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 (*see section 9.7*) 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 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.

$$\text{ATP} + \text{glucose 1-phosphate} \longrightarrow \text{ADP-glucose} + PP_i$$
$$(\text{Glucose})_n + \text{ADP-glucose} \longrightarrow (\text{glucose})_{n+1} + \text{ADP}$$

Nucleoside diphosphate sugars also participate in the synthesis of complex molecules such as bacterial cell walls (pp. 221–23).

1. Briefly describe the three stages of the Calvin cycle.
2. What is gluconeogenesis and how does it usually occur? Describe the formation of mannose, galactose, starch, and glycogen. Why are nucleoside diphosphate sugars important?

10.4 The Assimilation of Inorganic Phosphorus, Sulfur, and Nitrogen

Besides carbon and oxygen, microorganisms also require large quantities of phosphorus, sulfur, and nitrogen for biosynthesis. Each of these is assimilated, or incorporated into organic molecules, by different routes. Microbial nutrition (chapter 5); Microbial participation in biogeochemical cycles (section 28.4)

Phosphorus Assimilation

Phosphorus is found in nucleic acids, 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 (*see pp. 196–99*), (2) oxidative phosphorylation (*see pp. 187–89*), and (3) substrate-level phosphorylation. Glycolysis provides an example of the latter process. Phosphate is joined with glyceraldehyde 3-phosphate to give 1,3-bisphosphoglycerate, which is next used in ATP synthesis.

$$\text{Glyceraldehyde 3-P} + P_i + NAD^+ \longrightarrow \text{1,3-bisphosphoglycerate} + \text{NADH} + H^+$$

$$\text{1,3-bisphosphoglycerate} + \text{ADP} \longrightarrow \text{3-phosphoglycerate} + \text{ATP}$$

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 between their cell wall and the plasma membrane, which allows phosphate to be taken up immediately after release. On the other hand, protozoa can directly use organic phosphates after ingestion or hydrolyze them in lysosomes and incorporate the phosphate.

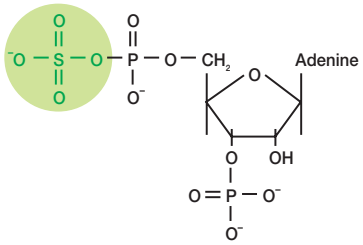

Figure 10.8 Phosphoadenosine 5′-phosphosulfate (PAPS). The sulfate group is in color.

Sulfur Assimilation

Sulfur is needed for the synthesis of amino acids (cysteine and methionine) and several coenzymes (e.g., coenzyme A and biotin) and may be obtained from two sources. Many microorganisms use cysteine and methionine, obtained 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** that takes place when sulfate acts as an electron acceptor during anaerobic respiration (*see figure 28.21*). Anaerobic respiration (pp. 190–91)

Assimilatory sulfate reduction involves sulfate activation through the formation of **phosphoadenosine 5′-phosphosulfate** (**figure 10.8**), followed by reduction of the sulfate. The process is a complex one (**figure 10.9**) in which 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 (process 1), whereas many bacteria join hydrogen sulfide with O-acetylserine instead (process 2).

(1) $H_2S + \text{serine} \longrightarrow \text{cysteine} + H_2O$

(2) $\text{Serine} \xrightarrow{\text{acetyl-CoA CoA}} \text{O-acetylserine} \xrightarrow{H_2S\ \text{acetate}} \text{cysteine}$

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

Nitrogen Assimilation

Because nitrogen is a major component of proteins, nucleic acids, coenzymes, and many other cell constituents, the cell's ability to assimilate inorganic nitrogen is exceptionally important. Al-

