulose 1,5-Bisphosphate Carboxylase Reaction.** This enzyme catalyzes the addition of carbon dioxide to ribulose 1,5-bisphosphate, forming an unstable intermediate, which then breaks down to two molecules of 3-phosphoglycerate.

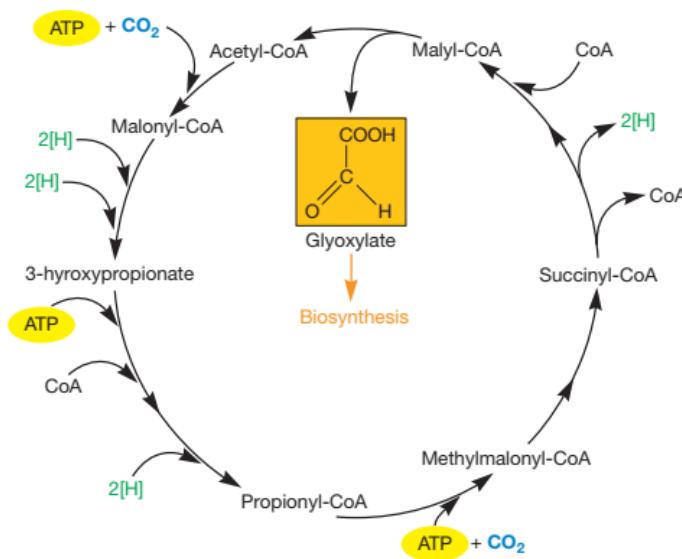
1. Briefly describe the three phases of the Calvin cycle. What other pathways are used to fix  $\text{CO}_2$ ?
2. Which two enzymes are specific to the Calvin cycle?

## 10.4 SYNTHESIS OF SUGARS AND POLYSACCHARIDES

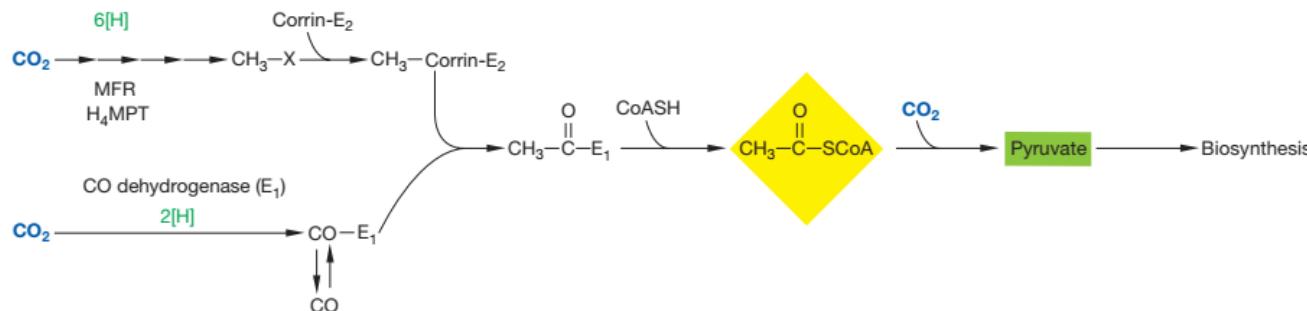
Autotrophs using  $\text{CO}_2$ -fixation processes other than the Calvin cycle and heterotrophs growing on carbon sources other than sugars must be able to synthesize glucose. The synthesis of glucose from noncarbohydrate precursors is called **gluconeogenesis**. The gluconeogenic pathway shares seven enzymes with the Embden-Meyerhof pathway. However, the two pathways are not identical (**figure 10.9**). Three glycolytic steps are irreversible in the cell: (1) the conversion of phosphoenolpyruvate to pyruvate, (2) the formation of fructose 1,6-bisphosphate from fructose 6-phosphate, and (3) the phosphorylation of glucose. These must be bypassed when the pathway is operating biosynthetically. For example, the formation of fructose 1,6-bisphosphate by phosphofructokinase is reversed by a different enzyme, fructose bisphosphatase, which hydrolytically removes a phosphate from fructose bisphosphate. Usually at least two enzymes are involved in the conversion of pyruvate to phosphoenolpyruvate (the reversal of the pyruvate kinase step).



**Figure 10.6 The Reductive TCA Cycle.** This cycle is used by green sulfur bacteria and some chemolithotrophic archaea to fix  $\text{CO}_2$ . The cycle runs in the opposite direction as the TCA cycle. ATP and reducing equivalents [H] power the reversal. In green sulfur bacteria, the reducing equivalents are provided by reduced ferredoxin. The product of this process is acetyl-CoA, which can be used to synthesize other organic molecules and precursor metabolites.



**Figure 10.7** The 3-Hydroxypropionate Pathway. This pathway functions in green nonsulfur bacteria, a group of anoxygenic phototrophs. The product of the cycle is glyoxylate, which is used in biosynthesis by mechanisms that have not been definitively elucidated.



**Figure 10.8** The Acetyl-CoA Pathway. Methanogens reduce two molecules of  $\text{CO}_2$ , each by a different mechanism, and combine them to form acetyl and then acetyl-CoA. Acetogenic bacteria use a slightly different version of the pathway.

### Synthesis of Monosaccharides

As can be seen in figure 10.9, gluconeogenesis synthesizes fructose 6-phosphate and glucose 6-phosphate. Once these two precursor metabolites have been formed, other common sugars can be manufactured. For example, mannose comes directly from fructose 6-phosphate by a simple rearrangement.

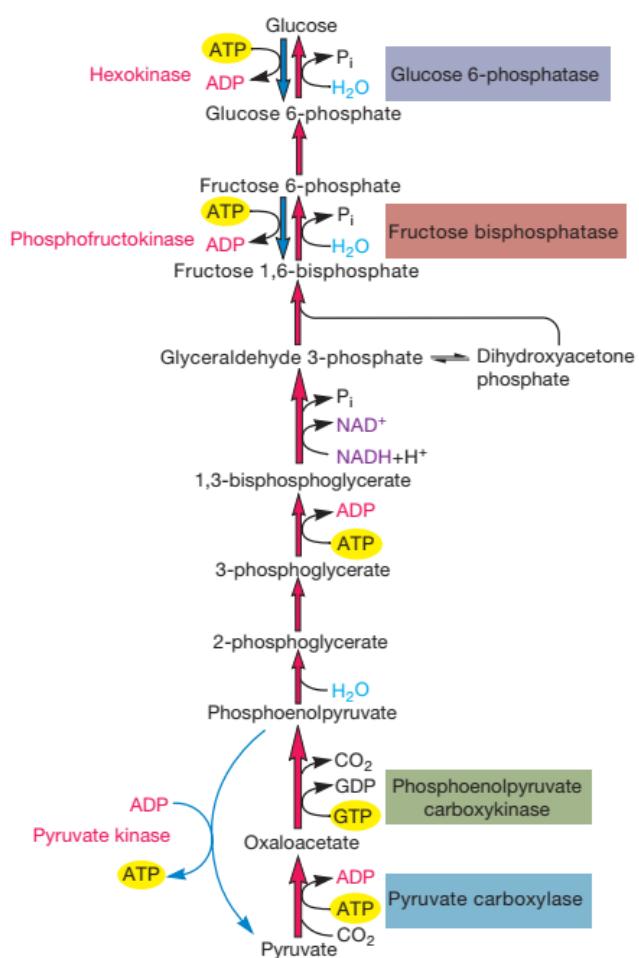


Several sugars are synthesized while attached to a nucleoside diphosphate. The most important nucleoside diphosphate sugar is **uridine diphosphate glucose (UDPG)**. Glucose is activated by attachment to the pyrophosphate of uridine diphosphate through a reaction with uridine triphosphate (figure 10.10). The UDP portion of UDPG is recognized by enzymes and carries glucose around the

cell for participation in enzyme reactions much like ADP bears phosphate in the form of ATP. UDP-galactose is synthesized from UDPG through a rearrangement of one hydroxyl group. A different enzyme catalyzes the synthesis of UDP-glucuronic acid through the oxidation of UDPG (figure 10.11).

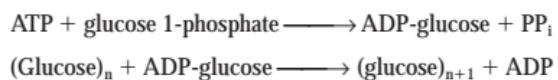
### Synthesis of Polysaccharides

Nucleoside diphosphate sugars also play a central role in the synthesis of polysaccharides such as starch and glycogen. Again, biosynthesis is not simply a direct reversal of catabolism. Glycogen and starch catabolism proceeds either by hydrolysis to form free sugars or by the addition of phosphate to these polymers with the production of glucose 1-phosphate. Nucleoside diphosphate sugars are not involved. In contrast, during the synthesis of



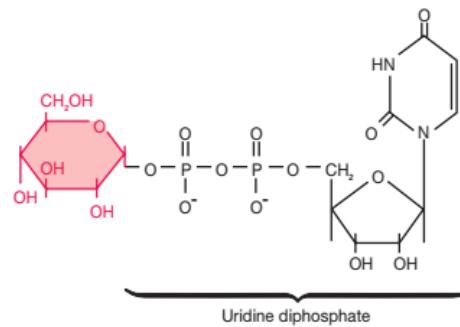
**Figure 10.9 Gluconeogenesis.** The gluconeogenic pathway used in many microorganisms. The names of the four enzymes catalyzing reactions different from those found in the Embden-Meyerhof pathway (EMP) are in shaded boxes. EMP steps are shown in blue for comparison.

glycogen and starch in bacteria and algae, adenosine diphosphate glucose is formed from glucose 1-phosphate and then donates glucose to the end of growing glycogen and starch chains.

