Chapter 10 Microbial Metabolism: Energy Production

OUTLINE **Some Principles of Bioenergetics**

Oxidation-Reduction Reactions

The Respiratory Chain

Energy Production by Anaerobic Processes
Glycolysis • The Pentose Phosphate Pathway • The Entner-Doudoroff Pathway • Fermentation

Energy Production by Aerobic Processes
The Tricarboxylic Acid Cycle • Energy Yield in Aerobic Respiration • Catabolism of Lipids • Catabolism of Proteins • Respiration without Oxygen in Some Bacteria • Heterotrophic CO_2 Fixation • The Glyoxylate Cycle

Energy Production by Photosynthesis
Cyclic and Noncyclic Photophosphorylation

The Mechanism of ATP Synthesis

The multiplicity of processes performed by all biological systems can be traced, directly or indirectly, to certain chemical reactions. Even the shape of a bacterium depends on such reactions: shape is determined by the geometric structure of the rigid peptidoglycan component of the cell wall, the peptidoglycan in turn being determined by the series of chemical reactions involved in its synthesis. The term **metabolism** denotes all the organized chemical activities performed by a cell, which comprise two general types, **energy production** and **energy utilization.** Energy is the ability to do work, and the work of a bacterial cell is extensive and varied. Energy is utilized for the construction of the physical parts of the cell such as wall or membrane; it is required for synthesis of enzymes, nucleic acids, polysaccharides, and other chemical components; it is required for repair of damage and mere maintenance of the status quo, as well as for growth and multiplication; it is required for accumulating certain nutrients in high concentration in the cell and for keeping certain other substances out of the cell; and it is required for motility. To support such extensive activities, vast amounts of energy must be provided. Under certain optimal conditions,

some bacteria have been found capable of metabolizing an amount of nutrient equivalent to their own weight *every few seconds* to provide such energy! A bacterial cell can be pictured as a dynamo of tremendous energy production. In this chapter, some basic principles of energetics will be discussed, followed by descriptions of just a few of the energy-producing mechanisms used by bacteria. Many of these mechanisms are also used by other microorganisms and by higher organisms, including human beings.

SOME PRINCIPLES OF BIOENERGETICS

Most cells obtain energy by carrying out chemical reactions which liberate energy. Some cells are able to use light as a source of their energy, but even here the light energy must be converted into chemical energy to be in a form useful for the work of the cell.

In the course of any chemical reaction, energy available for the performance of *useful* work is either released or absorbed. The amount of energy liberated or taken up during the course of a reaction is referred to as the *free-energy change* (ΔG) of the reaction. Thus free-energy change can be defined as useful energy. ΔG is expressed in terms of calories; however, this is merely a convenience since the free energy is not always in the form of heat but can, instead, be in the form of chemical energy. If the ΔG of a chemical reaction has a negative value (such as -8000 cal), the reaction releases energy (an *exergonic* reaction). If the ΔG of a reaction has a positive value (such as $+3000$ cal), the reaction requires energy (an *endergonic* reaction).

Concentration of reactants affects the value of ΔG for a chemical reaction, and to make valid comparisons between the energetics of various reactions, a basis of reference must be used. For purposes of comparison, it is assumed that the concentration of all reactants is 1.0 M in the steady state; this is referred to as *standard concentration*.

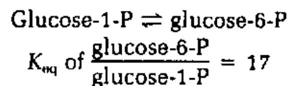
Under conditions of standard concentration, the free-energy change (ΔG) of a reaction is referred to by a special term, $\Delta G°$. In other words, $\Delta G°$ is the amount of free energy released (or absorbed) when one mole of the reactant is converted to one mole of product at 25°C and one atmosphere of pressure, and, under (hypothetical) conditions, where all reactants and products are maintained at 1 M concentration.

The $\Delta G°$ or standard free-energy change is related to the equilibrium constant, K_{eq}, of a chemical reaction by the equation

$$\Delta G° = -RT \ln K_{eq}$$

where R is the gas constant and T is the absolute temperature. If $\Delta G°$ is a negative value, the K_{eq} is greater than 1.0 and the formation of products is favored. If $\Delta G°$ is positive in value, K_{eq} is less than 1.0 and the chemical reaction tends to proceed in the reverse direction.

The value of $\Delta G°$ for a reaction can be calculated from the equilibrium constant of the reaction by using the above equation. For example, let us calculate $\Delta G°$ for the reaction catalyzed by phosphoglucomutase at 25°C:



$$\text{Glucose-1-P} \rightleftharpoons \text{glucose-6-P}$$

$$K_{eq} \text{ of } \frac{\text{glucose-6-P}}{\text{glucose-1-P}} = 17$$

The equation $\Delta G^\circ = -RT \ln K_{eq}$ mav he rewritten as:

$$\Delta G^\circ = -2.303RT \log K_{eq}$$

Substituting values for these terms, we get

$$\Delta G^\circ = -2.303RT \log 17$$
$$\Delta G^\circ = -2.303(1.987)\,(298) \log 17$$
$$\Delta G^\circ = -1{,}680 \text{ cal/mole}$$

Since the value of ΔG° is negative, the reaction can proceed from left to right under standard conditions.

Let us now calculate ΔG under physiological conditions. (ΔG is the actual free-energy change of a given chemical reaction under the conditions of concentration, pH, and temperature actually prevailing during the reaction, which are not necessarily the standard conditions as defined above.) Suppose that at 38°C the concentrations of glucose-6-P is 1×10^{-4} M and glucose-1-P are 3×10^{-5} M. We substitute these values in the following equation:

$$\Delta G = \Delta G^\circ + 2.303RT \log K_{eq}$$

We get

$$\Delta G = -1{,}680 + 2.303(1.987)\,(311) \log \frac{(1 \times 10^{-4})}{(3 \times 10^{-5})}$$
$$\Delta G = -1{,}680 + 1{,}423 \log 3.3$$
$$\Delta G = -1{,}680 + 740,$$
$$\Delta G = -940 \text{ cal/mole}$$

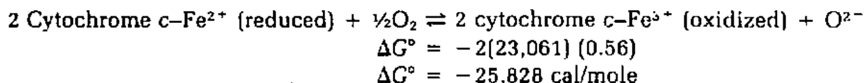
Thus, under physiological conditions, the reaction still proceeds from left to right.

It is also possible to obtain energy from a chemical reaction in the form of electric potential. Conversely, it is possible to use electric potential energy to drive a chemical reaction. Electric energy is generated when oxidations occur by the removal of electrons (as we will soon see). When the electrons fall through a potential difference or drop, energy is produced. The relationship between an oxidation-reduction potential difference and the standard free-energy change is

$$\Delta G^\circ = -nFE^\circ$$

where n is the number of moles of electrons transferred in the reaction, F is Faraday's constant (23,061 cals/V per equivalent), and E° is the standard oxidation-reduction potential difference.

For example, let us calculate ΔG° for the reaction in which cytochrome c is oxidized by oxygen from the ferrous to the ferric state with $E^\circ = +0.56$V:

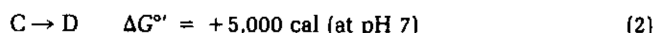


$$2 \text{ Cytochrome c–Fe}^{2+} \text{ (reduced)} + \tfrac{1}{2}O_2 \rightleftharpoons 2 \text{ cytochrome c–Fe}^{3+} \text{ (oxidized)} + O^{2-}$$
$$\Delta G^\circ = -2(23{,}061)\,(0.56)$$
$$\Delta G^\circ = -25{,}828 \text{ cal/mole}$$

In order for life to continue, it is essential that the energy released from exergonic reactions be used to drive endergonic reactions, and living organisms have developed characteristic ways of *coupling* exergonic reactions with endergonic reactions. The basic principle involved is that there be a *common reactant*. This can be best understood by the following example.

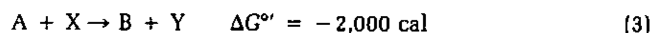
Consider the two general reactions

$$A \rightarrow B \qquad \Delta G^{\circ\prime} = -10{,}000 \text{ cal (at pH 7)} \qquad (1)$$

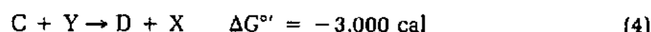


$$C \rightarrow D \qquad \Delta G^{\circ\prime} = +5{,}000 \text{ cal (at pH 7)} \qquad (2)$$

(ΔG° at pH 7 is designated $\Delta G^{\circ\prime}$.) The energy liberated by the first reaction (exergonic) can be used to drive the second reaction (endergonic) by coupling the two reactions in the following manner.



$$A + X \rightarrow B + Y \qquad \Delta G^{\circ\prime} = -2{,}000 \text{ cal} \qquad (3)$$

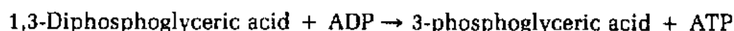


$$C + Y \rightarrow D + X \qquad \Delta G^{\circ\prime} = -3{,}000 \text{ cal} \qquad (4)$$

Here, Y is a reactant common to both reactions. In the first reaction (3), the overall $\Delta G^{\circ\prime}$ of $-2{,}000$ cal indicates that 8,000 of the original 10,000 cal was used for the conversion of X to Y. In the second reaction, Y was converted back to X, thereby releasing the previously trapped 8,000 cal to drive the endergonic conversion of C→D. Thus, the overall $\Delta G^{\circ\prime}$ of the second reaction (4) is $+5{,}000 - 8{,}000$, or $-3{,}000$ cal. The common reactant Y is referred to as an *energy-rich* or *energy-transfer compound*.

The common reactants of greatest use to the cell are those capable of transferring large amounts of free energy, called *high-energy-transfer compounds*. A variety of such compounds exists in cells; and although such compounds may possess no more total energy than other compounds, the energy is distributed in such a way within the molecule that one portion opposes another, resulting in considerable molecular strain. Triggering the breakdown of the molecule (by the catalytic action of an enzyme) causes release of the energy no longer held in check. A high-energy-transfer molecule is analogous to a mousetrap. When set, the trap has great energy, but the energy of the spring is opposed by the action of the catch. Tripping the catch is analogous to catalyzing the breakdown of the transfer molecule, with subsequent release of energy of the spring.

Table 10-1 lists some of the high-energy-transfer compounds found in cells, of which ATP is by far the most important. Just as money constitutes a common medium of exchange in our society, so ATP constitutes the "energy currency" of the cell in the exchange of energy between exergonic and endergonic reactions. It should be noted that all the compounds in Table 10-1 can transfer their energy directly or indirectly to ATP synthesis; for example:



$$\text{1,3-Diphosphoglyceric acid} + \text{ADP} \rightarrow \text{3-phosphoglyceric acid} + \text{ATP}$$

Energy is released from ATP by hydrolysis (Fig. 10-1). The amount of energy so

Table 10-1. Some High-Energy-Transfer Compounds Found in Cells with Their Standard Free-Energy Changes upon Hydrolysis

Compound	$\Delta G^{\circ\prime}$, kcal mol^{-1}
Adenosine triphosphate (ATP)	−7.3
Guanosine triphosphate (GTP)	−7.3
Uridine triphosphate (UTP)	−7.3
Cytidine triphosphate (CTP)	−7.3
Acetyl phosphate	−10.1
1,3-Diphosphoglyceric acid	−11.8
Phosphoenolpyruvic acid (PEP)	−14.8

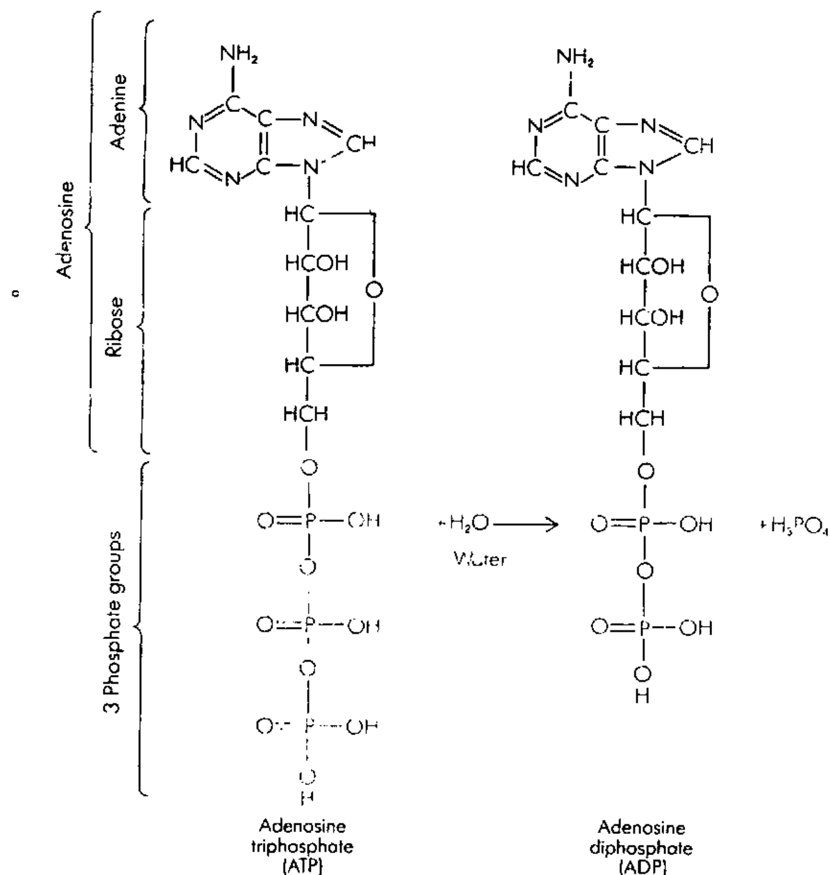


Figure 10-1. Hydrolysis of adenosine triphosphate.

Overall reaction:
$ATP + H_2O \rightarrow ADP + H_3PO_4$; $\Delta G°' = -7.3$ kcal mol^{-1}

released is a measure of the transfer energy capacity of ATP. It should be remembered, however, that the $\Delta G°'$ of -7.3 kcal for ATP is not necessarily that existing in the intact cell. The pH and the concentrations of ATP, ADP, Mg^{2+} etc., in the cell are not identical to the standard conditions employed for determining $\Delta G°'$. If appropriate corrections are made, the free energy of hydrolysis within the cell is closer to -12.5 kcal, although this value can vary depending upon intracellular concentrations of various materials. However, for consistency and comparison, thermodynamic calculations of biological energy exchanges must be carried out under arbitrarily defined standard conditions.

The compound ADP (adenosine diphosphate) is also a high-energy-transfer compound, since its hydrolysis also liberates a large quantity of energy:

$$ADP + H_2O \rightarrow AMP + H_3PO_4 \qquad \Delta G°' = -7.3 \text{ kcal}$$

where AMP stands for adenosine monophosphate. AMP, however, is a low-

energy compound; its hydrolysis yields only a small quantity of energy:

$$AMP + H_2O \rightarrow adenosine + H_3PO_4 \qquad \Delta G^{\circ\prime} = -2\ kcal$$

Several types of chemical reactions are involved in energy production, but oxidation-reduction is probably the commonest. A discussion of some of the basic aspects of oxidation-reduction reactions will clarify their relationship to energy production.

OXIDATION-REDUCTION REACTIONS

Oxidation is the loss of electrons; reduction is the gain of electrons. Frequently, oxidation reactions are dehydrogenations (reactions involving the loss of hydrogen atoms); since a hydrogen atom consists of a proton plus an electron, a compound which loses a hydrogen atom has essentially lost an electron and therefore has been oxidized.

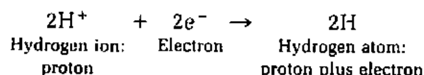
An oxidizing agent (oxidant) will absorb electrons and will therefore become reduced, as illustrated by the following examples.

The ferric ion is an oxidizing agent; it absorbs electrons and becomes reduced to ferrous ion:

$$Fe^{3+} + e^- \rightarrow Fe^{2+}$$

Ferric ion, Electron, Ferrous ion

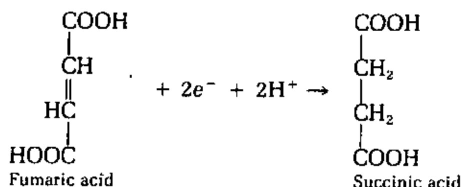
The hydrogen ion is an oxidizing agent; it absorbs electrons and becomes reduced to atomic hydrogen:



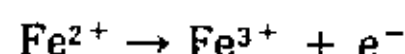
$$2H^+ + 2e^- \rightarrow 2H$$

Hydrogen ion: proton, Electron, Hydrogen atom: proton plus electron

Fumaric acid is an oxidizing agent; it absorbs hydrogen atoms (which contain electrons) and becomes reduced to succinic acid:



A reducing agent (reductant) donates electrons, becoming oxidized in the process. The ferrous ion is a reducing agent, it donates electrons and becomes oxidized to ferric ion:



$$Fe^{2+} \rightarrow Fe^{3+} + e^-$$

From this example, one can see that the reverse of each oxidation reaction is a reduction and the reverse of a reduction reaction is an oxidation. Moreover, in each reaction, a *pair* of substances is involved: one is the *reduced form*, the other the *oxidized form*, e.g., ferrous ion and ferric ion, succinic acid and fumaric acid. Each such pair of substances is referred to as an oxidation-reduction (O/R) system.

One O/R system may tend to absorb electrons from another O/R system; i.e., the first system will oxidize the second. On the other hand, the tendency of the

first system to absorb electrons may be so low that the second system may oxidize the first. This power (the tendency to absorb electrons) is expressed by the standard oxidation-reduction potential or the electromotive potential (E_0') of an O/R system, which is measured electrically under standardized conditions of comparison (electron donor and its conjugate at 1.0 M concentration, 25°C, and pH 7.0) and expressed in volts. The more positive the E_0', the greater the oxidizing ability of the system. Consequently, any system listed in Table 10-2 can oxidize any other system listed above it, but not below it, under the standard conditions. Such relationships are very important in understanding the orderly sequence in which biological oxidations occur.

As discussed previously, when one O/R system oxidizes another, energy is released. It is important to know the values of E_0' for each system, because the $\Delta G^{\circ\prime}$ of the overall reaction is directly proportional to the difference in E_0' values. If the voltage difference is large, an amount of free energy sufficient to drive the synthesis of ATP may be liberated.

In respiration, an oxidizable substrate is the primary electron donor. In aerobic respiration the terminal electron acceptor is oxygen; in anaerobic respiration the final electron acceptor is a compound like fumarate, NO_3^-, SO_4^{2-}, or CO_3^{2-} In fermentation, an organic compound is the final electron acceptor; an oxidizable substrate is the electron donor. In photosynthesis carried out by bacteria, bacteriochlorophylls serve as both electron donors and acceptors. In photosynthesis by green plants, algae, and cyanobacteria, water serves as a primary electron donor and $NADP^+$ (nicotinamide adenine dinucleotide phosphate) as a terminal electron acceptor. The paths through which these electrons flow in the various processes are called electron-transport chains.

Electron-transport chains are sequences of oxidation-reduction reactions that occur in cells. These reactions are mediated by a number of electron carriers and electron-carrier enzymes (discussed later). As the electrons flow through the chains, much of their free energy is conserved in the form of ATP; this process is called **oxidative phosphorylation.**

The multicomponent electron-transport chains are always associated with membranes. In eucaryotes, they are in mitochondrial or chloroplast membranes; in procaryotes, they are in the cytoplasmic membrane.

THE RESPIRATORY CHAIN

A respiratory chain is an electron-transport chain. When a pair of electrons or hydrogen atoms (which contain electrons) from an oxidizable substrate is coupled with the reduction of an ultimate electron acceptor, such as oxygen, there is a large free-energy change (ΔG°). The flow of electrons through the transport chain allows a stepwise release of this energy, some of which is conserved in the form of ATP at several steps in the chain. At these specific steps the difference in E_0' values is great enough to permit sufficient energy to be liberated for oxidative phosphorylation to occur.

The component O/R systems of a common type of respiratory chain are shown in Table 10-2 and are illustrated in Fig. 10-2.

A respiratory chain consists of enzymes having prosthetic groups or coenzymes. These can be regarded as the working parts of the enzymes, and in the case of the respiratory chain each is in fact an O/R system. The oxidized form of each prosthetic group or coenzyme has an absorption spectrum different from

Table 10-2. Component O/R Systems of a Respiratory Chain, with Their Corresponding E_0' Values

O-R System	E_0',V
$NAD^+/NADH + H^+$*	−0.32
Flavoprotein/flavoprotein-H_2	−0.03
CoQ/CoQ-H_2	+0.04
Cyt b–Fe^{3+}/cyt b–Fe^{2+}	+0.07
Cyt c_1–Fe^{3+}/cyt c_1–Fe^{2+}	+0.21
Cyt c–Fe^{3+}/cyt c–Fe^{2+}	+0.23
Cyt a–Fe^{3+}/cyt a–Fe^{2+}	+0.29
Cyt a_3–Fe^{3+}/cyt a_3–Fe^{2+}	+0.53
Oxygen/water	+0.82

* $NADH + H^+$ may also be designated as $NADH_2$ or simply as NADH since the other hydrogen atom appears as a free H^+ ion.

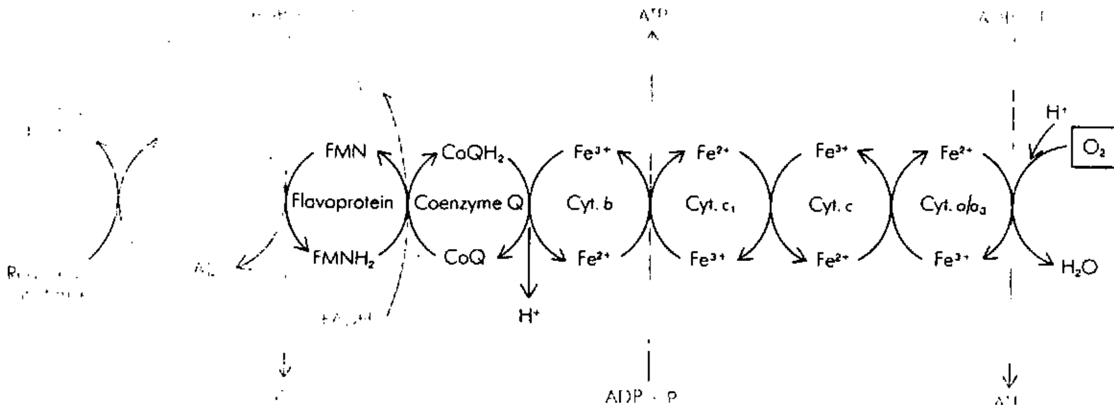
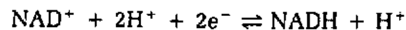


Figure 10-2. A respiratory chain, showing sequential oxidation steps and points where sufficient energy is liberated to permit synthesis of ATP. Electron transfer is accompanied by a flow of protons (H^+) from $NADH_2$ through coenzyme Q but not in later steps involving cytochromes. Note that three ATP are formed per molecule of $NADH_2$ reoxidized but only two ATP per molecule of $FADH_2$ reoxidized. (Note that this is an abbreviated representation. Actually, there are more than 15 chemical substances in the chain.)

that of the reduced form, so that the two states can be distinguished by spectrophotometry. A summary of these coenzymes follows.

Nicotinamide Adenine Dinucleotide (NAD) and Nicotinamide Adenine Dinucleotide Phosphate (NADP)

Certain enzymes which remove electrons and hydrogen ions from reduced substrates (referred to as dehydrogenases) have NAD^+ or $NADP^+$ as their coenzyme. NAD^+ can exist in a reduced form, $NADH + H^+$, to form an O/R system:



$$NAD^+ + 2H^+ + 2e^- \rightleftharpoons NADH + H^+$$

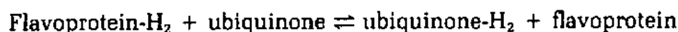
In the same way, $NADP^+$ can exist in a reduced state.

The vitamin niacin (nicotinic acid) forms part of the structure of NAD and NADP and is a precursor (building block) in their biosynthesis.

Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN)

Another class of dehydrogenases known as flavoproteins exists and contains either FAD or FMN as prosthetic groups. One of the basic parts of their coenzyme structure is the vitamin riboflavin. Riboflavin can exist in either an oxidized or

reduced form:



$$\text{Riboflavin} + 2H \rightleftharpoons \text{riboflavin-}H_2$$

The reduced forms of the coenzymes are $FADH_2$ and $FMNH_2$.

Coenzyme Q

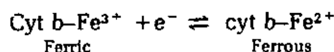
Coenzyme Q is also called **ubiquinone** because it is a quinone and is present in all cells. Coenzyme Q is a fat-soluble coenzyme. It functions as an acceptor of reducing power from the flavin-linked dehydrogenases:

$$\text{Flavoprotein-}H_2 + \text{ubiquinone} \rightleftharpoons \text{ubiquinone-}H_2 + \text{flavoprotein}$$

NAD^+, $NADP^+$, flavoproteins, and ubiquinones carry $2H^+$ and $2e^-$ but the cytochromes (discussed below) transfer only electrons, the protons being associated with an $-NH_2$ group or a $-COO^-$ group and eventually transferred to O_2 (see Fig. 10-2).

Cytochromes

Another major class of oxidative enzymes in the respiratory chain is the cytochromes. The prosthetic group of a cytochrome is a derivative of heme and contains a single iron atom, which is responsible for the oxidative or reductive properties of the enzyme. On the basis of differences in absorption spectra, cytochromes can be divided into three main categories: cytochromes *a*, cytochromes b, and cytochromes c. Each of these groups has a different function in the respiratory chain and can be further subdivided on the basis of minor differences in absorption spectra, e.g., cytochromes *c* and c_1 or cytochromes *a* and a_3. Each cytochrome type can exist in either an oxidized or reduced form, depending on the state of the iron atom contained in their structure:



$$\underset{\text{Ferric}}{\text{Cyt b-}Fe^{3+}} + e^- \rightleftharpoons \underset{\text{Ferrous}}{\text{cyt b-}Fe^{2+}}$$

The cytochromes act sequentially to transport electrons from coenzyme Q to O_2. Cytochromes *a* and a_3 together are called cytochrome oxidase. Both of them also contain copper. But only cytochrome a_3 can react directly with oxygen.

Sequence of Oxidation

The arrangement of O/R systems in Table 10-2 according to E_0' values is based on the experimentally determined sequence of oxidation reactions in the respiratory chain illustrated in Fig. 10-2. Sufficient energy for ATP synthesis is liberated at three points along the chain. The incremental release of energy in the respiratory chain results in a more efficient trapping of energy in ATP than would be true of direct oxidation of the reduced substrate by oxygen.

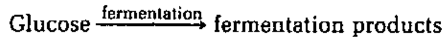
The respiratory chain of bacteria is associated with the *cytoplasmic membrane*; that of eucaryotes is present in mitochondrial membranes. Much of the electron transfer in membranes is accomplished within highly integrated particles or complexes.

ENERGY PRODUCTION BY ANAEROBIC PROCESSES

Heterotrophic bacteria can use a variety of organic compounds as energy sources. These compounds include carbohydrates, organic and fatty acids, and amino acids. For many microorganisms the preferred compounds are carbohydrates, especially the 6-carbon sugar glucose.

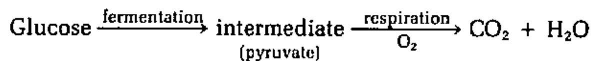
Glycolysis

The most common pathway of glucose catabolism is the Embden-Meyerhof pathway of glycolysis ("splitting of sugar"). This process occurs very widely and is found in microorganisms as well as in animals and plants. Glycolysis does not require the presence of oxygen and therefore can occur in both aerobic and anaerobic cells. Aerobic cells degrade glucose by glycolysis, and this process constitutes the preparatory stage for the aerobic phase of glucose oxidation. Thus, under anaerobic conditions this situation prevails:



$$\text{Glucose} \xrightarrow{\text{fermentation}} \text{fermentation products}$$

whereas under aerobic conditions, the following occurs:

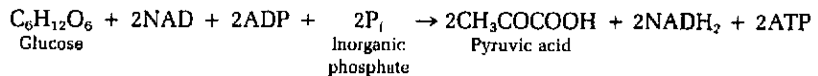


$$\text{Glucose} \xrightarrow{\text{fermentation}} \underset{\text{(pyruvate)}}{\text{intermediate}} \xrightarrow[O_2]{\text{respiration}} CO_2 + H_2O$$

In glycolysis, as shown in Fig. 10-3, fructose-1,6-diphosphate formed from glucose is split into two 3-carbon units (dihydroxyacetone phosphate and glyceraldehyde-3-phosphate), and they are subsequently oxidized to pyruvic acid. At the step where glyceraldehyde-3-phosphate is oxidized, a pair of electrons (two hydrogen atoms) is removed. In the absence of oxygen, this pair of electrons may be used to reduce pyruvic acid to lactic acid or ethanol. In the presence of oxygen, this pair of electrons may enter the respiratory chain.

Many of the reactions of the glycolytic pathway are freely reversible and can be used for the synthesis of glucose as well as for its breakdown. Only three of the reactions are not reversible by common enzymes; but the presence of other enzymes can reverse them for glucose synthesis to occur. Thus phosphoenolpyruvate is synthesized from pyruvate by the action of phosphoenolpyruvate synthase and specific phosphatases hydrolyze fructose-1,6-diphosphate and glucose-6-phosphate in the biosynthetic direction. The enzymes at these steps in the degradative direction are kinases and require ATP. (See Fig. 10-3.) For each molecule of glucose metabolized, two molecules of ATP are used up and four molecules of ATP are formed. Therefore for each molecule of glucose metabolized by glycolysis, there is a net yield of two ATP molecules. This is shown in Fig. 10-3.

The overall reaction of glycolysis can be summarized as follows:



$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 2NAD + 2ADP + \underset{\text{Inorganic phosphate}}{2P_i} \rightarrow \underset{\text{Pyruvic acid}}{2CH_3COCOOH} + 2NADH_2 + 2ATP$$

The Pentose Phosphate Pathway

The pentose phosphate pathway, like the glycolytic one, is another catabolic reaction pathway that exists in both procaryotic and eucaryotic cells. Since it involves some reactions of the glycolytic pathway, it has been viewed as a "shunt" of glycolysis; hence it may also be called the hexose monophosphate shunt. Its other synonym is the phosphogluconate pathway.

Glucose can be oxidized by the pentose phosphate pathway with the liberation of electron pairs, which may enter the respiratory chain. However, this cycle is not generally considered a major energy-yielding pathway in most microorganisms. It provides reducing power in the form of NADPH + H^+, which is required in many biosynthetic reactions of the cell, and it provides pentose phosphates

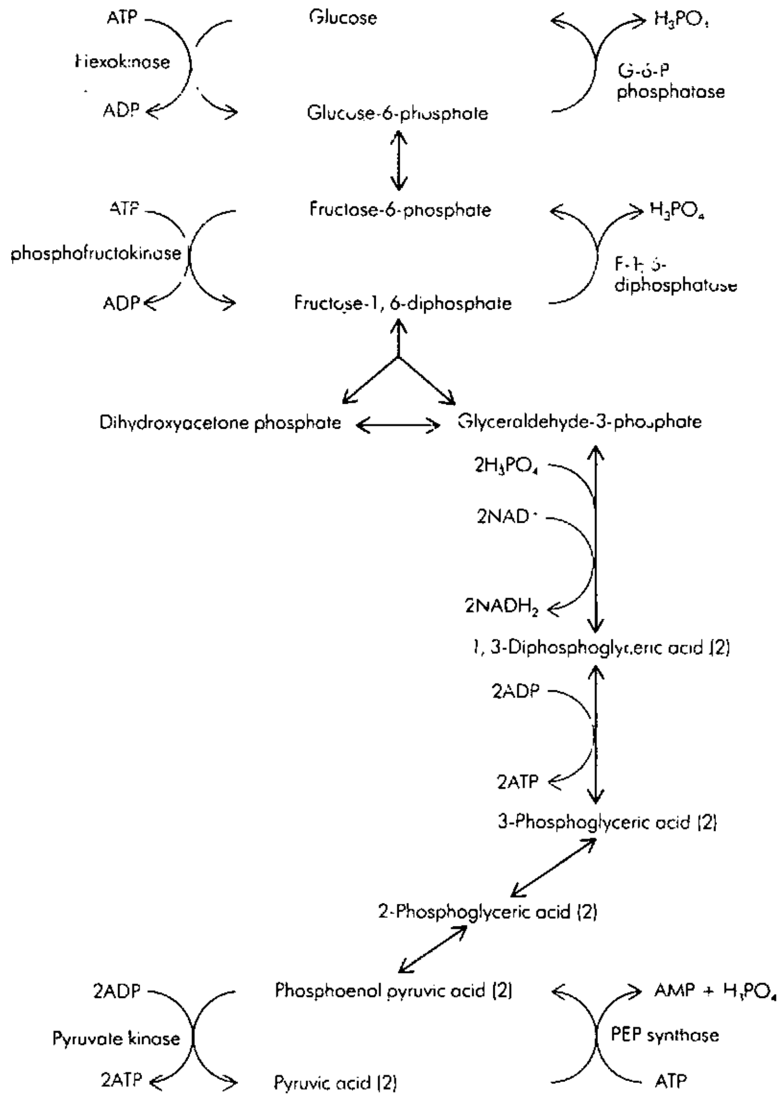


Figure 10-3. The Embden-Meyerhof (glycolytic) pathway of glucose catabolism. Enzymes shown are for those steps which are not freely reversible by a common enzyme.

for use in nucleotide synthesis. Although it can produce energy for the cell as an alternate pathway for the oxidation of glucose, it is also a mechanism for obtaining energy from 5-carbon sugars.

As seen in Fig. 10-4, the pentose phosphate pathway involves the initial phosphorylation of glucose to form glucose-6-phosphate; the latter is oxidized to 6-phosphogluconic acid with the simultaneous production of NADPH. Decarboxylation of 6-phosphogluconic acid, together with a yield of NADPH, produces ribulose-6-phosphate. Epimerization reactions yield xylulose-5-phosphate and ribose-5-phosphate. These two compounds are the starting point for

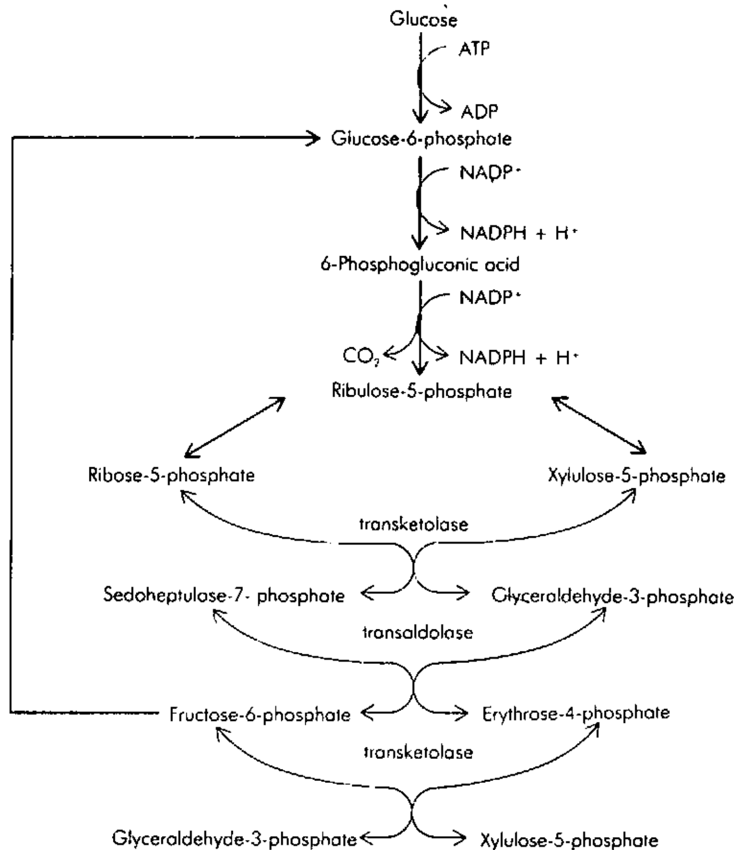
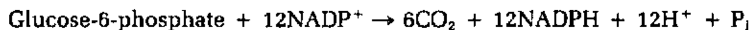


Figure 10-4. The pentose phosphate pathway of glucose catabolism yielding ribose-5-phosphate and NADPH + H^+.

a series of transketolase reactions and transaldolase reactions leading subsequently to the initial compound of the pathway, 6-phosphogluconic acid, thus completing the cycle. Note that two intermediates of glycolysis—fructose-6-phosphate and glyceraldehyde-3-phosphate—are generated. Theoretically, by means of this cycle, the cell can carry out the complete oxidation of glucose-6-phosphate to CO_2. Specifically, six molecules of glucose-6-phosphate are oxidized to six molecules each of ribulose-5-phosphate and CO_2; five molecules of glucose-6-phosphate are then regenerated from the six molecules of ribulose-5-phosphate. The overall equation is as follows:

$$6 \text{ Glucose-6-phosphate} + 12\text{NADP}^+ \rightarrow 5 \text{ glucose-6-phosphate} + 6\text{CO}_2 + 12\text{NADPH} + 12\text{H}^+ + \text{P}_i$$

The net equation is therefore:



$$\text{Glucose-6-phosphate} + 12\text{NADP}^+ \rightarrow 6\text{CO}_2 + 12\text{NADPH} + 12\text{H}^+ + \text{P}_i$$

In the real situation, it is more probable that the pentose phosphate pathway feeds into the glycolytic pathway by means of fructose-6-phosphate and glyceraldehyde-3-phosphate.

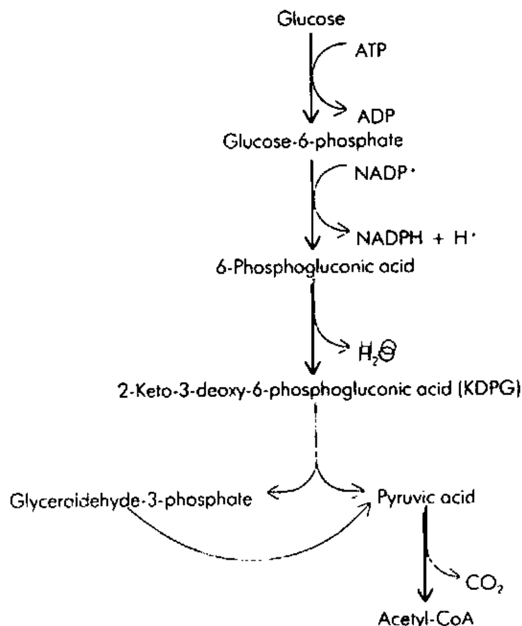


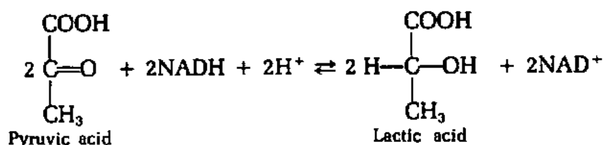
Figure 10-5. The Entner-Doudoroff pathway of glucose catabolism for some bacteria.