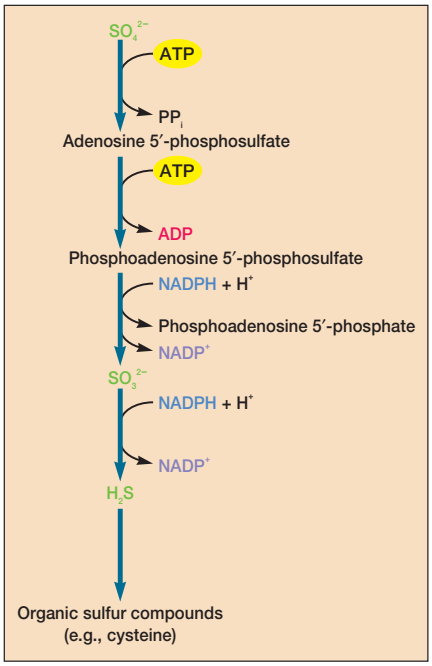

Figure 10.9 **The Sulfate Reduction Pathway.**

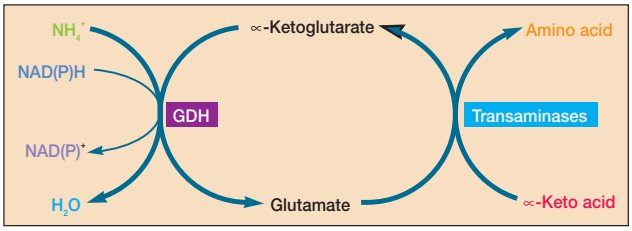

Figure 10.10 **The Ammonia Assimilation Pathway.** Ammonia assimilation by use of glutamate dehydrogenase (GDH) and transaminases. Either NADP- or NAD-dependent glutamate dehydrogenases may be involved. This route is most active at high ammonia concentrations.

though 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.

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. Some microorganisms form the amino acid alanine in a reductive amination reaction catalyzed by alanine dehydrogenase.

$$\text{Pyruvate} + NH_4^+ + \text{NADH (NADPH)} + H^+ \rightleftharpoons \text{L-alanine} + \text{NAD}^+ (\text{NADP}^+) + H_2O$$

The major route for ammonia incorporation often is the formation of glutamate from α-ketoglutarate (a TCA cycle intermediate). Many bacteria and fungi employ **glutamate dehydrogenase,** at least when the ammonia concentration is high.

$$\alpha\text{-ketoglutarate} + NH_4^+ + \text{NADPH (NADH)} + H^+ \rightleftharpoons \text{glutamate} + \text{NADP}^+ (\text{NAD}^+) + H_2O$$

Different species vary in their ability to use NADPH and NADH as the reducing agent in glutamate synthesis.

Once either alanine or glutamate has been synthesized, the newly formed α-amino group can be transferred to other carbon skeletons by transamination reactions (*see section 9.9*) 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 10.10**).

A second route of ammonia incorporation involves two enzymes acting in sequence, **glutamine synthetase** and **glutamate synthase** (**figure 10.11**). Ammonia is used to synthesize glutamine from glutamate, then the amide nitrogen of glutamine is transferred to α-ketoglutarate to generate a new glutamate molecule. 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 (**figure 10.12**). Both ATP and a source of electrons, such as NADPH or reduced ferredoxin, are required. This route is present in *Escherichia coli, Bacillus megaterium,* and other bacteria. The two enzymes acting in sequence operate very effectively at low ammonia concentrations, unlike the glutamate dehydrogenase pathway. As we saw earlier, glutamine synthetase is tightly regulated by reversible covalent modification and allosteric effectors (*see pp. 168–69*).

Assimilatory Nitrate Reduction

The nitrogen in nitrate (NO_3^-) is much more oxidized than that in ammonia. 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 and dissimilatory nitrate reduction (*see sections 9.6 and 28.4*). 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 algae.

Assimilatory nitrate reduction takes place in the cytoplasm in bacteria. The first step in nitrate assimilation is its reduction to nitrite