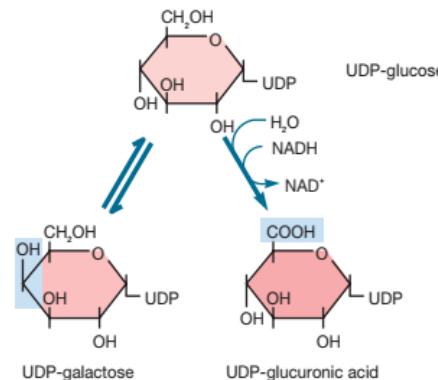


#### Synthesis of Peptidoglycan

Nucleoside diphosphate sugars also participate in the synthesis of peptidoglycan. Recall that peptidoglycan is a large, complex molecule consisting of long polysaccharide chains made of alternating *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) residues. Pentapeptide chains are attached to the NAM



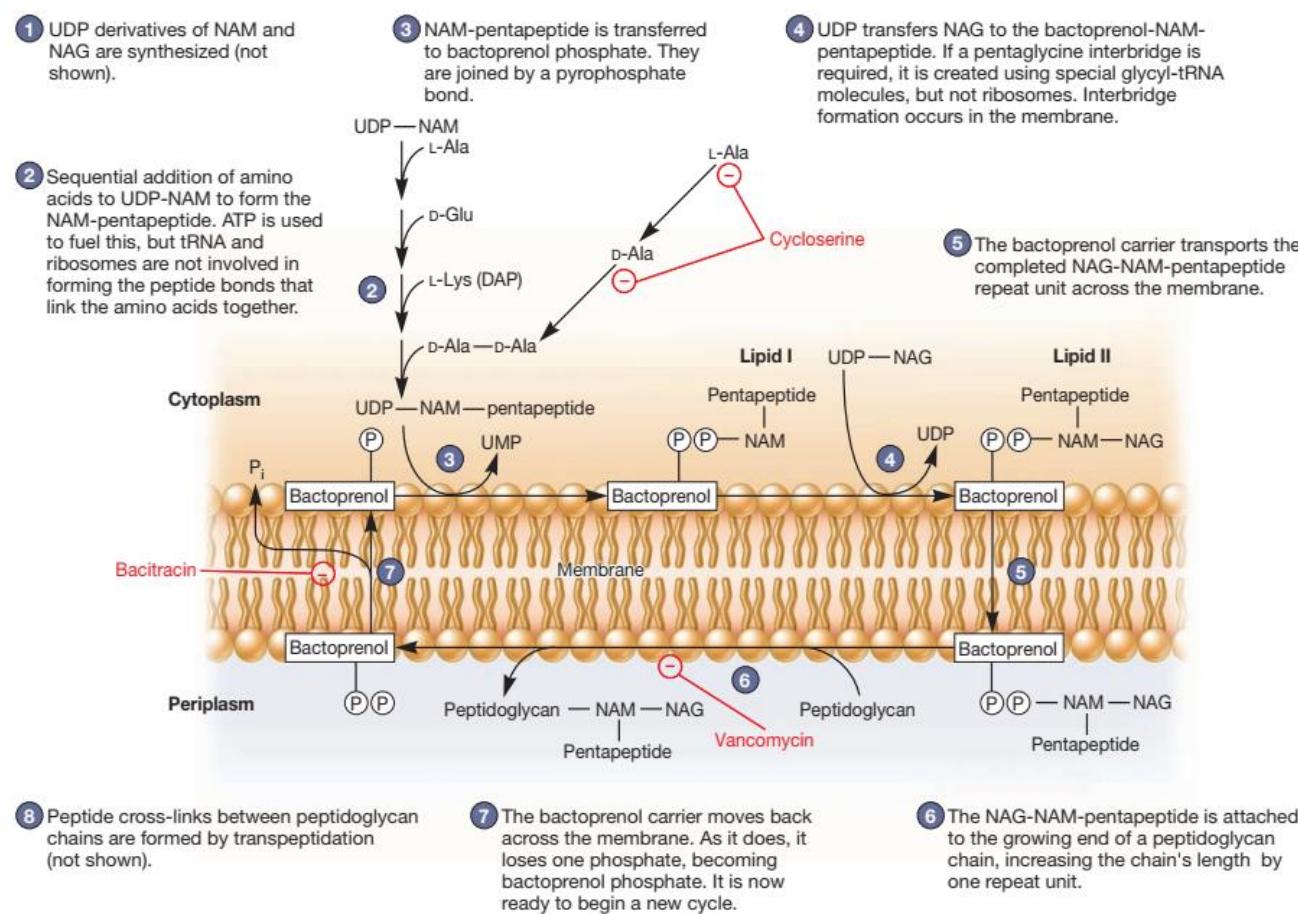
**Figure 10.10 Uridine Diphosphate Glucose.** Glucose is in color.



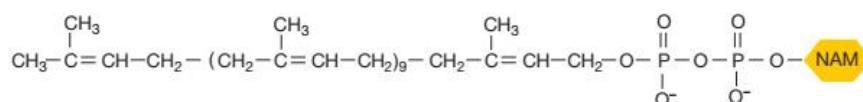
**Figure 10.11 Uridine Diphosphate Galactose and Glucuronate Synthesis.** The synthesis of UDP-galactose and UDP-glucuronic acid from UDP-glucose. Structural changes are indicated by blue boxes.

groups. The polysaccharide chains are connected through their pentapeptides or by interbridges (see figures 3.20 and 3.21). [The bacterial cell wall \(section 3.6\)](#)

Not surprisingly, such an intricate structure requires an equally intricate biosynthetic process, especially because some reactions occur in the cytoplasm, others in the membrane, and others in the periplasmic space. Peptidoglycan synthesis involves two carriers (**figure 10.12**). The first, uridine diphosphate (UDP) functions in the cytoplasmic reactions. In the first step of peptidoglycan synthesis, UDP derivatives of *N*-acetylmuramic acid and *N*-acetylglucosamine are formed. Amino acids are then added sequentially to UDP-NAM to form the pentapeptide chain. NAM-pentapeptide is then transferred to the second carrier, bactoprenol phosphate, which is located at the cytoplasmic side of the plasma membrane. The resulting intermediate is often called **Lipid I. Bactoprenol** (**figure 10.13**) is a 55-carbon alcohol and is linked to NAM by a pyrophosphate group. Next, UDP transfers NAG to the bactoprenol-NAM-pentapeptide complex (Lipid I), to generate **Lipid II**. This creates the peptidoglycan repeat unit. The



**Figure 10.12 Peptidoglycan Synthesis.** NAM is *N*-acetylmuramic acid and NAG is *N*-acetylglucosamine. The pentapeptide contains L-lysine in *Staphylococcus aureus* peptidoglycan, and diaminopimelic acid (DAP) in *E. coli*. Inhibition by bacitracin, cycloserine, and vancomycin also is shown. The numbers correspond to six of the eight stages discussed in the text. Stage eight is depicted in figure 10.14.

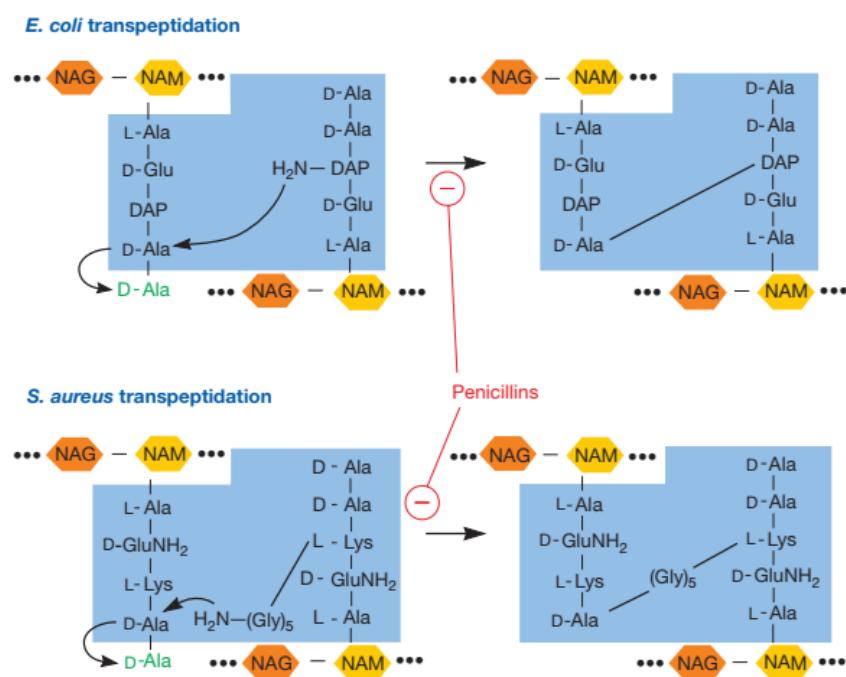


**Figure 10.13 Bactoprenol Pyrophosphate.** Bactoprenol pyrophosphate connected to *N*-acetylmuramic acid (NAM).

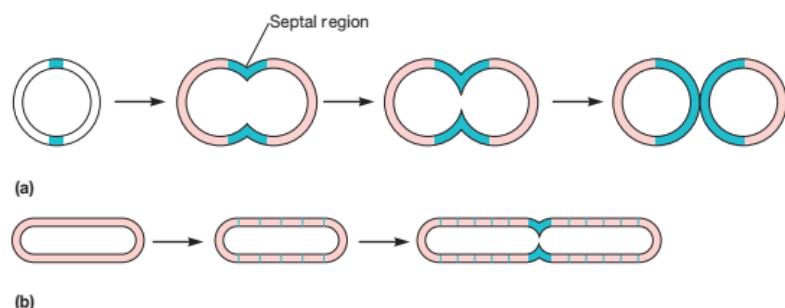
repeat unit is transferred across the membrane by bactoprenol. If the peptidoglycan unit requires an interbridge, it is added while the repeat unit is within the membrane. Bactoprenol stays within the membrane and does not enter the periplasmic space. After releasing the peptidoglycan repeat unit into the periplasmic space, bactoprenol-pyrophosphate is dephosphorylated and returns to the cytoplasmic side of the plasma membrane, where it can function in the next round of synthesis. Meanwhile, the peptidoglycan repeat unit is added to the growing end of a peptidoglycan chain. The final step in peptidoglycan synthesis is **transpeptidation**

(figure 10.14), which creates the peptide cross-links between the peptidoglycan chains. The enzyme that catalyzes the reaction removes the terminal D-alanine as the cross-link is formed.