The Entner-Doudoroff Pathway

Another pathway of glucose catabolism is called the Entner-Doudoroff pathway. It is found in both aerobic and anaerobic procaryotes but not in eucaryotes. It is fairly widespread, particularly among Gram-negative bacteria. As shown in Fig. 10-5, glucose is phosphorylated to glucose-6-phosphate. It is then oxidized to 6-phosphogluconic acid. A dehydration step follows to yield 2-keto-3-deoxy-6-phosphogluconic acid (KDPG); the latter is cleaved to pyruvic acid and glyceraldehyde-3-phosphate, which is metabolized via some Embden-Meyerhof pathway enzymes to produce a second molecule of pyruvic acid. In the aerobic pseudomonads the catabolism is completed via acetyl-CoA and the tricarboxylic acid cycle (see later in this chapter).

Fermentation

Anaerobes also produce energy by reactions called fermentations, which use organic compounds as electron donors and acceptors. Facultative anaerobic bacteria and obligately anaerobic bacteria employ many different kinds of fermentations to produce energy. The lactic fermentation is a typical example. *Streptococcus lactis*, the bacterium responsible for the normal souring of raw milk, dissimilates glucose to lactic acid, which accumulates in the medium as the sole fermentation product. How does this happen? By glycolysis (Fig. 10-3), one molecule of glucose is converted to two molecules of pyruvic acid with concomitant production of two NADH + H^+. The pyruvic acid is converted to lactic acid in the following reaction:



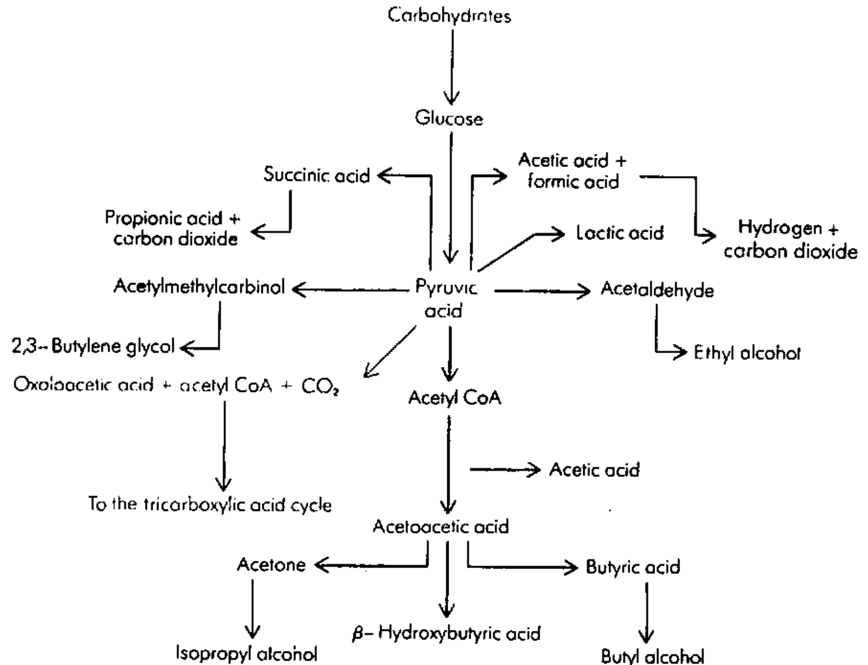


Figure 10-6. Pyruvic acid is regarded as the key compound in the dissimilation of glucose, as shown in this schematic illustration.

Insufficient energy for ATP synthesis results from this reaction; however, NAD^+ is regenerated for further use as an oxidant.

In other carbohydrate fermentations, the initial stages of glucose dissimilation frequently, but not always, follow the scheme of glycolysis. Differences in carbohydrate fermentations usually occur in the ways the resulting pyruvic acid is used. Thus pyruvic acid is the "hub" of carbohydrate fermentations. Figure 10-6 illustrates the variety of products resulting from the metabolism of pyruvic acid.

Most heterotrophic bacteria produce several end products of the types indicated in Fig. 10-6 from glucose dissimilation, but no single species produces all these end products. The types listed represent a summary of what can be expected when one takes an inventory of the end products of glucose dissimilation by all heterotrophs. Actually, it is possible to group microorganisms on the basis of their products of fermentation (the lactic acid group or the propionic acid group of bacteria, for example, as shown in Table 10-3). Such designations are established on the basis of the major end products of carbohydrate fermentation. From this it is evident that not all microorganisms metabolize the same substrate in exactly the same manner. For example, *Streptococcus lactis* and *Escherichia coli* both ferment glucose but by quite different pathways of fermentation, as shown in Fig. 10-7.

However, some anaerobes do not have a functional glycolytic system. They may have carbohydrate fermentation pathways that use the pentose phosphate pathway and the Entner-Doudoroff pathway. Fermentations of noncarbohydrate substrates, such as amino acids, involve highly specific pathways.

Table 10-3. Bacteria Grouped According to Major Products of Glucose Dissimilation

Groups with Examples of Some Genera	Representative Products
Lactic acid bacteria *Streptococcus* *Lactobacillus* *Leuconostoc*	Lactic acid only or lactic acid plus acetic acid, formic acid, and ethyl alcohol; species producing only lactic acid are homofermentative, and those producing lactic acid plus other compounds are heterofermentative
Propionic acid bacteria *Propionibacterium* *Veillonella*	Propionic acid plus acetic acid and carbon dioxide
Coli-aerogenes-typhoid bacteria *Escherichia* *Enterobacter* *Salmonella*	Formic acid, acetic acid, lactic acid, succinic acid, ethyl alcohol, carbon dioxide, hydrogen, 2,3-butylene glycol (produced in various combinations and amounts depending on genus and species)
Acetone, butyl alcohol bacteria *Clostridium* *Eubacterium* *Bacillus*	Butyric acid, butyl alcohol, acetone, isopropyl alcohol, acetic acid, formic acid, ethyl alcohol, hydrogen, and carbon dioxide (produced in various combinations and amounts depending on species)
Acetic acid bacteria *Acetobacter*	Acetic acid, gluconic acid, kojic acid

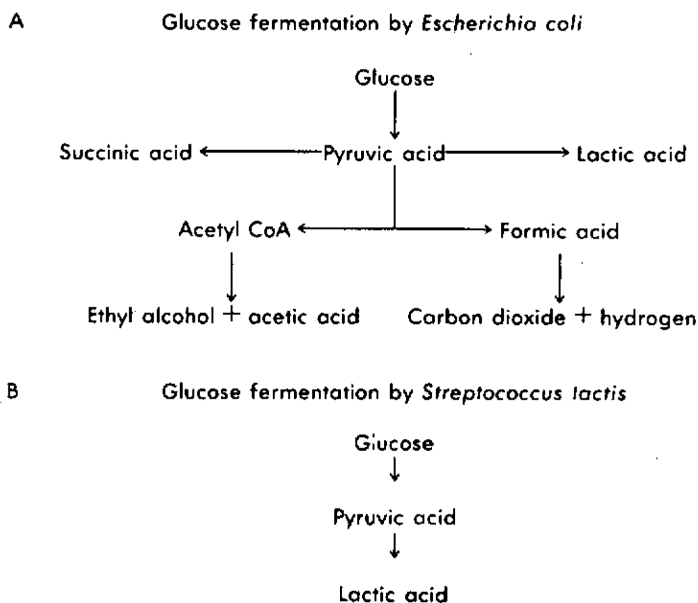
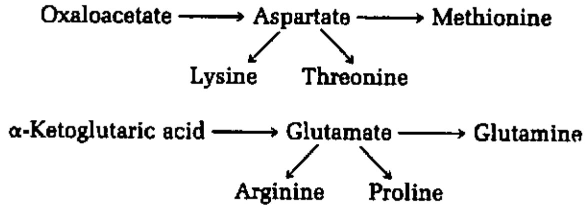


Figure 10-7. Glucose is fermented by many different bacteria and in many different ways. (A) *Escherichia coli* fermentation of glucose results in a mixture of products, whereas (B) *Streptococcus lactis* fermentation of glucose produces lactic acid almost exclusively.

ENERGY PRODUCTION BY AEROBIC PROCESSES

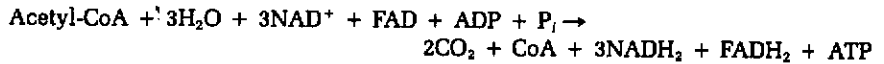
The Tricarboxylic Acid Cycle

The tricarboxylic acid (TCA) cycle is a sequence of reactions that generate energy in the form of ATP and reduced coenzyme molecules ($NADH_2$ and $FADH_2$). It also performs other functions. Many intermediates in the cycle are precursors in the biosynthesis of amino acids, purines, pyrimidines, etc. For example, oxaloacetic acid and α-ketoglutaric acid are amino acid precursors as shown in the following:



Thus the TCA cycle is an amphibolic cycle, which means that it functions not only in catabolic (breakdown) but also in anabolic (synthesis) reactions. The cycle is shown in Fig. 10-8.

The overall reaction of the TCA cycle can be summarized as follows:



$$\text{Acetyl-CoA} + 3H_2O + 3NAD^+ + FAD + ADP + P_i \rightarrow 2CO_2 + CoA + 3NADH_2 + FADH_2 + ATP$$

Since the breakdown of glucose by glycolysis yields two acetyl-CoA molecules

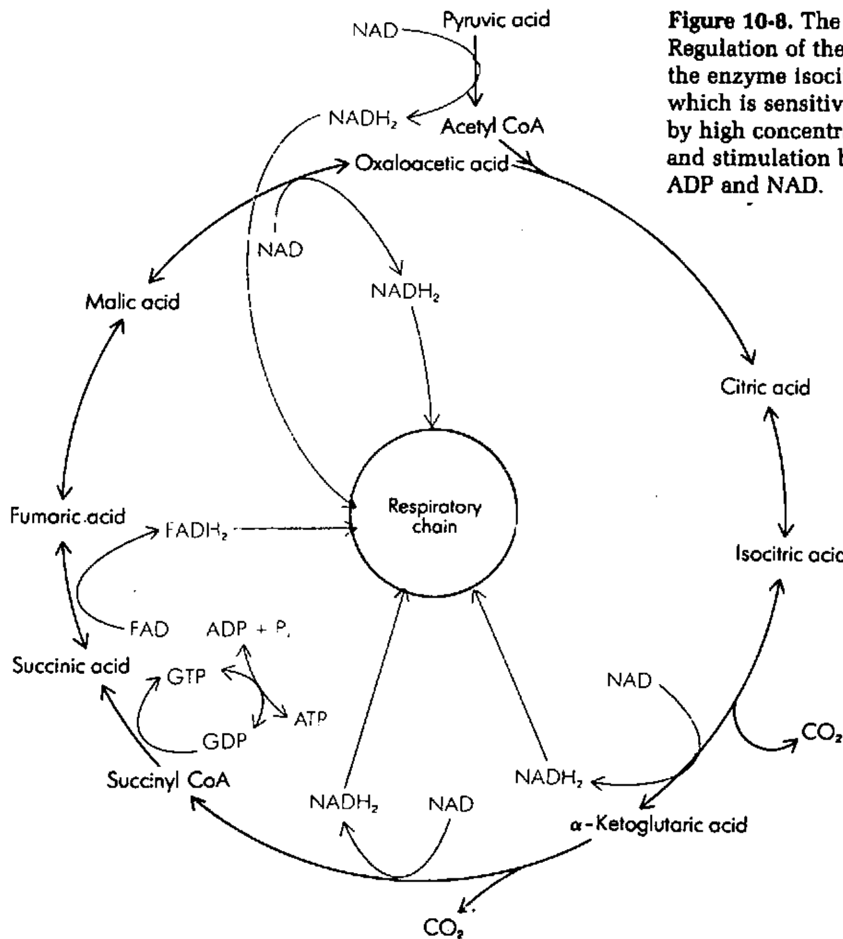


Figure 10-8. The tricarboxylic acid cycle. Regulation of the TCA cycle focuses on the enzyme isocitrate dehydrogenase, which is sensitive to feedback inhibition by high concentrations of ATP and $NADH_2$ and stimulation by high concentrations of ADP and NAD.

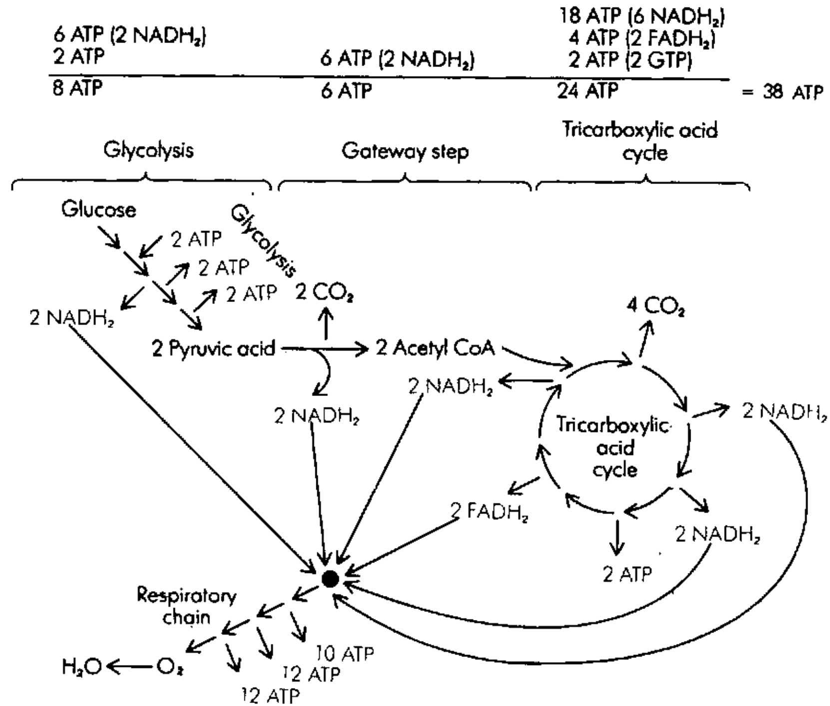


Figure 10-9. ATP yield per glucose molecule broken down in aerobic respiration.

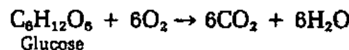
which can enter this cycle, the overall equation for the cycle, per glucose molecule broken down, is twice the above.

Energy Yield in Aerobic Respiration

We may now look at the energy yield from the aerobic breakdown of one molecule of glucose when the electrons stored in the reduced coenzyme molecules are fed into the electron-transport chain. As shown previously, the electrons are transferred stepwise from the coenzyme carriers to molecular oxygen, and this transfer is coupled to the generation of ATP by oxidative phosphorylation.

For each glucose molecule broken down, there are 12 reduced coenzymes to be oxidized: 2 $FADH_2$ (1 from each turn of the TCA cycle) and 10 $NADH_2$ (2 from glycolysis; 2 from the gateway step between glycolysis and the TCA cycle, i.e., pyruvic acid to acetyl-CoA; and 6 from two turns of the TCA cycle). Since 3 ATP are produced from each $NADH_2$ and 2 ATP from each $FADH_2$, there are 34 ATP generated from the reduced coenzymes via oxidative phosphorylation through the respiratory chain. But the total yield of ATP from the aerobic respiration of 1 glucose molecule is 38: 34 from the oxidation of reduced coenzymes, 2 from glycolysis, and 2 from the side reaction of the TCA cycle, that is, from 2 GTP. The total ATP yield per glucose molecule from aerobic respiration is summarized in Fig. 10-9.

The complete oxidation of glucose via glycolysis, the TCA cycle, and the respiratory chain is summarized in this overall reaction:

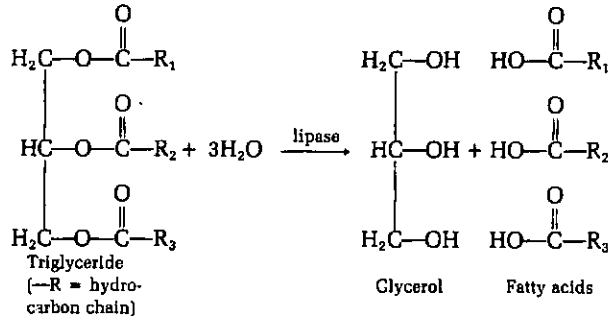


$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

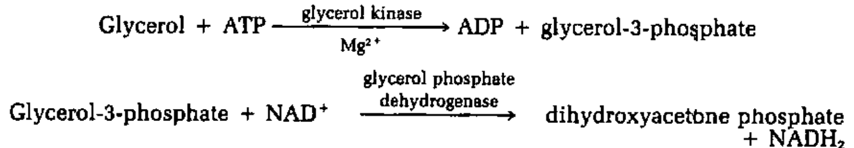
Catabolism of Lipids

Glucose is the single most important source of energy for most cells. However, for many microorganisms, other substances, such as lipids and proteins, may be used as alternate sources of energy. There is a general rule that governs their utilization: they are converted as quickly and efficiently as possible into intermediates of the glycolytic and TCA pathways so that a minimum number of additional enzymes is required to effect complete breakdown. This rule highlights the fact that the glycolytic pathway and the TCA cycle serve as a common center around which other catabolic pathways are built.

The breakdown of lipids or fats begins with the cleavage of triglycerides by the addition of water to form glycerol and fatty acids by means of enzymes called lipases:



Glycerol as a component of fats can be converted into an intermediate of the glycolytic pathway (dihydroxyacetone phosphate) by the following reactions:



$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Mg}^{2+}]{\text{glycerol kinase}} \text{ADP} + \text{glycerol-3-phosphate}$$

$$\text{Glycerol-3-phosphate} + \text{NAD}^+ \xrightarrow{\text{glycerol phosphate dehydrogenase}} \text{dihydroxyacetone phosphate} + \text{NADH}_2$$

The dihydroxyacetone phosphate formed would be broken down by the mechanisms shown in Fig. 10-3. Fatty acids are oxidized by the successive removal of 2-carbon fragments in the form of acetyl-CoA, a process known as β-oxidation. The acetyl-CoA formed can then enter the TCA cycle, and the hydrogen atoms and their electrons enter the respiratory transport chain, leading to oxidative phosphorylation.

There is more energy yield per gram of fat than per gram of carbohydrate. However, relatively few microbial species are effective in breaking down lipids of either simple or complex types, partly because of the limited solubility of lipids.

Catabolism of Proteins

Many heterotrophic microorganisms can degrade exogenous proteins, using the products as carbon and nitrogen energy sources. Since protein molecules are too large to pass into the cell, bacteria secrete exoenzymes called proteases that hydrolyze exogenous proteins to peptides, which are then transported into the cell cytoplasm.

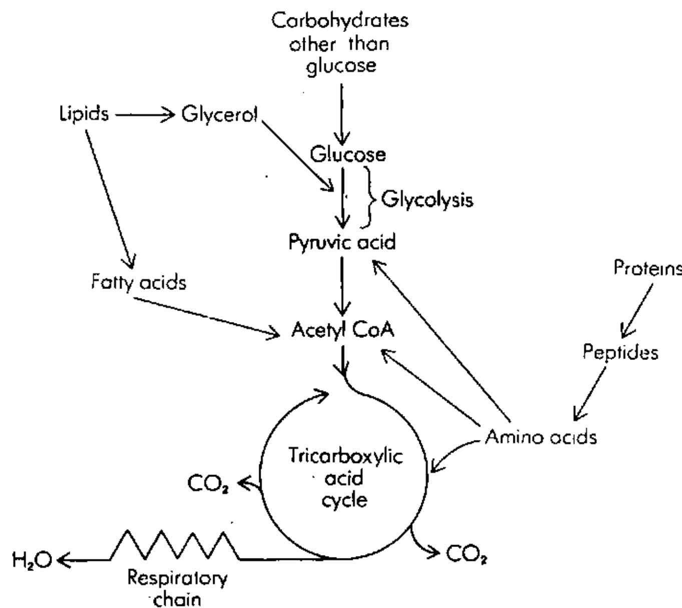


Figure 10-10. Metabolism of carbohydrates, lipids, and amino acids. As can be seen from the diagram, acetyl-CoA is a common intermediate of carbohydrate and lipid metabolism, and the TCA cycle is the common pathway for oxidation of carbohydrates, lipids, and amino acids.

Bacteria produce peptidases that break down peptides to the individual amino acids, which are then broken down according to the specific amino acid and the species or strain of bacteria breaking it down. This process may be shown as follows:

$$\text{Proteins} \xrightarrow{\text{proteases}} \text{peptides} \xrightarrow{\text{peptidases}} \text{amino acids}$$

Where amino acids are broken down, the carbon skeletons of the amino acids undergo oxidation to compounds that may enter the TCA cycle for further oxidation. Entry into the TCA cycle can be via acetyl-CoA, α-ketoglutaric acid, succinic acid, fumaric acid, or oxaloacetic acid.

An overall view of the dissimilation of carbohydrates, lipids, and proteins is shown in Fig. 10-10.

Respiration without Oxygen in Some Bacteria

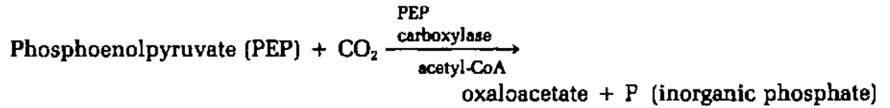
Some bacteria which are ordinarily aerobic can grow anaerobically if nitrate is present. For example, *Aquaspirillum itersonii*, an aquatic bacterium, is dependent on oxygen unless potassium nitrate is added to the medium. In such cases nitrate essentially substitutes for oxygen as the final electron acceptor in the respiratory chain. This process is termed **anaerobic respiration.** The pathways for the dissimilation of the carbon and energy sources are identical with those in aerobic respiration, and electron transport occurs via a respiratory chain similar to that in aerobic cells. Oxygen is replaced as the terminal electron acceptor by nitrate. However, in some strict anaerobes, other compounds, such as carbon dioxide, or ions, such as sulfate ion, can be the terminal electron acceptors.

Heterotrophic CO_2 Fixation

This phenomenon (unrelated to autotrophic CO_2 fixation) is important because it provides a mechanism for synthesis of compounds of the TCA cycle from the

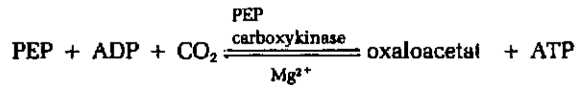
products of carbohydrate metabolism. Two types of CO_2- ixing reactions occur in heterotrophic bacteria.

1 This first type of reaction is essentially irreversible and occurs in many bacteria:



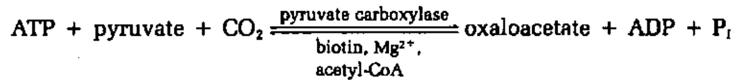
$$\text{Phosphoenolpyruvate (PEP)} + CO_2 \xrightarrow[\text{acetyl-CoA}]{\text{PEP carboxylase}} \text{oxaloacetate} + P \text{ (inorganic phosphate)}$$

A variation of this reaction requires a nucleoside diphos; hate:



$$\text{PEP} + \text{ADP} + CO_2 \xrightleftharpoons[Mg^{2+}]{\text{PEP carboxykinase}} \text{oxaloacetat} \quad + \text{ATP}$$

2 The second type requires the vitamin biotin for activity:



$$\text{ATP} + \text{pyruvate} + CO_2 \xrightleftharpoons[\text{biotin, } Mg^{2+}, \text{ acetyl-CoA}]{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$$

The Glyoxylate Cycle

The glyoxylate cycle is used by some microorganisms when acetate is the sole carbon source or during oxidation of primary substrates (such as higher fatty acids) that are cleaved to acetyl-CoA without the intermediate formation of pyruvic acid. This pathway does not occur in higher organisms because they are never forced to feed on 2-carbon molecules alone.

The specific enzymes of the glyoxylate cycle are isocitrate lyase and malate synthase. Figure 10-11 shows how these two enzymes fit together with other

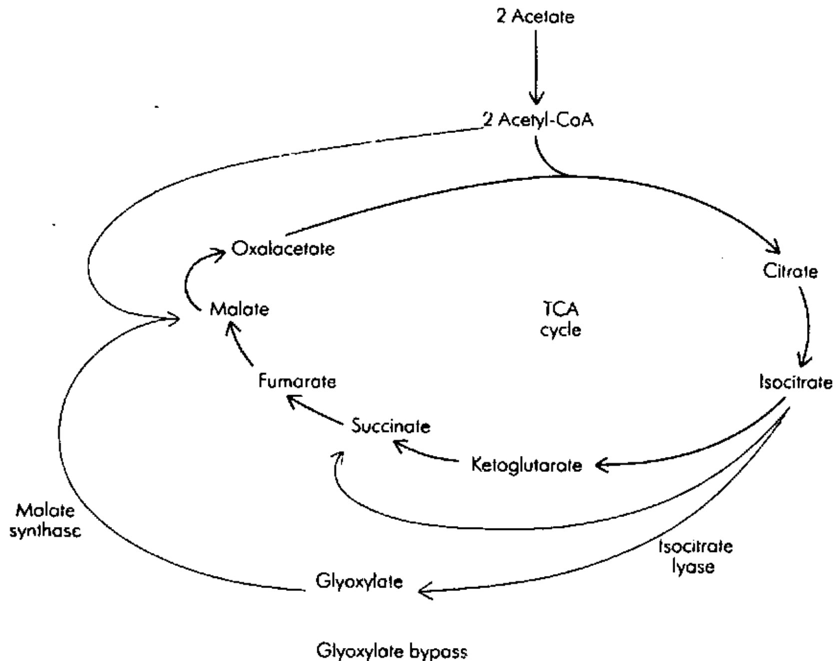


Figure 10-11. The glyoxylic acid cycle or bypass. Its reactions permit the replenishment of the pool of intermediates of the TCA cycle. The specific enzymes are isocitrate lyase and malate synthase.

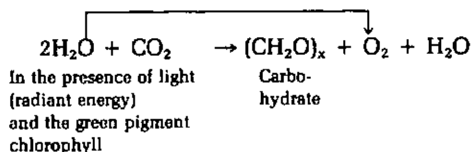
reactions of the tricarboxylic acid cycle to provide a bypass around some of the TCA-cycle reactions. The overall reaction of the glyoxylate cycle is

$$2\ \text{Acetyl-CoA} \rightarrow \text{succinate} + 2H + 2\text{CoA}$$

As seen in Fig. 10-11, acetyl-CoA enters the cycle at two places. It condenses with oxalacetate to give citrate, which is the entry point for the TCA cycle, and the further reaction leads to the formation of isocitrate. Isocitrate lyase is a splitting enzyme that produces succinate and glyoxylate. The second acetyl-CoA molecule condenses with glyoxylate to give malate by the action of malate synthase. Enzymes which carry out replenishment reactions such as this are known as *anaplerotic* enzymes; their function is to maintain the pool of essential intermediates for biosynthesis.

ENERGY PRODUCTION BY PHOTOSYNTHESIS

Plants, algae, and cyanobacteria are photoautotrophs. They use light as their source of energy and carbon dioxide as their sole source of carbon. In order for carbon dioxide to be useful for metabolism, it must first be reduced to carbohydrate. This process, by which light is used to convert carbon dioxide to carbohydrate, is called *photosynthesis*. The overall reaction can be written as

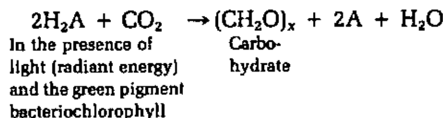


$$2H_2O + CO_2 \rightarrow (CH_2O)_x + O_2 + H_2O$$

Here $(CH_2O)_x$ is a formula representing any carbohydrate.

Photosynthesis has two important requirements: (1) a large amount of energy in the form of ATP, and (2) a large quantity of a chemical reductant, in this case water.

Several groups of bacteria—the photoautotrophic green and purple bacteria—are also characterized by their ability to perform photosynthesis. But unlike plants, algae, and cyanobacteria, they do not use water as their chemical reductant, nor do they produce oxygen as one of their end products of photosynthesis. The general equation for bacterial photosynthesis is:



$$2H_2A + CO_2 \rightarrow (CH_2O)_x + 2A + H_2O$$

Here H_2A represents the chemical reductant, such as the inorganic compounds H_2, H_2S, or $H_2S_2O_3$, or the organic compounds lactate or succinate. If H_2A in this equation stood for H_2S, then A would stand for S.

Both of the preceding equations represent the overall results of photosynthesis. A great deal has been learned about the specific chemical reactions involved in bacterial and plant photosynthesis. What follows is a look at the light-dependent energy-yielding processes involving bacteriochlorophyll in bacteria and chlorophyll in plants, algae, and cyanobacteria. What is presented is

in accord with the latest results of many investigators but may require modification as further evidence is accumulated.

Cyclic and Noncyclic Photophosphorylation

Anoxygenic photosynthetic bacteria possess chlorophylls, called bacteriochlorophylls, that differ from the chlorophylls of plants in structure and in light-absorbing properties. Bacteriochlorophylls absorb light in the infrared region (725 to 1,035 nm). They are not contained in chloroplasts but are found in extensive membrane systems throughout the bacterial cell.

When a molecule of bacteriochlorophyll absorbs a quantum of light, the energy of the light raises the molecule to an excited state. In this excited state an electron is given off by bacteriochlorophyll. Bacteriochlorophyll thus becomes positively charged. It then serves as an electron trap or strong oxidizing agent.

The electron, carrying some of the energy absorbed from light, is transferred to an iron-containing heme protein known as ferredoxin. From there it is passed successively to ubiquinone, to cytochrome *b*, and to cytochrome *f*, and finally back to the positively charged bacteriochlorophyll. Essentially, the electron has gone around in a cycle, beginning with, and returning to, bacteriochlorophyll. This relatively simple process is illustrated in Fig. 10-12.

The energy released in the step between cytochrome *b* and cytochrome *f* is used for photophosphorylation —the generation of ATP from ADP and inorganic phosphate.

Note that no $NADP^+$ has been reduced in these reactions. The reduction of $NADP^+$ in photosynthetic bacteria is accomplished not by photosynthesis but by using reducing power from constituents of the environment, such as H_2S and other inorganic and organic compounds. Such reduced compounds usually abound in the anaerobic environment of photosynthetic bacteria.

It may be added that light of higher energy than that absorbed by bacteriochlorophylls can contribute to bacterial photosynthesis since there are carotenoids and other accessory pigments in the bacterial cells which absorb light at shorter wavelengths and transfer the energy to the bacteriochlorophylls.

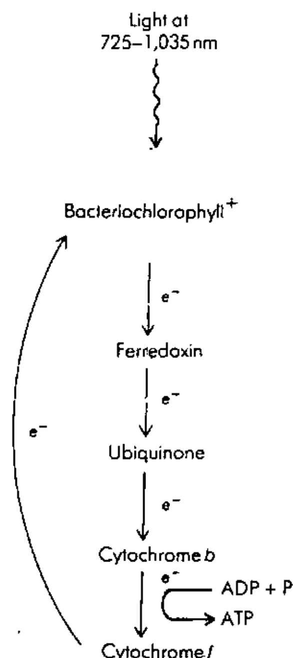


Figure 10-12. Cyclic photophosphorylation as it occurs in anoxygenic photosynthetic bacteria. The electron returns, at a lower energy state, to the bacteriochlorophyll, which had become positively charged after the initial ejection of the electron. No NADP is reduced and no external donor is necessary for this process.

In plants, algae, and cyanobacteria (oxygenic photosynthetic bacteria), noncyclic photophosphorylation occurs in photosynthesis. In this process, when a molecule in pigment system II (one of two systems of light reactions) absorbs light, this energy raises the molecule to an excited state and the molecule releases an electron. This electron is transferred to plastoquinone, to cytochrome *b*, to cytochrome *f*, and finally to pigment system I. Photophosphorylation occurs with generation of ATP from ADP and inorganic phosphate in the step between cytochrome *b* and cytochrome *f*. When pigment system I absorbs light, it releases an electron. This electron is transferred from ferredoxin, to flavoprotein, to $NADP^+$. Photophosphorylation occurs again between the release of the electron from pigment system I to ferredoxin. Also note that $NADP^+$ is reduced in this part of the process (see Fig. 10-13). This process differs from cyclic photophosphorylation because the electron lost by pigment system II is not cycled back to it. Instead, electrons are replaced in pigment system II by the light-generated breakdown of water, called photolysis. There is some evidence that this scheme of noncyclic photophosphorylation, shown in Fig. 10-13, may have to be modified. It appears that system II pigments alone can carry out the entire process

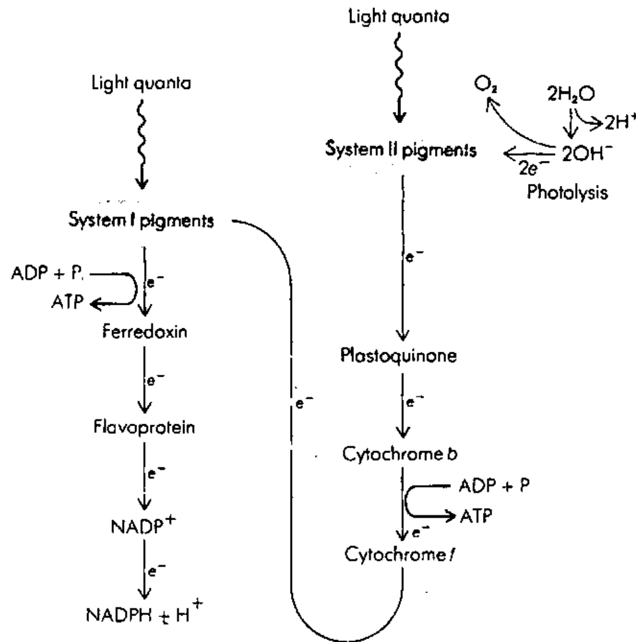


Figure 10-13. Noncyclic photophosphorylation as it occurs in green plants, algae, and cyanobacteria. In this process, electrons raised to a high energy state ultimately reduce $NADP^+$ and are not recycled to the light-pigment systems. The protons necessary for reduction come from the dissociation of water, which results in evolution of oxygen. Electrons are restored to the pigments of system II from the OH^- ion of H_2O. The OH^- ion is split to e^-, H^+, and ½ O_2 by photolysis.

of noncyclic photophosphorylation. Thus the noncyclic reduction of ferredoxin need not involve system I pigments. Further, the most important role of plastoquinone is in the transport of protons originating from water. This modified process has been termed oxygenic photophosphorylation. Further evidence is needed for its confirmation.

THE MECHANISM OF ATP SYNTHESIS

The chemical reactions that lead to the synthesis of ATP are now well understood. But how the transfer of electrons through the respiratory transport chain is coupled to the synthesis of ATP is not very clear. Several alternate hypotheses have been proposed to explain how energy released during electron transport is conserved in the form of ATP. The prevailing theory is the chemiosmotic hypothesis advanced in 1961 by Peter Mitchell, a British biochemist. Mitchell was awarded the Nobel prize for his work in this field in 1978. According to this theory, the flow of electrons through the system of carrier molecules releases energy which drives positively charged hydrogen ions (H^+), or protons, across the membranes of chloroplasts, mitochondria, and bacterial cells (Fig. 10-14). This movement of hydrogen ions results in the acidification of the surrounding medium and the generation of a pH gradient (a difference in pH) across the organelle or cell membrane. In addition, such hydrogen-ion movements lead to the formation of an electric potential gradient (a difference in charge) across the membrane (since an electric charge is carried by the proton). In this way, energy released during the transfer of electrons through the respiratory chain is conserved as a "protonmotive force"; the electric potential gradients are produced by pumping hydrogen ions across the membrane.

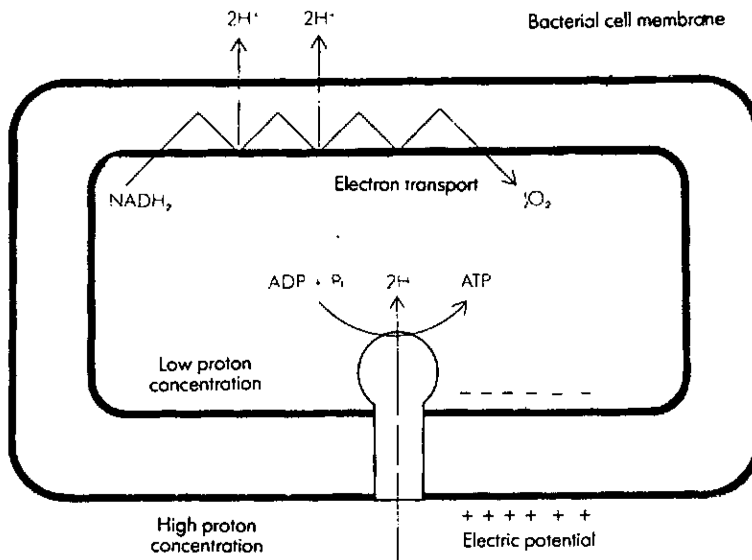


Figure 10-14. Mechanism of ATP synthesis. Flow of electrons through the respiratory chain drives hydrogen ions across the membrane. This results in a high hydrogen-ion concentration outside the cell and a low concentration inside the cell. This produces a pH and electrochemical gradient. ATP synthesis at the site of the ATPase complex (a knobbed structure on the membrane) is driven by the release of energy when hydrogen reenters the bacterial cell.

Following this first energy-conservation step, when the hydrogen ions reenter the organelle or cell, they are transported by the membrane-bound enzyme adenosine triphosphatase. The energy released on reentry drives the synthesis of ATP, the second energy-conservation step. This process is shown in Fig. 10-14.

QUESTIONS

1 What is meant by the following?
   (a) Free-energy change
   (b) Exergonic reaction
   (c) Endergonic reaction
   (d) ΔG, ΔG°, $\Delta G^{\circ\prime}$
2 Explain the relationship between ΔG° and the equilibrium constant, and the relationship between ΔG° and an oxidation-reduction potential difference.
3 What role does ATP play in energy exchanges in cells?
4 In what way is the coupling of exergonic reactions with endergonic reactions important in living organisms?
5 Define the meaning of a high-energy-transfer compound. Name those that occur in the glycolytic pathway.
6 Explain what is meant by an oxidation-reduction system.
7 What is oxidative phosphorylation? Where does it occur in the respiratory chain?
8 Briefly explain how glycolysis fits into the metabolism of glucose in aerobic cells.
9 Compare the disposition of electrons (or hydrogen atoms) obtained from the oxidation of glyceraldehyde-3-phosphate in aerobic and anaerobic cells.
10 Identify the three reactions in the glycolytic pathway that are not freely reversible by the same specific enzymes.

11 Account for the ATP yield per glucose molecule in glycolysis.
12 Describe the various ways in which the pentose phosphate cycle is useful to a cell.
13 Is the Entner-Doudoroff pathway found in (a) both aerobes and anaerobes; (b) both eucaryotes and procaryotes?
14 Explain why fermentation is a less efficient process for obtaining energy than aerobic respiration.
15 Explain how fermentation products can be used for the identification of bacteria. Provide specific examples to support your answer.
16 Why is the TCA cycle called an amphibolic cycle?
17 What general rule governs the utilization of substances other than carbohydrates for the production of energy?
18 Name the specific enzymes of the glyoxylate cycle and describe the reactions they catalyze.
19 What are the essential differences between photosynthesis by bacteria and by algae?
20 Explain why photophosphorylation in procaryotes is termed *cyclic*.
21 Describe the prevailing model for the mechanism of ATP synthesis.