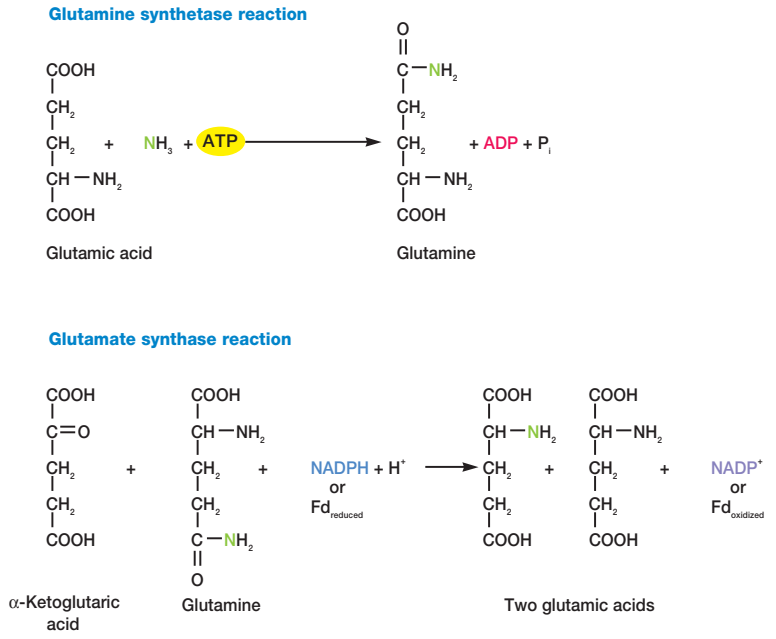

Figure 10.11 **Glutamine Synthetase and Glutamate Synthase.** The glutamine synthetase and glutamate synthase reactions involved in ammonia assimilation. Some glutamine synthases use NADPH as an electron source; others use reduced ferredoxin (Fd). The nitrogen being incorporated and transferred is shown in green.

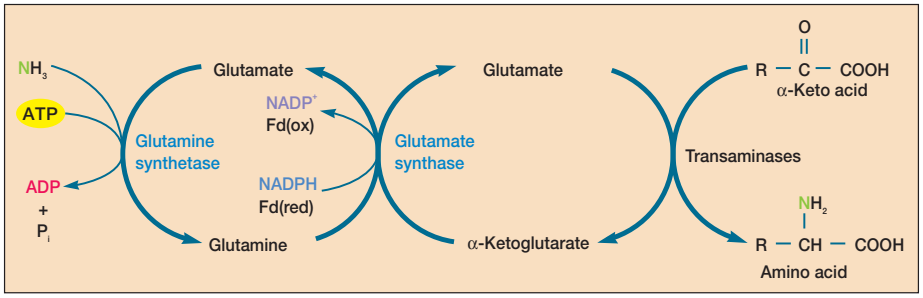

Figure 10.12 **Ammonia Incorporation Using Glutamine Synthetase and Glutamate Synthase.** This route is effective at low ammonia concentrations.

by **nitrate reductase,** an enzyme that contains both FAD and molybdenum (**figure 10.13**). NADPH is the electron source.

$$NO_3^- + NADPH + H^+ \longrightarrow NO_2^- + NADP^+ + H_2O$$

Nitrite is next reduced to ammonia with a series of two electron additions catalyzed by **nitrite reductase** and possibly other enzymes. Hydroxylamine may be an intermediate. 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 procaryotes 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 (e.g., *Azotobacter, Klebsiella, Clostridium,* and *Methanococcus*), (2) bacteria living in

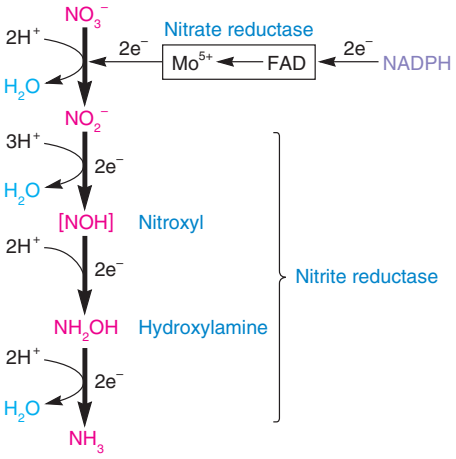

Figure 10.13 **Assimilatory Nitrate Reduction.** This sequence is thought to operate in bacteria that can reduce and assimilate nitrate nitrogen. See text for details.