To grow and divide efficiently, a bacterial cell must add new peptidoglycan to its cell wall in a precise and well-regulated way while maintaining wall shape and integrity in the presence of high osmotic pressure. Because the cell wall peptidoglycan is essentially a single, enormous network, the growing bacterium must be able to degrade it just enough to provide acceptor ends for the incorporation of new peptidoglycan units. It must also



**Figure 10.14 Transpeptidation.** The transpeptidation reactions in the formation of the peptidoglycans of *Escherichia coli* and *Staphylococcus aureus*.



**Figure 10.15 Wall Synthesis Patterns.** Patterns of new cell wall synthesis in growing and dividing bacteria. (a) Streptococci and some other gram-positive cocci. (b) Synthesis in rod-shaped bacteria (*E. coli*, *Salmonella*, *Bacillus*). The zones of growth are in turquoise. The actual situation is more complex than indicated because cells can begin to divide again before the first division is completed.

reorganize peptidoglycan structure when necessary. This limited peptidoglycan digestion is accomplished by enzymes known as **autolysins**, some of which attack the polysaccharide chains, while others hydrolyze the peptide cross-links. Autolysin inhibitors are produced to keep the activity of these enzymes under tight control.

Although the location and distribution of cell wall synthetic activity varies with species, there seem to be two general patterns (figure 10.15). Many gram-positive cocci (e.g., *Enterococcus faecalis* and *Streptococcus pyogenes*) have only one to a few

zones of growth. The principal growth zone is usually at the site of septum formation, and new cell halves are synthesized back-to-back. The second pattern of synthesis occurs in the rod-shaped bacteria *Escherichia coli*, *Salmonella*, and *Bacillus*. Active peptidoglycan synthesis occurs at the site of septum formation, but growth sites also are scattered along the cylindrical portion of the rod. Thus growth is distributed more diffusely in rod-shaped bacteria than in the streptococci. Synthesis must lengthen rod-shaped cells as well as divide them. Presumably this accounts for the differences in wall growth pattern. [The prokaryotic cell cycle \(section 6.1\)](#)

Because of the importance of peptidoglycan to cell wall structure and function, its synthesis is a particularly effective target for antimicrobial agents. Inhibition of any stage of synthesis weakens the cell wall and can lead to osmotic lysis. Many commonly used antibiotics interfere with peptidoglycan synthesis. For example, penicillin inhibits the transpeptidation reaction (figure 10.14), and bacitracin blocks the dephosphorylation of bactoprenol pyrophosphate (figure 10.12). [Antibacterial drugs \(section 34.4\)](#)

1. What is gluconeogenesis? Why is it important?
2. Describe the formation of mannose, galactose, starch, and glycogen. What are nucleoside diphosphate sugars? How do microorganisms use them?
3. Suppose that a microorganism is growing on a medium that contains amino acids but no sugars. In general terms, how would it synthesize the pentoses and hexoses it needs? How might it generate all the precursor metabolites it needs?
4. Diagram the steps involved in the synthesis of peptidoglycan and show where they occur in the cell. What are the roles of bactoprenol and UDP? What is unusual about the synthesis of the pentapeptide chain?
5. What is the function of autolysins in cell wall synthesis? Describe the patterns of peptidoglycan synthesis seen in gram-positive cocci and in rod-shaped bacteria such as *E. coli*.

## 10.5 SYNTHESIS OF AMINO ACIDS

Many of the precursor metabolites (figure 10.3) serve as starting substrates for the synthesis of amino acids. In the amino acid biosynthetic pathways, the carbon skeleton is remodeled and an amino group, and sometimes sulfur, are added. In this section, we first examine the mechanisms by which nitrogen and sulfur are assimilated and incorporated into amino acids. This is followed by a brief consideration of the organization of amino acid biosynthetic pathways.

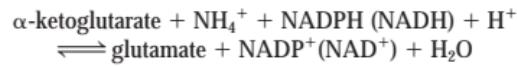
### Nitrogen Assimilation

Nitrogen is a major component not only of proteins, but of nucleic acids, coenzymes, and many other cell constituents as well. Thus the cell's ability to assimilate inorganic nitrogen is exceptionally important. Although nitrogen gas is abundant in the atmosphere, few microorganisms can reduce the gas and use it as a nitrogen source. Most must incorporate either ammonia or nitrate. We examine ammonia and nitrate assimilation first, and then briefly discuss nitrogen assimilation in microbes that fix N<sub>2</sub>.

#### Ammonia Incorporation

Ammonia nitrogen can be incorporated into organic material relatively easily and directly because it is more reduced than other forms of inorganic nitrogen. Ammonia is initially incorporated into carbon skeletons by one of two mechanisms: reductive amination or by the glutamine synthetase-glutamate synthase system. Once incorporated, the nitrogen can be transferred to other carbon skeletons by enzymes called transaminases. The major reductive amination pathway involves the formation of glutamate

from α-ketoglutarate, catalyzed in many bacteria and fungi by **glutamate dehydrogenase** when the ammonia concentration is high.

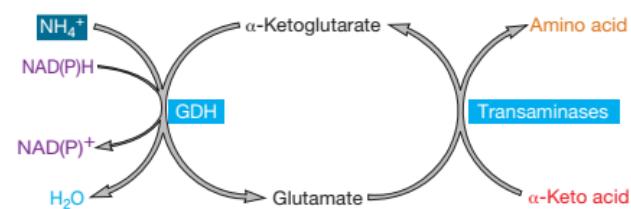


Once glutamate has been synthesized, the newly formed α-amino group can be transferred to other carbon skeletons by transamination reactions to form different amino acids. **Transaminases** possess the coenzyme pyridoxal phosphate, which is responsible for the amino group transfer. Microorganisms have a number of transaminases, each of which catalyzes the formation of several amino acids using the same amino acid as an amino group donor. When glutamate dehydrogenase works in cooperation with transaminases, ammonia can be incorporated into a variety of amino acids (**figure 10.16**).

The **glutamine synthetase-glutamate synthase (GS-GOGAT) system** is observed in *E. coli*, *Bacillus megaterium*, and other bacteria (**figure 10.17**). It functions when ammonia levels are low. Incorporation of ammonia by this system begins when ammonia is used to synthesize glutamine from glutamate in a reaction catalyzed by **glutamine synthetase** (**figure 10.18**). Then the amide nitrogen of glutamine is transferred to α-ketoglutarate to generate a new glutamate molecule. This reaction is catalyzed by **glutamate synthase**. Because glutamate acts as an amino donor in transaminase reactions, ammonia may be used to synthesize all common amino acids when suitable transaminases are present.

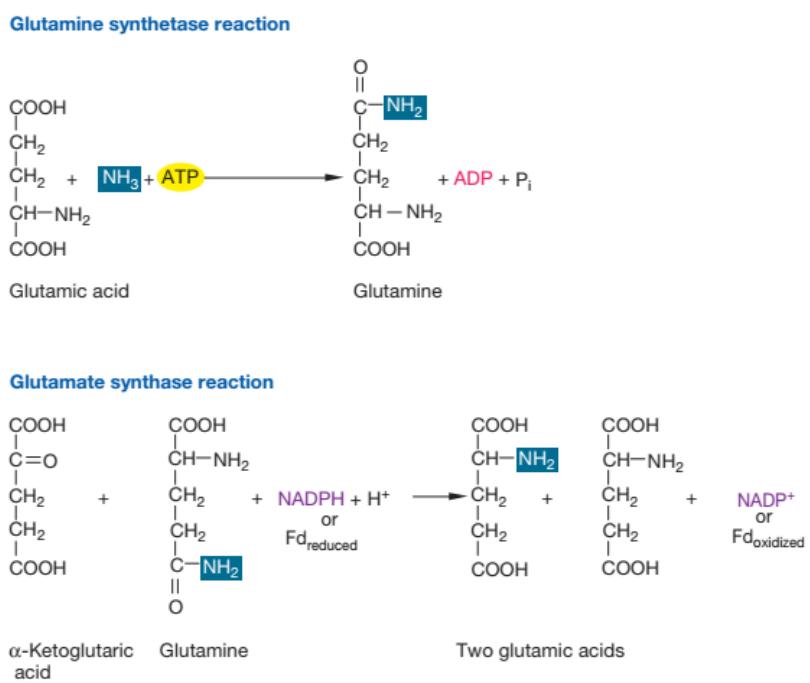
#### Assimilatory Nitrate Reduction

The nitrogen in nitrate (NO<sub>3</sub><sup>-</sup>) is much more oxidized than that in ammonia. Therefore nitrate must first be reduced to ammonia before the nitrogen can be converted to an organic form. This reduction of nitrate is called **assimilatory nitrate reduction**, which is not the same as that occurring during anaerobic respiration (dissimilatory nitrate reduction). In assimilatory nitrate reduction, nitrate is incorporated into organic material and does not participate in energy generation. The process is widespread among bacteria, fungi, and photosynthetic protists. [Anaerobic respiration \(section 9.6\); Biogeochemical cycling: Nitrogen cycle \(section 27.2\)](#)

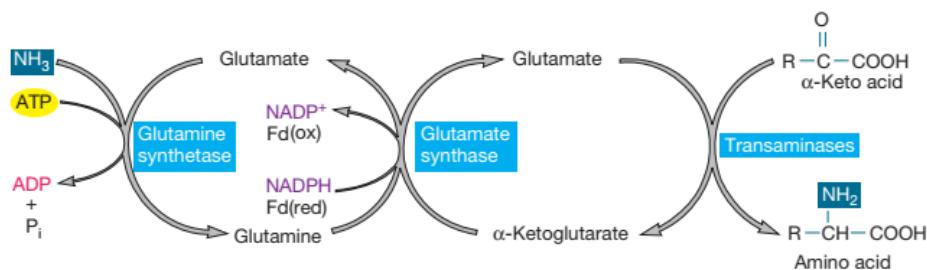


**Figure 10.16** The Ammonia Assimilation Pathway.

Ammonia assimilation by use of glutamate dehydrogenase (GDH) and transaminases. Either NADP- or NAD-dependent glutamate dehydrogenases may be involved. This route is most active at high ammonia concentrations.