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Chapter 11 Microbial Metabolism: Utilization of Energy and Biosynthesis

OUTLINE **Utilization of Energy in Nonbiosynthetic Processes**
Bacterial Motility • Transport of Nutrients by Bacteria

Utilization of Energy in Biosynthetic Processes
Synthesis of Small Molecules: The Amino Acids • Synthesis of Macromolecules: The Structure and Biosynthesis of a Cell-Wall Peptidoglycan • Synthesis of Organic Cell Material in Chemoautotrophic Bacteria

The Biosynthesis of Deoxyribonucleic Acid
The Structure of Deoxyribonucleic Acid • The Structure of Ribonucleic Acid • The Biosynthesis of Nucleotides in DNA Synthesis • Semiconservative Replication of DNA Strands • Replication of the DNA Molecule

Transcription and Translation of Genetic Information
The Building Blocks of Proteins • Transcription • Translation

The Process of Protein Synthesis
Assembly of the Protein Chain on the Ribosome

In the preceding chapter, we discussed some of the chemical mechanisms by which microorganisms obtain energy. In this chapter, we provide the sequel by considering some of the ways in which energy, once obtained, is utilized by microorganisms. We shall look at some examples of how this is done.

As we have seen in Chap. 10, energy is stored in the form of high-energy-transfer compounds (of which ATP is the most important). But energy is also available in the form of the **protonmotive force** (or electrochemical proton gradient). In these forms energy is used to drive the many endergonic reactions required for the life of the cell.

The principle of coupling exergonic reactions to endergonic reactions requires the utilization of high-energy-transfer compounds like ATP. An electrochemical proton gradient may result in ATP synthesis (see Fig. 10-14), but it can also be used for other biological purposes without the synthesis of ATP. For example, it can be used to generate heat and the rotation of bacterial flagella.

UTILIZATION OF ENERGY IN NONBIOSYNTHETIC PROCESSES

The ATP formed by the energy-producing reactions of the bacterial cell is expended in various ways. Much of it is used in the biosynthesis of new cell components, including energy-storage inclusion granules such as glycogen and

poly-β-hydroxybutyrate. Other metabolic processes which require phosphate-bound energy or the energy of the protonmotive force include maintenance of the physical and chemical integrity of the cell, transport of solutes across membranes, and activity of locomotor organelles.

Maintenance of the physical and chemical integrity of the cell is mainly through reactions that lead to biosynthesis of macromolecules, such as nucleic acids and proteins, that are continuously being broken down and need replacement. The extent of this degradation varies with the environmental conditions. Transport of solutes across membranes also requires energy, as does mechanical work such as motility by means of flagella. In general, the rate of utilization of energy in ATP determines the rate at which ATP is regenerated from ADP at the expense of energy from the environment.

Bacterial Motility

Bacterial flagella filaments appear to have no machinery for interconverting chemical and mechanical energy. For example, flagellin, the flagellar protein molecule, has no enzymatic activity, i.e., no detectable ATPase activity (such as is present in cilia and flagella of eucaryotic microorganisms). Bacterial flagella thus differ markedly from the much larger and more complex cilia and flagella that propel eucaryotic cells such as protozoa.

It is therefore not surprising that ATP has been demonstrated *not* to be the immediate source of energy for flagella rotation. Instead the flagellar motor (that rotates the flagellum) is driven by the protonmotive force, i.e., the force derived from the electric potential and the hydrogen gradient across the cytoplasmic membrane.

The rotary motor is believed to be the two elements in the basal body, the M ring and the S ring (see Chap. 5). The rod (which is connected to the filament by the hook) is fixed rigidly to the M ring, which rotates freely in the cytoplasmic membrane. The S ring is mounted rigidly on the cell wall. The inward flux of protons drives the flagellar motor (Fig. 11-1). Exactly what molecular events cause the conversion of protonmotive force into mechanical rotation are still unknown. However, it is clear that in the case of flagellar rotation, proton movements, and not ATP, constitute the energy currency.

Transport of Nutrients by Bacteria

We shall now give an account of the various processes by which ions or molecules cross the cytoplasmic membrane. It is the cytoplasmic membrane that allows the passive passage of certain small molecules and actively concentrates others within the cell.

Passive Diffusion

Except for water and some lipid-soluble molecules, few compounds can pass through the cytoplasmic membrane (a lipid-protein, semipermeable cell mem-

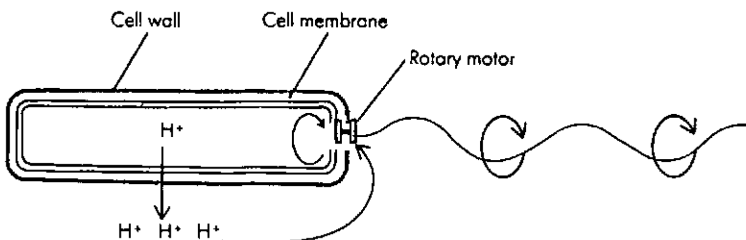


Figure 11-1. The flagellum of bacteria is driven by the protonmotive force. Protons flowing back into the cell through the basal body rings of each flagellum cause it to rotate; these rings constitute the rotary motor.

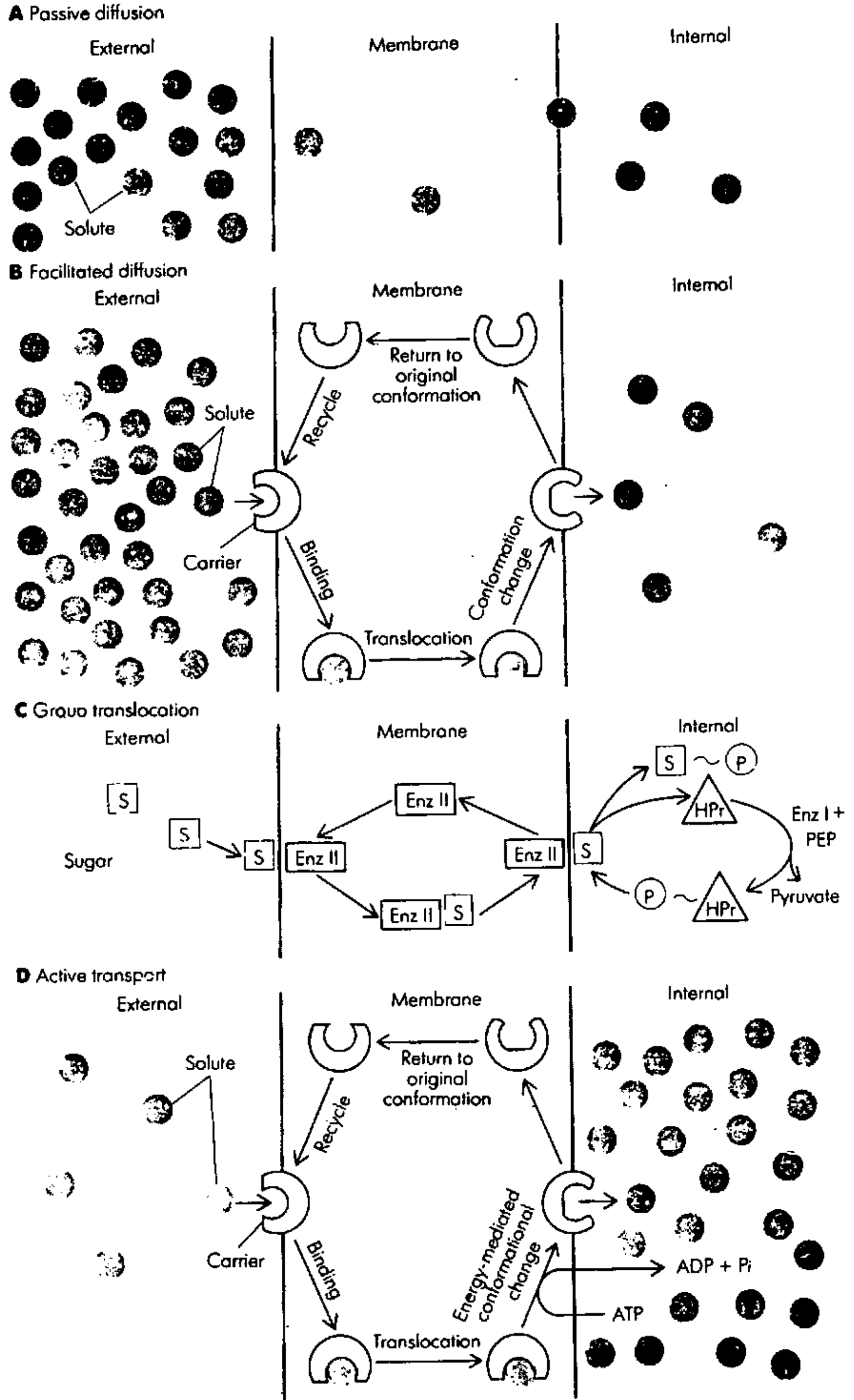


Figure 11-2. Mechanisms of nutrient transport into cells: (A) passive diffusion; (B) facilitated diffusion; (C) group translocation; (D) active transport.

brane) by simple, or passive, diffusion. In this process solute molecules cross the membrane as a result of a difference in concentration of the molecules across the membrane. The difference in concentration (higher outside the membrane than inside) governs the rate of inward flow of the solute molecule. With time, this concentration gradient diminishes until equilibrium is reached. In passive diffusion no substance in the membrane interacts specifically with the solute molecule as illustrated in Fig. 11-2A.

Facilitated Diffusion

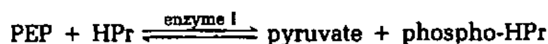
Another mechanism by which substances cross the semipermeable cell membrane is facilitated diffusion. This process is similar to passive diffusion in that the solute molecule also flows from a higher to a lower concentration. But it is different from passive diffusion because it involves a specific protein carrier molecule (called a porter or permease) located in the cytoplasmic membrane. The carrier molecule combines reversibly with the solute molecule, and the carrier-solute complex moves between the outer and inner surfaces of the membrane, releasing one solute molecule on the inner surface and returning to bind a new one on the outer surface. This process is shown in Fig. 11-2B. The entry of glycerol into bacterial cells is by facilitated diffusion. Although this mechanism of transport is common in eucaryotic cells (e.g., sugars enter them in this way), it is relatively rare in procaryotic cells. Neither of the above two mechanisms, passive diffusion or facilitated diffusion, require metabolic energy. Nor do they result in *concentration* or *accumulation* of solute against an electrochemical (with ions) or osmotic (with nonelectrolytes) gradient. Of greater interest to us in the context of this chapter are the two other mechanisms by which solutes cross membranes, both of which require metabolic energy and accumulate substrates against concentration gradients. Solutes can be concentrated within the cell several thousand times greater than outside the cell. These two mechanisms are group translocation and active transport.

Group Translocation

In group translocation the solute is altered chemically during transport. The best-studied group-translocation system is the phosphoenolpyruvate–dependent sugar–phosphotransferase system. It is widely distributed in many bacterial genera and mediates the translocation of many sugars and sugar derivatives. These solutes enter the cell as sugar phosphates and are accumulated in the cell in this form.

Phosphotransferase system (PTS) sugar uptake and phosphorylation require the participation of several soluble and membrane-bound enzymes. These proteins catalyze the transfer of the phosphoryl group of phosphoenolpyruvate to the sugar molecule. The products formed are therefore sugar phosphate and pyruvate; the overall reaction requires Mg^{2+}.

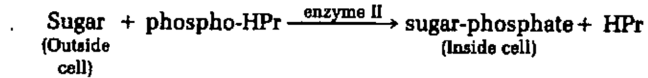
Specifically, a relatively heat-stable carrier protein (HPr) is activated first by transfer of a phosphate group from the high-energy compound phosphoenolpyruvate (PEP) inside the cell, as shown in Fig. 11-2C.



$$\text{PEP} + \text{HPr} \xrightleftharpoons{\text{enzyme I}} \text{pyruvate} + \text{phospho-HPr}$$

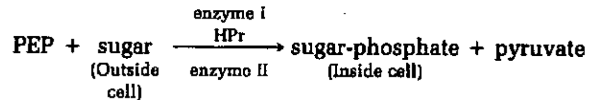
Enzyme I and HPr are soluble proteins and are nonspecific components of the process.

At the same time, the sugar combines with enzyme II at the outer membrane surface and is transported to the inner membrane surface (see Fig. 11-2C). Enzyme II is specific for a particular sugar and is an integral component of the cytoplasmic membrane. Here it combines with the phosphate group carried by the activated HPr. The sugar-phosphate is released by enzyme II and enters the cell. This is illustrated by the reaction equation shown below. Some investigators have reported a peripheral membrane enzyme III that mediates between enzyme II and phospho-HPr in translocating the sugar.



Enzyme I has been partially purified from several bacteria including *Escherichia coli* and *Salmonella typhimurium*. HPr has been purified to homogeneity from several bacteria. Mannitol enzyme II has been purified from *E. coli*.

The net chemical reaction of PTS sugar uptake is therefore:



Other known group-translocation processes include the uptake of adenine and butyrate at the exterior surface of the cell and their conversion at the interior membrane surface to adenosine monophosphate and butyryl-coenzyme A, respectively.

Active Transport

Almost all solutes, including sugars, amino acids, peptides, nucleosides, and ions, are taken up by cells through active transport. The three steps of active transport are:

1 Binding of a solute to a receptor site on a membrane-bound carrier protein.
2 Translocation of the solute-carrier complex across the membrane.
3 Coupling of translocation to an energy-yielding reaction to lower the affinity of the carrier protein for the solute at the inner membrane surface so that the carrier protein will release solute to the cell interior. This process is illustrated in Fig. 11-2D.

Several mechanisms have been proposed to explain the molecular basis of active transport of solutes in microorganisms. The accumulated evidence suggests that active transport may also be explained by Mitchell's chemiosmotic theory (see Chap. 10). In this case, energy released during the flow of electrons through the electron-transport chain or the splitting of a phosphate group from ATP drives protons out of the cell. This generates a difference in pH value and electric potential between the inside and the outside of the cell or across the membrane. This proton gradient gives rise to a protonmotive force which can be used to pump the solutes into the cell. When protons reenter the cell, the energy released on reentry drives the transport mechanism in the cell membrane, probably by inducing a conformational change in the carrier molecule so that its affinity for the solute is decreased and the solute is released into the cell interior. The link between active transport and metabolic energy generation is illustrated in Fig. 11-3.

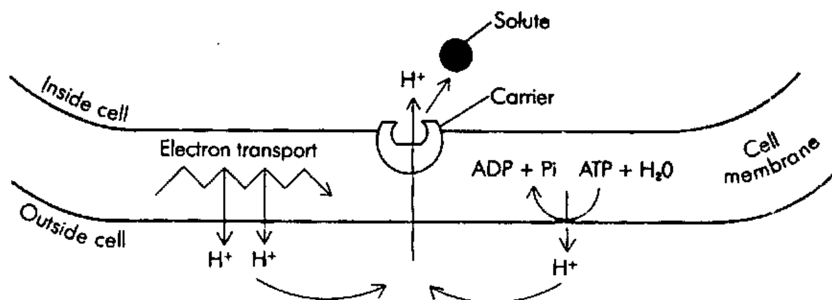


Figure 11-3. How release of metabolic energy is coupled to and drives active transport.

Many active-transport systems of Gram-negative bacteria are associated with binding proteins in the periplasmic space. These binding proteins have very high affinities for specific nutrients, including amino acids, sugars, and inorganic ions. Over one hundred different binding proteins have been isolated and characterized. They are essential for active transport of their specific substrates. However, they are not porters since they are located in the periplasmic space rather than in the cell membrane itself. But binding proteins function in conjunction with porters in the active transport of specific nutrients.

UTILIZATION OF ENERGY IN BIOSYNTHETIC PROCESSES

We have seen how energy is utilized for motility and transport of nutrients into bacterial cells. These are nonbiosynthetic processes. Biosynthetic processes in the cell also require energy; energy from ATP is used to convert one chemical substance into another and to synthesize complex substances from simpler ones.

Synthesis of Small Molecules: The Amino Acids

Amino acids, of which there are about 20 (Table 11-1), are the building blocks of proteins. The sequence and manner in which they are linked (i.e., their three-dimensional structure) determine the type of protein they form.

A microorganism growing in a medium may have all 20 of the amino acids present in the medium; that is, they are available for the microbe, preformed in the medium. If they are not available freely in the medium, the microorganism may have to liberate amino acids from proteins by the action of intracellular or extracellular proteolytic enzymes. In this way, the amino acids become available for use as nutritional building blocks. Sometimes, only a few amino acids are present in a medium, in which case the microbe has to convert other amino acids from the available ones into those that are missing. In yet other instances, the medium contains only inorganic sources of nitrogen, such as ammonium salts: The microorganism then has to synthesize all the required amino acids from these sources of available nitrogen, provided, of course, that it has this

Table 11-1. Amino Acid Building Blocks of Protein, with Standard Abbreviations

Alanine (Ala)	Glycine (Gly)	Proline (Pro)
Arginine (Arg)	Histidine (His)	Serine (Ser)
Asparagine (Asp-NH_2, Asn)	Isoleucine (Ile)	Threonine (Thr)
Aspartic acid (Asp)	Leucine (Leu)	Tryptophan (Trp)
Cysteine (Cys)	Lysine (Lys)	Tyrosine (Tyr)
Glutamic acid (Glu)	Methionine (Met)	Valine (Val)
Glutamine (Glu-NH_2, Gln)	Phenylalanine (Phe)	

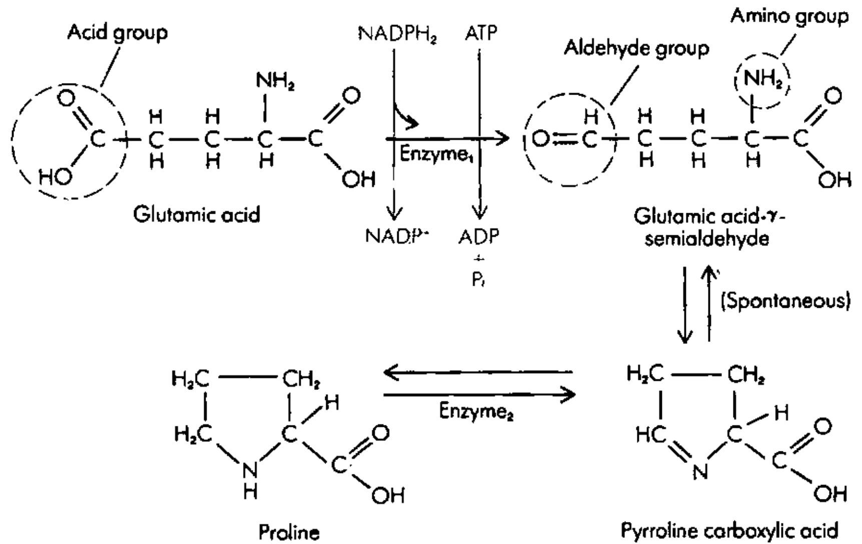


Figure 11-4. The biosynthesis of proline from glutamic acid in *E. coli*. Note the utilization of metabolic energy in the form of ATP in the initial step. (See text for details of synthetic steps.) Sections of the molecule reacting in each step are enclosed in color dashed lines.

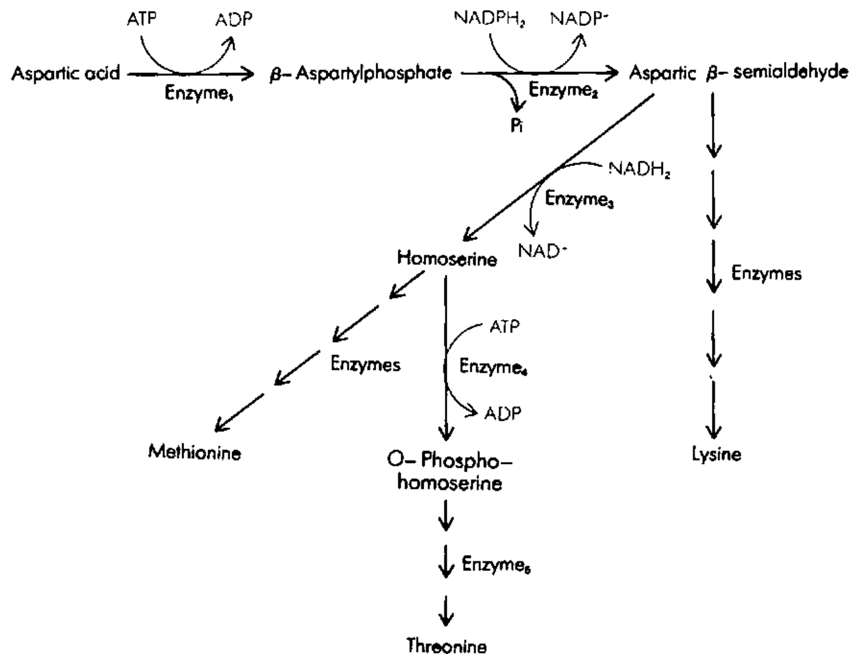


Figure 11-5. The biosynthesis of methionine, threonine, and lysine: another example of metabolic energy utilization in the form of ATP in the interconversion of substances.

ability. All these processes, the interconversion and biosynthesis of chemical substances, require the expenditure of energy.

Consider the specific example of the synthesis of the amino acid proline by the bacterium *E. coli*. Glutamic acid is the initial reactant, itself formed from

the reductive amination of α-ketoglutaric acid derived from the citric acid cycle. The steps involved are shown in Fig. 11-4. In the first step an acid group (—COOH) is reduced to an aldehyde group (—CHO). This requires two electrons from $NADPH_2$ and energy from ATP. The aldehyde group then spontaneously reacts with the amino group ($—NH_2$) on the same molecule, forming a ring. This step is followed by ring reduction to form proline.

Another example is the pathway for conversion of aspartic acid to lysine, methionine, and threonine. The conversion utilizes metabolic energy in the form of ATP, as shown in Fig. 11-5. These two examples serve to illustrate the expenditure of energy in the synthesis of amino acids. It may be added that the biosynthetic pathways for each of the 20 amino acids are well understood.

Just as amino acids are used by microorganisms to form proteins (see below for the process of protein synthesis), other low-molecular-weight organic precursors are polymerized to form other macromolecules. Nucleotides form nucleic acids (discussed later) and monosaccharides form polysaccharides. The precursors of lipids, especially the complex ones, include fatty acids, polyalcohols, amines, simple sugars, and even amino acids. It has been estimated that about 150 different small molecules are used to synthesize a new cell.

These small molecules are, in turn, synthesized from intermediates in the catabolic pathways of the microbes (Chap. 10). The most important of these intermediates are pyruvate, acetate, oxalacetate, succinate, α-ketoglutarate, and the sugar-phosphates.

Synthesis of Macromolecules: The Structure and Biosynthesis of a Cell-Wall Peptidoglycan

In all cells the major end products of biosynthesis are proteins and nucleic acids. However, there are other macromolecules peculiar to the procaryotes which require specialized biosynthetic processes. The utilization of energy in one of these processes is illustrated by the biosynthesis of bacterial cell-wall peptidoglycan. This particular biosynthetic process also serves as an example of how polymers are synthesized outside the membrane. Synthesis of cell-wall components is of interest because polymerization takes place outside the cell membrane by enzymes located on the membrane's outer surface.

Structure of Peptidoglycan

As discussed in Chap. 5, the rigid portion of a bacterial cell wall is a polymeric structure known as a murein, peptidoglycan, or mucopeptide. The walls of Gram-positive bacteria contain a large proportion of peptidoglycan; those of Gram-negative bacteria have a much smaller proportion. Peptidoglycans vary in their chemical composition and structure from species to species, but there are basic similarities. Peptidoglycans are very large polymers composed of three kinds of building blocks: (1) *acetylglucosamine* (AGA or GlcNAc), (2) *acetylmuramic acid* (AMA or MurNAc), and (3) a *peptide* consisting of *four* or *five* amino acids of limited variety. Several of the amino acids exist in the D configuration, not usually found elsewhere in nature. A peptidoglycan can best be thought of as consisting of polysaccharide backbone chains composed of alternating units of AGA and AMA linked by $\beta(1\rightarrow4)$ bonds, with the short peptide chains projecting from the AMA units. Many of these peptide chains are cross-linked with each other, imparting great rigidity to the total structure. Figure 11-6A illustrates the basic structure of peptidoglycans, and Fig. 11-6B shows a building block of the *E. coli* peptidoglycan. Some peptidoglycans differ in that

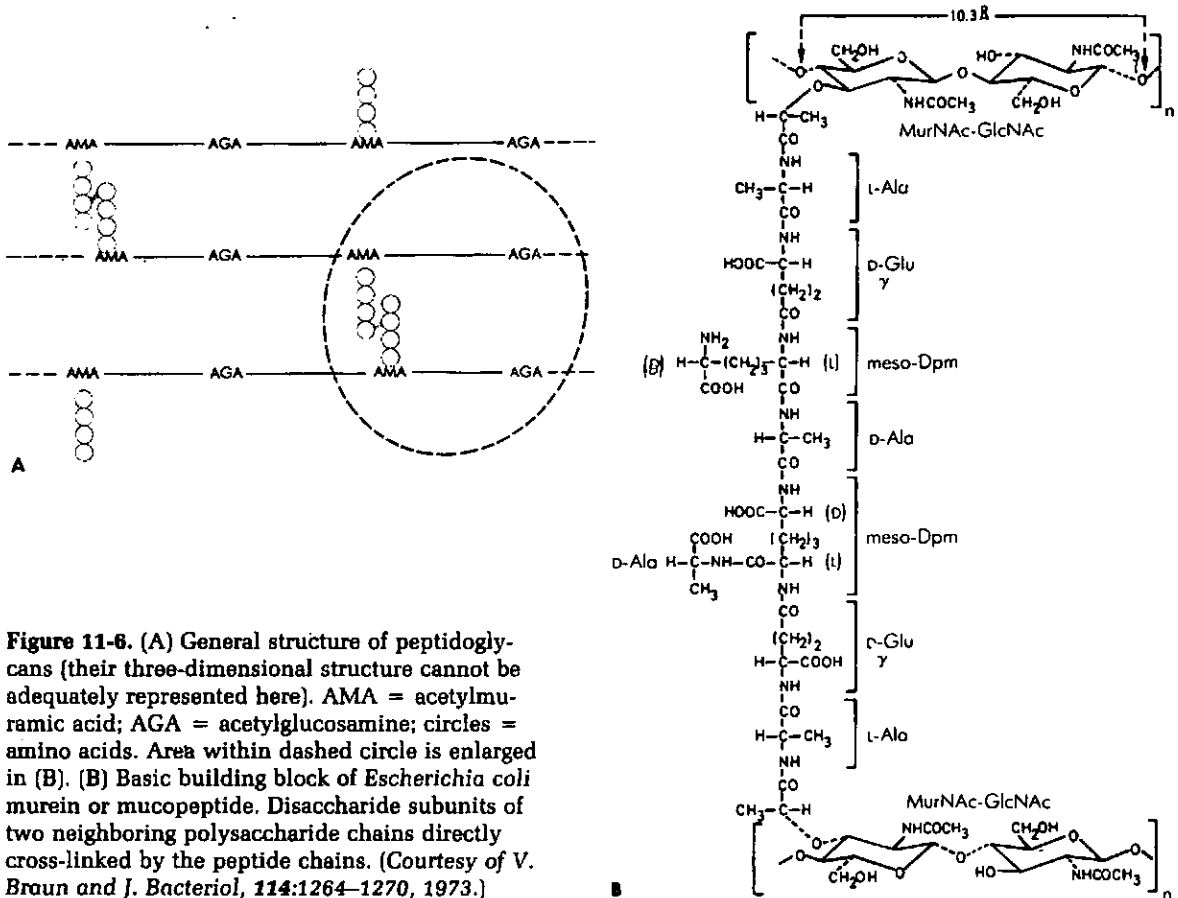


Figure 11-6. (A) General structure of peptidoglycans (their three-dimensional structure cannot be adequately represented here). AMA = acetylmuramic acid; AGA = acetylglucosamine; circles = amino acids. Area within dashed circle is enlarged in (B). (B) Basic building block of *Escherichia coli* murein or mucopeptide. Disaccharide subunits of two neighboring polysaccharide chains directly cross-linked by the peptide chains. (Courtesy *of V. Braun and J. Bacteriol,* ***114****:1264–1270, 1973.*)

the peptide chains may not be directly cross-linked to each other, being linked instead by another kind of peptide which forms a bridge between the terminal carboxyl group of one side chain with the free amino group of lysine or diaminopimelic acid (DPM or DAP) on the other side chain; e.g., in *Staphylococcus aureus* a bridge composed of five glycine molecules can link two muramic acid peptides together. This is shown in Fig. 11-7.

Activation of a Peptidoglycan Precursor

Escherichia coli can synthesize cell-wall peptidoglycan when grown in a simple medium of glucose, ammonium sulfate, and mineral salts. One of the early steps in this synthesis is the formation of an activated derivative of AMA. This process, which is shown in Fig. 11-8, requires energy at several points and occurs in the cytoplasm. The activation of sugars, such as acetylglucosamine, by the attachment of a uridine diphosphate (UDP) to form a sugar-UDP precursor is not peculiar to AMA but is a general method involved in the biosynthesis of many kinds of polysaccharides.

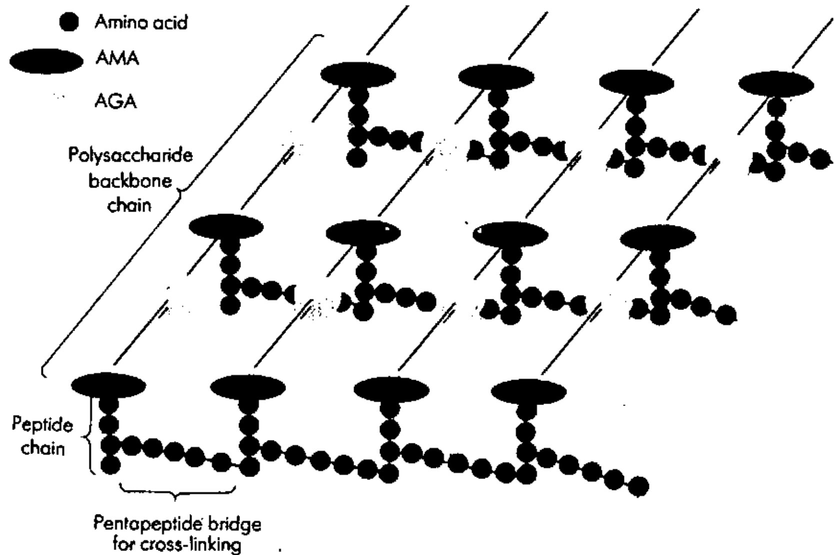


Figure 11-7. General structure of peptidoglycans. Note the pentapeptide bridge for cross-linking. In some peptidoglycans, peptides extending from AMA are linked directly to each other without pentapeptide bridges. AMA = acetylmuramic acid; AGA = acetylglucosamine.

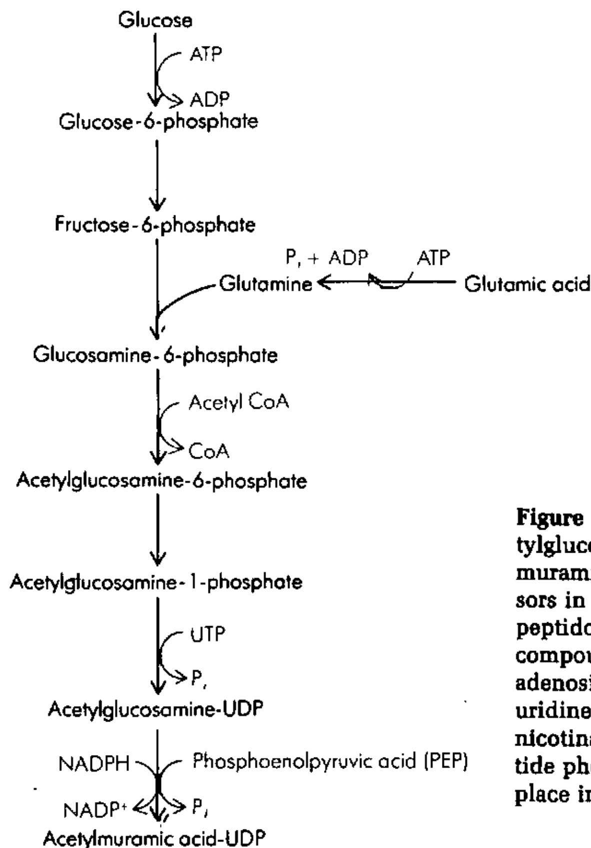


Figure 11-8. Biosynthesis of acetylglucosamine-UDP and acetylmuramic acid–UDP, key precursors in the synthesis of peptidoglycans. All high-energy compounds are in color. ATP = adenosine triphosphate; UTP = uridine triphosphate; NADP = nicotinamide adenine dinucleotide phosphate. This process takes place in the cytoplasm.

Synthesis of Peptidoglycan

After formation of the activated AMA, the synthesis of peptidoglycan proceeds as follows:

1 Amino acids are sequentially linked to the AMA portion of the activated precursor to form a short pentapeptide chain. Ribosomes are not involved, but each amino acid addition requires energy from the breakdown of ATP and the presence of Mg^{2+} or Mn^{2+} and a specific enzyme. These reactions occur in the cytoplasm.
2 The AMA-UDP precursor is coupled to a membrane phospholipid called bactoprenol (undecaprenol phosphate).
3 The AGA couples with AMA of the AMA-UDP precursor. This reaction requires the *activated* form of AGA, that is, the AGA-UDP derivative. In some organisms, the addition of bridging peptides takes place at this step. Reactions of steps 2 and 3 occur in the cell membrane.
4 The precursor, still linked to bactoprenol, is carried out of the cell through the cell membrane and is linked to a growing peptidoglycan chain in the cell wall. Peptide cross-linking may now occur, and the incorporation of the precursor into the growing peptidoglycan is thus completed. These reactions occur in the periplasm. Figure 11-9 illustrates the steps in a typical peptidoglycan biosynthesis.

The synthesis of peptidoglycan illustrates the utilization of energy in joining together smaller molecules into larger ones. Note that all the energy needed for polymerization is used in the cytosolic (cytoplasmic) reactions in synthesizing the activated precursors. Later on in the chapter, we will discuss the biosyntheses of those macromolecules that require a *template* which, acting like a tape, provides information about the order in which the smaller pieces are assembled into larger ones. Such processes include DNA synthesis (another piece of DNA is the template) and protein synthesis (a molecule of RNA serves as template).

Synthesis of Organic Cell Material in Chemoautotrophic Bacteria

Chemoautotrophic bacteria utilize carbon dioxide as their sole source of carbon. These bacteria oxidize inorganic nutrients such as hydrogen, ammonia, nitrite, and thiosulfate to produce metabolic energy (in the form of ATP) and reducing power (in the form of $NADPH_2$) in order to reduce CO_2 and convert it to organic cell material.

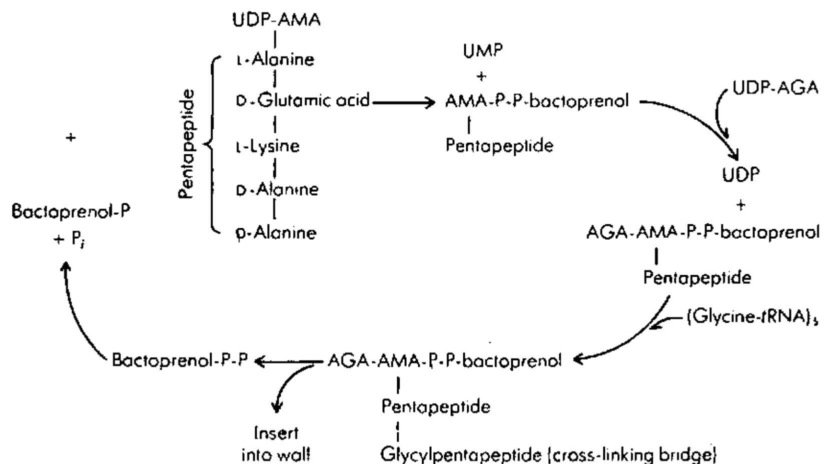


Figure 11-9. Biosynthesis of peptidoglycan in *Staphylococcus aureus*. AMA = acetylmuramic acid; AGA = acetylglucosamine. Note that unlike *E. coli* (Fig. 11-6B) *S. aureus* has a cross-linking pentaglycine bridge.

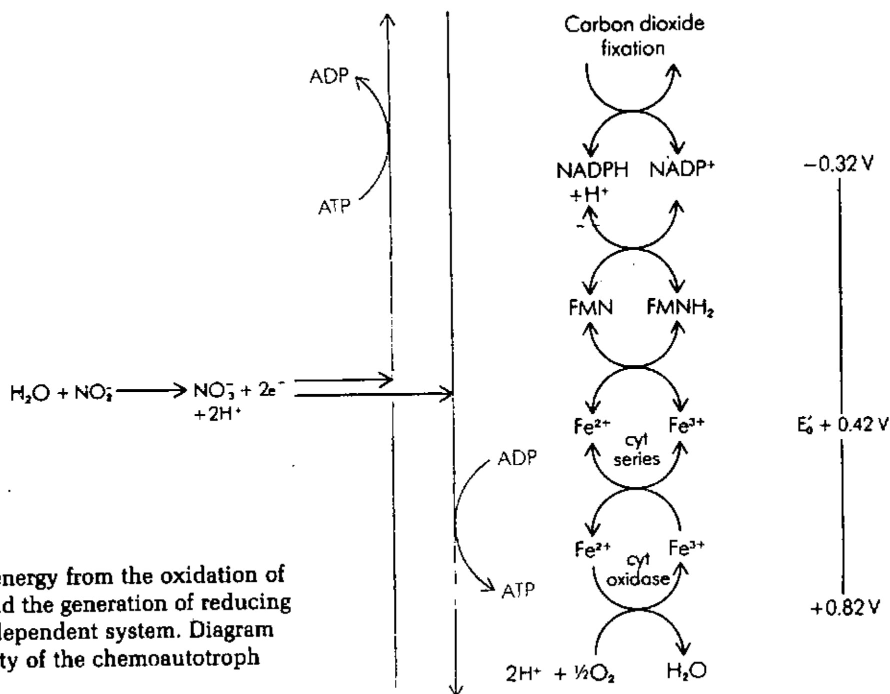


Figure 11-10. Production of energy from the oxidation of an inorganic source (NO_2^-) and the generation of reducing power (NADPH) by an ATP-dependent system. Diagram is representative of the activity of the chemoautotroph *Nitrobacter*.