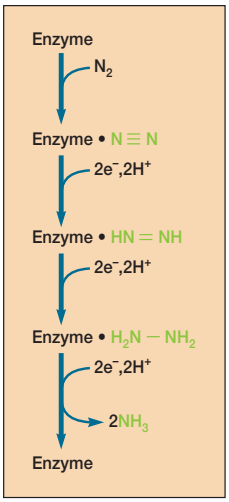

Figure 10.14 **Nitrogen Reduction.** A hypothetical sequence of nitrogen reduction by nitrogenase.

symbiotic association with plants such as legumes (*Rhizobium*), and (3) cyanobacteria (*Nostoc* and *Anabaena*). The biological aspects of nitrogen fixation are discussed in chapter 30. The biochemistry of nitrogen fixation is the focus of this section. The biology of nitrogen-fixing microorganisms (pp. 492, 616, 675–78)

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 10.14.** The reduction of molecular nitrogen to ammonia is

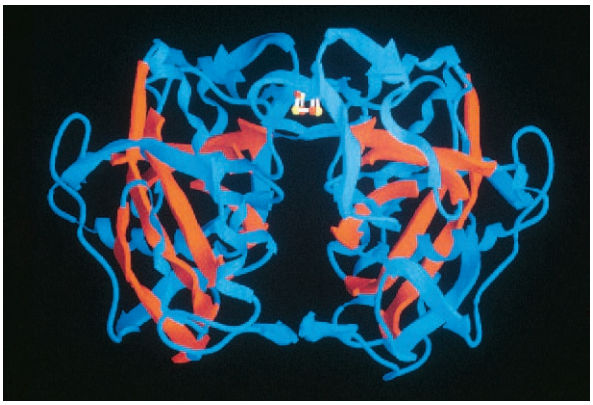

Figure 10.15 **Structure of the Nitrogenase Fe Protein.** The Fe protein's two subunits are arranged like a pair of butterfly wings with the iron sulfur cluster between the wings and at the "head" of the butterfly. The iron sulfur cluster is very exposed, which helps account for nitrogenase's sensitivity to oxygen. The oxygen can readily attack the exposed irons.

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.

$$N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

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 10.15**). Fe protein is first reduced by ferredoxin, then it binds ATP (**figure 10.16**). 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 sensitive to O_2 and must be protected from O_2 inactivation within the cell. In many cyanobacteria, this protection against oxygen is provided by a special structure called the heterocyst (*see p. 473*).

The reduction of N_2 to NH_3 occurs in three steps, each of which requires an electron pair (figures 10.14 and 10.16). Six electron transfers take place, and this requires a total 12 ATPs per N_2 reduced. The overall process actually requires at least 8 electrons

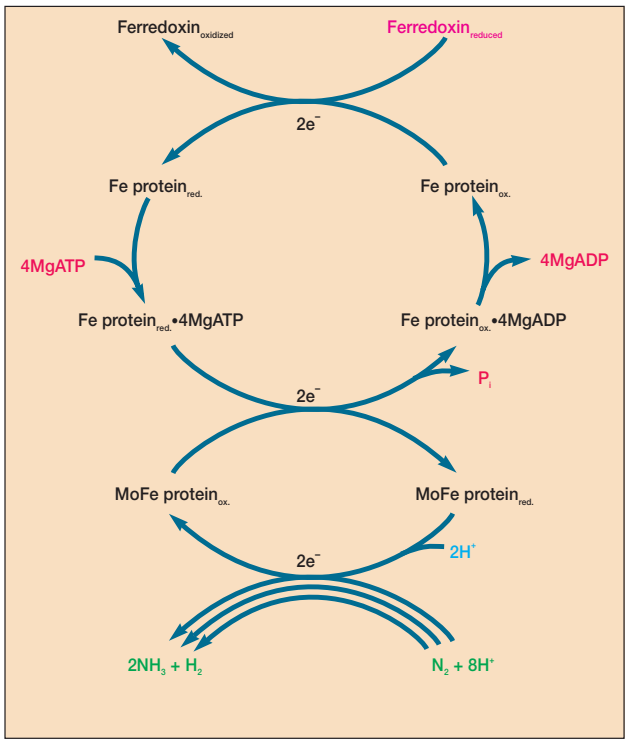

Figure 10.16 Mechanism of Nitrogenase Action. The flow of two electrons from ferredoxin to nitrogen is outlined. This process is repeated three times in order to reduce N_2 to two molecules of ammonia. The stoichiometry at the bottom includes proton reduction to H_2. See the text for a more detailed explanation.