**Figure 10.17 Glutamine Synthetase and Glutamate Synthase.** The glutamine synthetase and glutamate synthase reactions involved in ammonia assimilation. Some glutamine synthases use NADPH as an electron source; others use reduced ferredoxin (Fd). The nitrogen being incorporated and transferred is shown in turquoise.



**Figure 10.18 Ammonia Incorporation Using Glutamine Synthetase and Glutamate Synthase.** This route is effective at low ammonia concentrations.

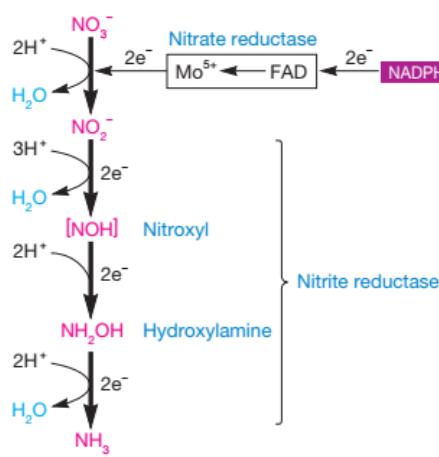
Assimilatory nitrate reduction takes place in the cytoplasm in bacteria. The first step in nitrate assimilation is its reduction to nitrite by **nitrate reductase**, an enzyme that contains both FAD and molybdenum (figure 10.19). NADPH is the electron source.



Nitrite is next reduced to ammonia with a series of two electron additions catalyzed by **nitrite reductase** and possibly other enzymes. The ammonia is then incorporated into amino acids by the routes already described.

#### Nitrogen Fixation

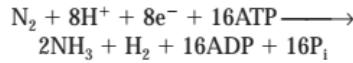
The reduction of atmospheric gaseous nitrogen to ammonia is called **nitrogen fixation**. Because ammonia and nitrate levels often are low and only a few bacteria and archaea can carry out nitrogen fixation (eucaryotic cells completely lack this ability), the rate of this process limits plant growth in many situations. Nitrogen fixation occurs in (1) free-living bacteria and archaea (e.g., *Azotobacter*, *Klebsiella*, *Clostridium*, and *Methanococcus*), (2) bacteria living in symbiotic association with plants such as legumes (*Rhizobium*), and (3) cyanobacteria (*Nostoc*, *Anabaena*, and *Trichodesmium*). The biological aspects of nitrogen fixation



**Figure 10.19 Assimilatory Nitrate Reduction.** This sequence is thought to operate in bacteria that can reduce and assimilate nitrate nitrogen. See text for details.

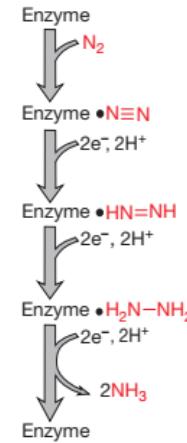
are discussed in chapters 28 and 29. The biochemistry of nitrogen fixation is the focus of this section.

The reduction of nitrogen to ammonia is catalyzed by the enzyme **nitrogenase**. Although the enzyme-bound intermediates in this process are still unknown, it is believed that nitrogen is reduced by two-electron additions in a way similar to that illustrated in figure 10.20. The reduction of molecular nitrogen to ammonia is quite exergonic, but the reaction has a high activation energy because molecular nitrogen is an unreactive gas with a triple bond between the two nitrogen atoms. Therefore nitrogen reduction is expensive and requires a large ATP expenditure. At least 8 electrons and 16 ATP molecules, 4 ATPs per pair of electrons, are required.

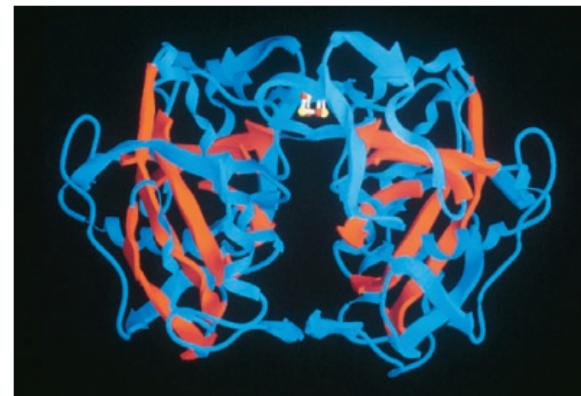


The electrons come from ferredoxin that has been reduced in a variety of ways: by photosynthesis in cyanobacteria, respiratory processes in aerobic nitrogen fixers, or fermentations in anaerobic bacteria. For example, *Clostridium pasteurianum* (an anaerobic bacterium) reduces ferredoxin during pyruvate oxidation, whereas the aerobic *Azotobacter* uses electrons from NADPH to reduce ferredoxin.

Nitrogenase is a complex system consisting of two major protein components, a MoFe protein (MW 220,000) joined with one or two Fe proteins (MW 64,000). The MoFe protein contains 2 atoms of molybdenum and 28 to 32 atoms of iron; the Fe protein has 4 iron atoms (figure 10.21). Fe protein is first reduced by ferredoxin, then it binds ATP (figure 10.22). ATP binding changes the conformation of the Fe protein and lowers its reduction potential, enabling it to reduce the MoFe protein. ATP is hydrolyzed when this electron transfer occurs. Finally, reduced MoFe protein donates electrons to atomic nitrogen. Nitrogenase is quite sensi-



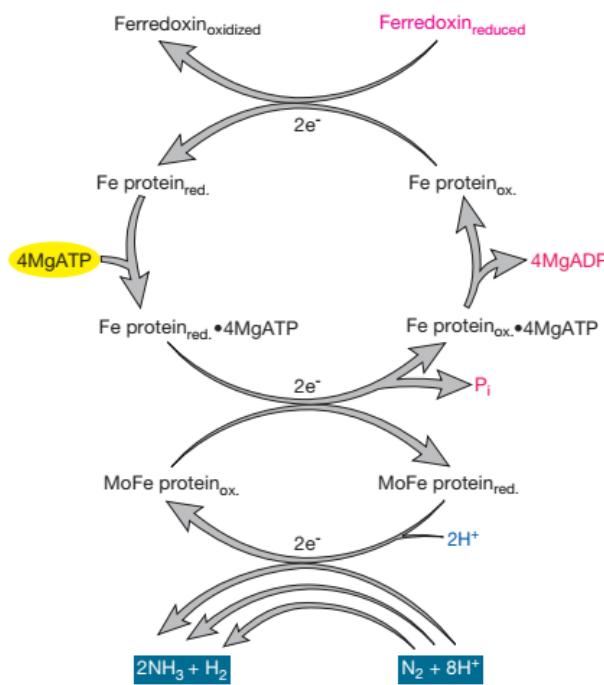
**Figure 10.20 Nitrogen Reduction.** A hypothetical sequence of nitrogen reduction by nitrogenase.



**Figure 10.21 Structure of the Nitrogenase Fe Protein.** The Fe protein's two subunits are arranged like a pair of butterfly wings with the iron sulfur cluster between the wings and at the "head" of the butterfly. The iron sulfur cluster is very exposed, which helps account for nitrogenase's sensitivity to oxygen. The oxygen can readily attack the exposed iron atoms.

tive to O<sub>2</sub> and must be protected from O<sub>2</sub> inactivation within the cell. In many cyanobacteria, this protection against oxygen is provided by a special structure called the heterocyst (see figure 21.9).

The reduction of N<sub>2</sub> to NH<sub>3</sub> occurs in three steps, each of which requires an electron pair (figures 10.20 and 10.22). Six electron transfers take place, and this requires a total 12 ATPs per N<sub>2</sub> reduced. The overall process actually requires at least 8 electrons and 16 ATPs because nitrogenase also reduces protons to H<sub>2</sub>. The H<sub>2</sub> reacts with diimine (HN = NH) to form N<sub>2</sub> and H<sub>2</sub>. This futile cycle produces some N<sub>2</sub> even under favorable conditions and makes nitrogen fixation even more expensive. Symbiotic



**Figure 10.22 Mechanism of Nitrogenase Action.** The flow of two electrons from ferredoxin to nitrogen is outlined. This process is repeated three times in order to reduce  $N_2$  to two molecules of ammonia. The stoichiometry at the bottom includes proton reduction to  $H_2$ . See the text for a more detailed explanation.

nitrogen-fixing bacteria can consume almost 20% of the ATP produced by the host plant.

Nitrogenase can reduce a variety of molecules containing triple bonds (e.g., acetylene, cyanide, and azide).



The rate of reduction of acetylene to ethylene is often used to estimate nitrogenase activity.