Compared with other bacteria, such as the heterotrophs, chemoautotrophic bacteria are at a considerable energetic disadvantage for the following reason. Electrons entering the respiratory chain from oxidations of inorganic substrates by chemoautotrophic bacteria usually enter the chain at a higher point (E_0') than those electrons from oxidations of organic substrates by chemoheterotrophs. Consider the typical example of inorganic substrate oxidation by *Nitrobacter*. Since electrons enter the respiratory chain at cytochrome a_1 from the oxidation of nitrite, *Nitrobacter* produces much less ATP than heterotrophs and no reducing power in the form of $NADPH_2$.

Since the E_0' of NO_3^-/NO_2^- is higher than the E_0' of $NADP/NADPH_2$ (+0.35 V and −0.32 V, respectively), it is not possible for oxidation of nitrite by *Nitrobacter* to be coupled with the production of reducing power in the form of $NADPH_2$ at the beginning of the electron-transport chain. How then do chemoautotrophs like *Nitrobacter* generate $NADPH_2$ for use with ATP in carbon dioxide fixation—the beginning of the biosynthesis of all organic compounds they require? These chemoautotrophs use a process called reversed electron flow, or ATP-dependent $NADPH_2$ production. In this process, energy released on breakdown of ATP is used to drive electrons from the oxidation of the inorganic energy source to an E_0' at which they can subsequently reduce NAD^+ or $NADP^+$ In this case of *Nitrobacter*, $NADP^+$ is reduced by ATP-driven electron transport from cytochrome a_1 via cytochromes *c*, *b*, and flavoprotein (FMN); this process is shown in Fig. 11-10.

The principal method of carbon dioxide fixation in autotrophic bacteria is the

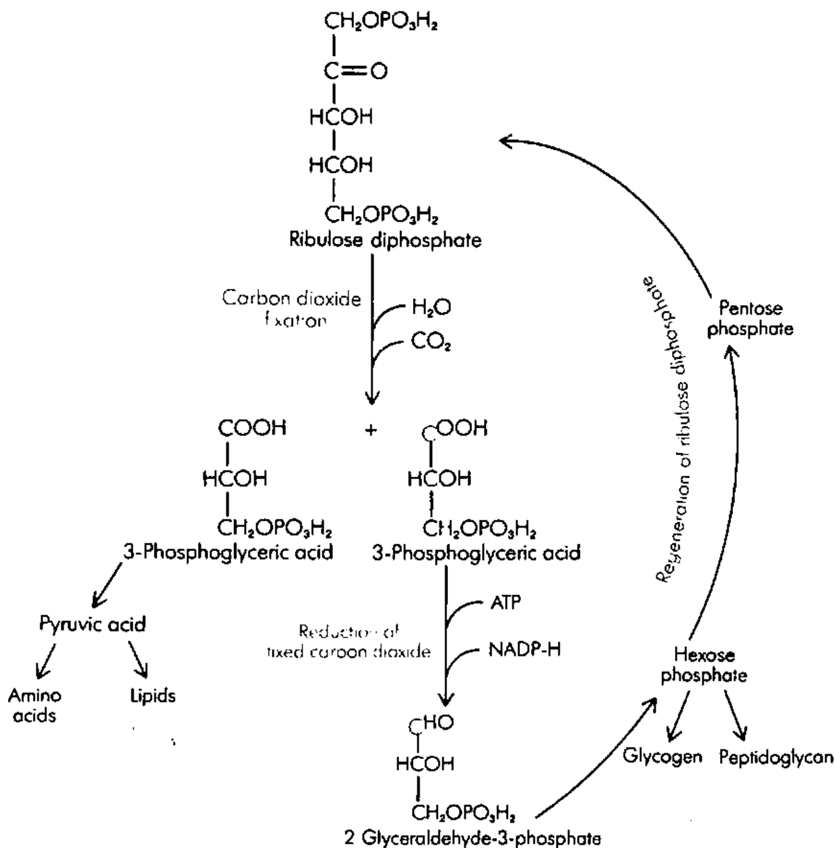
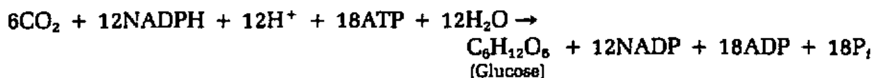


Figure 11-11. The Calvin cycle for carbon dioxide fixation in autotrophic organisms.

Calvin cycle, which is illustrated in Fig. 11-11. In the Calvin cycle, carbon dioxide is fixed in a reaction with the acceptor molecule ribulose diphosphate. The primary product of carbon dioxide fixation is 3-phosphoglyceric acid, from which all other organic molecules of the cell are synthesized. However, carbon dioxide fixation is dependent on a supply of the acceptor molecule, ribulose diphosphate, and so most of the 3-phosphoglyceric acid produced must be used to regenerate ribulose diphosphate. Thus the process of carbon dioxide fixation is cyclic. Each turn of the cycle results in the fixation of one molecule of carbon dioxide. Various intermediates of the cycle are drawn off and enter different biosynthetic pathways.

This cycle of carbon dioxide fixation is complex. It shares certain reactions of the glycolytic and pentose phosphate pathways discussed in Chap. 10. Two reactions are specific to the cycle: the carbon dioxide fixation reaction and the reaction which generates the carbon dioxide acceptor ribulose diphosphate.

The overall reaction for the Calvin cycle is



$$6CO_2 + 12NADPH + 12H^+ + 18ATP + 12H_2O \rightarrow \underset{\text{(Glucose)}}{C_6H_{12}O_6} + 12NADP + 18ADP + 18P_i$$

Note the high utilization of reducing power and energy in this cycle.

THE BIOSYNTHESIS OF DEOXYRIBONUCLEIC ACID

We now begin a discussion on the biosynthesis of those macromolecules that require a template, which provides information on the order in which smaller pieces are assembled into larger ones. In the biosynthesis of deoxyribonucleic acid (DNA) another DNA is the template; in the biosynthesis of protein a molecule of ribonucleic acid (RNA) is the template. A discussion of these biosynthetic processes must be preceded by an account of the structures of the specific macromolecules.

The Structure of Deoxyribonucleic Acid

Deoxyribonucleic acid (DNA) from any cell is a long ropelike molecule (Fig. 11-12) composed of two strands, each wound around the other to form a double helix (Fig. 11-13). The model for this structure was first proposed by James Watson and Francis Crick in 1953 when they published an article entitled "A Structure for Deoxyribonucleic Acid." They received the Nobel prize for these studies in 1962. Each strand of the DNA helix is made up of **nucleotides** linked together to form a chain, a **polynucleotide**.

Each nucleotide is constructed of three parts:

1 A heterocyclic (with more than one kind of atom) ring of carbon and nitrogen atoms called a nitrogenous base which is either a *purine* or a *pyrimidine*

Figure 11-12. A disrupted E. *coli* cell showing the ropelike DNA that has spilled out. Note the plasmid (top center pointer), a circular piece of DNA which is not part of the E. *coli* chromosome and which replicates separately from it. (*By permission from J. D. Griffith, The University of North Carolina.*)

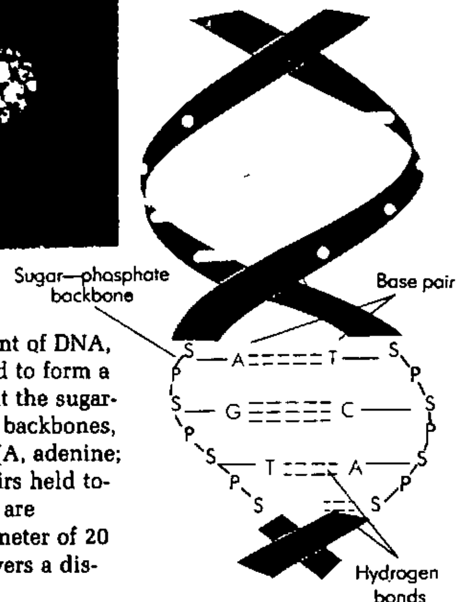


Figure 11-13. Diagram of a short segment of DNA, showing how its two strands are wound to form a double helix. The two ribbons represent the sugar-phosphate (S = sugar; P = phosphate) backbones, and the horizontal bars represent base (A, adenine; G, guanine; C, cytosine; T, thymine) pairs held together by hydrogen bonds. The strands are wrapped around an axis that has a diameter of 20 A. Every complete turn of the helix covers a distance of 34 Å.

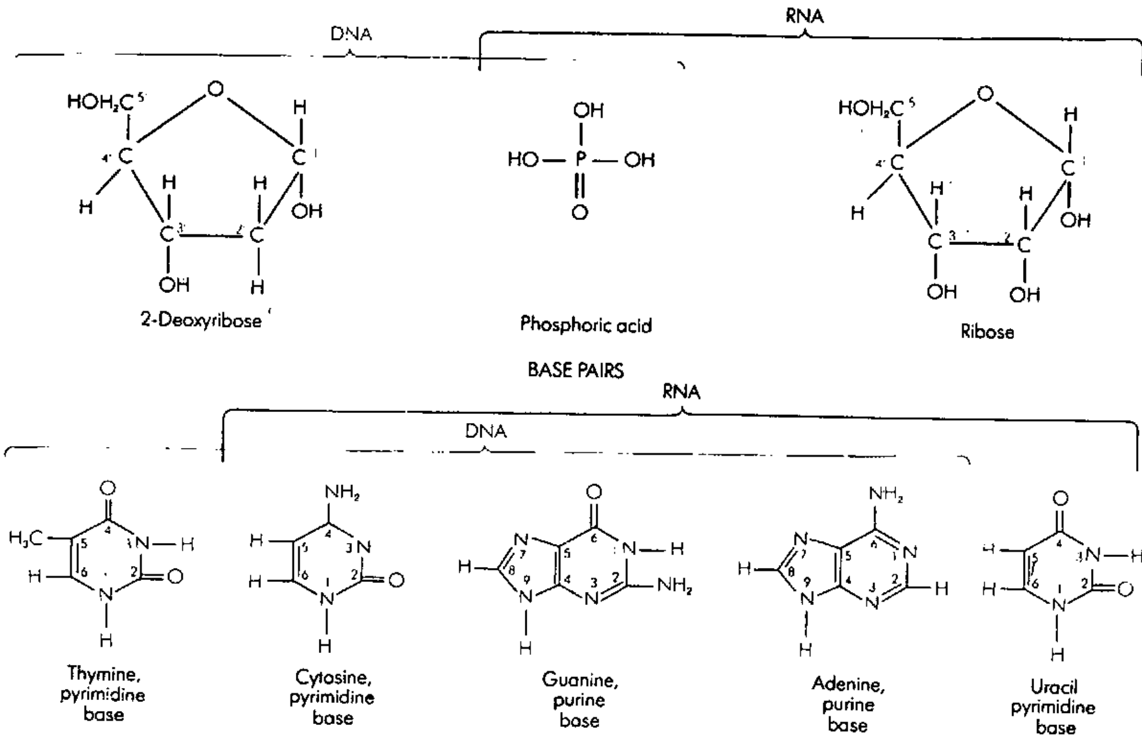
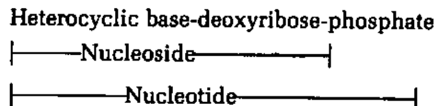


Figure 11-14. Building blocks of the nucleotides of DNA and RNA. Note that 2-deoxyribose, the sugar found in DNA, differs from ribose, the sugar found in RNA, because of the absence of an —OH group on carbon 2. RNA uses uracil as a building block instead of thymine found in DNA.

2 A 5-carbon sugar (pentose) called *deoxyribose*
3 A *phosphoric acid*

These parts are linked together as follows:



In DNA two kinds of purines, adenine and guanine, and two kinds of pyrimidines, cytosine and thymine, are found. The structures of these bases, as well as the structures of deoxyribose and phosphoric acid, are shown in Fig. 11-14. Since there are four kinds of bases, four kinds of nucleotides are found in DNA:

Deoxyadenosine-5′-monophosphate (adenine + deoxyribose + phosphate)
Deoxyguanosine-5′-monophosphate (guanine + deoxyribose + phosphate)
Deoxycytidine-5′-monophosphate (cytosine + deoxyribose + phosphate)
Thymidine-5′-monophosphate (thymine + deoxyribose + phosphate)

These four kinds of nucleotides are joined together in the polynucleotide strands of DNA by **phosphodiester linkages**; that is, each phosphate group links the number-3 carbon atom of one deoxyribose of a nucleotide to the number-5 carbon atom of the deoxyribose of the next nucleotide, with the phosphate

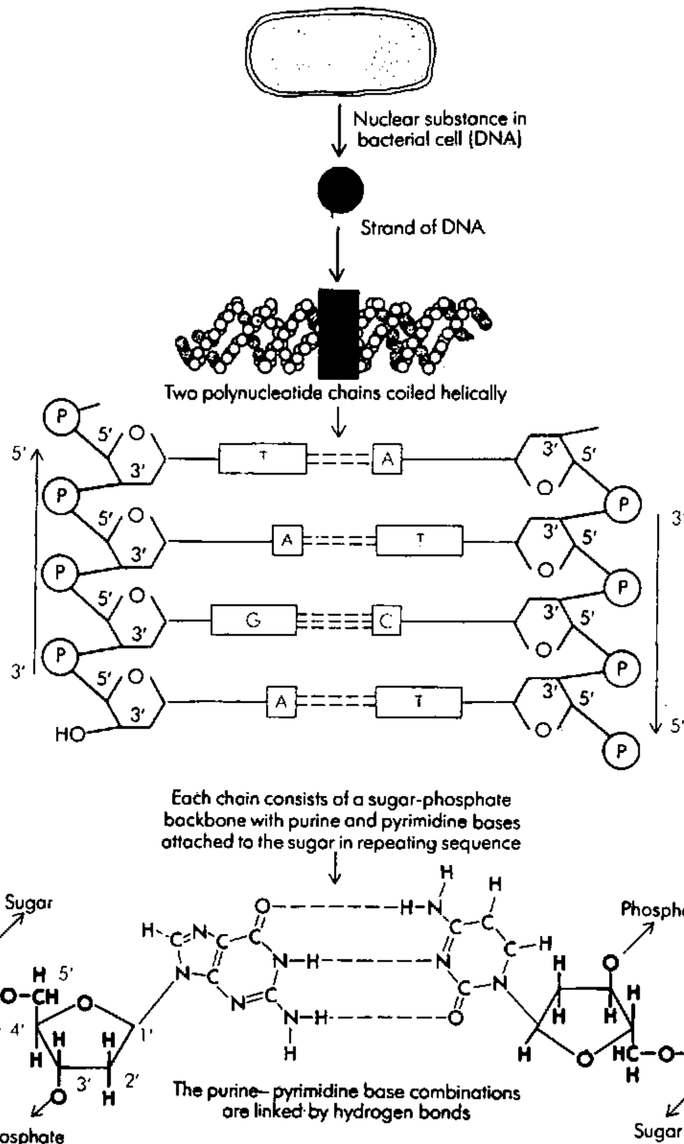


Figure 11-15. DNA location in the cell, molecular configuration, and chemical structure as viewed in progressively greater detail. The double helix maintains a constant width because purines always face pyrimidines in specific G-C and A-T base pairs.

group on the outside of the chain (Fig. 11-15). The result is a chain of alternating phosphate and sugar groups, with the nitrogenous bases projecting from the sugar groups (see Fig. 11-15). Weak bonds, known as hydrogen bonds, link the base on one chain and the base on the other chain. Two bases so linked are called a complementary base pair. Only two kinds of complementary base pairs are found in double-stranded DNA because of their hydrogen-bonding properties: adenine (A) and thymine (T), and guanine (G) and cytosine (C). As a consequence, the ratio of adenine to thymine or of guanine to cytosine in double-

stranded DNA is always 1:1. That is, the amount of purines is equal to the amount of pyrimidines.

The DNA of each species shows a characteristic composition that is not affected by age, growth, conditions, environmental changes, etc. The molar ratio $\frac{[A] + [T]}{[G] + [C]}$ indicates a characteristic composition of DNA of each species. For example, in humans this value is 1.52; in sheep, 1.36; in wheat germ, 1.19. Indeed, these values can even be used at the species level in bacteria for identification or taxonomic grouping: *Escherichia coli*, 0.93; *Staphylococcus aureus*, 1.50; *Clostridium perfringens*, 2.70; *Micrococcus lutea*, 0.35. (However, in bacterial taxonomy, the differences in base composition between species are more commonly expressed as [G + C] percent of total bases. This was explained in Chap. 3.)

The failure to find a 1:1 ratio between adenine and thymine or guanine and cytosine in certain viruses led to the discovery of single-stranded DNA in these organisms. For example, phage ϕX174 contains a single strand of DNA in a ring form. However, these cases are rare exceptions to the general occurrence of double-stranded DNA.

The complementary base pairs hold the two strands of the DNA helix together by hydrogen bonding. As shown in Figs. 11-13 and 11-15, there are two hydrogen bonds formed between each A-T pair, whereas there are three hydrogen bonds formed between each G-C pair. The complementarity of the purines and pyrimidines means that the sequence of bases on one strand dictates the sequence on the other strand. This is of critical importance in the synthesis, or replication, of new strands of DNA during cell division. A consequence of the formation of the A-T and G-C pairs is that the two strands of the DNA helix are said to be antiparallel, or to have opposite polarities. This means that each strand runs in opposite directions so that one is terminated by a free 3'-hydroxyl group and the other by a 5'-phosphate group (Fig. 11-15), where 3' and 5' refer to the numbering of the carbon atoms of the deoxyribose molecule. If you examine the nucleotides in Fig. 11-15, you will find that on the left strand the phosphate on the fifth carbon (5') of the sugar points up. On the right strand the phosphate on the fifth carbon points down. Each strand keeps the same polarity as it winds around the molecule.

The relationship of DNA to its low-molecular-weight components is shown

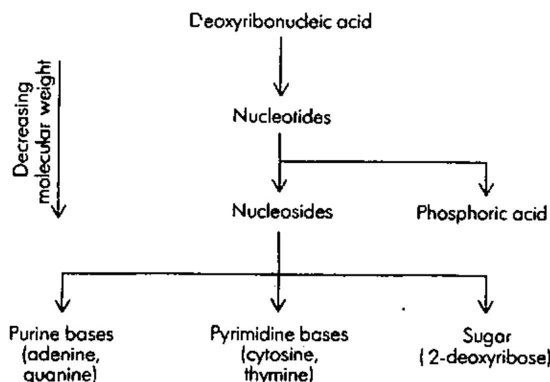


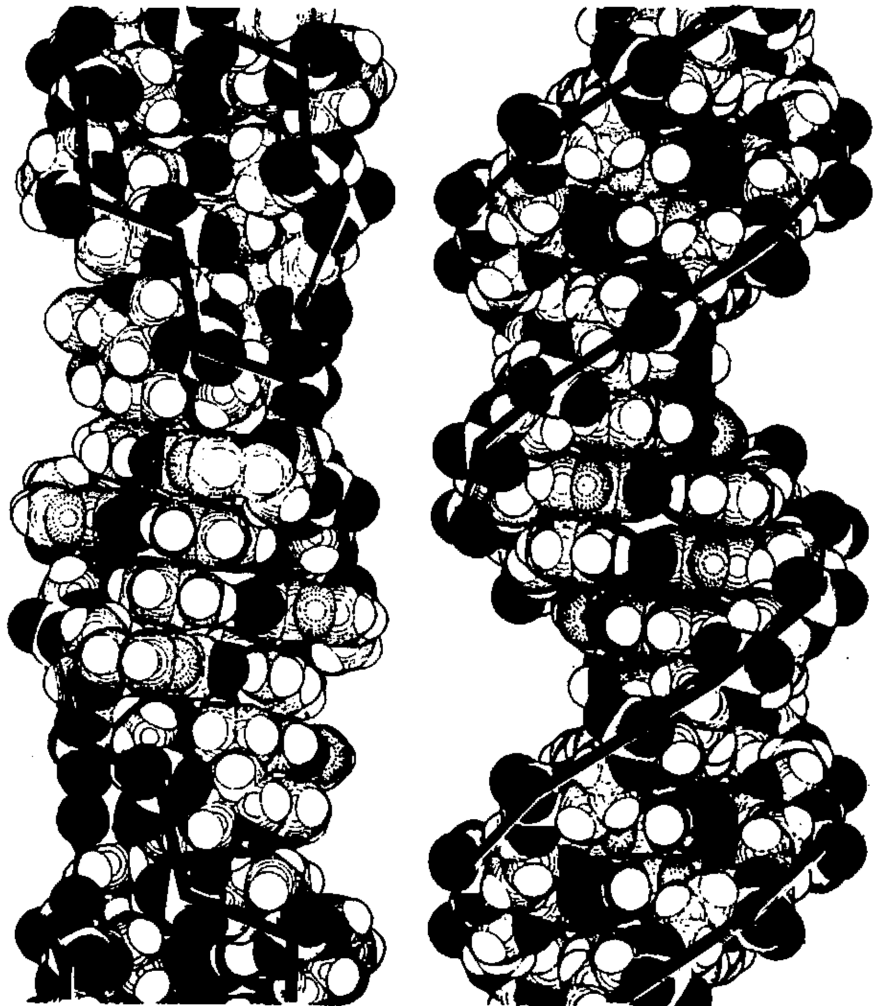
Figure 11-16. Breakdown of deoxyribonucleic acid into lower-molecular-weight components. DNA is made up of nucleotides which, when you remove phosphoric acid, yield nucleosides. Nucleosides can be broken down into bases and sugar (2-deoxyribose).

in Fig. 11-16. The removal of the phosphate group from the nucleotide yields a nucleoside consisting of a pentose sugar linked to a heterocyclic base.

The helical structure of the DNA molecule proposed by Watson and Crick is a right-handed, double-stranded one. This double helix is right-handed because the turns run clockwise looking along the helical axis. It represents what is known as the B form of DNA. But recent evidence indicates that DNA may be able to exist in other types of double-helical structures. There are A and C forms of the DNA helix; they differ from the B form in several features, such as the numbers of base pairs per turn, the vertical rise per base pair, or the helical diameter.

However, the Z form exhibits the most contrast with the other forms because it is a left-handed helix (sinistral DNA). It has the most base pairs per turn. Its name is taken from the anticlockwise zigzag path that the sugar-phosphate backbone follows along the helix. The structures of Z-DNA and B-DNA are compared in Fig. 11-17.

Figure 11-17. Left-handed (Z-form) and right-handed (B form) DNA models. The superimposed line traces the sugar-phosphate backbone; in the B form it is a continuous helix, while in the Z form the backbone zig-zags. (*Reproduced from Mosaic,* ***14****:2, 1983.*)



Z-DNA has been found in a variety of living organisms, including rats, rabbits, several species of plants, and protozoa. It appears that wherever there is regulation of gene action there is found Z-DNA. That is, Z-DNA seems to be important for biological development and control.

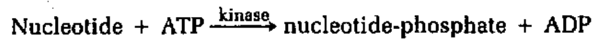
The Structure of Ribonucleic Acid

The other naturally occurring nucleic acid is ribonucleic acid (RNA). It plays a fundamental role by making it possible for the sequence of chemical groups in DNA to dictate the sequence of amino acids in proteins. It differs from DNA in these respects:

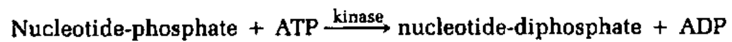
1 The sugar component of the nucleotides which make up RNA is ribose, instead of deoxyribose as in DNA. Ribose is similar to deoxyribose except for the presence of a hydroxyl group at the number-2 (2′) carbon atom (see Fig. 11-14).
2 The pyrimidine nitrogenous base uracil, instead of thymine, is found in the nucleotides that make up RNA (see Fig. 11-14).
3 It is usually single-stranded. The single strands can bend themselves backward and base-pair to form three-dimensional structures.
4 RNA is degraded to its nucleotides much more easily than DNA.

The Biosynthesis of Nucleotides in DNA Synthesis

Before the polynucleotide chains of DNA can be synthesized by bacteria (or any other organisms), an intracellular pool of nucleotides must be available. In some bacteria, these nucleotides must be supplied preformed in the medium, but in bacteria with relatively simple nutritional requirements, this reservoir of nucleotides can be synthesized from glucose, ammonium sulfate, and some minerals. The conversion of simple nutrients into nucleotides for DNA synthesis involves a complex series of enzymatically catalyzed reactions, several of which require energy in the form of ATP. Some of these reactions form *activated* nucleotides as direct precursors for synthesis of the polynucleotide chains of double stranded DNA:

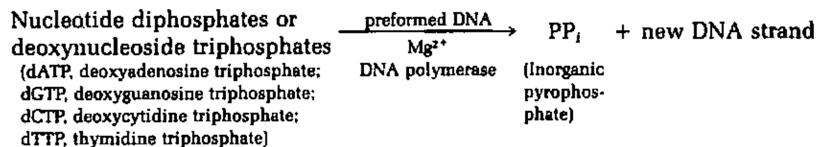


$$\text{Nucleotide} + \text{ATP} \xrightarrow{\text{kinase}} \text{nucleotide-phosphate} + \text{ADP}$$



$$\text{Nucleotide-phosphate} + \text{ATP} \xrightarrow{\text{kinase}} \text{nucleotide-diphosphate} + \text{ADP}$$

As seen in the above equations, energy in the form of ATP is utilized.
The overall chemical reaction is



The new DNA strand is complementary to the preformed or template DNA strand.

Semiconservative Replication of DNA Strands

Prior to cell division, the two DNA chains of a DNA molecule separate by unwinding, and each serves as a template for the synthesis of a new complementary chain, thus forming two new helices, each exactly like the original. Each half of the dividing cell then receives one of these helices. This type of

duplication in which one polynucleotide chain acts as a template to direct the synthesis of a new chain complementary to itself is termed semiconservative replication; it results in two daughter helices, each containing one old template strand and one new complementary strand. In other words, only one of the old strands is conserved in each daughter helix.

Replication of the DNA Molecule

Bacteria are almost always haploid, which means that their chromosomes are unpaired. In contrast, most eucaryotic cells (except for gametes) are diploid: they have paired homologous chromosomes which may be heterozygous (carry different alleles or genes occupying the same relative locus on homologous chromosomes). All bacteria studied to date have their genetic loci in a single linkage group; that is, they have a single chromosome per genome.

The chromosome of a typical bacterium is a circular double-stranded DNA molecule; that is, the double helix for a complete genome has no free ends. It has an approximate molecular weight of 2.5×10^9 daltons (a dalton is equal to the mass of one hydrogen atom) and has about 4×10^6 base pairs. The circular chromosome is further twisted on itself in the bacterial cell to form a supercoil. (This is also the case in many viruses.) If, instead, the chromosome were extended linearly, it would measure approximately 1250 μm (1.25 mm), which is several hundred times longer than the bacterial cell that contains it. A circular form is typical of the DNA molecules of procaryotic microorganisms, of viruses, and of organelles in eucaryotic organisms. However, not all DNA molecules are circular; the chromosomes of eucaryotic organisms and of many viruses consist of linear DNA molecules.

There are three general methods of replication of the DNA molecule.

θ (Theta) Mode

The replication of a circular DNA molecule is initiated at a certain point called the origin, which is specific for each bacterial species. Replication proceeds in two directions around the chromosome, leading to the formation of a "bubble," which increases in size as replication proceeds. This mode is called theta because intermediate structures resemble the Greek letter θ (see Fig. 11-18). In this process, a circular parental chromosome is replicated to two circular daughter chromosomes, in each of which one strand of the parental DNA molecule is conserved and a complementary strand is newly synthesized. Figure 11-18 illustrates this θ mode of replication of the DNA molecule.

σ (Sigma) or "Rolling Circle" Mode

Replication begins with the cleavage of a phosphodiester bond in one strand of the circular DNA molecule to produce a nick with 3′-OH and 5′-PO_4 ends on that strand. The complementary circular strand then serves as a template for the synthesis of a new strand, which is covalently linked to the 3′-OH end of the nicked parental strand. As this strand grows at the 3′-OH end, the 5′-PO_4 end of the same strand is displaced to form a "tail" on the circle. As replication proceeds, a circular parental molecule is converted to two daughter molecules, one circular and the other linear. This mode is called sigma because intermediate structures have the Greek letter σ conformation (see Fig. 11-19). The sigma mode of DNA molecule replication is carried out by some bacteriophages, such as λ and φX174 whose progeny viral DNA is linear; by bacteria involved in sexual conjugation; and by certain eucaryotes during oogenesis.

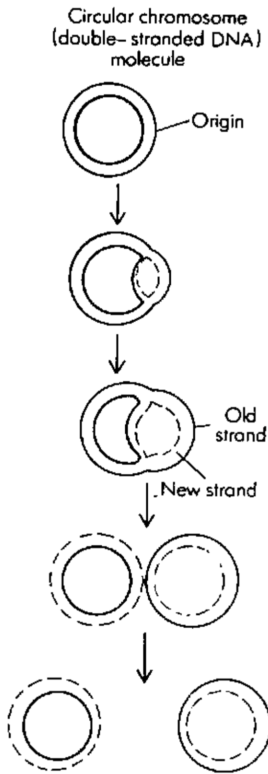


Figure 11-18. Replication of bacterial DNA by the theta mode. As shown, there is one origin of replication and two directions of replication from the origin; i.e., there are two growing points. (Note that the DNA strands are not drawn in double-helix form for the sake of simplicity.) In *E. coli* the average rate at which these two growing points, or *replication forks*, move during replication is about 45 kilobases per minute per fork at 37°C. Since there are 10 base pairs per turn of the helix, the rate of unwinding of the parental duplex at each fork must be approximately 4500 turns per minute.

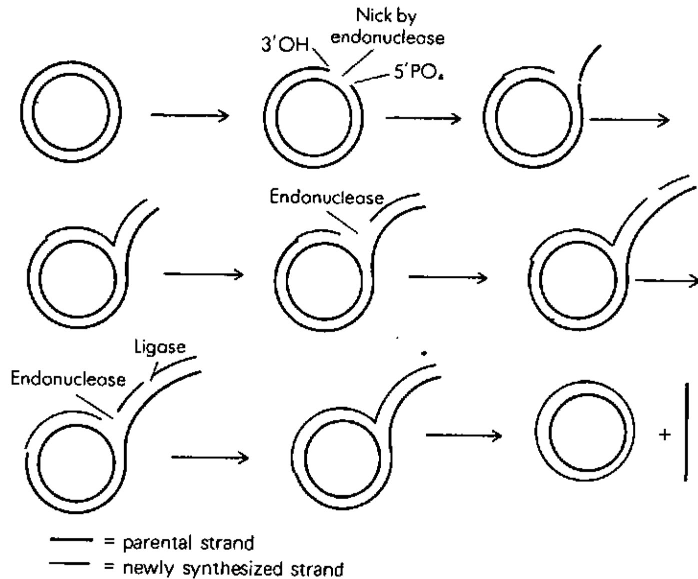


Figure 11-19. Sigma mode of DNA replication. The double-stranded circular DNA molecule is nicked at a specific point exposing 3'-hydroxyl and 5'-phosphate terminal groups. DNA replication begins at the 3'-hydroxyl terminus with the addition of nucleotides by DNA polymerase; therefore, synthesis is in the 5' → 3' direction. It then switches over to its complementary strand. After a short period an endonuclease nicks the new DNA strand at the growing point with further unwinding of unreplicated portions of parental helix. The process is repeated again and again. The short fragments are joined together by polynucleotide ligase. A specific nuclease then cuts unit-length segments. (For the sake of simplicity, the double-helix form of DNA strands is not shown.)

Linear Mode

All eucaryotic organisms and some viruses have linear DNA molecules. Replication of these chromosomes is initiated at specific sites by the formation of replication bubbles. Small viral linear DNA molecules may have only one point of initiation per molecule. Large DNA molecules of eucaryotes may have hundreds of initiation points per molecule. A replication bubble grows in size as DNA replication proceeds from the point of initiation. Adjacent bubbles fuse to

form larger ones as replication proceeds. Upon completion of replication, two linear double-helical daughter molecules are formed from one linear parental molecule. Each daughter molecule, of course, contains a conserved strand from the parental molecule and a newly synthesized strand. The linear mode of replication is illustrated in Fig. 11-20.

It may be noted that procaryotes replicate their DNA from one origin or growing point per molecule while eucaryotes replicate from many origins per molecule. Replication may occur in either a unidirectional or bidirectional manner from each origin.

Events at the Growing Point (Replication Fork)

It is at the growing point, or replication fork (see Fig. 11-20), that both DNA strands are duplicated. Let us examine the events that take place at the growing point.

Procaryotic DNA strands are synthesized at the rate of about 1000 nucleotides per second at a replication fork. Eucaryotic DNA strands are synthesized more slowly at about 100 nucleotides per second. Many enzymes are involved in this synthesis. Initial enzymatic activities are carried out by a helix-unwinding protein (ATP-dependent), a helix-destabilizing protein, and a helix-relaxing protein (DNA gyrase). These enzymes participate in opening the parental DNA helix ahead of the replication fork.

DNA replication is **discontinuous**: that is, the strands are replicated in small fragments, called **Okazaki fragments** after their discoverer, in the 5′ → 3′ direction. This process is shown schematically in Fig. 11-21.

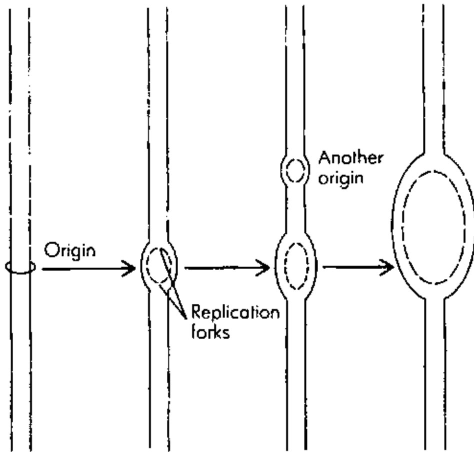


Figure 11-20. Linear mode of DNA replication and bidirectional replication from multiple origins. (Double-helical form of DNA strands is not shown, for the sake of simplicity.)

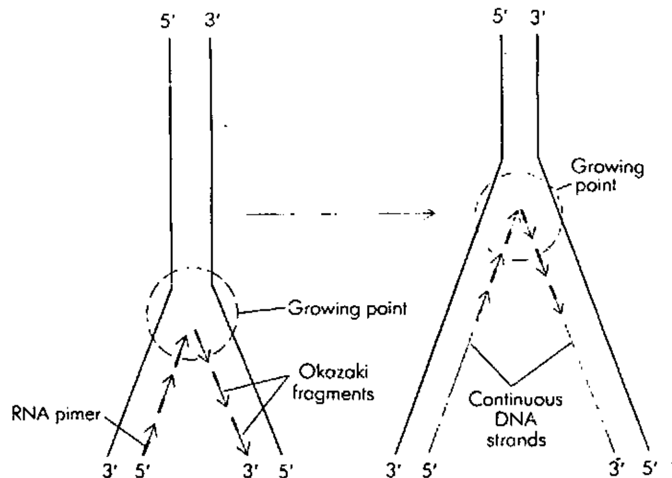


Figure 11-21. The discontinuous synthesis of DNA. Short segments, Okazaki fragments, of DNA are synthesized in the 5′ → 3′ direction. These fragments are represented by short colored arrows. Subsequently the fragments are linked together by ligase to form continuous strands as the growing point moves farther along the DNA. (See text for details.)

Initiation of DNA replication requires a primer, a short sequence of RNA that is synthesized by RNA polymerase and is complementary to the DNA which serves as a template. (These RNA primers are used because RNA polymerase, unlike DNA polymerase, requires only template but not primer. "Polymerase" implies a polymerization action.) The RNA primer's 3'-OH end serves for the addition of nucleotide diphosphates by DNA polymerase III. DNA polymerase I then removes the RNA primer with its 5'-nuclease activity; simultaneously it fills in the gap with DNA via its 3'-polymerase activity. This DNA polymerase I uses the 3'-OH end of the preceding DNA fragment as its primer. Thus as it removes the RNA, it extends or elongates the preceding DNA molecule that served as its primer. When the entire RNA is removed, a single-stranded nick remains between the two DNA fragments and is sealed by DNA ligase, a DNA-joining enzyme. (DNA polymerase II is found also in *E. coli*, but, as yet, no specific function has been ascribed to it.)

TRANSCRIPTION AND TRANSLATION OF GENETIC INFORMATION

We have examined how DNA is replicated as a cell grows and before it divides into two cells. One of these DNA copies is then transmitted to the progeny or sister cell. In this way genetic information programmed in the genes is passed on from an organism to its offspring. Thus, DNA, or the genes, is a very important molecule in the cell. It is carefully guarded from damage, and it is repaired when damage is detected. (On the other hand, RNA and proteins are subject to degradation and replacement rather than to repair; that is, they undergo turnover.) We will now see how genetic information is coded in the DNA, and how the genetic code is deciphered and used in the production of proteins. All these processes constitute what is known as the central dogma of molecular genetics, which may be described in three steps. The first is DNA replication (which we have just studied); the second is transcription, the process in which the genetic message on DNA is transcribed to RNA; and the third is translation, in which the genetic message coded by RNA is translated by the ribosomes into a protein structure. The outline of the central dogma is shown in Fig. 11-22.

The Building Blocks of Proteins

Just as nucleotides are building blocks of DNA, amino acids are building blocks of proteins. However, DNA consists of only four kinds of nucleotides, whereas proteins consist of about 20 kinds of amino acids. Microorganisms differ widely in their ability to synthesize amino acids. For example, *E. coli* can synthesize

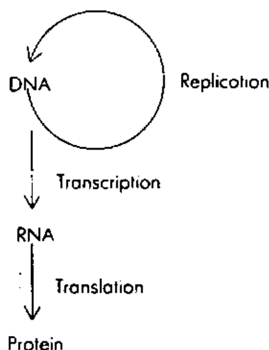


Figure 11-22. Processes in the central dogma of molecular genetics.

all of the amino acids required for protein synthesis, but lactic acid bacteria cannot and therefore must be supplied with preformed amino acids.