and 16 ATPs because nitrogenase also reduces protons to H_2. The H_2 reacts with diimine (HN = NH) to form N_2 and H_2. This futile cycle produces some N_2 even under favorable conditions and makes nitrogen fixation even more expensive. Symbiotic 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).

$$HC \equiv CH + 2H^+ + 2e^- \longrightarrow H_2C = CH_2$$

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

Once molecular nitrogen has been reduced to ammonia, the ammonia can be incorporated into organic compounds. In the symbiotic nitrogen fixer *Rhizobium,* it appears that ammonia diffuses out of the bacterial cell and is assimilated in the surrounding legume cell. The primary route of ammonia assimilation seems to be the synthesis of glutamine by the glutamine synthetase–glutamate synthase system (figure 10.11). However, substances such as the purine derivatives allantoin and allantoic acid also are synthesized and used for the transport of nitrogen to other parts of the plant.

10.5 The Synthesis of Amino Acids

Microorganisms vary with respect to the type of nitrogen source they employ, but most can assimilate some form of inorganic nitrogen by the routes just described. Amino acid synthesis also requires construction of the proper carbon skeletons, and this is often a complex process involving many steps. Because of the need to conserve nitrogen, carbon, and energy, amino acid synthetic pathways are usually tightly regulated by allosteric and feedback mechanisms (*see section 8.9*). Although individual amino acid biosynthetic pathways are not described in detail, a survey of the general pattern of amino acid biosynthesis is worthwhile. Further details of amino acid biosynthesis may be found in introductory biochemistry textbooks.

The relationship of amino acid biosynthetic pathways to amphibolic routes is shown in **figure 10.17.** Amino acid skeletons are derived from acetyl-CoA and from intermediates of the TCA cycle, glycolysis, and the pentose phosphate pathway. To maximize efficiency and economy, the precursors for amino acid biosynthesis are provided by a few major amphibolic pathways. Sequences leading to individual amino acids branch off from these central routes. Alanine, aspartate, and glutamate are made by transamination directly from pyruvate, oxaloacetate, and α-ketoglutarate, respectively. Most biosynthetic pathways are more complex, and common intermediates often are used in the synthesis of families of related amino acids for the sake of further economy. For example, the amino acids lysine, threonine, isoleucine, and methionine are synthesized from oxaloacetate by such a branching anabolic route (**figure 10.18**). The biosynthetic pathways for the aromatic amino acids phenylalanine, tyrosine, and tryptophan also share many intermediates (**figure 10.19**).

1. How do microorganisms assimilate sulfur and phosphorus?
2. Describe the roles of glutamate dehydrogenase, glutamine synthetase, glutamate synthase, and transaminases in ammonia assimilation. How is nitrate incorporated by assimilatory nitrate reduction?
3. What is nitrogen fixation? Briefly describe the structure and mechanism of action of nitrogenase.
4. Summarize in general terms the organization of amino acid biosynthesis.