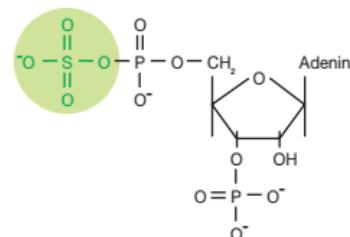
Once molecular nitrogen has been reduced to ammonia, the ammonia can be incorporated into organic compounds. The mechanisms by which heterocystous cells exchange  $NH_3$  with the vegetative cyanobacterial cells, as well as how symbiotic nitrogen-fixing rhizobia share ammonia with host plants, comprise an area of active research. [Microorganism associations with vascular plants: Nitrogen fixation \(section 29.5\)](#)

### Sulfur Assimilation

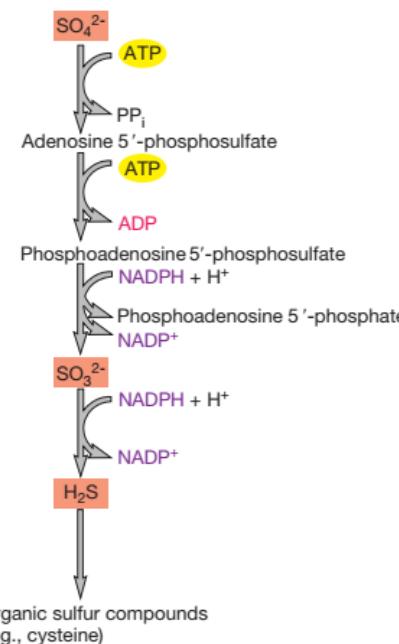
Sulfur is needed for the synthesis of the amino acids cysteine and methionine. It is also needed for the synthesis of several coenzymes (e.g., coenzyme A and biotin). Sulfur is obtained from two sources. Many microorganisms use cysteine and methionine, ob-

tained from either external sources or intracellular amino acid reserves. In addition, sulfate can provide sulfur for biosynthesis. The sulfur atom in sulfate is more oxidized than it is in cysteine and other organic molecules; thus sulfate must be reduced before it can be assimilated. This process is known as **assimilatory sulfate reduction** to distinguish it from the **dissimilatory sulfate reduction**, which takes place when sulfate acts as an electron acceptor during anaerobic respiration. [Anaerobic respiration \(section 9.6\); Biogeochemical cycling: Sulfur cycle \(section 27.2\)](#)

Assimilatory sulfate reduction involves sulfate activation through the formation of **phosphoadenosine 5'-phosphosulfate** (**PAPS**) (figure 10.23), followed by reduction of the sulfate. The process is complex (figure 10.24). Sulfate is first reduced to sulfite ( $SO_3^{2-}$ ), then to hydrogen sulfide. Cysteine can be synthesized from hydrogen sulfide in two ways. Fungi appear to combine hydrogen sulfide with serine to form cysteine

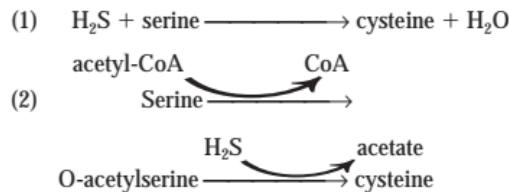


**Figure 10.23 Phosphoadenosine 5'-phosphosulfate (PAPS).** The sulfate group is in color.



**Figure 10.24 The Sulfate Reduction Pathway.**

(process 1), whereas many bacteria join hydrogen sulfide with O-acetylserine instead (process 2).



Once formed, cysteine can be used in the synthesis of other sulfur-containing organic compounds including the amino acid methionine.

### Amino Acid Biosynthetic Pathways

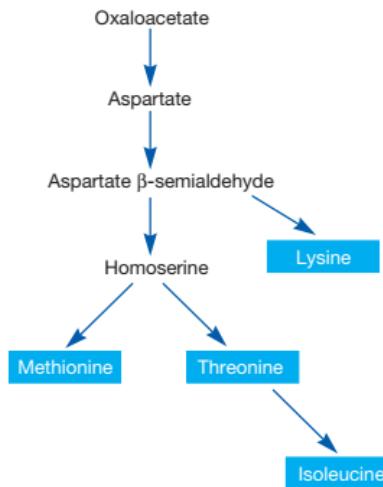
Some amino acids are made directly by transamination of a precursor metabolite. For example, alanine and aspartate are made directly from pyruvate and oxaloacetate, respectively, using glutamate as the amino group donor. However, for most amino acids, the precursor metabolite from which they are synthesized must be altered by more than just the addition of an amino group. In many cases, the carbon skeleton must be reconfigured, and for cysteine and methionine, the carbon skeleton must be amended by the addition of sulfur. These biosynthetic pathways are more complex. They often involve many steps and are branched. By using branched pathways, a single precursor metabolite can be used for the synthesis of a family of related amino acids. For example, the amino acids lysine, threonine, isoleucine, and methionine are synthesized from oxaloacetate by a branching anabolic route (figure 10.25). The biosynthetic pathways for the aromatic amino acids phenylalanine, tyrosine, and tryptophan also share many intermediates (figure 10.26). Because of the need to conserve nitrogen, carbon, and energy, amino acid synthetic pathways are usually tightly regulated by allosteric and feedback mechanisms.

[Control of enzyme activity \(section 8.10\)](#)

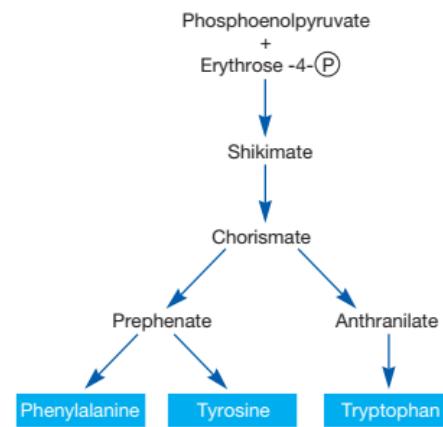
### Anaplerotic Reactions and Amino Acid Biosynthesis

When an organism is actively synthesizing amino acids, a heavy demand for precursor metabolites is placed on the central metabolic pathways. Because many amino acid biosynthetic pathways begin with TCA cycle intermediates, it is critical that they be readily available. This is especially true for organisms carrying out fermentation, where the TCA cycle does not function in the catabolism of glucose. To ensure an adequate supply of TCA cycle-generated precursor metabolites, microorganisms use reactions that replenish TCA cycle intermediates. Reactions that replace cycle intermediates are called **anaplerotic reactions** [Greek *anaplerotic*, filling up].

Most microorganisms can replace TCA cycle intermediates using two reactions that generate oxaloacetate from either phosphoenolpyruvate or pyruvate, both of which are intermediates of the Embden-Meyerhof pathway (figure 10.3). These 3-carbon molecules are converted to oxaloacetate by a carboxylation reac-



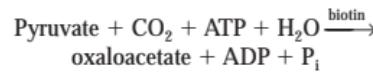
**Figure 10.25 A Branching Pathway of Amino Acid Synthesis.** The pathways to methionine, threonine, isoleucine, and lysine. Although some arrows represent one step, most interconversions require the participation of several enzymes. Also not shown is the consumption of reducing power and ATP. For instance, the synthesis of isoleucine consumes two ATP and three NADPH.



**Figure 10.26 Aromatic Amino Acid Synthesis.** The synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Most arrows represent more than one enzyme reaction.

tion (i.e.,  $\text{CO}_2$  is added to the molecule, forming a carboxyl group).

The conversion of pyruvate to oxaloacetate is catalyzed by the enzyme **pyruvate carboxylase**, which requires the cofactor **biotin**.

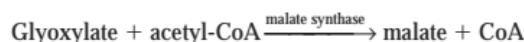
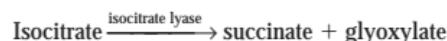


Biotin is often the cofactor for enzymes catalyzing carboxylation reactions. Because of its importance, biotin is a required growth factor for many species. The pyruvate carboxylase reaction is observed in yeasts and some bacteria. Other microorganisms, such as the bacteria *E. coli* and *Salmonella* spp., have the enzyme **phosphoenolpyruvate carboxylase**, which catalyzes the carboxylation of phosphoenolpyruvate.

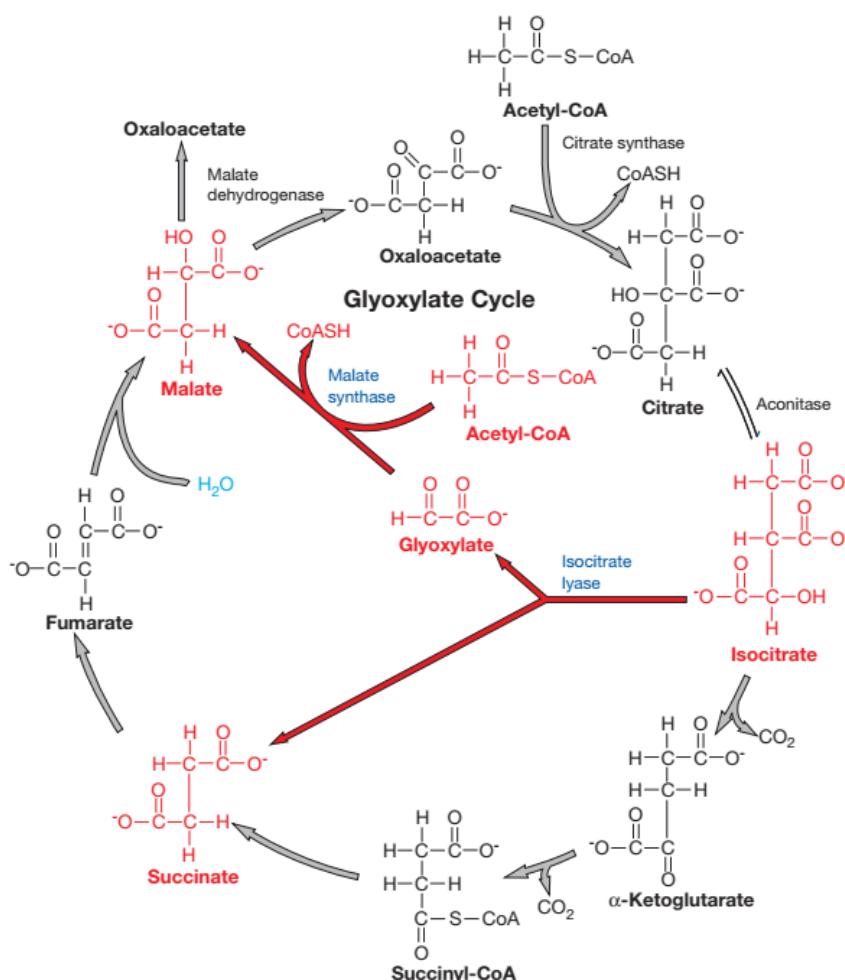


Other anaplerotic reactions are part of the **glyoxylate cycle** (figure 10.27), which functions in some bacteria, fungi, and protists.