There are thousands of different proteins in a bacterial cell. Each type of protein has its own specific sequence of amino acids and three-dimensional structure. The amino acids are joined together by *peptide bonds* to form a long chain. A peptide bond is formed as shown:

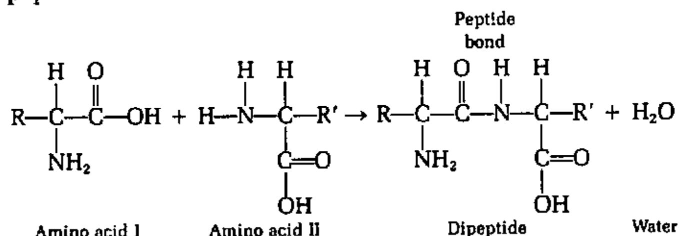
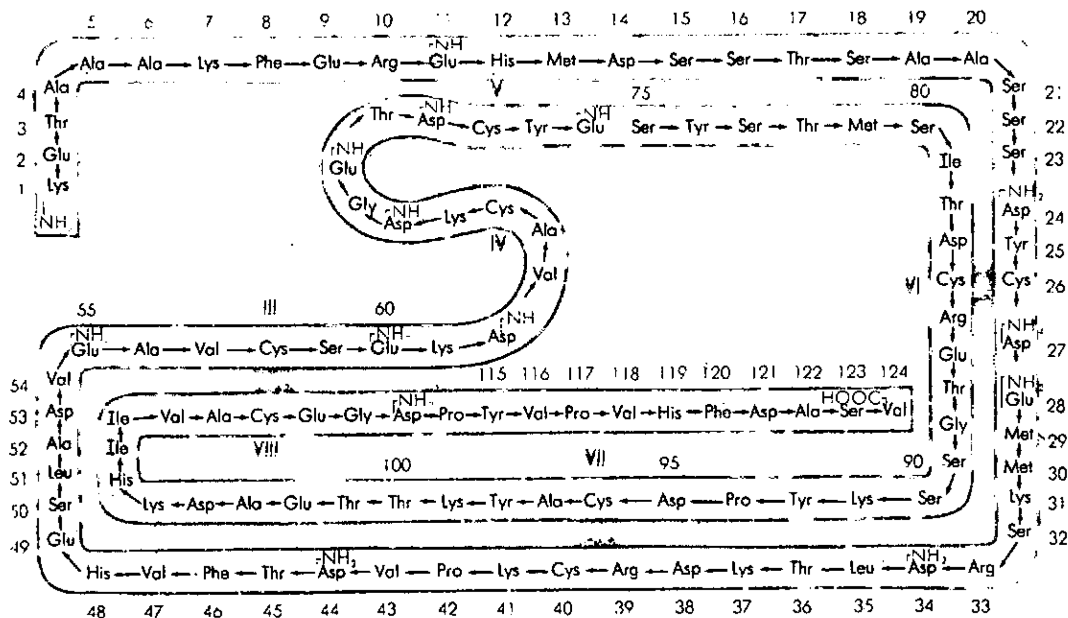


Figure 11-23. The amino acid sequence of the enzyme ribonuclease. The shaded areas between cysteines represent disulfide bridges. This illustration is diagrammatic; the polypeptide chain is actually folded to give a complex three-dimensional configuration. (*Courtesy of D. G. Smyth, W. H. Stein, and S. Moore, J. Biol. Chem.,* ***238****:227,* 1963.)

The chain of amino acids formed when a large number of amino acids are joined together by peptide bonds is called a polypeptide chain. Proteins consist of one or more polypeptide chains. Polypeptide chains range from fewer than a hundred amino acid monomers or residues (e.g., the hormone insulin) to over a thousand residues (e.g., DNA polymerase). The sequence of amino acids in the enzyme ribonuclease (RNAase) is shown in Fig. 11-23.

The sequence of amino acids is characteristic for each protein. It is determined by the sequence of bases in the DNA of a gene. That is, each kind of polypeptide is specified by a particular gene, giving rise to the dictum "one gene, one polypeptide chain."

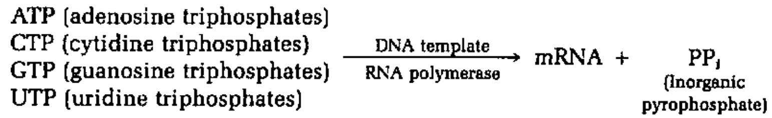
The sequence of amino acid residues in a polypeptide constitutes its primary structure. Polypeptide chains can take on specific shapes by folding; this folding



pattern constitutes the secondary structure of the protein. The amino acid side chains or groups of a polypeptide (e.g., cysteine residues) contribute further to the folding process by forces of attraction and repulsion; this process gives a protein its tertiary structure. In Fig. 11-23, the tertiary structure of RNAase is seen to be contributed by four disulfide bridges. As may be observed, RNAase is a single polypeptide chain of 124 amino acid residues; it is folded, bent, and twisted into a globular shape in its active form. Finally, the overall shape resulting from the interaction of two or more polypeptide chains constitutes the quaternary structure of a protein.

Transcription

Protein synthesis takes place on the ribosomes, which are large RNA-protein particles in the cytoplasm of the bacterial cell. (In eucaryotic cells the ribosomes are attached to the endoplasmic reticulum.) Ribosomal RNA (*r*RNA) constitutes about 90 percent of the total cellular RNA. Before protein synthesis can proceed, the coding of DNA must first be transferred to a substance that passes information from the DNA in the nuclear region to the ribosomes in the cytoplasm. This substance is known as messenger RNA (*m*RNA). The process or step in which a single-stranded mRNA is synthesized complementary to one DNA strand is called transcription. The synthesis of the polynucleotide chain of mRNA is catalyzed by the enzyme RNA polymerase. Just as activated deoxyribonucleotides are required in DNA synthesis, activated ribonucleotides are required as substrates for this enzyme:



The synthesis of RNA is different from that of DNA in the following features:

1 Only one of the two strands of any given segment of DNA serves as the template.
2 Only specific, relatively short lengths of DNA are transcribed; i.e., an RNA chain is a transcript of a short section of DNA.

Transcription is the first step in gene expression. This process, as shown in Fig. 11-24, involves separation of the two DNA strands, one of which serves as a template for the synthesis of a complementary strand of mRNA by DNA-dependent RNA polymerase. When a short RNA chain is completed, the DNA double helix closes again. The strand of DNA selected for transcription in a given segment is called the "sense" strand and contains a specific initiation site, which is a regulatory sequence of DNA nucleotides called the promoter region. (The other strand may be the "sense" strand for another group of genes

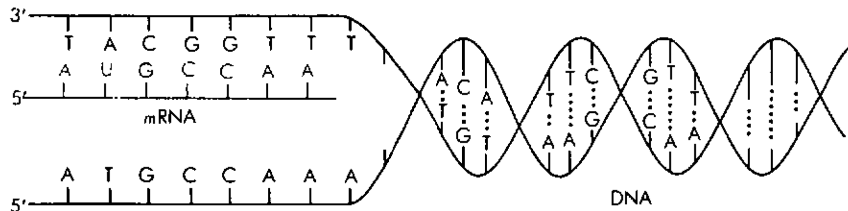


Figure 11-24. Transcription of DNA by DNA-dependent RNA polymerase. The chain of mRNA is being synthesized from the information in DNA. Note how complementarity is maintained. For example, where there is a G in DNA, a C is inserted in mRNA, and where there is an A in DNA, a U is inserted in mRNA. The mRNA will serve as a pattern for protein synthesis. The growth of a mRNA chain proceeds in a 5' → 3' direction in a manner similar to DNA replication. (A, adenine; T, thymine; C, cytosine, G, guanine; U, uracil.)

in another segment.) In bacteria, the initiation of RNA polymerase activity at this site is due to an initiation factor called the sigma factor, which is a component of the enzyme. Termination of mRNA synthesis is also at specific regulatory sequences of DNA nucleotides along the DNA molecule which are recognized by the RNA polymerase. Furthermore, a tetrameric protein factor called the rho factor binds to RNA polymerase and promotes its termination. When transcription has been completed, rho dissociates from the RNA polymerase-DNA complex.

Translation

Translation, the next step in gene expression, is the process in which the genetic information now present in the mRNA molecule directs protein synthesis.

When the four different bases of the nucleotides of mRNA are arranged in sequences of three, each base triplet, called a codon, is capable of specifying a particular amino acid. Since there are four different bases, the number of sequences of three of them is 4^3, or 64. These base triplets, each of which specifies a particular amino acid, constitute the genetic code (Table 11-2). The code is probably universal for all species of living organisms.

How is this code translated? Using Table 11-2, suppose the base sequence of mRNA is

CUUAGAAAAUUUAGUGGGACUUCU

The translation of this code into amino acids in a polypeptide chain, at a ribosome, would be

Leu-Arg-Lys-Phe-Ser-Gly-Thr-Ser

Table 11-2. The Genetic Code for the Base Triplets of mRNA and the Amino Acids They Code for*

First Base	Second Base: U		Second Base: C		Second Base: A		Second Base: G		Third Base
U	UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine	UGU	Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine	UCA		UAA	"Ochre"	UGA	"Umber"	A
	UUG		UCG		UAG	"Amber"	UGG	Tryptophan	G
C	CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Isoleucine	ACU	Threonine	AAU	Asparagine	AGU	Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lysine	AGA	Arginine	A
	AUG	Methionine	ACG		AAG		AGG		G
G	GUU	Valine	GCU	Alanine	GAU	Aspartic acid	GGU	Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

* The codons read in the 5′ to 3′ direction (left to right) on the mRNA. Codons UAA (ochre), UAG (amber), and UGA (umber) cause termination of synthesis of a protein chain. AUG and GUG are chain-initiating codons. Note that the same amino acid may be coded for by more than one codon (such a code is called *degenerate*). But no codon codes for more than one amino acid.

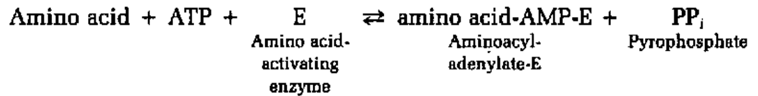
AUG and GUG are polypeptide-chain-initiating codons, and UAA, UAG, and UGA are polypeptide-chain-terminating codons. The latter three are called nonsense codons.

Another distinctive property of the genetic code is that the same amino acid may be coded for by more than one codon; that is, the code is degenerate. Furthermore, no "punctuation," or signal, is necessary to indicate the end of one codon and the beginning of the next. Therefore, the reading frame, or the sequence in which the genetic code is deciphered, must be correctly set at the beginning of the readout of a *m*RNA molecule. Reading then moves sequentially from one triplet to the next one without pause. If the reading frame is incorrectly set in the beginning, all codons will be out of step and lead to the formation of a missense protein with a deranged amino acid sequence.

The events occurring from DNA to RNA to protein are shown in Fig. 11-25. This figure leads us to a more detailed discussion of protein synthesis, which is a very elaborate biosynthetic process.

THE PROCESS OF PROTEIN SYNTHESIS

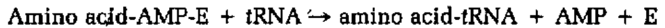
The first step in protein synthesis is the activation of amino acids. The amino acids are activated by amino acid–activating enzymes called aminoacyl-tRNA synthetases. This activation reaction requires energy in the form of ATP:



$$\text{Amino acid} + \text{ATP} + \underset{\text{Amino acid-activating enzyme}}{\text{E}} \rightleftarrows \underset{\text{Aminoacyl-adenylate-E}}{\text{amino acid-AMP-E}} + \underset{\text{Pyrophosphate}}{\text{PP}_i}$$

There is a specific activating enzyme for each kind of amino acid. The activated amino acid remains tightly bound to the enzyme after activation.

Next the activated amino acid binds to an RNA molecule called transfer RNA (*t*RNA). This reaction is catalyzed by the enzyme that was originally bound to the amino acid:



$$\text{Amino acid-AMP-E} + t\text{RNA} \rightarrow \text{amino acid-}t\text{RNA} + \text{AMP} + \text{E}$$

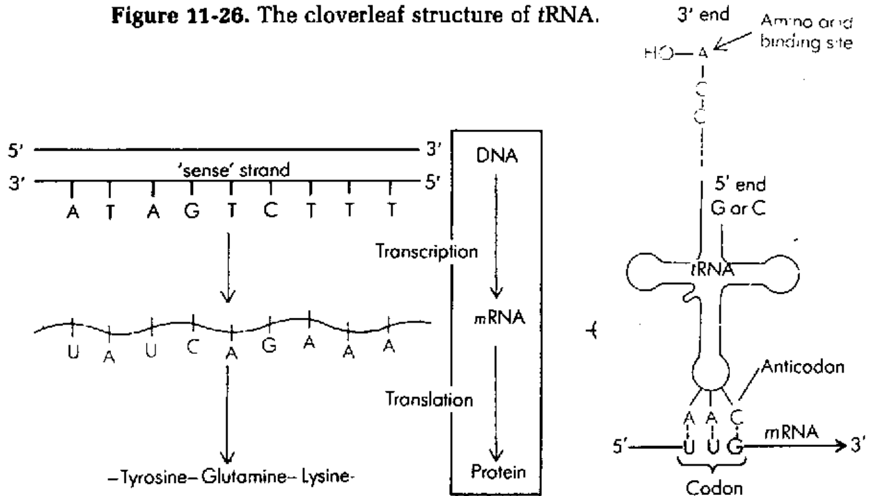


Figure 11-25. The events from DNA to mRNA to protein.

Figure 11-26. The cloverleaf structure of *t*RNA.

The tRNA functions in protein synthesis to carry amino acids to, and recognize codons in, mRNA.

Transfer RNA is a single chain of about 80 nucleotides that is folded back upon itself and held in a cloverleaf arrangement by means of hydrogen bonding due to complementary base pairing. The general structure of tRNA is shown in Fig. 11-26. Three of the unpaired bases in tRNA form an anticodon triplet which specifically recognizes the complementary codon in mRNA for a specific amino acid. The terminal sequence of nucleotides is adenylic-cytidylic-cytidylic (ACC) and is found in all tRNA. The amino acid to be carried is linked to the terminal nucleotide containing adenine.

Like mRNA, tRNA is transcribed from a certain region of the DNA molecule by RNA polymerase. The tRNA molecules function as "adapters" into which specific amino acids are "plugged" so that they can be adapted to the nucleotide triplet language of the genetic code which is transcribed on the mRNA.

The tRNA now carries the amino acid to the mRNA attached to the surface of the ribosome. Here the amino acid is added to a growing polypeptide chain. The surface of the ribosome may be viewed as the assembly point for protein synthesis.

Assembly of the Protein Chain on the Ribosome

As mentioned before, the ribosome is the site of protein synthesis. Its rRNA is transcribed from certain portions of DNA by the same energy-requiring process used for synthesis of mRNA and tRNA. A ribosome is analogous to a videotape-playing machine; just as the latter will produce any kind of image, depending on the videotape played, the ribosome will manufacture any kind of protein, depending on the kind of mRNA supplied.

The ribosomes of *E. coli* have been studied extensively and have been found to consist of two subunits, each of which is identified by a sedimentation constant (S) determined by ultracentrifugation studies. The larger subunit is a 50S particle, while the smaller unit is a 30S particle. Ribosomal subunits may associate or dissociate with each other. A 30S and a 50S subunit associate to form a 70S ribosome. (S is the Svedberg unit, a measure of how fast a particle sediments during ultracentrifugation. The association of a 30S and a 50S subunit to form a 70S ribosome shows that the sedimentation behavior of the 70S ribosome is not a simple addition of the units of the smaller particles.) Each subunit is made up of ribosomal RNA molecules and numerous proteins.

The synthesis of a protein chain on a ribosome is carried out as follows:

- Step 1 A ribosome binds to one end of a mRNA molecule at a specific site. (The specificity here is important because it starts the translation of the mRNA in the correct reading sequence. See Fig. 11-27.)
- Step 2 A charged tRNA carrying the first amino acid molecule then attaches to the chain-initiating codon (X) of the mRNA.
- Step 3 Another tRNA carrying the second amino acid binds at the next codon (Y).
- Step 4 The amino group of the amino acid on the second tRNA reacts with the active terminal carboxyl group on the amino acid of the first tRNA to form a dipeptide; the first tRNA is then released. The mRNA is moved along the ribosome to position the next codon (Z) in readiness for the tRNA carrying the third amino acid.

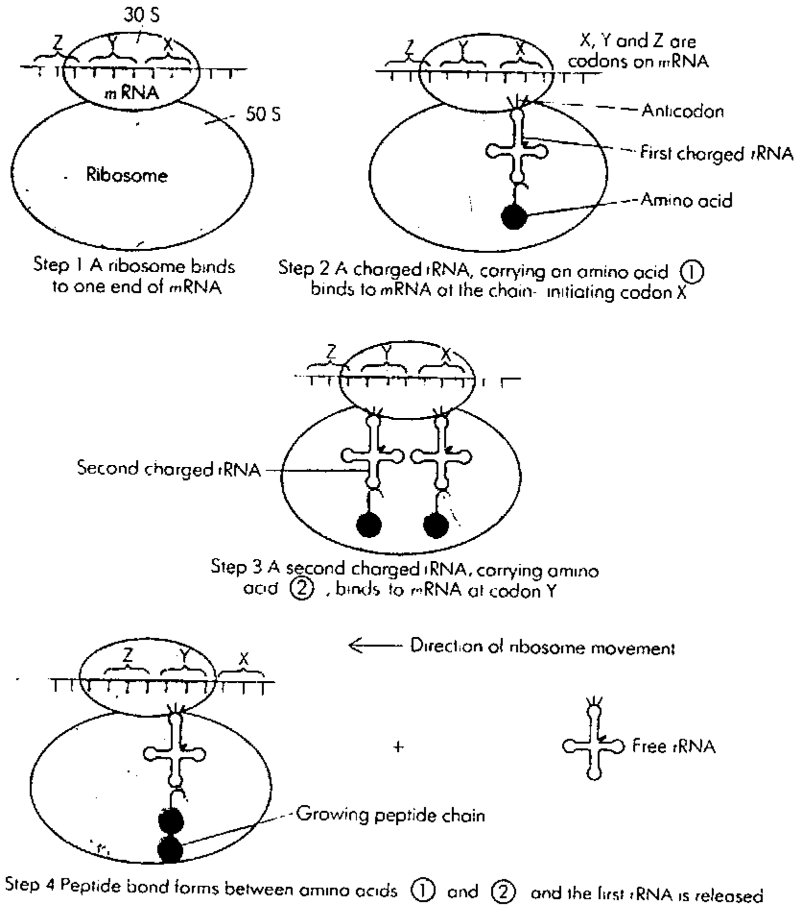


Figure 11-27. Synthesis of a protein chain on a ribosome.

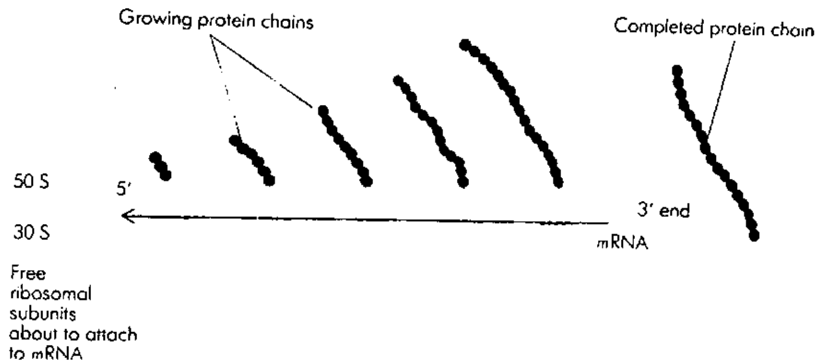


Figure 11-28. Schematic representation of a polysome during protein synthesis. The mRNA moves from right to left. The ribosomes move from the 5′ end to the 3′ end (left to right) of the mRNA, all reading simultaneously. In procaryotes, the life span of mRNA is very short, only about 2 min. Solid dots (circles) are amino acids.

The process is continued until the peptide chain is complete. Termination takes place at one of the nonsense codons UAA, UAG, or UGA, and the chain dissociates from the last tRNA molecule.

A single molecule of mRNA is long enough for several ribosomes to read the molecule at the same time. When a number of 70S ribosomes are actively engaged in protein synthesis on a strand of mRNA, this is called a polysome (see Fig. 11-28).

Protein synthesis in eucaryotic cells differs in some details from protein synthesis in procaryotic cells, but the main features are similar.

QUESTIONS

1 Describe in general terms the ways in which bacteria utilize energy other than for biosynthesis.
2 What form of energy drives the rotation of the bacterial flagellum? Explain how this is accomplished.
3 Describe the process of passive diffusion.
4 How is passive diffusion differentiated from facilitated diffusion?
5 How is group translocation similar to and different from active transport of solutes?
6 What are binding proteins?
7 Explain how the molecular mechanisms of active transport and ATP synthesis are coupled on the basis of Mitchell's chemiosmotic theory.
8 Give an example to illustrate the expenditure of energy in the biosynthesis of amino acids.
9 Draw a diagram to show the structure of peptidoglycan in the bacterial cell wall.
10 What is meant by an activated precursor? What activated precursors are involved in bacterial peptidoglycan biosynthesis?
11 Is a template involved in bacterial peptidoglycan synthesis? Explain.
12 How is energy utilized in the biosynthesis of peptidoglycan?
13 Why are chemolithotrophs at a considerable energetic disadvantage compared with chemoheterotrophs in the oxidation of substrates?
14 How is energy used in the synthesis of organic cell material by chemoautotrophic bacteria?
15 Explain why reducing power is required by chemoautotrophs in the fixation of carbon dioxide.
16 What is reversed electron flow? Why is this process important for carbon dioxide fixation?
17 What two chemical reactions are specific to the Calvin cycle?
18 What are the three parts of a nucleotide?
19 Explain how nucleotides serve as building blocks of DNA.
20 What are complementary base pairs in DNA and how are they bonded together?
21 Explain how molar base ratios are used in the taxonomic grouping of bacteria. (Refer also to Chap. 3.)
22 Why are the two strands in a DNA molecule said to be antiparallel?
23 Write a general description of the nature of the bacterial chromosome.
24 Compare and contrast the mode of DNA replication in a bacterial cell, the phage lambda (λ), and a mammalian cell.
25 Discuss the role of primers in DNA replication.
26 What is meant by the primary, secondary, tertiary, and quaternary structures of a protein?

27 What is meant by the central dogma of molecular genetics?
28 Why is the genetic code said to be degenerate? How does it determine the initiation and termination of protein synthesis?
29 Describe the different kinds of RNA that participate in protein synthesis.
30 Outline the process of protein synthesis on a ribosome.

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Note that some references of Chaps. 9 and 10 are also relevant to this chapter.

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Chapter 12 Bacterial Genetics

OUTLINE **The Study of Microbial Genetics**

The Inheritance of Characteristics and Variability

Phenotypic Changes Due to Environmental Alterations

Genotypic Changes
Types of Mutations • How Mutations Occur • How Mutations Are Repaired • Mutation Rate • Phenotypes of Bacterial Mutants • Designation of Bacterial Mutants

Bacterial Recombination

Bacterial Conjugation
Extrachromosomal Genetic Elements (Plasmids)

Transduction
Generalized Transduction • Specialized Transduction

Bacterial Transformation

The Regulation and Expression of Gene Activity
The *lac* Operon

Genetic Engineering

It was only a little more than 100 years ago that the first serious study of genetics was undertaken by the Austrian monk Gregor Mendel in his pea patch. He crossed strains of peas and studied the results of these crosses—changes in color, shape, size, and other properties of peas. He published his work entitled *Experiments with Plant Hybrids* in 1865. In it he provided the first description of the segregation of parental characters from one generation to the next. From these studies he developed the basic laws of heredity. The laws of heredity, first formulated by Mendel, are common to all forms of life. They apply to humans just as well as to the "lowly" bacterium.

Genetics is the study of the **inheritance** (heredity) and the **variability** of the characteristics of an organism. Inheritance concerns the exact transmission of genetic information from parents to their progeny. Variability of the inherited characteristics can be accounted for by a change either in the genetic makeup of a cell or in environmental conditions.

Another great step forward was the recognition of deoxyribonucleic acid (DNA) as the chemical substance responsible for heredity in all cells. It was

identified as a compound bearing genetic information when, in 1944, Avery, MacLeod, and McCarty discovered that a nonvirulent strain of the bacterium *Streptococcus pneumoniae* could be transformed in a heritable manner into a virulent strain by simply adding DNA extracted from a dead virulent strain into the medium. That is, the now virulent bacterium could transmit that virulence indefinitely to its progeny. The DNA derived from dead nonvirulent bacteria had no effect under the same conditions.

THE STUDY OF MICROBIAL GENETICS

Like biochemical principles, genetic principles are universal. The study of microbial genetics has contributed much to what we know about the genetics of all organisms. At first, geneticists were very reluctant to believe that procaryotes, being so small and devoid of complex structures, were also genetic beings like higher organisms. But it was not long before bacteria (and their viruses) became important subjects for genetic research. Indeed, they became the principal experimental tools for unraveling the basic knowledge of genetics at the molecular level.

There are distinct advantages in the use of bacteria for genetic experiments. Bacterial cultures contain millions of individual cells. Therefore, by appropriate selective techniques, rare genetic events can be discovered. Further, a procaryotic cell contains a single chromosome; thus a change in the genetic material of a procaryote results in an immediate, observable change in characteristics. (There is no masking effect due to the presence of an unaffected member of a paired chromosome.) Other obvious advantages in using microbes in genetic studies include the rapid growth rates of microbes, the relative ease of growing bacteria and their viruses in a constant, controlled environment, and the great diversity of metabolic types among microorganisms.

THE INHERITANCE OF CHARACTERISTICS AND VARIABILITY

A characteristic of all forms of life, from the standpoint of genetics, is the general stability or "likeness" in the characteristics of progeny and parent. We readily observe in our own species, for example, that some families regularly have black hair, brown eyes, and a certain shape of nose and chin, whereas other families have blond hair, blue eyes, and a different facial structure. In the same way, and in spite of their small dimension, bacteria and other protists also transmit characteristics to their progeny. The very fact that we can identify species and even strains of bacteria implies that they are capable of transmitting genetic information from generation to generation with great accuracy.

However, in addition to the inheritance of characteristics, which accounts for the constancy exhibited by biological species, there is variability or change expressed in the progeny. These changes are associated with two fundamental properties of the cell or organism, namely, the **genotype** and the **phenotype**. The genotype refers to the genetic constitution of the cell. The phenotype is the expression of the genotype in observable properties characteristic of the cell or organism.

The genotype of a culture of cells remains relatively constant during growth. However, it can change by mutation. This change can result in an alteration in the observable properties, or phenotype, of the cells. So the genotype represents

the heritable total *potential* characteristics of a cell, whereas the phenotype represents the characteristics expressed.

PHENOTYPIC CHANGES DUE TO ENVIRONMENTAL ALTERATIONS

Bacteria, like the cells of higher organisms, carry more genetic information—their genotype—than is utilized or expressed at any one time. The extent to which this information is expressed depends on the environment. For instance, a facultatively anaerobic bacterium will produce different end products of metabolism, depending on the presence or absence of oxygen during growth. The presence or absence of oxygen determines which enzymes function and which do not. Indeed in a given environment, knowledge of the factors which regulate genetic activity constitutes a very important aspect in the understanding of cell metabolism.

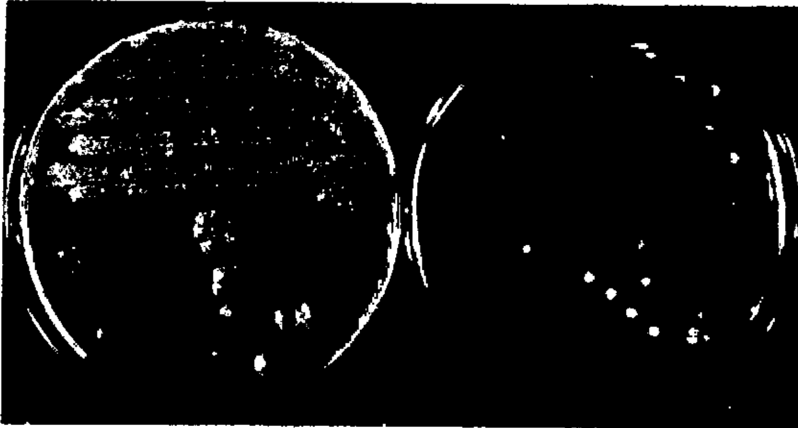


Figure 12-1. *Agrobacterium radiobacter* grown on two different media. Left: Mucoid colonies on sucrose-salts medium; right: nonmucoid colonies on trypticase-soy agar medium.

Figure 12-2. Morphological modifications (phenotypic changes) resulting from changes in media composition. (A) and (B) are phase-contrast micrographs of *Nocardia* sp. in (A) tryptone agar culture; (B) brain-heart infusion agar; both cultures 12 h at 30°C. (*Courtesy of B. L. Beaman and D. M. Shankel, and J. Bacteriol,* 99:876, 1969.) (C) and (D) are electron micrographs of thin sections of *Arthrobacter globiformis* 425 grown in (C) nutritionally complete medium and (D) biotin-deficient medium resulting in abnormal forms of the bacterium (several protoplasts embedded in an amorphous matrix). Note that the aberrant cells are devoid of cell walls and no longer exhibit the typical shape of the species as shown in (C). Incubation was at 25°C for 36 h. (*Courtesy of Margaret Gomersall and E. C. S. Chan.*)

The outstanding feature of a phenotypic change due to environment is that it involves most of the cells in the culture. A phenotypic change of this type is not inherited; rather, it occurs when some condition of the environment changes. A return to the original phenotype occurs when the original environmental conditions are restored.

Figures 12-1 and 12-2 show some phenotypic changes due to alterations in environmental conditions. Such phenotypic changes have also been called physiological adaptations (to the environment) and so are differentiated from phenotypic changes as a consequence of mutation (discussed below).

GENOTYPIC CHANGES

The genotype of a cell is determined by the genetic information contained in its chromosome (or chromosomes, in the case of a eucaryotic cell). The chromosome is divided into genes. A gene is a functional unit of inheritance; it specifies the formation of a particular polypeptide as well as various types of RNA. Each gene consists of hundreds of nucleotide pairs. For instance, if a polypeptide chain contains 300 amino acids, then the gene coding for this polypeptide must contain 900 base pairs (three bases for each amino acid). This is the basis for the one gene–one polypeptide relationship mentioned in Chap. 11. It has been estimated that the bacterial chromosome has the capacity to code for approximately 3,500 different proteins.

Any gene is capable of changing or *mutating* to a different form so that it specifies formation of an altered or new protein which may in turn change the characteristics of the cell (sometimes leading to its death). For example, the substitution of even one amino acid among several hundred in a polypeptide chain may cause the protein to be nonfunctional. A mutation is a change in the nucleotide sequence of a gene. This gives rise to a new genetic trait, or a changed genotype. A cell or an organism which shows the effects of a mutation is called a mutant. Thus we occasionally see sudden changes in familiar plants and animals. Now and then, an albino cat appears in a black litter, or a yellow pea appears among many green peas. The same sort of phenomenon occurs among microorganisms.

In nature, mutations are rare events which occur at random and arise spontaneously with no regard to environmental conditions. Spontaneous bacterial mutations may occur at a rate of only one mutation in 1 million bacterial cells to a rate of only one mutation in 10 billion bacterial cells. Generally, the mutants in a cell population are masked by the greater numbers of unmutated cells. Isolating a mutant cell is like looking for the proverbial needle in a haystack. However, microbiologists have developed techniques which facilitate isolation of the few mutants from a large population of nonmutated (wild-type) cells. For example, an antibiotic can be incorporated in a medium to select for antibiotic-resistant mutants.

As mentioned previously, for a long time many geneticists were very reluctant to believe that the observable changes in bacterial cultures were ever anything

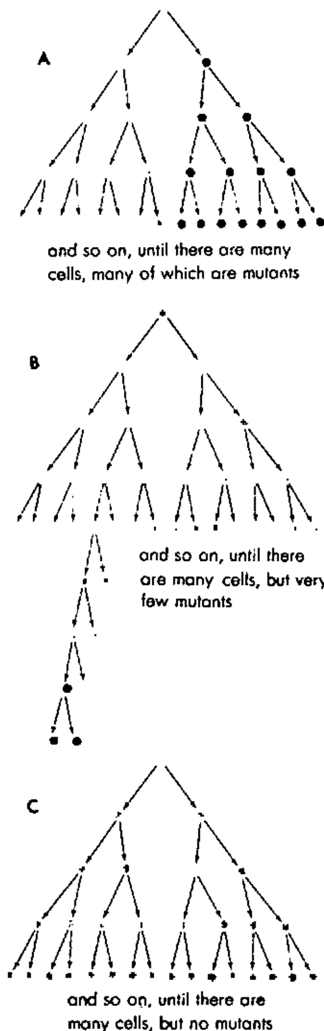


Figure 12-3. Formation of phage-resistant bacteria by mutation. In some cultures (A) a mutation occurs very early, and thus many of the cells are resistant. In others (B) a mutation may occur very late, so that there are few resistant cells. In still others (C) there is no mutation and hence no resistant cells.

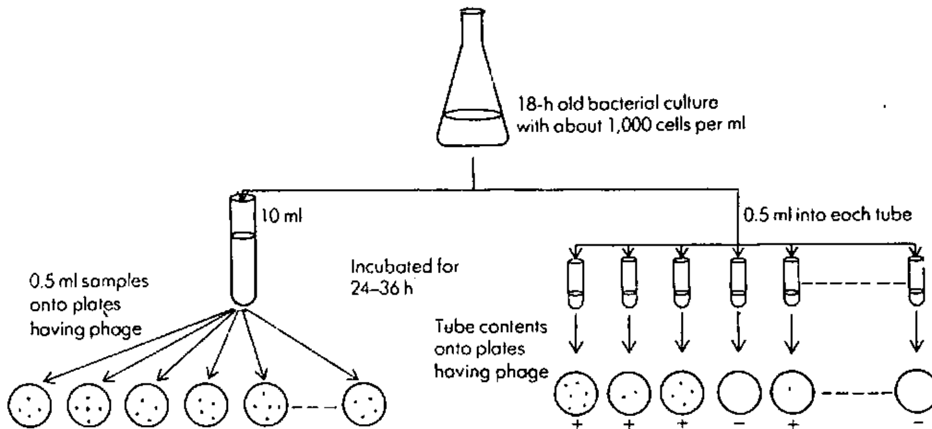


Figure 12-4. The fluctuation test was performed essentially as follows. A series of tubes containing 0.5 ml of cells was incubated without phage until a certain poulation size was reached. The cultures were then exposed to phage by pouring the contents of each tube into an agar plate containing phage. The number of phage-resistant mutants in each tube was thus determined. The colony counts from such a *series of similar cultures* were then compared with the results of a series of samples taken from *one culture* started with a similar density of cells per milliliter and allowed to reach a similar population number per milliliter. The results showed that resistant bacteria arise spontaneously prior to the exposure to phage since a series of similar cultures yielded results different from those obtained with a series of samples from one culture. (See text for further explanation.)

more than physiological adaptations or phenotypic changes. That is, they refused to believe that bacteria have stable hereditary systems and could undergo permanent changes or mutations and assumed that any changes in the characteristics of a culture were simply due to environmental influences. However, Max Delbrück and Salvador Luria believed that bacteria have stable hereditary mechanisms, and in 1943 they performed an elegant experiment that proved the point.

Bacterial viruses called **bacteriophages**, or simply **phages**, are capable of killing bacteria. When susceptible bacteria are exposed to a phage, some of the bacterial cells survive, and they and their descendants are resistant to the phage. Some microbiologists assumed that these cells were modified by their contact with the phage so they became resistant by physiological adaptation. But Luria and Delbrück believed that phage-resistant bacteria were the result of mutations that occurred before the bacteria came into contact with the phages.

Let us suppose that resistant cells are the result of contact with phages; then if we set up a large number of identical bacterial cultures and expose them all to identical batches of phage, approximately the same number of resistant cells should appear in all cultures. However, if the resistant cells are really the result of mutations and since mutations occur entirely at random, then when we grow many identical cultures and expose them all to identical batches of phage, we should find a great fluctuation in the numbers of resistant cells. In some cultures, there may be no mutations and therefore no resistant cells, but in other cultures a mutation might have occurred very early, so that nearly all the cells are resistant (Fig. 12-3).

Thus Luria and Delbrück predicted that physiological adaptation to the phage by the bacteria should give about the same number of resistant bacteria (within sampling error) in each culture. But if phage resistance was due to mutations, preexisting and merely selected by the addition of phage, the number of resistant bacteria should fluctuate widely in each culture. Luria and Delbrück found a much greater fluctuation in numbers than could be accounted for by physiological adaptation, and thus they proved statistically by their **fluctuation test** that phage resistance was really the result of mutation (see Fig. 12-4).