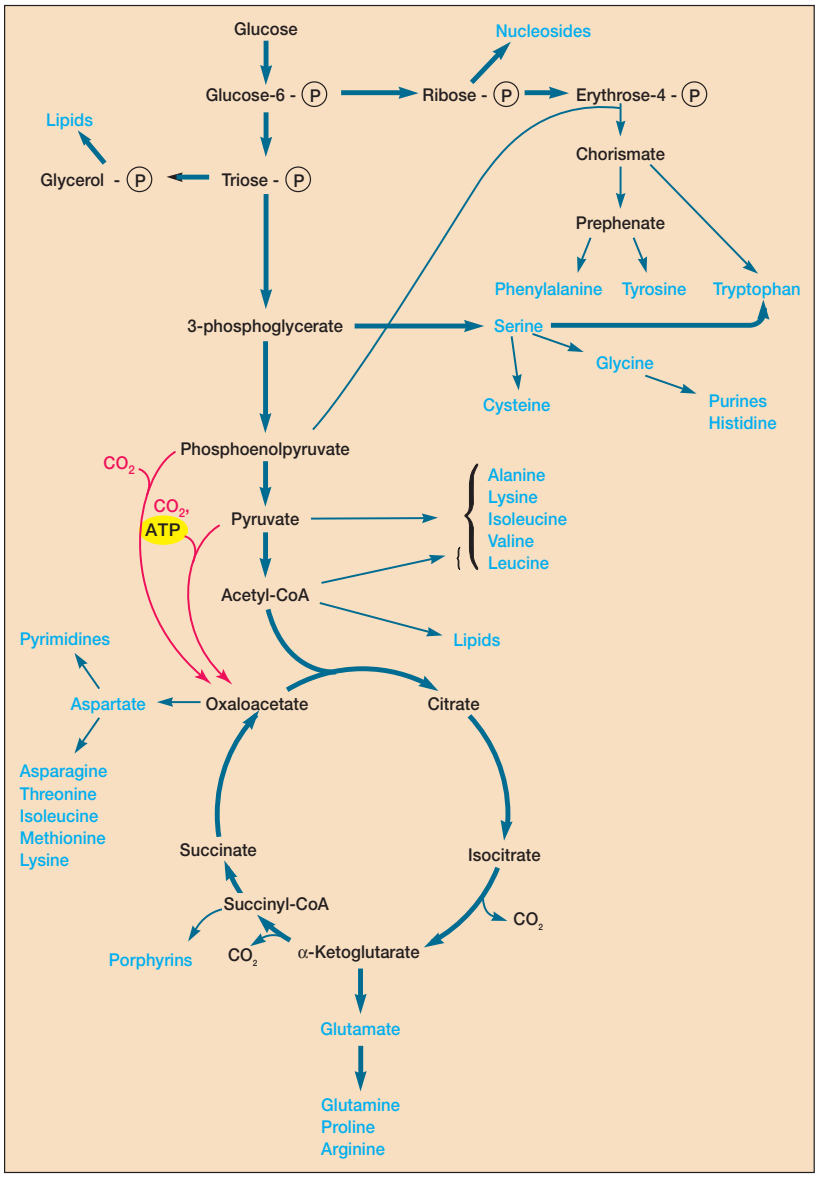

Figure 10.17 **The Organization of Anabolism.** Biosynthetic products (in blue) are derived from intermediates of amphibolic pathways. Two major anaplerotic CO_2 fixation reactions are shown in red.

10.6 Anaplerotic Reactions

Inspection of figure 10.17 will show that TCA cycle intermediates are used in the synthesis of pyrimidines and a wide variety of amino acids. In fact, the biosynthetic functions of this pathway are so essential that most of it must operate anaerobically to supply biosynthetic precursors, even though NADH is not required for electron transport and oxidative phosphorylation in the absence of oxygen. Thus there is a heavy demand upon the TCA cycle to supply carbon for biosynthesis, and cycle intermediates could be depleted if

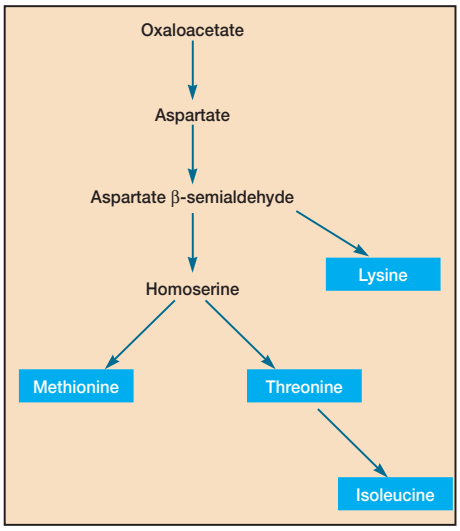

Figure 10.18 A Branching Pathway of Amino Acid Synthesis. The pathways to methionine, threonine, isoleucine, and lysine. Although some arrows represent one step, most interconversions require the participation of several enzymes.