This cycle is made possible by two unique enzymes, isocitrate lyase and malate synthase, that catalyze the following reactions.



The glyoxylate cycle is actually a modified TCA cycle. The two de-carboxylations of the TCA cycle (the isocitrate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase steps) are bypassed, making possible the conversion of acetyl-CoA to form oxaloacetate without loss of acetyl-CoA carbon as  $\text{CO}_2$ . In this fashion acetate and any mol-



**Figure 10.27 The Glyoxylate Cycle.** The reactions and enzymes unique to the cycle are shown in red. The tricarboxylic acid cycle enzymes that have been bypassed are at the bottom.

ecules that give rise to it can contribute carbon to the cycle and support microbial growth. [The tricarboxylic acid cycle \(section 9.4\)](#)

1. Describe the roles of glutamate dehydrogenase, glutamine synthetase, glutamate synthase, and transaminases in ammonia assimilation.
2. How is nitrate assimilated? How does assimilatory nitrate reduction differ from dissimilatory nitrate reduction? What is the fate of nitrate following assimilatory nitrate reduction versus its fate following denitrification?
3. What is nitrogen fixation? Briefly describe the structure and mechanism of action of nitrogenase.
4. How do organisms assimilate sulfur? How does assimilatory sulfate reduction differ from dissimilatory sulfate reduction?
5. Why is using branched pathways an efficient mechanism for synthesizing amino acids?
6. Define an anaplerotic reaction. Give three examples of anaplerotic reactions.
7. Describe the glyoxylate cycle. How is it similar to the TCA cycle? How does it differ from the TCA cycle?

## 10.6 SYNTHESIS OF PURINES, PYRIMIDINES, AND NUCLEOTIDES

Purine and pyrimidine biosynthesis is critical for all cells because these molecules are used in the synthesis of ATP, several cofactors, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and other important cell components. Nearly all microorganisms can synthesize their own purines and pyrimidines as these are so crucial to cell function. [DNA replication \(section 11.4\); Transcription \(section 11.6\)](#)

**Purines** and **pyrimidines** are cyclic nitrogenous bases with several double bonds and pronounced aromatic properties. Purines consist of two joined rings, whereas pyrimidines have only one. The purines **adenine** and **guanine** and the pyrimidines **uracil**, **cytosine**, and **thymine** are commonly found in microorganisms. A purine or pyrimidine base joined with a pentose sugar, either ribose or deoxyribose, is a **nucleoside**. A **nucleotide** is a nucleoside with one or more phosphate groups attached to the sugar.

As discussed in section 10.6, amino acids participate in the synthesis of nitrogenous bases and nucleotides in a number of ways, including providing the nitrogen that is part of all purines and pyrimidines. The phosphorus present in nucleotides is provided by other mechanisms. We begin this section by examining phosphorus assimilation. We then examine the pathways for synthesis of nitrogenous bases and nucleotides.

### Phosphorus Assimilation

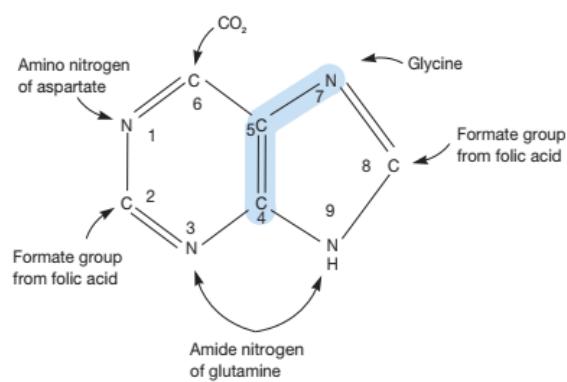
In addition to nucleic acids, phosphorus is found in proteins, phospholipids, ATP, and coenzymes like NADP. The most common phosphorus sources are inorganic phosphate and organic phosphate esters. Inorganic phosphate is incorporated through the formation of ATP in one of three ways: by (1) photophosphorylation, (2) oxidative phosphorylation, and (3) substrate-level phosphorylation. [The breakdown of glucose to pyruvate \(section 9.3\); Electron transport and oxidative phosphorylation \(section 9.5\); Phototrophy \(section 9.12\)](#)

Microorganisms may obtain organic phosphates from their surroundings in dissolved or particulate form. **Phosphatases** very often hydrolyze organic phosphate esters to release inorganic phosphate. Gram-negative bacteria have phosphatases in the periplasmic space, which allows phosphate to be taken up immediately after release. On the other hand, protists can directly use organic phosphates after ingestion or hydrolyze them in lysosomes and incorporate the phosphate.

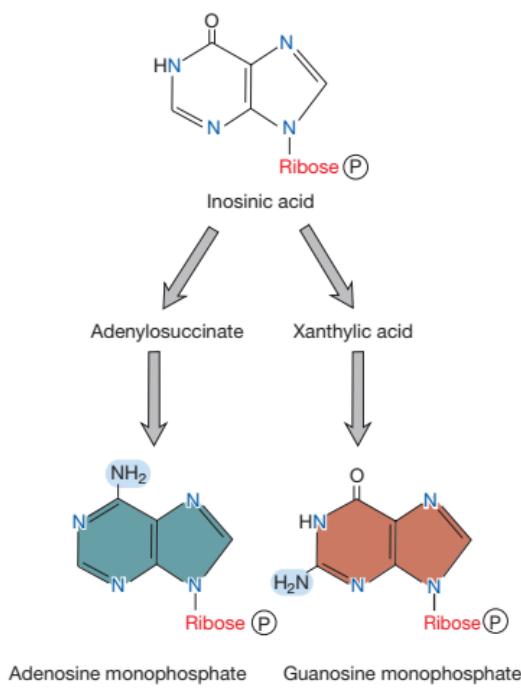
### Purine Biosynthesis

The biosynthetic pathway for purines is a complex, 11-step sequence (*see appendix II*) in which seven different molecules contribute parts to the final purine skeleton (**figure 10.28**). Because the pathway begins with ribose 5-phosphate and the purine skeleton is constructed on this sugar, the first purine product of the pathway is the nucleotide **inosinic acid**, not a free purine base. The cofactor folic acid is very important in purine biosynthesis. Folic acid derivatives contribute carbons two and eight to the purine skeleton. In fact, the drug sulfonamide inhibits bacterial growth by blocking folic acid synthesis. This interferes with purine biosynthesis and other processes that require folic acid. [Antibacterial drugs: Metabolic antagonists \(section 34.4\)](#)

Once inosinic acid has been formed, relatively short pathways synthesize adenosine monophosphate and guanosine monophosphate (**figure 10.29**) and produce nucleoside diphosphates and triphosphates by phosphate transfers from ATP. DNA contains deoxyribonucleotides (the ribose lacks a hydroxyl group on carbon two) instead of the ribonucleotides found in RNA. Deoxyribonucleotides arise from the reduction of nucleoside diphosphates or nucleoside triphosphates by two different routes. Some microorganisms reduce the triphosphates with a system requiring vitamin B<sub>12</sub> as a cofactor. Others, such as *E. coli*, reduce the ribose in nucleoside diphosphates. Both systems employ a small sulfur-containing protein called thioredoxin as their reducing agent.



**Figure 10.28 Purine Biosynthesis.** The sources of purine skeleton nitrogen and carbon are indicated. The contribution of glycine is shaded in blue.



**Figure 10.29 Synthesis of Adenosine Monophosphate and Guanosine Monophosphate.** The highlighted groups are the ones differing from those in inosinic acid.

### Pyrimidine Biosynthesis

Pyrimidine biosynthesis begins with aspartic acid and carbamoyl phosphate, a high-energy molecule synthesized from CO<sub>2</sub> and ammonia (figure 10.30). Aspartate carbamoyltransferase catalyzes the condensation of these two substrates to form carbamoyl aspartate, which is then converted to the initial pyrimidine product, orotic acid.

After synthesis of the pyrimidine skeleton, a nucleotide is produced by the addition of ribose 5-phosphate, using the high-energy intermediate 5-phosphoribosyl 1-pyrophosphate. Thus construction of the pyrimidine ring is completed before ribose is added, in contrast with purine ring synthesis, which begins with ribose 5-phosphate. Decarboxylation of orotidine monophosphate yields uridine monophosphate and eventually uridine triphosphate and cytidine triphosphate.

The third common pyrimidine is thymine, a constituent of DNA. The ribose in pyrimidine nucleotides is reduced in the same way as it is in purine nucleotides. Then deoxyuridine monophosphate is methylated with a folic acid derivative to form deoxythymidine monophosphate (figure 10.31).

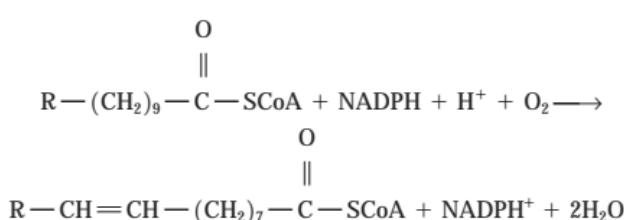
- How is phosphorus assimilated? What roles do phosphatases play in phosphorus assimilation? Why can phosphate be directly incorporated into cell constituents, whereas nitrate, nitrogen gas, and sulfate cannot?
- Define purine, pyrimidine, nucleoside, and nucleotide.
- Outline the way in which purines and pyrimidines are synthesized. How is the deoxyribose component of deoxyribonucleotides made?