Shortly after, in 1952, more direct proof of preexisting mutants was provided

by Joshua and Esther Lederberg. They introduced the replica plating technique (shown in Fig. 12-5) and provided a direct method for demonstrating the undirected spontaneous origin of bacterial mutants; i.e., the mutants occurred independently of any selective agent or environment. The procedure made it

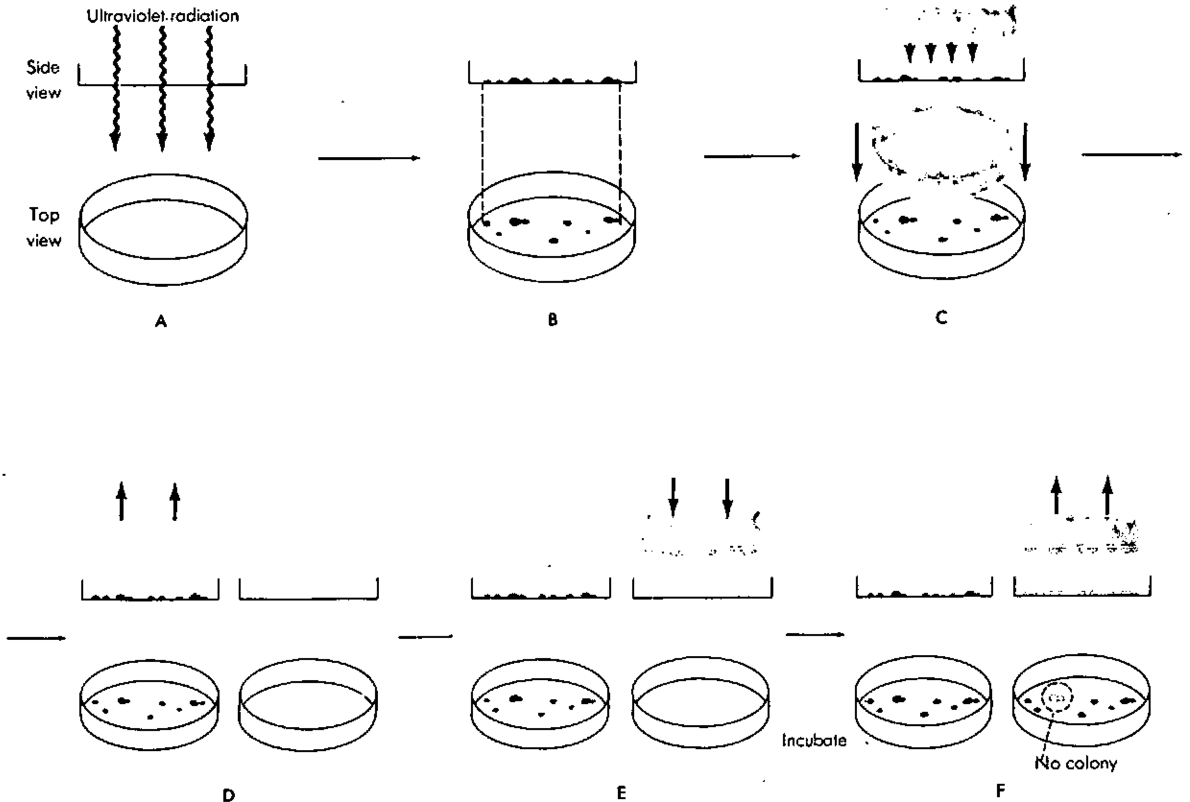


Figure 12-5. Replica plating is used for isolating nutritional mutants of *Escherichia coli*. (A) Bacterial suspension placed in open half of Petri dish and exposed to mutagenic agent, such as ultraviolet radiation. (B) Sample from (A) plated on surface of a "complete" medium such as nutrient agar. The plate is incubated; after incubation, the exact position of colonies on the plate is noted. (C) A sterile replica plating unit is gently pressed to the surface of plate (B), then raised (D), and then pressed to the surface of a plate of "minimal" agar medium (E). The positioning of the replica plating unit on the minimal agar must be precise, so that colony locations will be comparable on each of the two plates. The plates will be replicas of one another. The minimal agar in the plate in (E) consists of inorganic salts and glucose, nutrients which normally permit growth of *E. coli*. After incubation (F), colonies appear on the new plate at most, but not all, of the positions corresponding to locations of colonies on the original plate. It may be assumed that the organisms that failed to develop are nutritional mutants; that is, they are not able to grow on an inorganic salts–glucose medium, a characteristic which they originally possessed.

practical to examine large numbers of clones (populations of cells descending from a single cell) for a particular characteristic. By using sufficiently large samples, one could, for example, demonstrate the occurrence of phage-resistant mutants in a culture which was known to be phage-sensitive. The mutant types developed and could be located on an agar-plate culture which had not en-

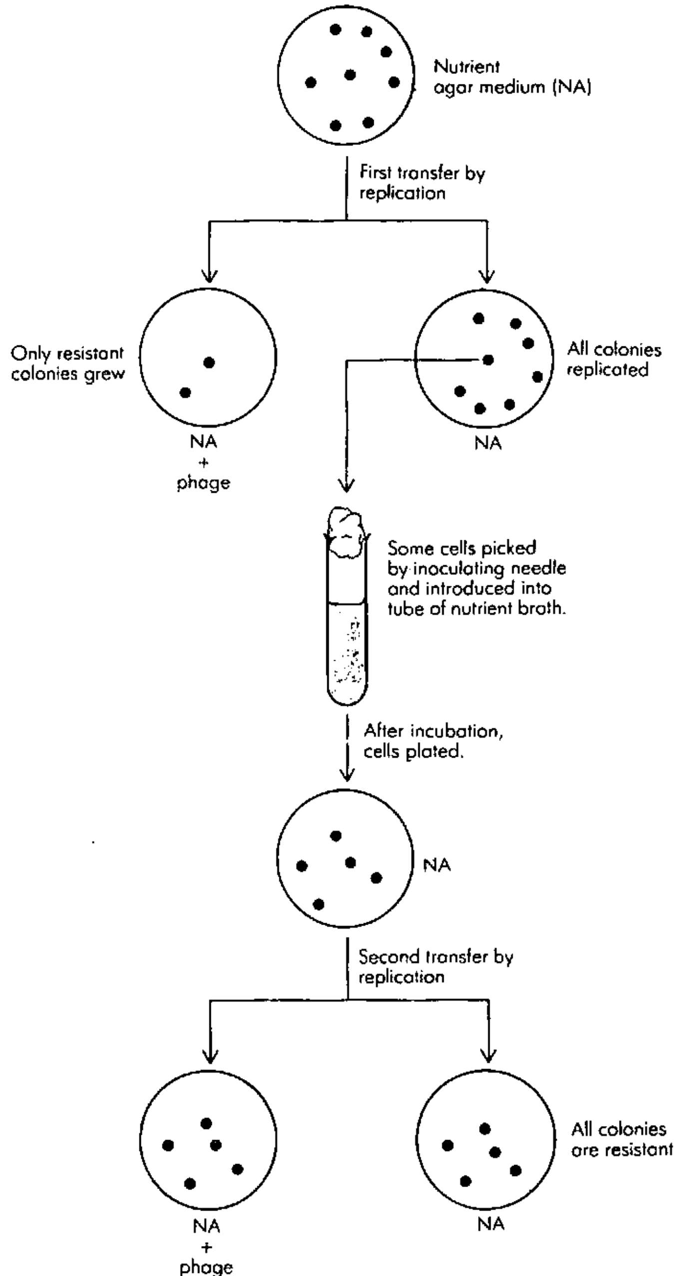


Figure 12-6. Simplified representation of the experiment of the Lederbergs showing the spontaneous nature of mutation in bacteria. The drawing shows that isolation of a pure colony of phage-resistant bacteria from a medium is possible without prior exposure to the virus.

countered phage previously. Similarly, the spontaneous appearance of antibiotic-resistant strains could be demonstrated without previous exposure of the culture to the antibiotic. As Fig. 12-5 shows, replica plating can also be used for isolating nutritional mutants. In essence the technique provides a practical means for finding the one cell in a million (more or less) which has mutated.

Thus the Lederbergs were able to isolate pure colonies of resistant *E. coli* mutants from many colonies on a medium plate that had *never been exposed* to lytic phage. This experiment demonstrated that mutation against the phage had its origin in *spontaneous* mutation. The growth of resistant colonies on replica plates arose from cells that were already present and were already resistant on the original nonselective plate *prior* to exposure to the selective agent, such as a lytic phage. Figure 12-6 shows a simplified representation of the experiment performed by the Lederbergs. Since then many other types of mutations have been found in bacteria, and it is now firmly established that bacteria have a hereditary system just like higher organisms.

Types of Mutations

At the molecular level there are several ways in which changes in the purine-pyrimidine base sequence of a gene can occur, resulting in mutation. Two common types are **point mutations** and **frameshift mutations**.

Point Mutations

Point mutations occur as a result of the substitution of one nucleotide for another in the specific nucleotide sequence of a gene. The substitution of one purine for another purine or one pyrimidine for another pyrimidine is termed a **transition** type of point mutation. A **transversion** is the replacement of a purine by a pyrimidine, or vice versa. This **base-pair substitution** may result in one of three kinds of mutations affecting the translation process:

1 The altered gene triplet produces a codon in the mRNA which specifies an amino acid different from the one present in the normal protein. This mutation is called a **missense mutation**. Such a protein may be functionally inactive or less active than the normal one. A good example of a missense mutation in humans is the disease sickle cell anemia. A single base substitution in the codon for the sixth amino acid of normal hemoglobin A changes the sixth amino acid from glutamic acid to valine, thus forming the characteristic hemoglobin S of sickle cell anemia. That is, GAG, which codes for glutamic acid, has changed to GUG for valine. Under low oxygen concentration the altered hemoglobin S molecules stack into crystals, giving the red blood cells a sickle shape.

2 The altered gene triplet produces a chain terminating codon in mRNA, resulting in premature termination of protein formation during translation. This is called a **nonsense mutation**. The result is an incomplete polypeptide which is nonfunctional.

3 The altered gene triplet produces a mRNA codon which specifies the same amino acid because the codon resulting from mutation is a synonym for the original codon. This is a **neutral mutation**.

Frameshift Mutations

These mutations result from an *addition* or *loss* of one or more nucleotides in a gene and are termed **insertion** or **deletion** mutations, respectively. This results in a shift of the reading frame. We saw earlier that during protein synthesis the reading of the genetic code starts from one end of the protein template, mRNA,

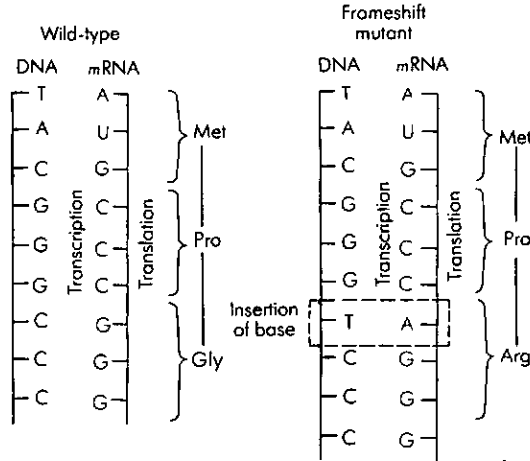


Figure 12-7. Frameshift mutation, as a result of insertion of a nucleotide in a gene. Insertion of a nucleotide in a gene results in the transcription of an additional nucleotide in mRNA. This results in a frameshift when codons are read during translation, so all codons following the insertion are altered and all amino acids coded for are changed. A frameshift mutation as a result of deletion of a nucleotide would have essentially the same effect.

and is read in consecutive blocks of three bases. Frameshift mutations, therefore, generally lead to nonfunctional proteins, because an entirely new sequence of amino acids is synthesized from a frameshift reading of the nucleotide sequences of mRNA (which was transcribed from a mutation in the DNA of the cell). This type of mutation is illustrated in Fig. 12-7.

How Mutations Occur

Mutations most commonly occur during DNA replication. Some mutations occur as the result of damages inflicted by ultraviolet (UV) light or x-rays. Since these agents are an inescapable part of the environment (for example, UV light is a component of sunlight), they probably account for many spontaneous mutations. However, mutation rates can be increased substantially by deliberately exposing a culture to such radiation. Any agent that increases the mutation rate is called a **mutagen.** Mutations obtained by use of a mutagen are said to be **induced,** rather than spontaneous, though they may differ only in frequency, not in kind. For example, UV light causes mutation under both natural and laboratory conditions. The number of mutants obtained by laboratory conditions is much higher, however, because of the high dosage of UV light used.

The major effect of UV light is to cause the formation of **dimers** by cross-linking between adjacent pyrimidine, especially thymine, residues in DNA. These cross-linked residues disrupt the normal process of replication by preventing the various polymerases from functioning. When x-rays interact with DNA, the result is usually a break in the phosphodiester backbone of the nucleic acid.

The most revealing findings about mutation in recent years have come from studies on the mutagenic effects of various chemicals. There are three main types of mutagenic chemicals. The first consists of *compounds that can react chemically with DNA.* Since specificity of DNA replication depends upon purine-pyrimidine bonding, which results from hydrogen bonding between the amino and hydroxyl groups of the purines and pyrimidines, chemical modification of these amino and hydroxyl groups can cause mutation. Nitrous acid, which can remove amino groups from purines and pyrimidines, is such a

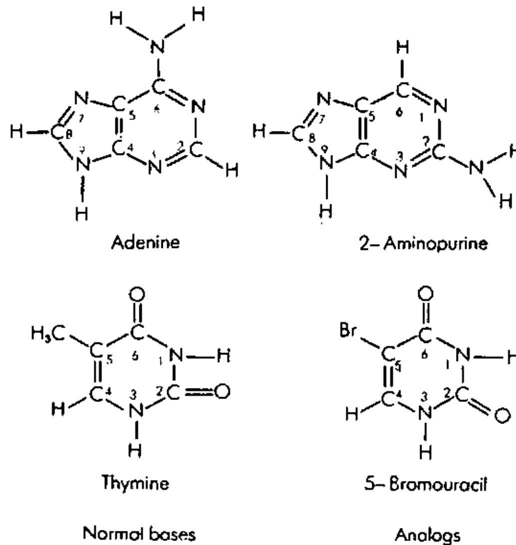


Figure 12-8. Two normal DNA bases and two base analog mutagens. 2-Aminopurine is an analog of adenine and can pair with thymine or cytosine. 5-Bromouracil is an analog of thymine and can pair with adenine or guanine. A color box highlights the part of the analog which differs from the normal base.

mutagen. The second type of mutagenic chemicals consists of *base analogs*. These are chemicals sufficiently similar in structure to normal DNA bases to be substituted for them during DNA replication (Fig. 12-8). Although similar in structure, base analogs do not have the same hydrogen-bonding properties as the normal bases. They can therefore introduce errors in replication which result in mutation. A third type of mutagenic chemicals is *intercalating agents*. These are flat molecules that can intercalate (slip in) between base pairs in the central stack of the DNA helix. By this means they distort the structures and cause subsequent replication errors. Examples of such agents are acridine orange, proflavin, and nitrogen mustards.

Recently, it was shown that mutations can occur because of transposons. **Transposons** are units of DNA which move from one DNA molecule to another, inserting themselves nearly at random. They are also capable of causing DNA rearrangements such as deletions or inversions. For example, one such transposon is from the bacterial virus called Mu, which may be considered a mutagen.

How Mutations Are Repaired

We have said that DNA damage can occur by UV radiation, x-rays, and certain chemicals. Fortunately, cells contain specific enzymes which can repair damaged DNA. In this way, some affected cells can continue to function normally.

Many kinds of bacterial cells and yeasts have been shown to possess an efficient photoreactivating mechanism for repairing damage caused by UV radiation. This **photoreactivation** occurs when cells exposed to lethal doses of UV light are immediately exposed to visible light. A special enzyme designated PRE, induced by visible light, splits or unlinks the dimers formed because of exposure to UV light and restores the DNA to its original state.

Some bacteria have enzymes, called **endonucleases** and **exonucleases**, that excise or cut out a damaged segment of DNA. Then the other enzymes, poly-

merases and ligases, repair the resulting break by filling in the gap and joining the fragments together. This mechanism is illustrated in Fig. 12-9 and is called **excision repair.**

The process by which *E. coli* repairs large amounts of DNA damage is called **inducible** or **SOS repair.** This process is not a single discrete mechanism but includes diverse responses such as the ability to repair pyrimidine dimers, to induce various prophages, to shut off respiration, and to delay septum formation during cell division. But all the responses are coordinately regulated. The process is a very efficient one; however, it tends to insert mismatched bases and thus is error-prone and introduces additional mutation.

Mutation Rate

The rate of mutation is the probability that a gene will mutate at any particular cell division. Thus the mutation rate is generally defined as the average number of mutations per cell per division. It is expressed as a negative exponent per cell division. For example, if there is one chance in a million that a gene will mutate when the cell divides, the mutation rate for any single gene equals 10^{-6} per cell division. Generally, the mutation rate for any single gene ranges between 10^{-3} and 10^{-9} per cell division. Thus a mutation rate for *E. coli* may be given as 5.8×10^{-8} mutations per bacterium per cell division.

The mutation rate has some practical implications. Since genes mutate at random and independently of each other, the chance of two mutations in the same cell is the product of the single mutation rates for each. So, for example, if the mutation rate to penicillin resistance is 10^{-8} per cell division and that to streptomycin resistance is 10^{-6} per cell division, the probability that both mutations will occur in the same cell is $10^{-6} \times 10^{-8}$, or 10^{-14}. This mutation rate is very low. For this reason, it is a common practice to give two antibiotics simultaneously in the treatment of some diseases. For example, a combination of penicillin G and streptomycin has been of proven value in treating streptococcal infections. A cell which has become resistant to one antibiotic is still likely to be inhibited or killed by the other.

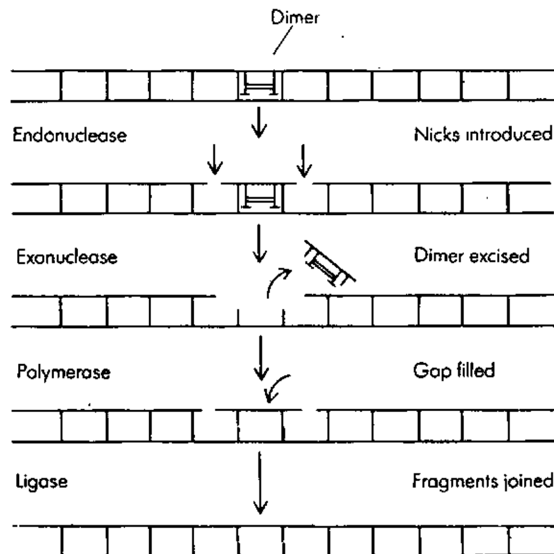


Figure 12-9. Excision repair of UV-light-damaged DNA containing a thymine-thymine dimer generated by covalent links between adjacent bases.

Phenotypes of Bacterial Mutants

Since all properties of living cells are ultimately gene-controlled, any cell characteristic may be changed by mutation. A large variety of bacterial mutants have been isolated and studied intensively. Some of the major phenotypes of mutants are as follows:

1 Mutants that exhibit an increased tolerance to inhibitory agents, particularly antibiotics (antibiotic- or drug-resistant mutants)
2 Mutants that demonstrate an altered fermentation ability or increased or decreased capacity to produce some end product
3 Mutants that are nutritionally deficient, that is, that require a more complex medium for growth than the original culture from which they were derived (auxotrophic mutants)
4 Mutants that exhibit changes in colonial form or ability to produce pigments
5 Mutants that show a change in the surface structure and composition of the microbial cell (antigenic mutants)
6 Mutants that are resistant to the action of bacteriophages
7 Mutants that exhibit some change in morphological features, for example, the loss of ability to produce spores, capsules, or flagella (Fig. 12-10)
8 Mutants that have lost a particular function but retain the intracellular enzymatic activities to catalyze the reactions of the function, for example, loss of a permease (cryptic mutants)
9 Mutants that yield a wild-type phenotype under one set of conditions and a mutant phenotype under another (conditionally expressed mutants)

It is evident from our list of bacterial mutants that all the characteristic features of bacteria are subject to alteration by the process of mutation. It is also apparent that some of the specific changes caused by mutation are similar or the same as those resulting from a change in environmental conditions. It is therefore necessary to ascertain experimentally that a change is really due to a mutation and not to a response to the environment.

There are many practical implications associated with the occurrence of microbial mutants. The following examples illustrate this.

1 Some microorganisms are known to develop resistance to certain antibiotics because of mutation. This fact is of great importance in the treatment of disease, since antibiotics originally effective for the control of a bacterial infection become less effective or ineffective as antibiotic-resistant mutants appear (Fig. 12-11).
2 It is possible to isolate biochemical mutants capable of producing large yields of an end product. This is important in industry. For example, the yield of penicillin in commercial production was dramatically increased through selection of mutant strains of *Penicillium*.

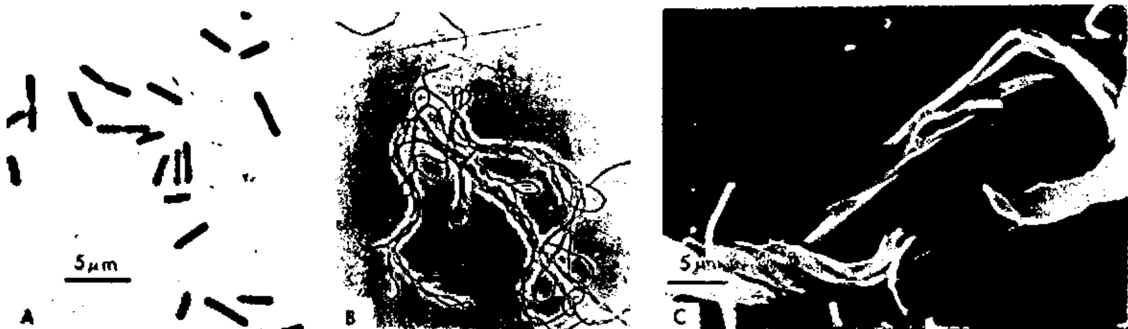


Figure 12-10. Some mutants exhibit morphological changes. Mutants of *Bacillus subtilis* that are grossly deficient in the enzymes needed to separate daughter cells after division grow at normal rates as very long chains of unseparated cells. Under certain growth conditions these mutants also form helical structures. (A) Wild-type, phase-contrast photomicrograph; (B) mutants, phase-contrast photomicrograph; (C) mutants, scanning electron micrograph. Note helical structures in (B) and (C). *(Courtesy of Jared E. Fein, McGill University.)*

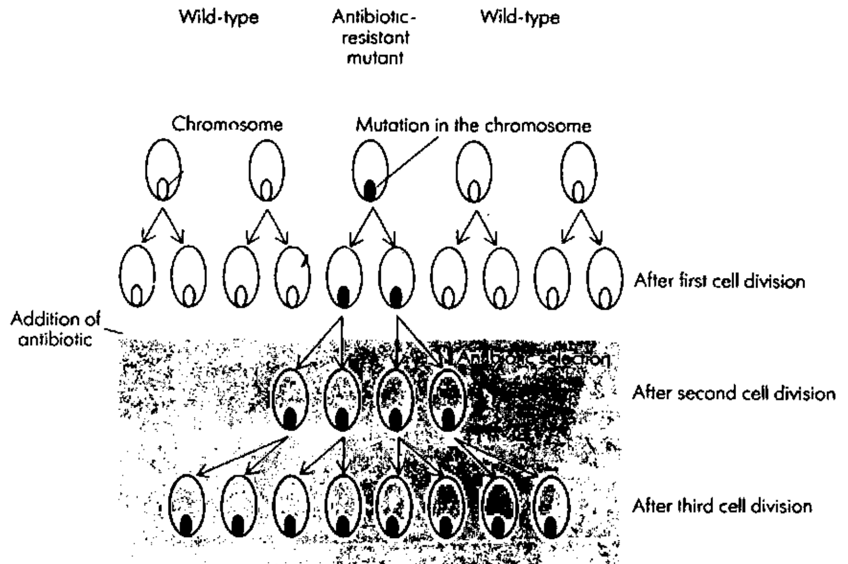


Figure 12-11. Antibiotics originally effective for control of a bacterial infection become less effective or ineffective as antibiotic-resistant mutants appear. Note how the use of antibiotics (color screen) actually selects for bacteria that have become antibiotic-resistant due to chromosomal mutation. Bacteria sensitive to the antibiotic are killed or prevented from reproducing. *(Courtesy of SANDORAMA 1978, Sandoz Ltd., Basel, Switzerland, and G. Lebek University of Berne.)*

3 The maintenance of pure cultures of typical microorganism species requires that occurrence of mutation be prevented; otherwise, the culture will no longer be typical.
4 Microbial mutants have been extensively used in the investigation of various biochemical processes, particularly biosynthetic reactions. For example, mutants with blocks or impairment at different enzymatic steps have been used to unravel metabolic sequences.

Many mutants, perhaps a majority, are able to revert to the wild-type condition by reverse mutation. This is a return to the original phenotype by the mutant cells. However, this may not *necessarily* be due to a precise reversal of the original mutation. Sometimes, the effect of the original mutation may be partially or entirely suppressed by a second mutation at a different site on the chromosome.

Designation of Bacterial Mutants

The conventional designations used for bacterial mutants may be described briefly as follows. Each genotype is given a lowercase, italicized, three-letter code. For instance, a mutation which affects proline synthesis is designated pro. Since mutation in a number of different genes may exhibit identical phenotypes, discrete genetic loci can be differentiated by means of capital letters, for example, *proA*, *proB*, and *proC*. Numbers may be added sequentially to designate particular mutations; that is, as each new mutation is isolated, it is assigned a number that identifies it in bacterial pedigrees, for example, *proA52* is the 52d isolate of the *Escherichia coli* Genetic Stock Center at Yale University. A further example is given in Fig. 12-12. Table 12-1 shows some frequently encountered genotype abbreviations.

In referring to the phenotype of a bacterium, we use the same three-letter abbreviation, except that it is not italicized and its first letter is capitalized.

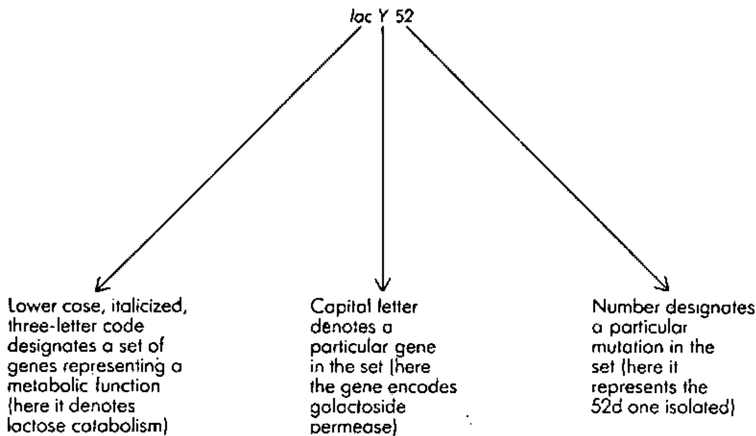


Figure 12-12. Designation of bacterial mutations. The example used here is that of a mutation in β-galactosidase synthesis.

Table 12-1. Some Frequently Encountered Genotypes

Genotype	Mutation
ala	Alanine requirement
azi	Azide resistance
div	Cell division
fla	Flagella biosynthesis
gal	Galactose utilization
lac	Lactose utilization
met	Methionine requirement
pur	Purine biosynthesis
str	Streptomycin resistance
thi	Thiamine requirement
ton	Phage T1 resistance
uvr	Ultraviolet radiation sensitivity

Thus a mutant strain designated *pro* would be phenotypically Pro^- (which means inability to synthesize the amino acid proline). The superscript "+" would designate the wild type, for example, Pro^+. The "−" superscript represents the mutant.

BACTERIAL RECOMBINATION

Genetic recombination is the formation of a new genotype by reassortment of genes following an exchange of genetic material between two different chromosomes which have similar genes at corresponding sites. These are called **homologous chromosomes** and are from different individuals. Progeny from recombination have combinations of genes different from those that are present in the parents. In bacteria, genetic recombination results from three types of gene transfer:

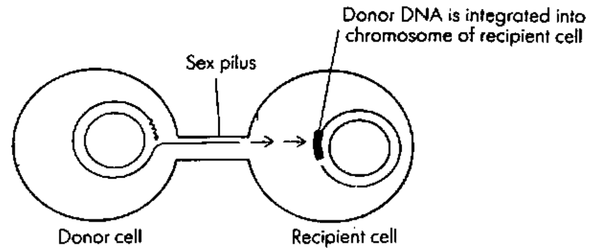
1 ***Conjugation.*** Transfer of genes between cells that are in physical contact with one another
2 ***Transduction.*** Transfer of genes from one cell to another by a bacteriophage
3 ***Transformation.*** Transfer of cell-free or "naked" DNA from one cell to another

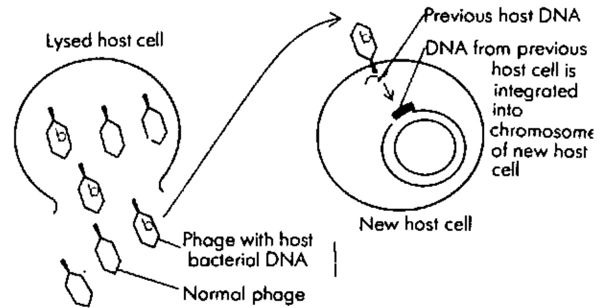
These three types of gene transfer are shown in Fig. 12-13.

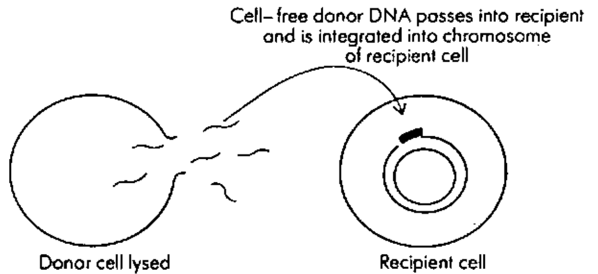
In bacterial recombination the cells do not fuse, and usually only a portion of the chromosome from the **donor cell** (male) is transferred to the **recipient cell** (female). The recipient cell thus becomes a **merozygote,** a zygote that is a partial diploid. Once merozygote transformation has occurred, recombination can take place.

The general mechanism for bacterial recombination is believed to take place as follows. Inside the recipient cell the donor DNA fragment is positioned alongside the recipient DNA in such a way that homologous genes are adjacent. Enzymes act on the recipient DNA, causing nicks and excision of a fragment.

Figure 12-13. (*Right*) Three types of gene transfer from which genetic recombination results. (A) Conjugation, the transfer of genes between cells in physical contact with each other, perhaps by a sex pilus; (B) transduction, the transfer of genes between cells by a bacteriophage; (C) transformation, the transfer of cell-free or "naked" DNA from one cell to another.







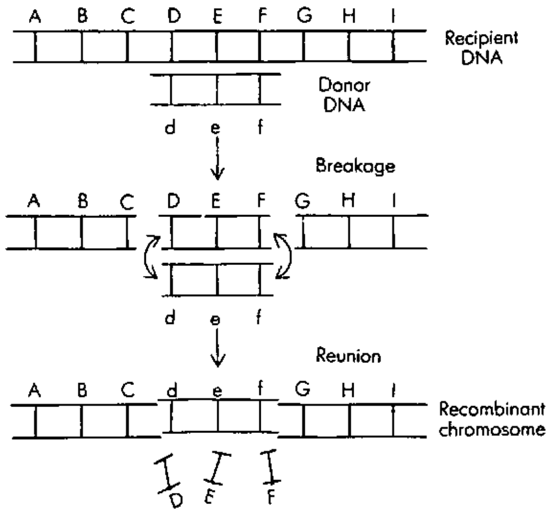


Figure 12-14. (*Above*) The breakage and reunion model of bacterial recombination. The donor DNA becomes integrated into the recipient DNA.

Then the donor DNA is integrated into the recipient chromosome in place of the excised DNA. The recipient cell then becomes the recombinant cell because its chromosome contains DNA of both the donor and the recipient cell. (The excised DNA pieces from the recipient chromosome are probably broken down by specific enzymes.) This general recombination mechanism is seen in Fig. 12-14.

BACTERIAL CONJUGATION

Even though Luria and Delbrück had demonstrated in 1943 that bacteria have a stable hereditary system, it was impossible at that time to explore the system experimentally because of the lack of knowledge of any mating system in bacteria. The genetics of plants and animals depends upon the regular cycle of sexual reproduction in these organisms; once each generation, there is an opportunity for different mutants of a species to mate with each other and produce

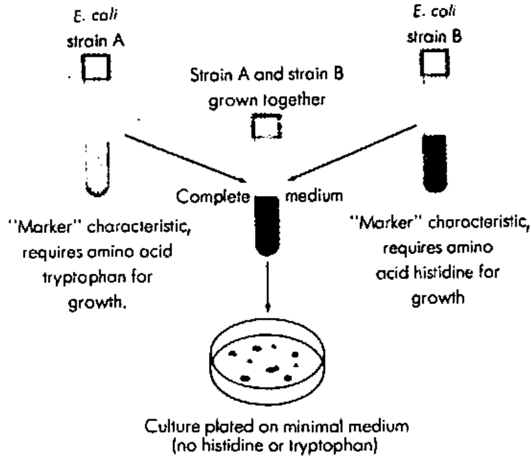


Figure 12-15. Evidence for conjugation in bacteria. The two specific characteristics of the *E. coli* strains are (A) trp^-, a tryptophan auxotroph, and (B) *his* , a histidine auxotroph. The mixture is plated on a minimal medium; growth allows for the selection of prototrophic recombinants (organisms which can synthesize all their amino acid requirements).

new individuals with new combinations of mutations, i.e., to *recombine* with each other, or to produce **recombinants**. For example, a plant that produces smooth, yellow peas can be bred with one that produces wrinkled, green peas. Some of the next generation will be plants that produce the parental types—smooth and yellow or wrinkled and green. But other plants will produce the recombinant types smooth and green or wrinkled and yellow. Only by performing such crosses and observing the progeny can genetic work be done. The first demonstration of recombination in bacteria was achieved by Lederberg and Tatum in 1946 in a brilliant and remarkable experiment that opened the door to a whole new world of microbiology. Lederberg and Tatum knew that conjugation in bacteria must be quite rare, since no one had found it in spite of many attempts, and so they determined to *select* the few possible recombinants out of a large population. They combined two different auxotrophic strains of *E. coli* and gave them an opportunity to mate. Then they plated the combined cultures on a minimal medium, where only prototrophs could grow; when they found prototrophic colonies growing there, they knew that these must have been the result of a recombination between the auxotrophs.

Figure 12-15 shows the principle of their experiments in simplified form. When Lederberg and Tatum did their experiments, they used **polyauxotrophs** (mutants with more than one nutritional requirement) so that back mutation or spontaneous reversion to the wild type would not occur to confuse their results. For example, the probability of simultaneous reversion of three different mutations is of the order of $(10^{-6})^3$.

It had to be shown also that the prototrophs which arose could not have arisen by the phenomenon of transformation (discussed in the next section) since transformation had been discovered by Griffith in 1928. Cell-to-cell contact as a precondition was shown by using a U tube where the auxotrophic parental cells could be cultured together yet separated physically by a microporous fritted glass disk. This disk was permeable to macromolecules like DNA but not to cells. Culture fluid and soluble material could pass freely between the two parent cultures. Prototrophs or recombinants were never recovered from such

cultures. Result like these indicated that bacterial recombination by conjugation is indeed a true sexual process.

It is apparent that mating or conjugation in *E. coli* is radically different from sexual mating in higher organisms. It is not a reproductive process that occurs regularly at each generation. It does not involve meiosis since bacterial cells are haploid, nor does it involve the fusion of gametes. Instead, it involves the transfer of some DNA from one cell to another followed by separation of the mating pair of cells. While only very small fragments of the bacterial chromosome are transferred in transduction and transformation (discussed later), in conjugation it is possible for large segments of the chromosome, and in special cases the entire chromosome, to be transferred.

Sex Factors

A clearer understanding of conjugation in bacteria came about with the discovery that there is sexual differentiation in *E. coli*; in other words, different mating types of the bacterium exist. Male cells contain a small circular piece of DNA, which is in the cytoplasm and not part of the chromosome, called the **sex factor** or **F factor** (fertility factor). These cells are referred to as F^+ and are donors in mating. Female cells lack this factor and are labeled F^-. They are recipient cells.

Crosses between two F^- strains do not yield recombinants. However, in $F^+ \times F^-$ crosses, the male replicates its sex factor, and one copy of it is almost always transferred to the female recipient. The F^- cell is converted to an F^+ cell and is itself capable of serving as a donor (see Fig. 12-16). Therefore, as long as the cells grow, the conjugation process can continue in an infectious way with repeated transfer of the sex factor. The transfer of the F factor in an $F^+ \times F^-$ cross occurs with a frequency that approaches 100 percent. But the formation of recombinants in an $F^+ \times F^-$ cross occurs at a low frequency—about one recombinant per 10^4 to 10^5 cells. Thus we see that the transfer of the F factor is independent of the transfer of chromosomal genes.

Since the transfer of the F factor is independent, it follows that the F factor DNA replicates independently of the F^+ donor cell's normal chromosome. The F factor DNA is only sufficient to specify about 40 genes which control sex-factor replication and synthesis of sex pili. One or more sex pili are produced by each F^+ cell (Fig. 12-17). Sex pili seem to act to bind an F^- cell to an F^+ cell and then to retract into the F^+ cell, pulling the F^- cell into close contact. There is also some evidence that sex pili are tubules through which DNA passes

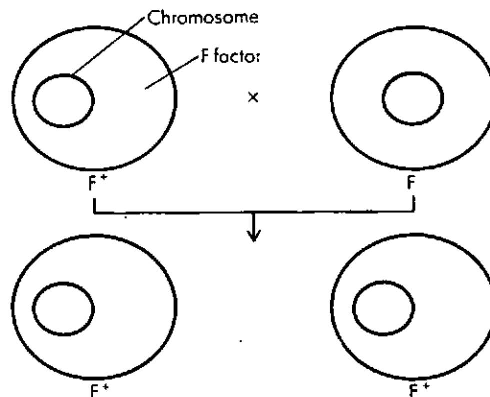


Figure 12-16. During mating of an F^+ and F^- cell, the F^+ cell replicates the sex or F factor and the copy is almost always transferred to the F^- cell. Thus an F^- cell usually becomes an F^+ cell during mating.

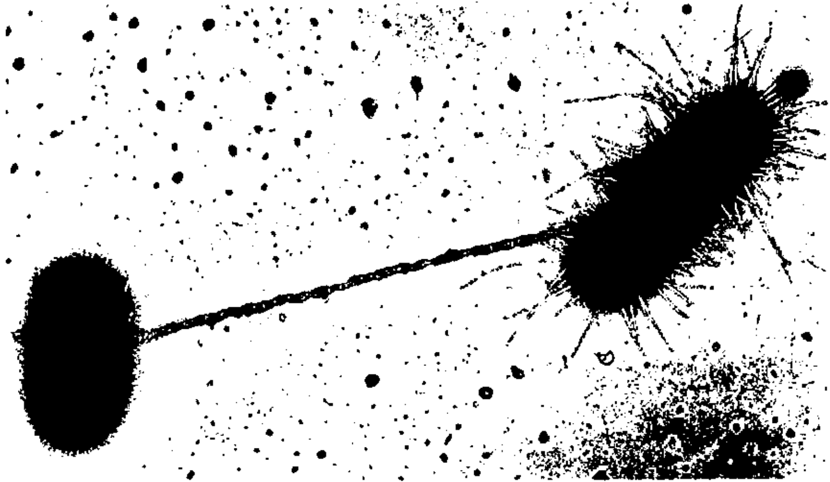


Figure 12-17. Sex pilus holding together a mating pair of *E. coli*. The male cell (on the right) also has another type of pili besides the sex pilus. Small RNA bacteriophages adsorbed to the sex pilus may be seen as dots. (X25,000.) *(Courtesy of C. Brinton, Jr., University of Pittsburgh.)*

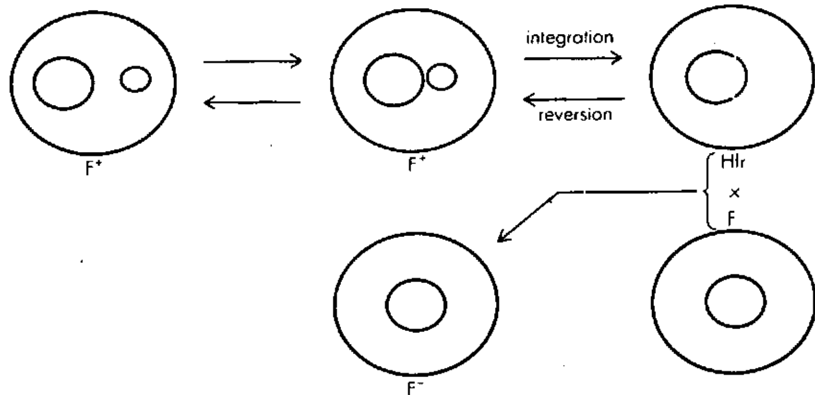


Figure 12-18. An Hfr cell arises from an F^+ cell in which the F factor becomes integrated into the bacterial chromosome. During mating of an Hfr and F^- cell, the F^- cell almost always remains F^-. This results because Hfr rarely transfers an entire F factor to the F^- cell. But the recombination frequency is high.

from an F^+ to an F^- cell during conjugation, although the DNA may be passed from one cell to another at sites of contact between them.

High-Frequency Recombination Strains

The study of conjugation in bacteria was made easier when new strains of cells were isolated from F^+ cultures which underwent sexual recombination with F^- cells at a rate at least 10^3 times greater than $F^+ \times F^-$ cells. These new donor strains were thus called **high-frequency recombination, or Hfr, strains.** Hfr cells arise from F^+ cells in which the F factor becomes integrated into the bacterial chromosome. They differ from F^+ cells in that the F factor of the Hfr is rarely transferred during recombination. Thus in an Hfr $\times$ F^- cross, the frequency of recombination is high and the transfer of F factor is low (Fig. 12-18); in an $F^+ \times F^-$ cross, the frequency of recombination is low and the transfer of F factor is high.