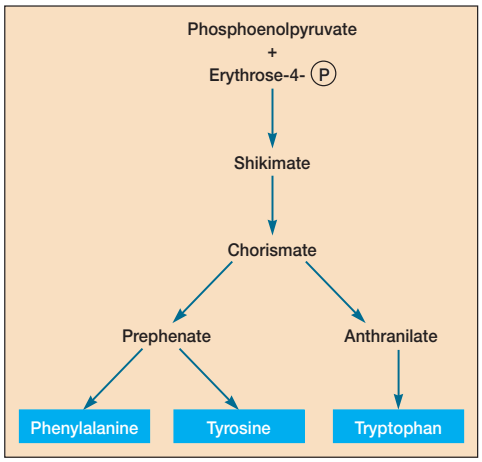

Figure 10.19 Aromatic Amino Acid Synthesis. The synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Most arrows represent more than one enzyme reaction.

nothing were done to maintain their levels. However, microorganisms have reactions that replenish cycle intermediates so that the TCA cycle can continue to function when active biosynthesis is taking place. Reactions that replace cycle intermediates are called **anaplerotic reactions** [Greek *anaplerotic,* filling up].

Most microorganisms can replace TCA cycle intermediates by **CO_2 fixation,** in which inorganic CO_2 is converted to organic carbon and assimilated. It should be emphasized that anaplerotic reactions do not serve the same function as the CO_2 fixation pathway that supplies the carbon required by autotrophs. In autotrophs CO_2 fixation provides most or all of the carbon required for growth. Anaplerotic CO_2 fixation reactions simply replace TCA cycle intermediates and maintain metabolic balance. Usually CO_2 is added to an acceptor molecule, either pyruvate or phosphoenolpyruvate, to form the cycle intermediate oxaloacetate (figure 10.17). Some microorganisms (e.g., *Arthrobacter globiformis,* yeasts) use pyruvate carboxylase in this role.

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \xrightarrow{\text{biotin}} \text{oxaloacetate} + \text{ADP} + P_i$$

This enzyme requires the cofactor biotin and uses ATP energy to join CO_2 and pyruvate. Biotin is often the cofactor for enzymes catalyzing carboxylation reactions. Because of its importance, biotin is a required growth factor for many species. Other microorganisms, such as the bacteria *Escherichia coli* and *Salmonella typhimurium,* have the enzyme phosphoenolpyruvate carboxylase, which catalyzes the following reaction.

$$\text{Phosphoenolpyruvate} + CO_2 \longrightarrow \text{oxaloacetate} + P_i$$

Some bacteria, algae, fungi, and protozoa can grow with acetate as the sole carbon source by using it to synthesize TCA cycle intermediates in the **glyoxylate cycle** (**figure 10.20**). This cycle is made possible by two unique enzymes, isocitrate lyase and malate synthase, that catalyze the following reactions.

$$\text{Isocitrate} \xrightarrow{\text{isocitrate lyase}} \text{succinate} + \text{glyoxylate}$$

$$\text{Glyoxylate} + \text{acetyl-CoA} \xrightarrow{\text{malate synthase}} \text{malate} + \text{CoA}$$

The glyoxylate cycle is actually a modified TCA cycle. The two decarboxylations of the latter pathway (the isocitrate dehydrogenase and α-ketoglutarate dehydrogenase steps) are bypassed, making possible the conversion of acetyl-CoA to form oxaloacetate without loss of acetyl-CoA carbon as CO_2. In this fashion acetate and any molecules that give rise to it can contribute carbon to the cycle and support microbial growth. The TCA cycle (pp. 183–84)

1. Define an anaplerotic reaction and give an example.
2. How does the glyoxylate cycle convert acetyl-CoA to oxaloacetate, and what special enzymes are used?

10.7 The 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 and RNA synthesis (pp. 235–39, 261–64)

Purines and **pyrimidines** are cyclic nitrogenous bases with several double bonds and pronounced aromatic properties. Purines