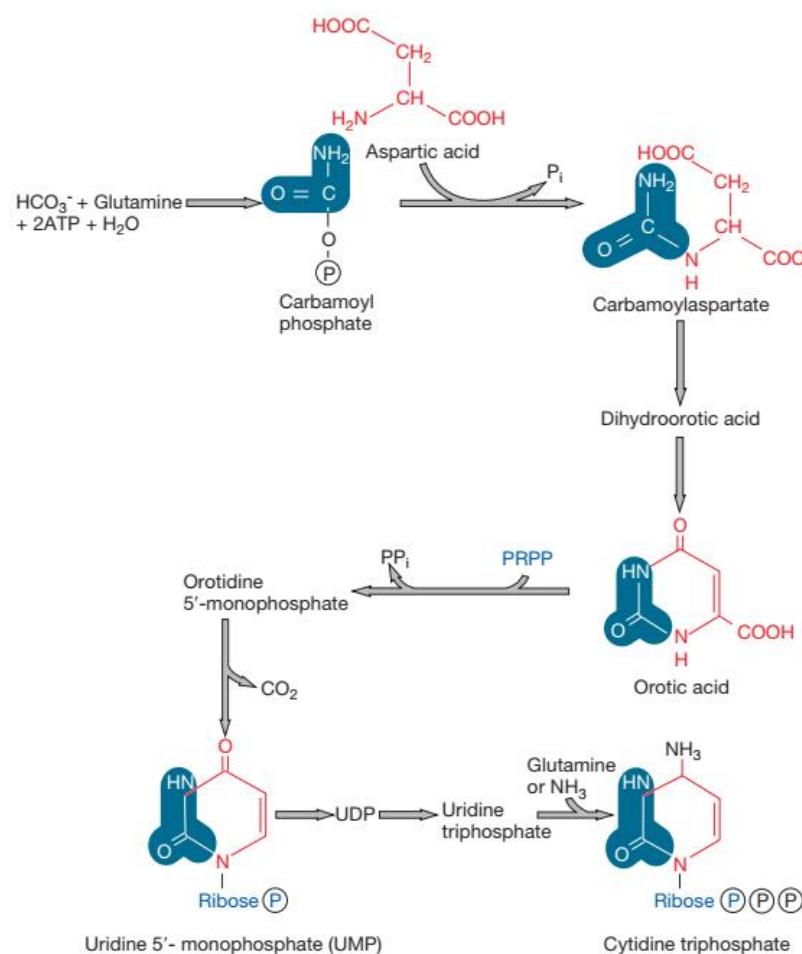
### 10.7 LIPID SYNTHESIS

A variety of lipids are found in microorganisms, particularly in cell membranes. Most contain **fatty acids** or their derivatives. Fatty acids are monocarboxylic acids with long alkyl chains that usually have an even number of carbons (the average length is 18 carbons). Some may be unsaturated—that is, have one or more double bonds. Most microbial fatty acids are straight chained, but some are branched. Gram-negative bacteria often have cyclopropane fatty acids (fatty acids with one or more cyclopropane rings in their chains). [Lipids \(appendix I\)](#)

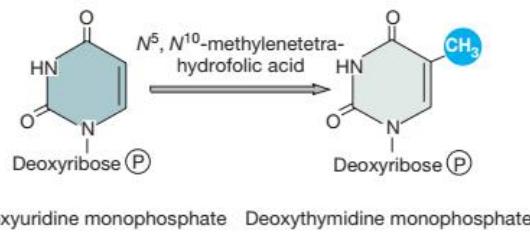
Fatty acid synthesis is catalyzed by the **fatty acid synthase** complex with acetyl-CoA and malonyl-CoA as the substrates and NADPH as the electron donor. Malonyl-CoA arises from the ATP-driven carboxylation of acetyl-CoA (figure 10.32). Synthesis takes place after acetate and malonate have been transferred from coenzyme A to the sulfhydryl group of the **acyl carrier protein (ACP)**, a small protein that carries the growing fatty acid chain during synthesis. The synthase adds two carbons at a time to the carboxyl end of the growing fatty acid chain in a two-stage process (figure 10.32). First, malonyl-ACP reacts with the fatty acyl-ACP to yield CO<sub>2</sub> and a fatty acyl-ACP two carbons longer. The loss of CO<sub>2</sub> drives this reaction to completion. Notice that ATP is used to add CO<sub>2</sub> to acetyl-CoA, forming malonyl-CoA. The same CO<sub>2</sub> is lost when malonyl-ACP donates carbons to the chain. Thus carbon dioxide is essential to fatty acid synthesis but it is not permanently incorporated. Indeed, some microorganisms require CO<sub>2</sub> for good growth, but they can do without it in the presence of a fatty acid like oleic acid (an 18-carbon unsaturated fatty acid). In the second stage of synthesis, the β-keto group arising from the initial condensation reaction is removed in a three-step process involving two reductions and a dehydration. The fatty acid is then ready for the addition of two more carbon atoms.

Unsaturated fatty acids are synthesized in two ways. Eucaryotes and aerobic bacteria like *B. megaterium* employ an aerobic pathway using both NADPH and O<sub>2</sub>.





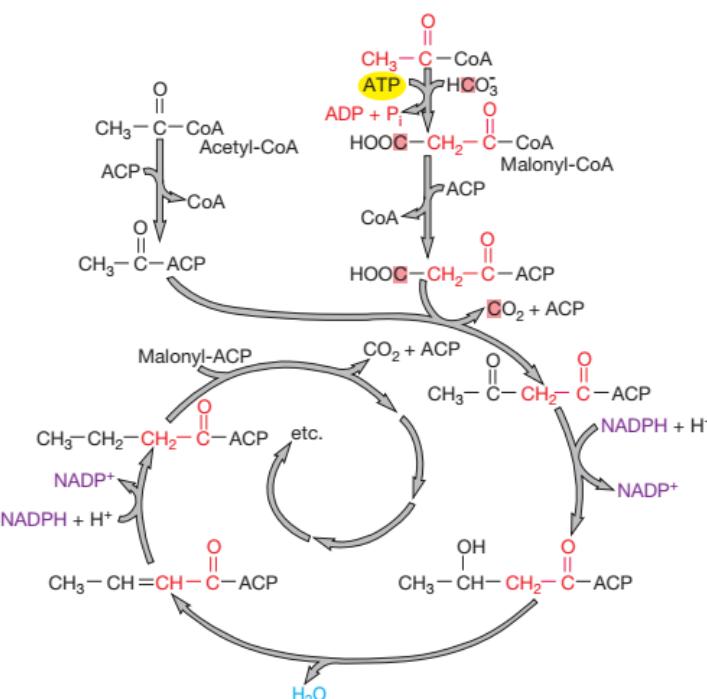
**Figure 10.30 Pyrimidine Synthesis.** PRPP stands for 5-phosphoribosyl 1-pyrophosphoric acid, which provides the ribose 5-phosphate chain. The part derived from carbamoyl phosphate is shaded in turquoise.



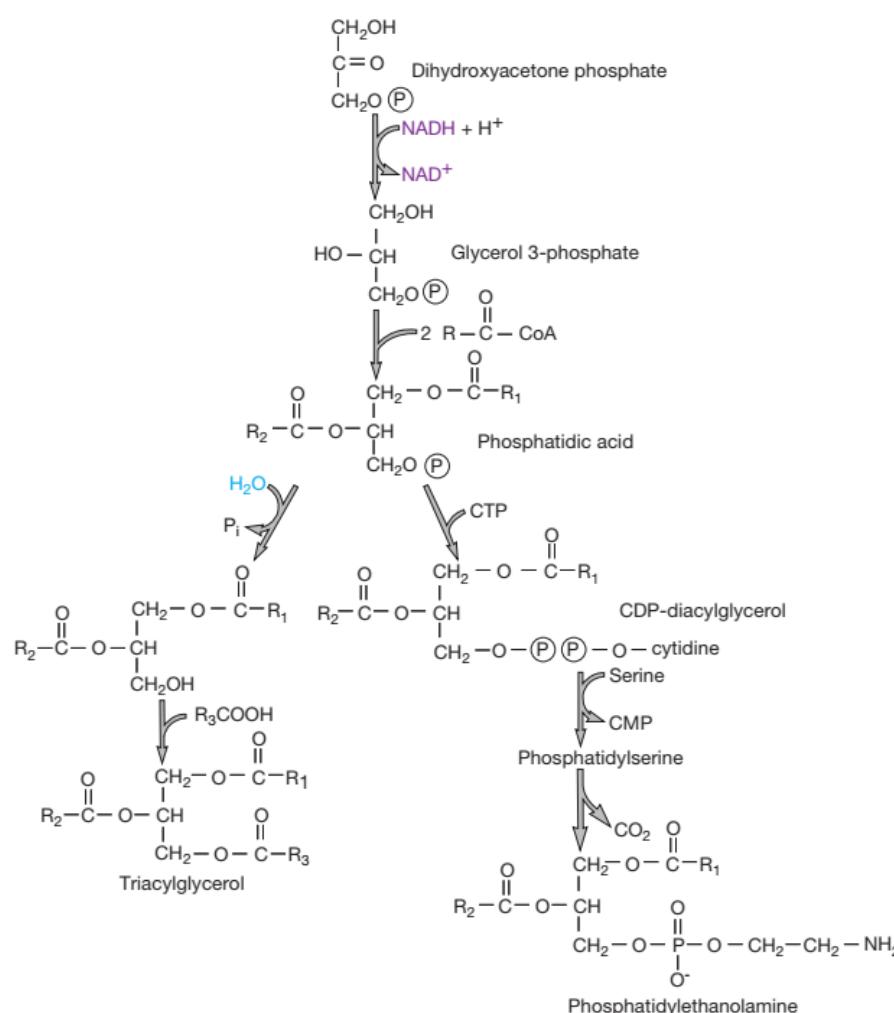
**Figure 10.31 Deoxythymidine Monophosphate Synthesis.** Deoxythymidine differs from deoxyuridine in having the shaded methyl group.