The order in which chromosomal material is transferred from an Hfr donor to an F^- recipient was determined by the *interrupted mating* experiments of

Elie Wollman and François Jacob. An Hfr strain was mixed with an F⁻ strain, and at various times the conjugation was interrupted by breaking the cells apart in a high-speed blender. The cells were then plated on various types of selective agar media in order to select for recombinant cells which had received donor genes before mating was interrupted.

Interrupted mating experiments reveal the order of genes on a chromosome by the time of entry and the frequency of recombination of each **marker**, which is a detectable mutation serving to identify the gene at the locus or site where it occurs. Each gene enters the F^- cell at a characteristic time, and a **linkage map** of the Hfr chromosome can be constructed using time of entry as a measure. This is the principal method of learning where the genes are on a bacterial chromosome (Fig. 12-19). This is all possible because the Hfr chromosome is transferred to the F^- cell in a *linear* fashion (Fig. 12-20) even though it is a circular chromosome. During transfer the Hfr chromosome begins replicating at the point of insertion of the F factor. Since the F factor can integrate in different positions of the Hfr bacterial chromosome, the first genes to enter an F^- cell will vary with different Hfr strains (Fig. 12-20). This means that the integrated F factor serves as the point of chromosomal opening, and *part* of it serves as the origin of transfer. The 5′ end of the single DNA strand enters the F^- cell first. Data from an interrupted mating experiment is shown in Fig. 12-21.

It takes about 100 min to inject a copy of the whole Hfr *E. coli* genome (i.e., the chromosome and the integrated F factor). Since conjugation is usually in-

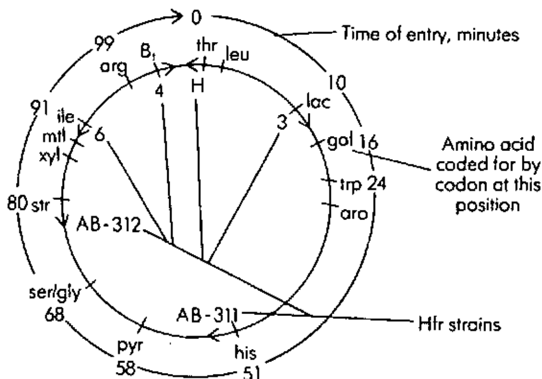


Figure 12-19. Simplified linkage map (color) of the circular chromosome of *E. coli* constructed from interrupted mating experiments using different Hfr strains. The arrows on the linkage map indicate the leading end and direction of entry of the chromosomes injected by each of the Hfr strains, the designations of which are shown inside the circle. This is determined by the position of the F factor in each of the Hfr strains. The numbers around the outside of the map show distances as a function of time, in minutes, based on time of entry of each codon in experiments. (Note that the map distances in minutes are drawn relative to the Hfr strain H so that the *thr* gene has been arbitrarily chosen as the origin.)

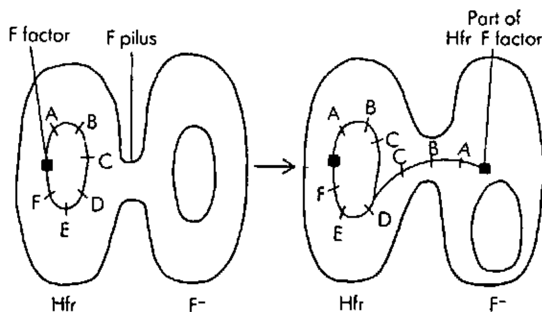


Figure 12-20. Mechanism for DNA transfer between Hfr and F^- cells. The Hfr chromosome begins replicating at the point of insertion of the F factor. Since the F factor can integrate in different positions of the Hfr bacterial chromosome, the first genes to enter an F^- cell will vary with different Hfr strains. As shown, the order of genes transferred is ABCDEF. In another Hfr strain, the F factor might be integrated between B and C. In this case the order of genes transferred would be CDEFAB.

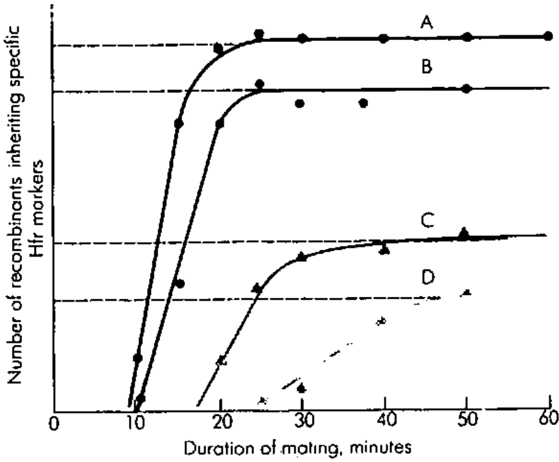
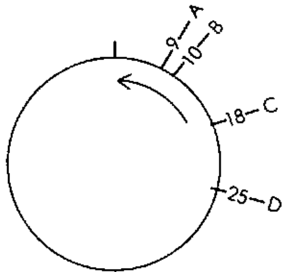


Figure 12-21. An interrupted mating experiment. (A) At intervals during the mating between Hfr and F⁻ cells, samples were removed from the culture and the conjugation interrupted. The cells were then plated on selective media that permitted growth of those F⁻ cells that inherited specific genetic markers received from the Hfr cells In the graph, the number of recombinant colonies is plotted as a function of the interval of mating allowed before interruption. Extrapolation of each curve to zero recombinants gives the time of entry of the Hfr marker. As may be seen in the graph, the order of genes on the DNA is ABCD, and that A is very close to B. (B) Analysis of the data in (A) allows the plotting of a map as shown. Numbers denote minutes.



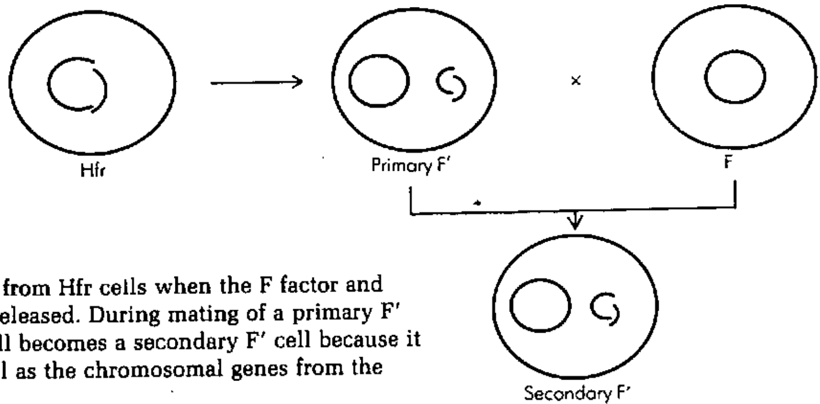


Figure 12-22. An F′ cell arises from Hfr cells when the F factor and some chromosomal genes are released. During mating of a primary F′ cell with an F⁻ cell, the F⁻ cell becomes a secondary F′ cell because it now carries the F factor as well as the chromosomal genes from the primary F′ cell.

terrupted by accident before this can occur, the distal Hfr genes are rarely transferred. Since *all* the Hfr chromosomal genes must be transferred before all the F factor genes are also transferred, most F⁻ recipients remain F⁻ after conjugation with Hfr cells.

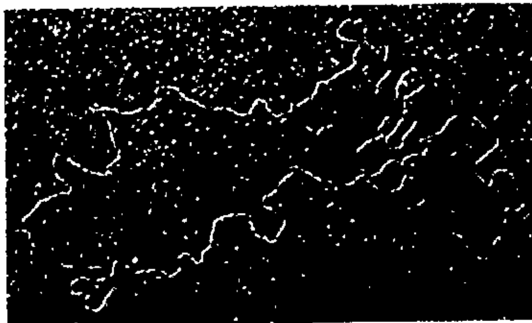


Figure 12-23. Bacterial plasmid shown as a molecule of looped DNA. The drug-resistant plasmid shown is called R28K, carries ampicillin resistance, and has a length of 21 μm. *(Courtesy of Michiko Egel-Mitani.)*

Hfr cells can revert to the F^+ state. When this occurs, the sex factor is released from the chromosome and resumes its autonomous replication. Sometimes this detachment is not cleanly accomplished, so the F factor carries along with it some chromosomal genes. In this state it is termed an F′ factor, and the cell in which this has occurred is called an F′ cell (see Fig. 12-22). When such primary F′ cells are mated with F^- recipients, the sex factor is transferred very efficiently together with the added bacterial genes. The recipient cell then becomes a secondary F′ cell; it is a partial diploid for those genes it receives from the primary F′ cell. This process whereby bacterial genes are transmitted from donor to recipient as part of the sex factor has been termed sexduction by Jacob and Wollman (Fig. 12-22).

Extrachromosomal Genetic Elements (Plasmids)

In addition to the normal DNA chromosome, extrachromosomal genetic elements are often found in bacteria. These elements are called plasmids and are capable of autonomous replication in the cytoplasm of the bacterial cell (Figs. 11-12 and 12-23). Plasmids are circular pieces of DNA that are extra genes. Some plasmids are capable of either replicating autonomously or integrating into the bacterial DNA chromosome and are called *episomes*. Thus the F factor of *E. coli* was called an episome because it can alternately exist in the F^+ or Hfr state.

Some bacteria have plasmids that are bacteriocinogenic factors. They determine the formation of bacteriocins, which are proteins that kill the same or other closely related species of bacteria. The bacteriocins of *E. coli* are called **colicins**; those of *Pseudomonas aeruginosa* are called pyocins, and so on. Bacteriocins have proved useful for distinguishing between certain strains of the same species of bacteria in medical bacteriological diagnosis. Bacteria possess other kinds of plasmids called R plasmids which confer resistance to a number of antibiotics. Some of the R plasmids can be transferred to other cells by conjugation, hence the term infectious resistance. Each form of resistance is due to a gene whose product is an enzyme that destroys a specific antibiotic.

TRANSDUCTION

Most bacteriophages, the virulent phages, undergo a rapid *lytic* growth cycle in their host cells. They inject their nucleic acid, usually DNA, into the bacterium, where it replicates rapidly and also directs the synthesis of new phage proteins.

Within 10 to 20 min, depending on the phage, the new DNA combines with the new proteins to make whole phage particles, which are released by destruction of the cell wall and lysis of the cell. However, some bacterial viruses, the **temperate** phages, which ordinarily do not lyse the cell, carry DNA that can behave as a kind of episome in bacteria; like other episomes, such as the F factor, these viral genomes can become integrated into the bacterial genome; they are then known as **prophages**. Bacteria that carry prophages (**lysogenic** bacteria) can be *induced* with ultraviolet light and other agents to make the prophages start to replicate rapidly and go through a lytic growth cycle, resulting in lysis of the cell with release of new phage particles. (Bacteriophages are discussed in greater detail in Chap. 20.) Phage particles may become filled with cell chromosomal DNA or a mixture of chromosomal and phage DNA (rather than completely with phage DNA, as is normally the case). Such aberrant phages can attach to other bacteria and introduce bacterial, rather than just phage, DNA into them. By this means they transfer bacterial DNA from one cell to another. Thus we can define **bacterial transduction** as the transfer by a bacteriophage, serving as a vector, of a portion of DNA from one bacterium (a donor) to another (a recipient).

This phenomenon was discovered by Zinder and Lederberg in 1952 when they searched for sexual conjugation among *Salmonella* species. They mixed auxotrophic mutants together and isolated prototrophic recombinant colonies from selective nutritional media. When the U-tube experiment was carried out with a parental auxotrophic strain in each arm and separated by a microporous fritted glass filter, prototrophs appeared in one arm of the tube. Since the filter prevented cell-to-cell contact but allowed free passage of fluid between the cultures, it could be concluded that some phenomenon other than conjugation was involved. Furthermore, the phenomenon could not be prevented by DNAase activity, thus eliminating transformation as the process for changing the recipient auxotrophs to prototrophy. Further experiments implicated a bacteriophage as the vector or transducing agent in the following manner. The bacteriophage was released from a lysogenic (recipient) culture. The phage passed through the filter and infected the other strain (donor), lysing it. During replication in the donor strain, the phage adventitiously included parts of the bacterial chromosome with it. It then passed through the filter again, carrying part of the donor's genetic information and imparting it to the recipient strain.

Generalized Transduction

If all fragments of bacterial DNA (i.e., from any region of the bacterial chromosome) have a chance to enter a transducing phage, the process is called **generalized transduction**. In this process, as the phage begins the lytic cycle, viral enzymes hydrolyze the bacterial chromosome into many small pieces of DNA. Any part of the bacterial chromosome may be incorporated into the phage head during phage assembly and is usually not associated with any viral DNA. For example, coliphage P1 can transduce a variety of genes in the bacterial chromosome. (This means that in a large population of phages there will be transducing phages carrying different fragments of the bacterial genome.) After infection a small proportion of the phages carry only bacterial DNA (see Fig. 12-24). The frequency of such defective phage particles is about 10^{-5} to 10^{-7} of the progeny phage produced. Since this DNA matches the DNA of the new

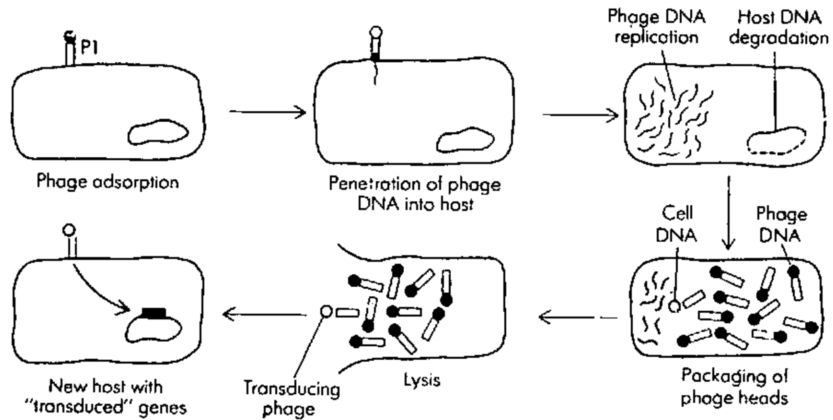


Figure 12-24. Generalized transduction. The phage P1 chromosome, after injection into the host cell, causes degradation of host chromosome into small fragments. During maturation of the virus particles, a few phage heads may envelop fragments of bacterial DNA instead of phage DNA. When this bacterial DNA is introduced into a new host cell, it can become integrated into the bacterial chromosome, thereby transferring several genes from one host cell to another.

bacterium infected, the recipient bacterium will not become lysogenic for P1 phage. Instead, the injected DNA will be integrated into the chromosome of the recipient cell. Defective P1 phages bearing *E. coli* DNA can be detected by the genetic markers present in that DNA. For instance, if a thr^- cell is infected by a phage carrying a fragment of *E. coli* DNA with a thr^+ gene, this thr^+ gene may be integrated (recombined) into the bacterial chromosome to result in a prototrophic recombinant detectable by growth in a medium devoid of threonine.

Generalized transduction, like bacterial conjugation and transformation, also provides a means for mapping bacterial genes, since the fragments transferred by a bacteriophage are often large enough to contain hundreds of genes. The mapping technique involves providing to the phage-infected bacteria a growth medium that *selects for* those recombinants that have inherited a given genetic marker from bacterial DNA carried by a transducing phage. Growth on other media can then be used to test how many of these recombinants have also inherited other donor markers. The closer two markers are together on the bacterial chromosome, the more likely they are to be inherited together by means of a single transducing phage. For example, when coliphage P1 is grown in a thr^+ $leu^+ azi^R$ host and then used to infect a thr^- leu^- azi^- recipient bacterial cell, only 3 percent of the selected Thr$^+$ recombinants are also Leu$^+$, and none are Az^R, or azide-resistant. But, if Leu$^+$ recombinants are selected, about 50 percent of these are also Az^R. This means that leu^+ is more closely linked to azi^R than it is to thr^+ and the suggested order is therefore thr^+ leu^+ azi^R. Thus the degree of linkage of genes can be measured by the frequencies of cotransduction of markers. The fact that only 3 percent of thr^+ transducing phages also carry leu^+ shows that these two genes are so far apart that they are rarely included at the same time in a DNA fragment that goes into the P1 head. (The P1 head carries a DNA molecule of slightly less than 10^5 nucleotide pairs.)

Specialized Transduction

Bacterial genes can also be transduced by bacteriophage in another process called **specialized transduction** in which certain temperate phage strains can transfer only a few restricted genes of the bacterial chromosome. More specifi-

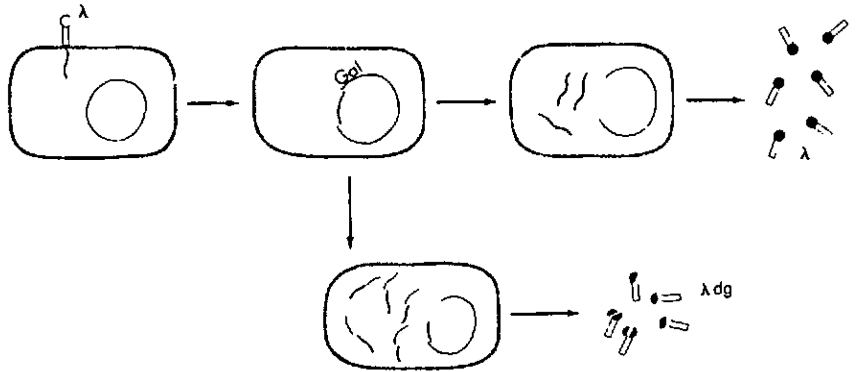


Figure 12-25. Specialized transduction. When phage λ infects a cell, its DNA is inserted into the bacterial genome next to the genes for galactose metabolism (*gal* genes). Usually when such a cell is induced, the λ DNA comes out, replicates, and makes normal phage. However, occasionally the λ DNA is excised imperfectly, taking *gal* genes with it and leaving some of itself behind, leading to λdg (defective, galactose transducing phage).

cally, the phages transduce only those bacterial genes adjacent to the prophage in the bacterial chromosome. Thus the process is also called **restricted transduction.** It occurs when a bacteriophage genome, after becoming integrated as prophage in the DNA of the host bacterium, again becomes free upon induction and takes with it into the phage head a small adjacent piece of the bacterial chromosome. In this way, when such a phage infects a cell, it carries with it the group of bacterial genes that has become part of it. Such genes can then recombine with the homologous DNA of the infected cell.

The best-studied specialized transducing phage is the phage lambda (λ) of *E. coli.* The location of the λ prophage in the bacterial chromosome is almost always between the bacterial genes *gal* and *bio.* Whenever the phage genome comes out of, or is *excised* from, the bacterial chromosome, it sometimes takes with it *gal* or *bio* genes. When phages carrying *gal* or *bio* genes infect a new host, recombination with the *gal* or *bio* genes of the host can occur. This process is illustrated in Fig. 12-25. It should be noted that almost all phages that carry some bacterial genes because of "incorrect" excision are defective in certain viral functions because they are missing a piece of phage genetic information taken up by the bacterial genes. They cannot proceed through their entire replicative cycle, but the cell will yield phages if it is also infected with a complete phage that can code for the missing functions of the defective phages.

BACTERIAL TRANSFORMATION

In 1928, an English health officer named Griffith injected mice with a mixture consisting of a few rough (noncapsulated and nonpathogenic) pneumococci and a large number of *heat-killed* smooth (capsulated and pathogenic) cells. (Living smooth pneumococci cause pneumonia in humans and other animals. "Rough" and "smooth" refer to the surface texture of the colonies of the respective cells.) The mice subsequently died of pneumonia, and *live* smooth cells were isolated from their blood. Apparently, some factor responsible for the pathogenicity of the smooth bacteria (even though they were dead) had been transferred to the living rough bacteria and had *transformed* them into pathogenic smooth ones. Griffith also showed that the transforming factor could be passed from the

transformed cells to their progeny and thus had the characteristics of a gene. This experiment of Griffith is illustrated in Fig. 12-26.

This "transforming principle" was identified as DNA by Avery, MacLeod, and McCarty in 1944. They defined DNA as the chemical substance responsible for heredity.

Thus transformation is the process whereby *cell-free*, or "naked," DNA containing a limited amount of genetic information is transferred from one bacterial cell to another. The DNA is obtained from the donor cell by natural cell lysis or by chemical extraction. Once the DNA is taken up by the recipient cell,

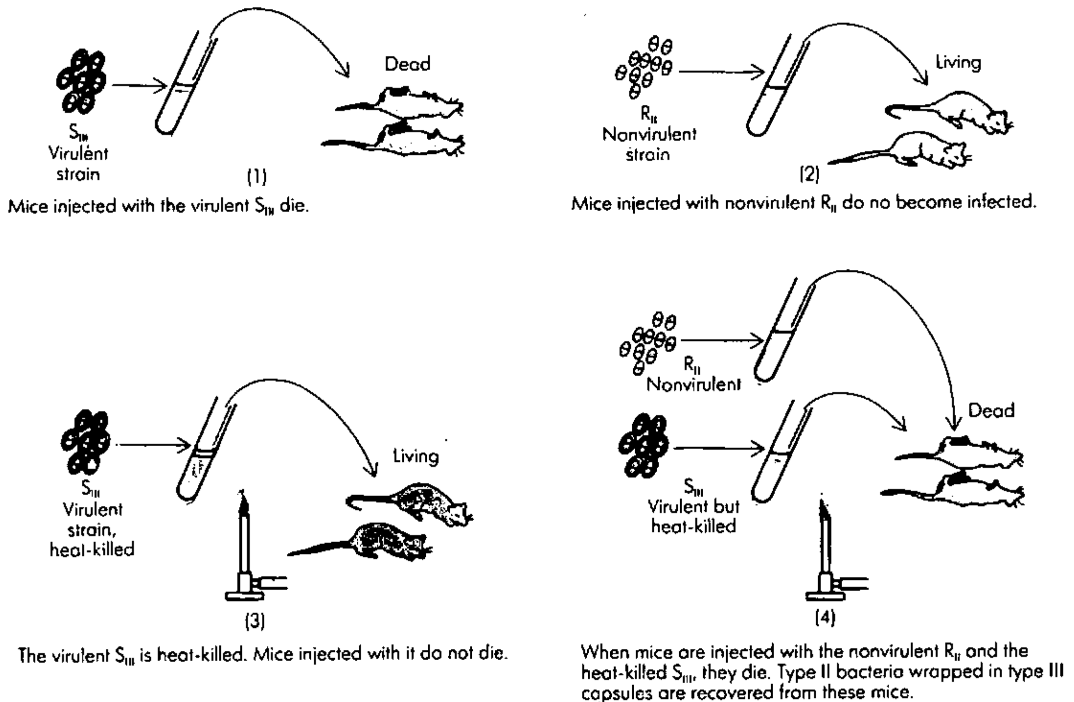
Figure 12-26. The Griffith experiment. *(From the Office of Technology Assessment.)*

There are two types of pneumococcus, each of which can exist in two forms:

where R represents the rough, nonencapsulated, benign form; and

S represents the smooth, encapsulated, virulent form.

The experiment consists of four steps:



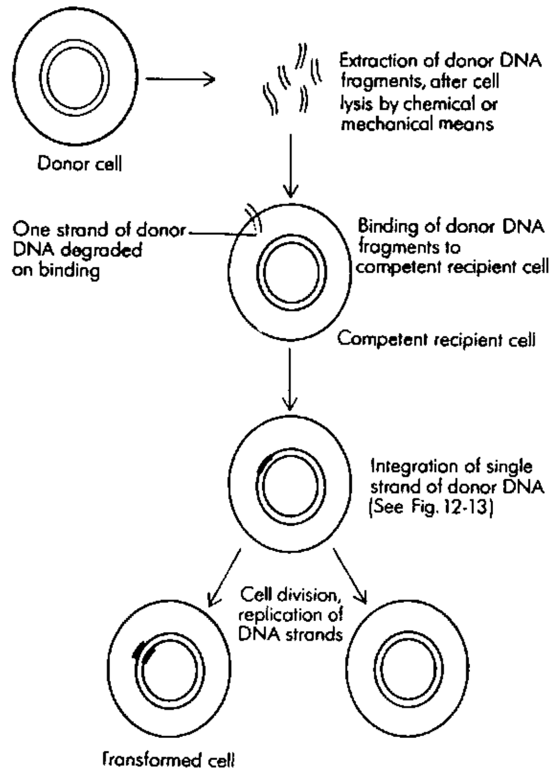


Figure 12-27. Principal steps in bacterial transformation.

recombination occurs. Bacteria that have inherited markers (specific characteristics) from the donor cells are said to be transformed. Thus certain bacteria, when grown in the presence of dead cells, culture filtrates, or cell extracts of a closely related strain, will acquire and subsequently transmit a characteristic(s) of the related strain.

The DNA is taken in through the cell wall and cell membrane of the recipient cell. The molecular size of the DNA affects transformation. Molecular weights of DNA in the range of 300,000 to 8 million daltons have been shown to result in successful transformation. The number of transformed cells increased linearly with increasing concentration of DNA. However, each transformation results from the transfer of a single DNA molecule of double-stranded DNA.

After DNA entry into a cell, one strand is immediately degraded by deoxyribonucleases, while the other strand undergoes base pairing with a homologous portion of the recipient cell chromosome; it then becomes integrated into the recipient DNA (see Fig. 12-27). Since complementary base pairing takes place between one strand of the donor DNA fragment and a specific region of the recipient chromosome, only closely related strains of bacteria can be transformed.

The principal steps of transformation are shown in Fig. 12-27. Bacterial species that have been transformed include, besides *Streptococcus pneumoniae*

(pneumococcus), those in the genera *Bacillus, Haemophilus, Neisseria*, and *Rhizobium*.

Properties of Recipient Cells

Conditions suitable for uptake of donor DNA into recipient cells occur only during the late logarithmic phase of growth. During this period, the transformable bacteria are said to be **competent** to take up and incorporate donor DNA. Competent cultures probably produce an extracellular protein factor that apparently acts by binding or trapping donor DNA fragments at specific sites on the bacterial surface. The uptake process has been found to be an energy-requiring mechanism because it can be inhibited by agents that interfere with energy metabolism.

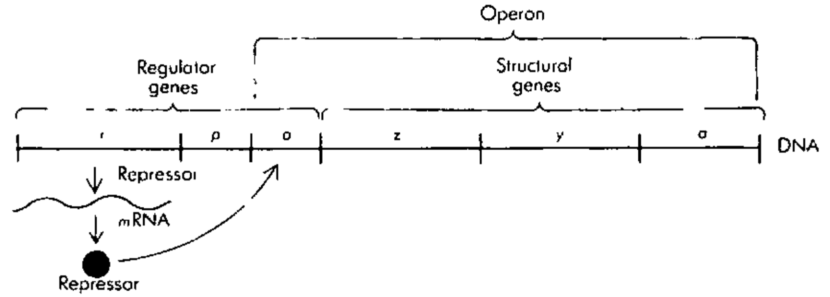
The significance of transformation as a natural mechanism of genetic change is questionable. It probably occurs following the lysis of a microbe and the release of its DNA into the environment. It is conceivable that transformation between bacterial strains of low **virulence** (disease-producing power of a microorganism) can give rise to transformed cells of high virulence. In any case, the phenomenon of transformation has proved to be extremely useful in genetic studies of bacteria in the laboratory, particularly in mapping the bacterial chromosome. This is because when DNA enters a recipient cell during transformation, the entering fragments of DNA are not unlike the DNA fragments transferred in a mating between Hfr and F^- cells of *E. coli*. They will undergo **crossing-over** (exchange of portions of homologous chromosomes) with the homologous DNA segments of the recipient cells, and recombinants will be formed. As in conjugation, the frequency of transformation of two genes at the same time is an indication of the distance between these genes on the chromosome.

THE REGULATION AND EXPRESSION OF GENE ACTIVITY

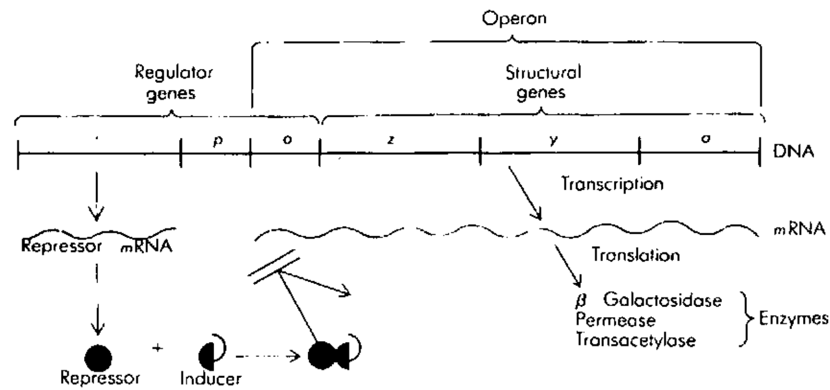
The regulation of gene activity is best controlled at the level of gene transcription. Many examples of such regulation have been discovered in *E. coli* and other bacteria.

Recall that in the bacterial chromosome the genes controlling the enzymes of a metabolic pathway are adjacent to each other. Several adjacent genes code for a single, long *m*RNA molecule that directs the synthesis of several enzymes of a specific metabolic pathway. The consequence of such an arrangement is that the amount of synthesis of gene products is **coordinately** regulated. Therefore, if a cell is stimulated to synthesize a large amount of one of the enzymes of a group, it will also make large amounts of the other enzymes of the same group. This kind of regulation involves the *induction* and *repression* of enzyme synthesis at the gene level and was discussed in Chap. 9. Maintenance of induction requires the continued synthesis of *m*RNA to balance its degradation. Thus this *m*RNA instability, coupled with transcriptional control, assumes that only necessary proteins are synthesized by the cell.

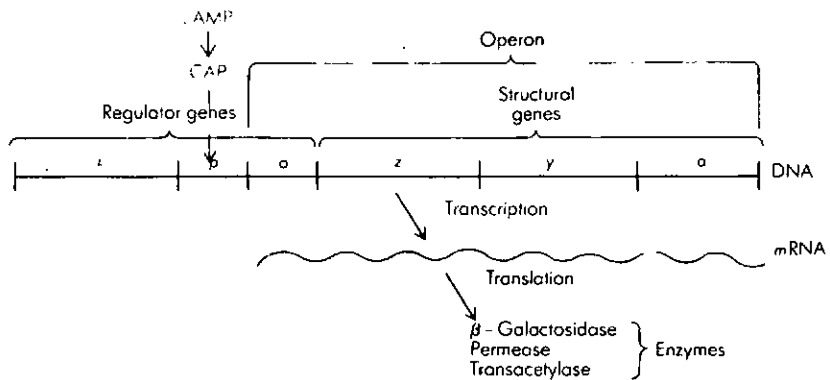
We can better understand the regulation of gene expression in procaryotes by discussing the lactose (*lac*) operon of *E. coli*. This operon is now by far the best understood part of any cellular genome. There are other bacterial operons, but they are less well understood and are different in detail from the *E. coli lac* operon.



A Repression of *m*RNA synthesis



B Induction of *m*RNA synthesis



C Positive control of enzyme synthesis

Figure 12-28. The Jacob-Monod model of gene control for the *lac* operon. (A) Repression of *m*RNA synthesis from *lac* operon. In the absence of inducer, the repressor (the product of the *i* gene) binds to the *o* gene to prevent transcription of the *z*, *y*, and *a* genes. (B) Induction of *m*RNA synthesis from *lac* operon. In the presence of inducer, the repressor binds to the inducer and can no longer combine with the *o* gene. The *lac* operon is no longer repressed, and transcription of the *z*, *y*, and *a* genes takes place. (C) Positive control of enzyme synthesis. Presence of cylic AMP (cAMP) activates the catabolite gene activator protein (CAP), which in turn activates transcription of the *lac* operon.

The *lac* Operon

When inducers such as lactose or other β-galactosides are added to a culture of *E. coli*, there is a 1,000-fold increase in the rate of synthesis of the enzymes β-galactosidase (which hydrolyzes lactose to glucose and galactose), β-galactoside permease (which transports lactose into the cell), and thiogalactoside transacetylase (which plays no role in lactose utilization but may play a role in detoxifying certain thiogalactosides). The genes for these proteins are linked together on the *E. coli* chromosome. These are shown in Fig. 12-28 as *z*, *y*, and *a*, coding for β-galactosidase, galactoside permease, and transacetylase, respectively. In the absence of control, the rate of enzyme production would be constant and depend only on the structural genes (such as *z*, *y*, and *a*), amino acid levels, activating enzymes, and other substances. However, the control of the rate of enzyme synthesis is directed by the regulator genes designated *i*, *p*, and *o*, shown in Fig. 12-28, where *i* is the repressor gene, *p* the promoter gene, and *o* the operator gene. The *i* gene codes for a repressor protein which binds to the DNA of the operator *o* gene, thus preventing transcription, that is, the synthesis of mRNA (see Fig. 12-28). The promoter gene *p* is considered to be the site on the DNA where the RNA polymerase enzyme, catalyzing the synthesis of mRNA, binds, and is thus the site where the specific *lac* mRNA (responsible for the biosynthesis of the specific enzymes of the operon) synthesis begins. Let us discuss the functioning of the Jacob-Monod model of gene control for the *lac* operon as it is now understood.

1 Genes function as templates or blueprints for the transcription of mRNA. Using the protein-synthesizing machinery of the cell (ribosomes), the mRNA directs the synthesis of polypeptides (long chains of amino acids) in a process called translation.
2 The genes *z*, *y*, and *a* operate as a single unit of transcription, which is initiated at *p*.
3 Transcription of the operon is both negatively and positively controlled.

Negative control is mediated by the *lac* repressor which binds to the *o* gene and blocks transcription. Inducers, such as lactose, stimulate *lac* mRNA synthesis by binding to the repressor and reducing its affinity for the operator (see Fig. 12-28). Both repression and induction of enzyme synthesis are negative control systems because, in either case, the synthesis of enzyme can proceed only when the repressor is removed from its blocking site on the *o* gene.

Positive control of enzyme synthesis is said to occur when an association between a protein and a part of the regulatory region of an operon is essential for expression of related structural genes in the operon. Expression of the *lac* operon is inhibited when a more efficient source of energy, such as glucose, is present in the medium. The presence of glucose results in a decreased concentration of intracellular cyclic AMP (adenosine-3′,5′-monophosphate). Cyclic AMP is necessary for efficient expression of the *lac* operon since it activates the catabolite gene activator protein (CAP), which in turn activates transcription of *lac* mRNA by RNA polymerase at the promoter site (see Fig. 12-28).

Thus both cyclic AMP and a specific inducer acting in concert are necessary for the synthesis of many inducible enzymes in *E. coli*. Little enzyme is made if either is absent.

The Jacob-Monod model of the *lac* operon has given biologists an insight into the molecular events of gene regulation. It shows up impressively the precision by which regulatory proteins modulate gene function: the repressors must recognize the specific nucleotide sequences of the operator gene on the one hand, and on the other hand they must recognize specific inducer molecules like lactose. An understanding of such regulatory mechanisms has been extended into the study of bacterial viruses.

Upon entering a bacterial host cell, the DNA genome of phage λ may either proceed to a developmental cycle leading to host-cell lysis or integrate into the chromosome of the host bacterium, making it lysogenic or temperate (see Fig. 12-25). Several phage genes are involved in deciding how fast a critical level of a specific repressor can be produced. When sufficient repressor is available, it blocks transcription of all the other phage genes by combining with two separate operators that control two important operons. In such a circumstance, no λ phage proteins are made, the host cell does not lyse, and the circular λ DNA is capable of being integrated into the host chromosome. If the repressors are destroyed or inactivated at any time during lysogeny of the bacterial cell, then the phage operons become derepressed and start functioning, the λ genome replicates, and the cell lyses. Thus it is seen that the repressor gene controls the fate of both the bacterial cell and the bacteriophage.

The mechanisms of the operon model of gene regulation are applicable only to procaryotic organisms and viruses. The same mechanisms have not been found to occur in eucaryotic cells in which the situation is more complex. For example, animal and human genes are full of "gibberish": segments of DNA that serve as coded genetic instructions are interrupted by other segments that have no function whatsoever. These extraneous pieces of DNA are called **introns** and often make up a larger portion of a gene than the actual code-bearing sequences (called **exons**). Thus the introns must all be spliced out of the genetic message before the cell can use it. It has been suggested that in eucaryotic cells regulation must involve controlling the functioning of the mRNA rather than its synthesis; that is, translation is controlled rather than transcription.

Furthermore, since cells with the same genome function differently in different organs in multicellular eucaryotic organisms, there must be some means for switching on and off whole sets of genes in particular cells. Since the chromosomes of eucaryotic cells do not exhibit any clustering of functionally related genes, any mechanism for controlling transcription must act, directly or indirectly, on many genes distant from each other. That is, there is coordinate control of many genes in different chromosomes.

In addition, it has been found that the mRNA that codes for a polypeptide does not always have the full sequence of nucleotides of the corresponding gene. Such mRNAs have been modified, after transcription, by *splicing* with specific enzymes.

Our knowledge of the mechanism of gene control in eucaryotic organisms is still fragmentary. But progress is slowly being made.

GENETIC ENGINEERING

Genetic engineering refers to the development of organisms with genetic structure altered by biochemical manipulation. This kind of biochemical procedure is termed **recombinant DNA technology** and involves the use of plasmids as

well as certain bacteriophages. In a nutshell, this technology, developed in the early 1970s, consists of isolating, purifying, and identifying genetic material from one source; tailoring it for insertion into a new host; and isolating a colony of cells with the desired new genes. Since much of the results of genetic engineering have been utilized in biotechnology, this subject will be discussed in greater detail in Chap. 29.

QUESTIONS

1. Explain how variability can arise in a bacterial culture.
2. Describe the advantages in using microorganisms for the study of genetics.
3. How do phenotypic changes differ from genotypic changes?
4. Compare the contribution of Max Delbrück and Salvador Luria with that of Esther and Joshua Lederberg in establishing that bacteria have a hereditary system.
5. What are point mutations, and what are their consequences?
6. What are frameshift mutations, and what causes them?
7. Explain the mechanism of photoreactivation.
8. What are transposons?
9. Why would it be desirable to give two antibiotics simultaneously in the treatment of some diseases?
10. Explain the notation used to describe bacterial mutants with respect to phenotypes and genotypes.
11. Describe some practical implications associated with the occurrence of microbial mutants.
12. What are the distinguishing characteristics of each of the three types of gene transfer?
13. How is sexual mating in bacteria like *Escherichia coli* different from that in higher organisms?
14. In what way did the discovery of sexual differentiation in *E. coli* contribute to an understanding of conjugation in bacteria?
15. Explain how interrupted mating experiments are used for determining the location of genes on a bacterial chromosome.
16. Describe the relationship between plasmids and episomes.
17. What are some conditions that facilitate the occurrence of transformation?
18. Differentiate between generalized transduction and specialized transduction.
19. Describe the events that take place during conjugation between an Hfr cell and an F^- cell of *E. coli*.
20. In what manner is the formation of a λ*gal* transducing phage similar to the formation of an F′ factor?
21. Briefly explain how gene activity is controlled at the level of gene transcription.
22. Describe the molecular mechanism which governs the fate of the host cell upon infection with λ phage.