A double bond is formed between carbons nine and ten, and  $O_2$  is reduced to water with electrons supplied by both the fatty acid and NADPH. Anaerobic bacteria and some aerobes create double bonds during fatty acid synthesis by dehydrating hydroxy fatty acids. Oxygen is not required for double bond synthesis by this pathway. The anaerobic pathway is present in a number of common gram-negative bacteria (e.g., *E. coli* and *Salmonella* spp.), gram-positive bacteria (e.g., *Lactobacillus plantarum* and *Clostridium pasteurianum*), and cyanobacteria.

Eucaryotic microorganisms frequently store carbon and energy as **triacylglycerol**, glycerol esterified to three fatty acids. Glycerol arises from the reduction of the precursor metabolites dihydroxyacetone phosphate to glycerol 3-phosphate, which is then esterified with two fatty acids to give **phosphatidic acid** (figure 10.33).



**Figure 10.32 Fatty Acid Synthesis.** The cycle is repeated until the proper chain length has been reached. Carbon dioxide carbon and the remainder of malonyl-CoA are shown in red. ACP stands for acyl carrier protein.



**Figure 10.33 Triacylglycerol and Phospholipid Synthesis.**

Phosphate is hydrolyzed from phosphatidic acid giving a diacylglycerol, and the third fatty acid is attached to yield a triacylglycerol.

Phospholipids are major components of eucaryotic and bacterial cell membranes. Their synthesis also usually proceeds by way of phosphatidic acid. A special cytidine diphosphate (CDP) carrier plays a role similar to that of uridine and adenosine diphosphate carriers in carbohydrate biosynthesis. For example, bacteria synthesize phosphatidylethanolamine, a major cell membrane component, through the initial formation of CDP-diacylglycerol (figure 10.33). This CDP derivative then reacts with serine to form the phospholipid phosphatidylserine, and decarboxylation yields phosphatidylethanolamine. In this way

a complex membrane lipid is constructed from the products of glycolysis, fatty acid biosynthesis, and amino acid biosynthesis.

1. What is a fatty acid? Describe in general terms how the fatty acid synthase manufactures a fatty acid.
2. How are unsaturated fatty acids made?
3. Briefly describe the pathways for triacylglycerol and phospholipid synthesis. Of what importance are phosphatidic acid and CDP-diacylglycerol?
4. Activated carriers participate in carbohydrate, peptidoglycan, and lipid synthesis. Briefly describe these carriers and their roles. Are there any features common to all the carriers? Explain your answer.

## Summary

In biosynthesis or anabolism, cells use energy to construct complex molecules from smaller, simpler precursors.

### 10.1 Principles Governing Biosynthesis

- a. Many important cell constituents are macromolecules, large polymers constructed of simple monomers.
- b. Although many catabolic and anabolic pathways share enzymes for the sake of efficiency, some of their enzymes are separate and independently regulated.
- c. Macromolecular components often undergo self-assembly to form the final molecule or complex.

### 10.2 The Precursor Metabolites

- a. Precursor metabolites are carbon skeletons used as the starting substrates for biosynthetic pathways. They are intermediates of glycolytic pathways and the TCA cycle (i.e., the central metabolic pathways) (figure 10.3).
- b. Most precursor metabolites are used for amino acid biosynthesis.

### 10.3 The Fixation of CO<sub>2</sub> by Autotrophs

- a. Four different CO<sub>2</sub>-fixation pathways have been identified in autotrophic microorganisms: the Calvin cycle, the reductive TCA cycle, the acetyl-CoA pathway, and the hydroxypropionate cycle.
- b. The Calvin cycle is used by most autotrophs to fix CO<sub>2</sub>. It can be divided into three phases: the carboxylation phase, the reduction phase, and the regeneration phase (figure 10.4). Three ATPs and two NADPHs are used during the incorporation of one CO<sub>2</sub>.

### 10.4 Synthesis of Sugars and Polysaccharides

- a. Gluconeogenesis is the synthesis of glucose and related sugars from nonglucose precursors.
- b. Glucose, fructose, and mannose are gluconeogenic intermediates or made directly from them; galactose is synthesized with nucleoside diphosphate derivatives. Bacteria and photosynthetic protists synthesize glycogen and starch from adenosine diphosphate glucose (figure 10.9).
- c. Peptidoglycan synthesis is a complex process involving both UDP derivatives and the lipid carrier bactoprenol, which transports NAG-NAM-pentapeptide units across the cell membrane. Cross-links are formed by transpeptidation (figures 10.12 and 10.14).
- d. Peptidoglycan synthesis occurs in discrete zones in the cell wall. Existing peptidoglycan is selectively degraded by autolysins so new material can be added (figure 10.15).

### 10.5 Synthesis of Amino Acids

- a. The addition of nitrogen to the carbon chain is an important step in amino acid biosynthesis. Ammonia, nitrate, or nitrogen can serve as the source of nitrogen.

- b. Ammonia nitrogen can be directly assimilated by the activity of transaminases and either glutamate dehydrogenase or the glutamine synthetase-glutamate synthase system (figures 10.16–10.18).
- c. Nitrate is incorporated through assimilatory nitrate reduction catalyzed by the enzymes nitrate reductase and nitrite reductase (figure 10.19).
- d. Nitrogen fixation is catalyzed by the nitrogenase complex. Atmospheric molecular nitrogen is reduced to ammonia, which is then incorporated into amino acids (figures 10.20 and 10.22).
- e. Microorganisms can use cysteine, methionine, and inorganic sulfate as sulfur sources. Sulfate must be reduced to sulfide before it is assimilated. This occurs during assimilatory sulfate reduction (figure 10.24).
- f. Although some amino acids are made directly by the addition of an amino group to a precursor metabolite, most amino acids are made by more complex pathways. Many amino acid biosynthetic pathways are branched. Thus a single precursor metabolite can give rise to several amino acids (figures 10.25 and 10.26).
- g. Anaplerotic reactions replace TCA cycle intermediates to keep the cycle in balance while it supplies biosynthetic precursors. The anaplerotic reactions include the glyoxylate cycle (figure 10.27).

### 10.6 Synthesis of Purines, Pyrimidines, and Nucleotides

- a. Purines and pyrimidines are nitrogenous bases found in DNA, RNA, and other molecules. The nitrogen is supplied by certain amino acids that participate in purine and pyrimidine biosynthesis. Phosphorus is provided by either inorganic phosphate or organic phosphate.
- b. Phosphorus can be assimilated directly by phosphorylation reactions that form ATP from ADP and P<sub>i</sub>. Organic phosphorus sources are the substrates of phosphatases that release phosphate from the organic molecule.
- c. The purine skeleton is synthesized beginning with ribose 5-phosphate and initially produces inosinic acid. Pyrimidine biosynthesis starts with carbamoyl phosphate and aspartate, and ribose is added after the skeleton has been constructed (figures 10.28–10.30).

### 10.7 Lipid Synthesis

- a. Fatty acids are synthesized from acetyl-CoA, malonyl-CoA, and NADPH by the fatty acid synthase system. During synthesis the intermediates are attached to the acyl carrier protein. Double bonds can be added in two different ways (figure 10.32).
- b. Triacylglycerols are made from fatty acids and glycerol phosphate. Phosphatidic acid is an important intermediate in this pathway (figure 10.33).
- c. Phospholipids like phosphatidylethanolamine can be synthesized from phosphatidic acid by forming CDP-diacylglycerol, then adding an amino acid.

## Key Terms

acetyl-CoA pathway 230	fatty acid 242	Lipid II 232	pyrimidine 241
acyl carrier protein (ACP) 242	fatty acid synthase 242	macromolecule 226	reductive TCA cycle 229
adenine 241	gluconeogenesis 230	monomers 226	ribulose-1,5-bisphosphate
anaplerotic reactions 239	glutamate dehydrogenase 235	nitrate reductase 236	carboxylase 229
assimilatory nitrate reduction 235	glutamate synthase 235	nitrite reductase 236	self-assembly 227
assimilatory sulfate reduction 238	glutamine synthetase 235	nitrogenase 237	thymine 241
autolysins 234	glutamine synthetase-glutamate synthase (GS-GOGAT) system 235	nitrogen fixation 236	transaminases 235
bactoprenol 232	glyoxylate cycle 240	nucleoside 241	transpeptidation 233
Calvin cycle 228	guanine 241	nucleotide 241	triacylglycerol 243
carboxysomes 229	3-hydroxypropionate cycle 229	phosphatase 241	turnover 225
central metabolic pathways 227	Lipid I 232	phosphatidic acid 243	uracil 241
cytosine 241		precursor metabolites 227	uridine diphosphate glucose (UDPG) 231
dissimilatory sulfate reduction 238		purine 241	

## Critical Thinking Questions

1. Discuss the relationship between catabolism and anabolism. How does anabolism depend on catabolism?
2. In metabolism, important intermediates are covalently attached to carriers, as if to mark these as important so the cell does not lose track of them. Think about a hotel placing your room key on a very large ring. List a few examples of these carriers and indicate whether they are involved primarily in anabolism or catabolism.
3. Intermediary carriers are in a limited supply—when they cannot be recycled because of a metabolic block, serious consequences ensue. Think of some examples of these consequences.

## Learn More

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