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Hayes, W.: The Genetics of Bacteria and Their Viruses, 2d ed., Wiley, New York, 1968. *An older but classic advanced text on the principles of microbial genetics.*

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Watson, J. D.: Molecular Biology of the Gene, 3d ed., W. A. Benjamin, Inc., New York, 1976. *Classical text on the structure and function of DNA. It is extremely well written with superb illustrations.*

PART FOUR THE WORLD OF BACTERIA

A mirror for the world of bacteria

Prior to 1923, many identification schemes for bacteria had been devised, but these schemes were usually fragmentary. There was need for a single, practical scheme that could cover all the described bacteria. From 1916 to 1918, Robert E. Buchanan was the first to prepare such a comprehensive scheme in a series of papers. In 1917, the Society for American Bacteriologists (now called the American Society for Microbiology) appointed a committee to coordinate all this information, and the final report from this committee in 1920, based largely on Buchanan's work, provided the beginning of a new outline for bacterial classification.

During this period, David H. Bergey began preparing a more complete review of the enormous literature of bacterial taxonomy. To aid the publication of this work, the Society of American Bacteriologists appointed an Editorial Board, chaired by Bergey. This resulted in the publication of the first edition of *Bergey's Manual of Determinative Bacteriology* in 1923.

Every taxonomic scheme for bacteria reflects the knowledge that exists at the time, and since knowledge constantly increases, bacterial taxonomy is subject to continual change. No one recognized this more clearly than Bergey and his colleagues. To cope with these changes, a second edition of the *Manual* was published in 1925, and a third edition in 1930. Five subsequent editions have appeared.

In 1934, the Society of American Bacteriologists transferred to Dr. Bergey all its rights, title, and interests in the *Manual* in order to allow Bergey to create an independent, nonprofit trust—The Bergey's Manual Trust. Throughout the years, this trust continues to prepare and publish successive editions of the *Manual* and promotes research in the field of bacterial taxonomy.

Until 1974, the *Manual* was perceived largely as an "American classification" for bacteria (although it was becoming used in other countries as well), and it was prepared by a relatively small group of microbiologists. It owed its popularity to its breadth of coverage: it was the only book available that attempted to describe all the genera and species of bacteria. But knowledge about the properties of bacteria continued to accumulate at a nearly exponential rate. No longer were just a few people able to cope with the enormous number of bacterial taxa. Thus in 1974 the *Manual* began to become a truly international cooperative effort. Authorities from all over the world were invited to prepare the descriptions of the various genera and species, and the eighth edition of the *Manual* contained contributions from 135 authors.

In 1984, another major change occurred: the scope of the *Manual* was greatly broadened to bring together information dealing with the ecology, enrichment, isolation, preservation, and characteristics of bacteria, all of which concerned bacterial classification and identification. The new breadth of coverage was reflected by a new name—*Bergey's Manual of Systematic Bacteriology*. The new edition of this work is presently being prepared as four volumes, with contributions from hundreds of microbiologists.

As knowledge about bacteria continues to increase, so will *Bergey's Manual* continue to change with it and to act as a "mirror" for the world of bacteria.

Preceding page. *Spirillum volutans* as seen by dark-field microscopy. *(Courtesy of N. R. Krieg.)*

Chapter 13 The World of Bacteria I: "Ordinary" Gram-Negative Bacteria

OUTLINE *Bergey's Manual of Systematic Bacteriology,* **Volume 1**

The Spirochetes

Aerobic/Microaerophilic, Motile, Helical/Vibrioid, Gram-Negative Bacteria

Nonmotile (or Rarely Motile), Gram-Negative Curved Bacteria

Aerobic Gram-Negative Rods and Cocci
The Family *PSEUDOMONADACEAE* • The Family *AZOTOBACTERACEAE* • The Family *RHIZOBIACEAE* • The Family *METHYLOCOCCACEAE* • The Family *ACETOBACTERACEAE* • The Family *LEGIONELLACEAE* • The Family *NEISSERIACEAE* • Other Genera of Aerobic Gram-Negative Rods and Cocci not Assigned to Any Family

Facultatively Anaerobic Gram-Negative Rods
The Family *ENTEROBACTERIACEAE* • The Family *VIBRIONACEAE* • The Family *PASTEURELLACEAE* • Other Genera of Facultatively Anaerobic Gram-Negative Rods not Assigned to Any Family

Anaerobic Gram-Negative Straight, Curved, and Helical Rods
The Family *BACTEROIDACEAE*

Dissimilatory Sulfate- or Sulfur-Reducing Bacteria

Anaerobic Gram-Negative Cocci

The Rickettsias and Chlamydias
The Order *RICKETTSIALES* • The Family *RICKETTSIACEAE* • The Family *BARTONELLACEAE* • The Family *ANAPLASMATACEAE* • The Order *CHLAMYDIALES*

The Mycoplasmas
The Family *MYCOPLASMATACEAE* • The Family *ACHOLEPLASMATACEAE* • The Family *SPIROPLASMATACEAE*

Endosymbionts

The most widely used reference for bacterial classification is *Bergey's Manual of Systematic Bacteriology*, now published in four volumes. Volume 1 includes mainly the familiar or "ordinary" Gram-negative chemoheterotrophic eubac-

teria, many of which have clinical, industrial, or agricultural importance. Most of the organisms have a relatively simple morphology and cellular arrangement. They do not form complex structures such as prosthecae (semirigid extensions of the cell wall and cytoplasmic membrane), sheaths (hollow tubes that enclose chains or trichomes), or, with rare exceptions, endospores (heat-resistant, thick-walled refractive forms) and cysts (thick-walled forms that are desiccation-resistant but not heat-resistant). The cells are not arranged in trichomes. They reproduce mainly by transverse binary fission rather than by budding, fragmentation, or spore formation. Motility, if present, is of the free-swimming type rather than the gliding type. The organisms are mainly heterotrophic, but some can grow autotrophically in the presence of H_2. Some are **saprophytes** (live on dead organic matter), others are **parasites** (living in association with a host). Some are **highly pathogenic** (often cause disease), others may be **opportunistic pathogens** (cause disease only in a patient whose defense mechanisms against infection have been weakened or compromised), and others are not known to cause disease. In this chapter we shall describe briefly some of the organisms included in the first volume of the *Manual*.

BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY, VOLUME 1

Each volume of *Bergey's Manual* is divided into a number of major sections, each bearing a descriptive common name rather than a formal taxonomic name. The major sections of Volume 1 are listed in Table 13-1. Within each section the bacteria are divided into formal taxa at various levels, most attention being given to families, genera, and species. In the remaining pages of this chapter we present a thumbnail sketch of the organisms included in the major sections of Volume 1. The purpose is to acquaint the student with the diversity of organisms that belong to the bacterial world.

THE SPIROCHETES

The distinguishing features of spirochetes are:

1 A helical shape
2 An ability to twist or contort their shape (i.e., flexibility)
3 The occurrence of a special kind of flagella termed *periplasmic flagella* (also called *axial fibrils* or *endoflagella*)

The major difference in structure from other Gram-negative eubacteria is in the location of the periplasmic flagella. As indicated in Fig. 13-1, they are located between the outer membrane (often termed the *outer sheath* in spirochetes) and the *protoplasmic cylinder* (i.e., the protoplast plus the overlying peptidoglycan layer); thus they are are located in the periplasmic space of the cell. The periplasmic flagella have an ultrastructure similar to that of ordinary flagella, including a basal body with disks, and they are responsible for the swimming motility of spirochetes; however, since periplasmic flagella do not extend outward from the cells as do ordinary flagella, the exact mechanism by which they accomplish this motility is not yet clear. Spirochetes swim best in media of high viscosity, whereas bacteria with ordinary flagella swim best in media of low viscosity; spirochetes can also exhibit a creeping or crawling motility when in contact with solid surfaces. Most spirochetes are so thin that they cannot be

easily seen by light microscopy, even when Gram-stained; however, dark-field microscopy does provide sufficient contrast and is the method of choice for visualizing these organisms.

Table 13-1. Gram-Negative Bacteria Included in *Bergey's Manual*, Volume 1

Section	Other Major Characteristics
THE SPIROCHETES	Flexible; helical; have periplasmic flagella; saprophytes or parasites
AEROBIC/MICROAEROPHILIC, MOTILE, HELICAL/VIBRIOID, GRAM-NEGATIVE BACTERIA	Rigid; motile by polar flagella; oxidative type of metabolism; saprophytes or parasites
NONMOTILE (OR RARELY MOTILE), GRAM-NEGATIVE CURVED BACTERIA	Rigid; curved, ring-shaped, or helical cells lacking flagella; saprophytes
AEROBIC GRAM-NEGATIVE RODS AND COCCI	Rigid; straight or slightly curved (but not helical) rods, and cocci; oxidative type of metabolism; saprophytes and parasites
FACULTATIVELY ANAEROBIC GRAM-NEGATIVE RODS	Rigid; straight or curved rods; have both an oxidative and a fermentative type of metabolism; saprophytes and parasites
ANAEROBIC GRAM-NEGATIVE STRAIGHT, CURVED AND HELICAL RODS	Rigid; obtain energy by fermentation or by an anaerobic respiration that does not use sulfur compounds as electron acceptors; parasites
DISSIMILATORY SULFATE- OR SULFUR-REDUCING BACTERIA	Rigid; anaerobic; use sulfur compounds as electron acceptors; saprophytes and parasites
ANAEROBIC GRAM-NEGATIVE COCCI	Rigid; nonmotile; fermentative; parasites
THE RICKETTSIAS AND CHLAMYDIAS	Rigid; tiny cells; intracellular parasites of humans, other animals, and arthropods, can be isolated and cultivated in host cells and sometimes on culture media
THE MYCOPLASMAS	Soft and plastic; nonmotile; lack cell walls; parasites and saprophytes
ENDOSYMBIONTS	Bacteria-like forms that are obligate parasites of protozoa, arthropods, or other hosts; often beneficial to their hosts; have not been isolated or cultivated

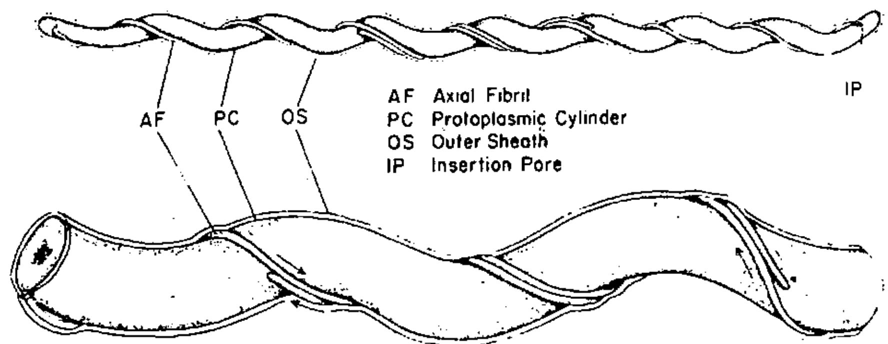


Figure 13-1. Basic anatomical components of spirochetes as interpreted from electron micrographs; surface views. *(Courtesy of S. C. Holt, Microbiol Rev 42:114, 1978.)*

The spirochetes comprise the order *SPIROCHAETALES*. This order is divided into two families, which are distinguished as follows:

THE FAMILY *SPIROCHAETACEAE*

1 They are stringent anaerobes, facultative anaerobes, or microaerophiles.
2 Carbohydrates or amino acids are used as carbon and energy sources.

THE FAMILY *LEPTOSPIRACEAE*

1 They are aerobes.
2 Long-chain fatty acids are used as the carbon and energy source.

Characteristics of the genera of these two families are indicated in Table 13-2.

AEROBIC/MICROAEROPHILIC, MOTILE, HELICAL/VIBRIOID, GRAM-NEGATIVE BACTERIA

These bacteria have the following characteristics:

1 The cells are rigid (unlike spirochetes) and range from vibrioid (having less than one turn or twist) to helical (having one to many turns or twists).
2 They swim by means of polar flagella.

Table 13-2. Characteristics of the Genera of Spirochetes

Family and Genus	Habitat	Oxygen Relationships	Major Characteristics
SPIROCHAETACEAE			
Spirochaeta	Harmless inhabitants of water, mud, and sediments of marine and freshwater environments	Anaerobic and facultatively anaerobic	Use carbohydrates but not amino acids as carbon and energy sources
Cristispira	Harmless parasites of freshwater and marine molluscs	Unknown	Have never been isolated; have unusually large number of periplasmic flagella (>100)
Treponema	Mouth, intestinal tract, and genital areas of humans and animals; some are pathogenic	Anaerobic and microaerophilic	Use carbohydrate and amino acids; some have been cultivated in vitro (on nonliving media) and are stringent anaerobes; these are mainly harmless parasites, but one species, *T. hyodysenteriae*, causes hog dysentery. Some species have not been cultivated in vitro, e.g., *T. pallidum* subsp. *pallidum*, which causes syphilis in humans and is microaerophilic
Borrelia	Parasites of wild rodents and small mammals, and also of the arthropods associated with these animals	Microaerophilic	Pathogenic, causing louseborne or tickborne relapsing fever in humans
LEPTOSPIRACEAE			
Leptospira	Some (*L. biflexa*) are harmless inhabitants of freshwater environments; others (*L. interrogans*) are parasites of wild and domestic animals	Aerobic	*L. interrogans* is pathogenic and causes leptospirosis in animals and humans

3 They are aerobic or microaerophilic.
4 They attack few or no carbohydrates.
5 The organisms usually give a positive reaction by the oxidase test (a laboratory test based on the presence of cytochrome c).

Most of the organisms are harmless saprophytes and occur in freshwater or marine environments, but a few are parasitic and can be pathogenic for humans and animals or for other bacteria. Some examples of genera in this section of *Bergey's Manual* are as follows:

Aquaspirillum

Aquaspirilla are helical or vibrioid organisms that typically possess bipolar tufts of flagella (see Fig. 13-2). These harmless saprophytes are aerobic to microaerophilic and occur in stagnant stream or pond water. No growth occurs in the presence of 3% NaCl or sea water.

Azospirillum

The cells are plump and vibrioid (see Fig. 13-3) with a single polar flagellum and, if grown on solid media, with numerous lateral flagella as well. Azospirilla occur within the roots of grasses, wheat, corn, and many other plants or as free-living soil organisms. They fix N_2 within plant roots or in laboratory cultures. Under N_2-fixing conditions they are microaerophilic, but they are aerobic if supplied with a source of fixed nitrogen such as an ammonium salt. One species, *A. lipoferum*, can grow autotrophically with hydrogen gas as the energy source.

Figure 13-2. *Aquaspirillum bengal* (X9,800). (Courtesy of R. Kumar, A. K. Banerjee, J. H. Bowdre, L. J. McElrov, and N. R. Krieg, *Int J Syst Bacteriol*, **42**:453, 1974.)

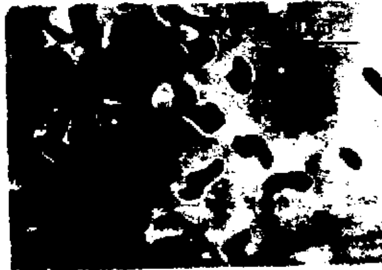


Figure 13-3. Plump vibrioid and straight cells of *Azospirillum brasilense* from a 48-h-old culture grown under nitrogen-fixing conditions. The cells are 1 μm in width. (Courtesy of N. R. Krieg, *Bacteriol Rev*, **40**:55, 1976.)

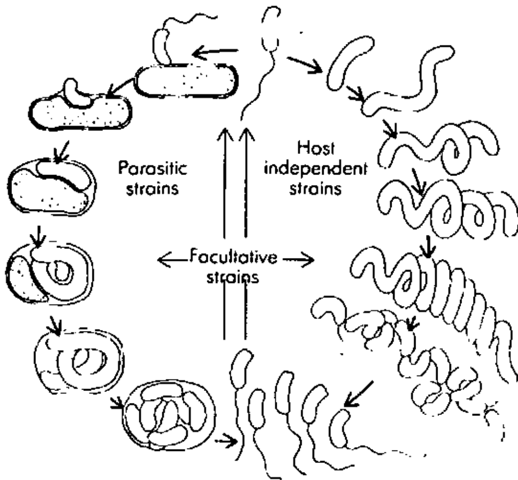


Figure 13-4. Schematic representation of the life cycles of *Bdellovibrio*. All strains isolated from nature require host bacteria for growth (i.e., they are obligately parasitic). In the cycle depicted at the left, a bdellovibrio attaches to a host cell, penetrates the wall, grows within the periplasmic space into a long, coiled form that eventually fragments into new bdellovibrio progeny. Certain mutants can grow only on nonliving culture media (host-independent cycle, shown on the right). Rare strains are apparently facultative parasites and will grow in culture media or in host cells. *(Courtesy of J. C. Burnham, T. Hashimoto, and S. F. Conti, J Bacteriol* **101***:997, 1970.)*

Oceanospirillum

The cells are helical, usually with bipolar tufts of flagella. Oceanospirilla are aerobic and are harmless saprophytes, occurring in coastal marine waters. Sea water is required for their growth.

Campylobacter

These vibrioid cells have a single flagellum at one or both poles (see Fig. 36-4). Campylobacters are microaerophilic parasites, occurring in the reproductive organs, intestinal tract, and oral cavity of humans and other mammals. Some species are pathogenic, e.g., *C. jejuni*, which causes diarrhea in humans, or *C. fetus* subspecies *venerealis*, which causes abortion in cattle.

Bdellovibrio

These aerobic, vibrioid cells possess a single polar flagellum. Bdellovibrios have the unique property of being parasitic on other Gram-negative bacteria. After attachment to a host bacterium, the bdellovibrio penetrates the outer membrane of the cell wall and grows within the periplasmic space. Eventually the host bacterium becomes an empty "ghost" cell. This life cycle is illustrated in Fig. 13-4. Bdellovibrios occur in soil, sewage, and in freshwater and marine environments. The genus *Vampirovibrio* has certain similarities to the genus *Bdellovibrio*, but the organisms attack eucaryotic algae, not bacteria.

NONMOTILE (OR RARELY MOTILE), GRAM-NEGATIVE, CURVED BACTERIA

The characteristics of the bacteria in this section are as follows:

1 Rigid cells that are curved to various degrees, forming coils, helical spirals, and sometimes rings (i.e., cells that are curved around so that the ends overlap (Fig. 13-5)
2 Nonmotile

These harmless saprophytes occur mainly in soil, freshwater, and marine environments. One example is the family *Spirosomaceae*, which contains three genera whose cells, which are aerobic, form no intracellular gas vacuoles, are catalase- and oxidase-positive, and form colonies that are yellow (the genus

Spirosoma) or pink (the genera *Runella* and *Flectobacillus*). Another common genus, not included in the family *Spirosomaceae*, is *Microcyclus*, which forms prominent intracellular gas vacuoles and whose colonies have no pigment.

AEROBIC, GRAM-NEGATIVE RODS AND COCCI

This section forms one of the largest and most diverse groups of bacteria. Two general features are as follows:

1 The cells are mainly straight or slightly curved (but not helical) rods, but some are cocci.
2 They have a strictly respiratory type of metabolism.

Several families and some additional genera that are not assigned to any family are represented.

The Family *PSEUDOMONADACEAE*

The features of this family include the following:

1 Straight or slightly curved rods
2 Motile by polar flagella (Fig. 13-6)
3 Catalase-positive and usually oxidase-positive

Some of the genera included in this family are described here.

Pseudomonas

These bacteria are widely distributed in soil and water. The genus contains five genetically distinct groups (each of which might be considered as a separate genus) as well as a large number of additional, less well studied organisms. All pseudomonads can grow aerobically, but some can also grow anaerobically by using nitrate as an electron acceptor. Several species are pathogenic for humans or animals; others are important plant pathogens. Some cause spoilage of meats and other foods. Species identification is based on many physiological and nutritional characteristics, such as the ability to use certain compounds as carbon sources for growth. Some species can use any of over 100 compounds as a carbon source. Sugar-containing media are acidified only weakly, and acid

Figure 13-6. (*Above*) Flagella stain (X2,000) of cells of a *Pseudomonas* strain showing the characteristic polar flagella. (*Courtesy of General Biological Supply House, Inc.*)

Figure 13-5. (*Below*) Phase-contrast photomicrograph of *Spirosoma* cells showing rings and coils. The bar indicates 10 μm. (*Courtesy of J. M. Larkin, P. M. Williams, and R. Taylor, In J Syst Bacteriol 27:147, 1977.*)

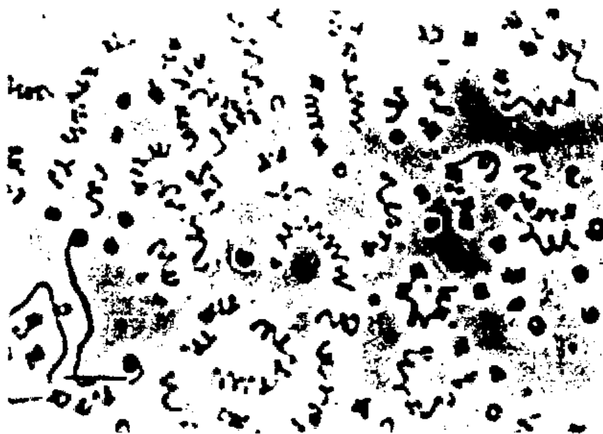


Figure 13-7. Cells of *Zoogloea ramigera* embedded in a matrix of exopolymer. The fingerlike projections of the slimy mass are characteristic of the genus. The bar indicates 50 μm. (*Courtesy of R. Unz, from N. Palleroni in The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, Springer-Verlag, 1981.*)

production from sugars is generally not useful in differentiating species (in contrast, for example, to the family *Enterobacteriaceae*). A few examples of *Pseudomonas* species are listed below:

P. aeruginosa produces a water-soluble blue pigment, pyocyanin, and a water-soluble fluorescent pigment, pyoverdin. The organism is mainly a soil and water saprophyte, but it is also frequently an opportunistic pathogen and can often be isolated from wound, burn, and urinary tract infections.

P. maltophilia is a nonfluorescent species that is also frequently isolated from clinical specimens.

P. fluorescens is a common saprophytic soil and water organism that makes only a fluorescent pigment.

P. syringae and several other species are important plant pathogens, causing diseases such as leaf spot, leaf stripe, wilt, and necrosis.

P. mallei is the causative agent of glanders and farcy, diseases of horses and donkeys that are transmissible to humans. *P. pseudomallei* causes melioidosis in animals and humans.

Xanthomonas

This genus forms characteristic yellow pigments called xanthomonadins. All species are pathogenic for plants, causing diseases such as spots, streaks, cankers, wilts, and rots. Xanthomonads make viscous exocellular polysaccharides (xanthan gums) which are useful for industrial applications such as stabilizers in foods and antidrip agents in paints.

Zoogloea

The outstanding characteristic of this genus is the embedment of the cells in a gelatinous matrix to form slimy masses with a fingerlike morphology (Fig. 13-7). These saprophytic organisms are commonly found coating the rocks on trickling-filter beds in sewage treatment plants, where they oxidize the soluble organic components of the sewage.

The Family *AZOTOBACTERACEAE*

This family has the following characteristic features:

1 The cells are large blunt rods, oval cells, or cocci.
2 Their motility and flagellar arrangement vary; some are nonmotile.
3 They are saprophytes that occur in soil, water, and sometimes the plant rhizosphere (soil region subjected to the influence of plant roots).
4 The organisms fix N_2 under aerobic conditions.

The unusually high respiratory rate of the cells serves to use up oxygen rapidly at the cell surface and maintain an anaerobic cell interior; this protects the oxygen-sensitive nitrogenase enzyme complex (responsible for N_2 fixation) from being inactivated under an air atmosphere.

One genus, *Azotobacter*, forms desiccation-resistant cysts (see Figs. 5-32 and 5-33).

The Family ***RHIZOBIACEAE***

This family contains rod-shaped cells that incite hypertrophies on plants (root nodules, leaf nodules, or tumors). Three genera of this family are described below.

Rhizobium and Bradyrhizobium

These bacteria fix N_2 by means of a complex, highly evolved symbiosis with the roots of leguminous plants. The bacteria attach to the root hairs, penetrate the root, and induce proliferation of the root cells (see Figs. 25-11 and 25-12). Within the resulting root nodules the bacteria exist as highly pleomorphic N_2-fixing forms called *bacteroids*. *Leghemoglobin* occurs within the root nodules and serves to protect the nitrogenase enzyme complex from being destroyed by excess oxygen. The species and strains of rhizobia and bradyrhizobia exhibit a range of specificities for various legumes.

Agrobacterium

Agrobacteria do not fix N_2. The organisms are plant pathogens that incite tumors when they invade the crown, roots, and stems of a great variety of dicotyledonous and some gymnospermous plants (see Fig. 36-20). Tumor induction is associated with the presence in the bacteria of a particular plasmid (see Chap. 36 for further details).

The Family ***METHYLOCOCCACEAE***

This family consists of a diverse group of rods, vibrios, and cocci having in common the ability to use methane gas as a sole carbon and energy source under aerobic or microaerophilic conditions. These harmless organisms occur in soil, mud, or water adjacent to or overlying the anaerobic environments where methane is formed. Some members of the family fix nitrogen under microaerophilic conditions. Some form *Azotobacter*-like cysts. The genera presently included—*Methylococcus* and *Methylomonas*—are all *obligate* methane-oxidizers (i.e., carbon sources such as glucose cannot be used for growth); however, the definition of the family permits inclusion of facultative methane-oxidizers as well.

The Family ***ACETOBACTERACEAE***

This family contains ellipsoidal to rod-shaped cells that oxidize ethanol to acetic acid in neutral or acidic (pH 4.5) media. Two genera are included, *Acetobacter* and *Gluconobacter*, which are differentiated by certain biochemical characteristics and by the occurrence of peritrichous flagella (*Acetobacter*) or polar flagella (*Gluconobacter*).

Members of these two genera are saprophytes that occur in sugar- or alcohol-enriched, acidic environments such as flowers, fruits, beer, wine, cider, vinegar, souring fruit juices, bees, and honey. Some have industrial importance: acetobacters are used to make vinegar, and gluconobacters are involved in the manufacture of chemicals such as dihydroxyacetone, sorbose, and 5-ketogluconic acid (see Table 29-1). Some strains of *Acetobacter* have the highly unusual ability (for bacteria) to make exocellular cellulose fibrils that accumulate around the cells.

The Family ***LEGIONELLACEAE***

These rod-shaped bacteria require L-cysteine, iron salts, and activated powdered charcoal for growth (the charcoal destroys toxic hydrogen peroxide in the medium). All belong to a single genus, *Legionella*. The organisms are motile by polar or lateral flagella. They occur in surface water, thermally polluted lakes and streams, water from air-conditioning cooling towers and evaporative con-

densers, and in moist soil adjacent to a body of water. All species are opportunistic pathogens of humans, causing legionellosis.

The Family *NEISSERIACEAE*

This family contains nonmotile rods and cocci that are catalase-positive and/or oxidase-positive. Examples are the genera *Neisseria* and *Acinetobacter*.

Neisseria

This genus has traditionally consisted of oxidase- and catalase-positive cocci that occur most often in pairs with the adjacent sides flattened (Fig. 13-8). However, one rod-shaped species (*N. elongata*) is now included because of its genetic relatedness. The neisseriae are parasites that inhabit the mucous membranes of humans and animals. Two species are highly pathogenic for humans, e.g., *N. gonorrhoeae*, the causative agent of gonorrhea, and *N. meningitidis*, the causative agent of epidemic cerebrospinal meningitis.

Acinetobacter

These diplobacilli are catalase-positive but oxidase-negative. They are saprophytes that occur in soil, water, and sewage, but they are also opportunistic human pathogens that can cause a variety of infections, particularly in hospitalized patients.

Other Genera of Aerobic Gram-Negative Rods and Cocci Not Assigned to Any Family

Several genera are not assigned to any family, yet are included in this general section of *Bergey's Manual*. Some of these are described in Table 13-3.

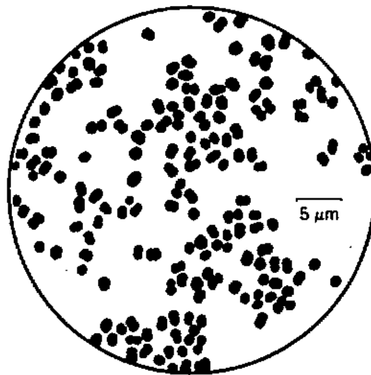


Figure 13-8. Drawing of *Neisseria gonorrhoeae*, showing the characteristic diplococcus arrangement of the cells. (*Erwin F. Lessel, illustrator.*)

FACULTATIVELY ANAEROBIC GRAM-NEGATIVE RODS

The organisms in this section form a very diverse group of straight or curved rods that can grow aerobically by respiring with oxygen and also under anaerobic conditions by fermenting various carbohydrates. Most genera are associated with animals or plants, but some occur in soil and water. The following are examples of some of the organisms included in this section.

The Family *ENTEROBACTERIACEAE*

Some distinctive features of this family are:

1 Cell diameter is 0.3 to 1.5 μm.
2 Cell shape is that of a straight rod.

Table 13-3. Some Genera of Aerobic Gram-Negative Rods and Cocci Not Affiliated with Any Family

Genus	Shape	Flagella	Major Characteristics
Beijerinckia and ***Derxia***	Rods	Peritrichous and polar, respectively	Tropical soil bacteria that can fix N_2 aerobically; *Derxia* can grow autotrophically with H_2 as the energy source
Xanthobacter	Rods	Usually none	Soil bacteria that fix N_2 under microaerophilic conditions; can grow autotrophically with H_2 as the energy source; form yellow colonies; cells stain Gram-positive or Gram-variable
Thermus and ***Thermomicrobium***	Rods	None	Occur in hot springs; obligate thermophiles; optimum temperature is 70–75°C
Alteromonas	Straight or curved rods	Polar	Harmless marine organisms; require sea water for growth; oxidase-positive
Flavobacterium	Rods	None	Mainly saprophytes, widely distributed in nature; may often occur in hospital environments; form yellow to orange colonies; oxidase-positive; one species, *F. meningosepticum*, can cause a severe meningitis in newborn infants
Alcaligenes	Very short rods	Peritrichous	Occur in soil, freshwater, and marine environments but can be opportunistic human pathogens. Form non-pigmented colonies. Oxidase-positive. Some can grow autotrophically with H_2 as the energy source.
Brucella	Very short rods	None	Parasites and pathogens of animals. Three species are highly pathogenic for animals and humans, causing brucellosis.
Bordetella	Very short rods	None	Parasitic and pathogenic for various mammalian hosts. *B. pertussis* occurs only in humans and causes whooping cough.
Francisella	Very short rods	None	The major species, *F. tularensis*, is a parasite of wild animals but can also cause tularemia in humans.
Lampropedia	Cocci, nearly cubical	None	Harmless saprophytes occurring in aquatic environments. Occur in distinctive, flat, square tablets of 16–64 cells (see Fig. 13-9).

3 Motility, if present, is by means of lateral flagella.
4 They are oxidase-negative.
5 Na^+ is not required or stimulatory for growth.
6 Cells contain a characteristic antigen, called the enterobacterial common antigen.
7 The organisms have simple nutritional requirements.

The family contains a large number of genera that are biochemically and genetically related to one another. Many of the more traditional or familiar bacteria are to be found in this family. Differentiation of the various genera is based on characteristic patterns obtained from a large number of biochemical tests; a few properties of some of the genera are listed in Table 13-4. Because

Figure 13-9. Negatively stained preparation of *Lampropedia hyalina* showing a sheet of actively growing tablets of cells. The bar indicates 5 μm. *(Courtesy of R. G. E. Murray, from Bergey's Manual of Determinative Bacteriology, 8th ed., Williams & Wilkins, Baltimore, 1974.)*

Table 13-4. Typical Characteristics of Some *Enterobacteriaceae*

Property	*Escherichia*	*Shigella*	*Salmonella*	*Enterobacter*	*Serratia*	*Proteus*	*Yersinia*
Motility	d	–	+	+	+	+	–
Voges-Proskauer test	–	–	–	+	d	d	–
Indole from tryptophan	d	d	–	–	–	d	d
Hydrogen sulfide produced	–	–	+	–	–	d	–
Lysine decarboxylase	+	–	+	d	d	–	–
Gas from glucose	+	–	+	+	d	+	–
Acid from lactose	d	–	–	+	d	–	d
Urease	–	–	–	d	–	+	d
Phenylalanine deaminase	–	–	–	–	–	+	–
Deoxyribonuclease	–	–	–	–	+	–	–

+ = most or all species positive; – = most or all species negative; d = different reactions occur among species.

sugar-containing media are strongly acidified, acidic reactions from various sugars are used extensively to differentiate the genera and species (in contrast, for example, to the genus *Pseudomonas*). Some selected genera are listed below.

Escherichia

(See Fig. 13-10.) The major species, *E. coli*, occurs in the lower portion of the intestine of humans and warm-blooded animals, where it is part of the normal flora. Some strains can cause gastroenteritis; others can cause urinary tract infections.

Shigella

This genus is very closely related to *Escherichia* but differs in a few characteristics (Table 13-4). Moreover, all strains are pathogenic, causing bacillary dysentery in humans.

Salmonella

(See Fig. 13-10.) This is a group of organisms that are closely related to one another and probably should be considered as a single species. All strains are pathogenic for humans, causing enteric fevers (such as typhoid and paratyphoid fevers), gastroenteritis, and septicemia; many strains also infect a variety of animals. Over 2,000 antigenic types of salmonellae occur.

Enterobacter

Unlike most other *Enterobacteriaceae*, *Enterobacter* species grow best at 30°C rather than at 37°C. They occur mainly in water, sewage, soil, meat, plants, and

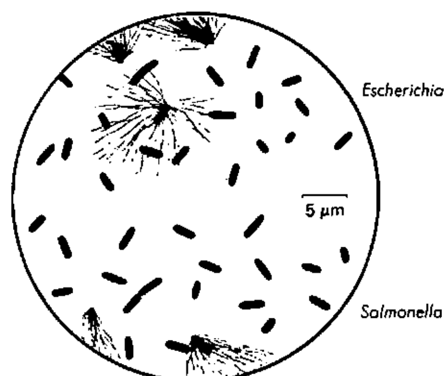


Figure 13-10. Drawing of cells of the genera *Escherichia* and *Salmonella*. The peritrichous flagella are not visible by ordinary staining. *(Erwin F. Lessel, illustrator.)*

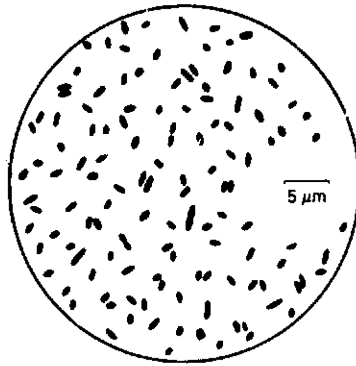


Figure 13-11. Drawing of cells of *Yersinia pestis*, the causative agent of plague. (*Erwin F. Lessel, illustrator.*)

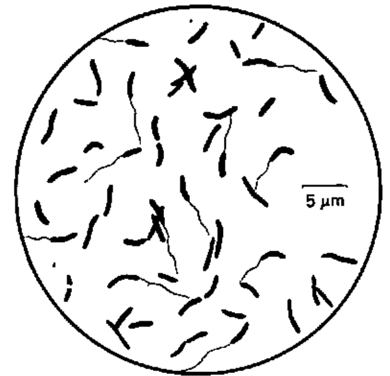


Figure 13-12. Drawing of cells of the genus *Vibrio*. The polar flagella are not visible by ordinary staining. (*Erwin F. Lessel, illustrator.*)

vegetables. Some species also occur in human and animal feces, and some can be opportunistic human pathogens.

Erwinia

These bacteria differ from most other *Enterobacteriaceae* by being mainly associated with plants. They are often plant pathogens, causing diseases such as blights, cankers, die back, leaf spot, wilts, discoloration of plant tissues, and soft rots. They are seldom isolated from animals or humans.

Serratia

The serratiae are widely distributed in soil, in water, and on plant surfaces. Many strains produce pink or red colonies. These organisms were once thought to be harmless; however, it is now clear that they can be opportunistic human pathogens and are particularly prone to infect hospitalized patients.

Proteus

These organisms can *swarm* on agar media; that is, they spread over the plates in a thin film resulting from periodic cycles of migration. Such swarming often makes it difficult to obtain pure cultures of other bacteria from streak plates. *Proteus* strains occur in the intestine of humans and a wide variety of animals, in polluted waters, and in soil, and they can be opportunistic human pathogens. Like *E. coli*, *P. mirabilis* is one of the leading causes of urinary tract infections in humans.

Yersinia

Yersiniae are parasites of animals but can also cause infections in humans. For example, *Y. pestis* (see Fig. 13-11) is the causative agent of plague, and *Y enterocolitica* is a frequent cause of gastroenteritis in children.

The Family *VIBRIONACEAE*

Some distinctive features of this family are:

1 Cell diameter is 0.3 to 1.3 μm.
2 Cell shape is that of curved or straight rods (Fig. 13-12).
3 The cells are motile by means of polar flagella.
4 They are usually oxidase-positive.

5 Na^+ is required or stimulatory for growth of some genera.
6 Cells do not contain the enterobacterial common antigen.
7 The organisms have simple nutritional requirements.

The *Vibrionaceae* occur in marine and freshwater environments or in association with animals living in those environments. Two genera are described below:

Vibrio

These are distinguished from other members of the family by having flagella that are covered by a membrane (sheathed flagella). The organisms occur in aquatic habitats with a wide range of salinities. Some species can emit light of a blue-green color *(bioluminescence)*, an oxygen-dependent reaction catalyzed by the enzyme luciferase. One such species, *V. fischeri*, occurs in the specialized luminous organs of certain deep-sea fishes. Most *Vibrio* species are harmless saprophytes, but some species are pathogenic; examples are *V. cholerae*, the causative agent of cholera in humans, *V. parahaemolyticus*, which causes gastroenteritis in humans, and *V. anguillarum*, which is a pathogen of marine fish and eels.

Aeromonas

Cells are straight rods that have nonsheathed flagella. The organisms occur in fresh water sources and sewage. Some species are pathogenic for frogs and fish; e.g., *A. salmonicida* is the causative agent of furunculosis in salmon and trout.

The Family *PASTEURELLACEAE*

Distinctive features of this family are:

1 The cell diameter is small (0.2 to 0.4 μm).
2 Cell shape is that of a straight rod.
3 The cells are nonmotile.
4 They are usually oxidase-positive.
5 Na^+ is not required or stimulatory for growth.
6 Cells do not contain the enterobacterial common antigen.
7 The organisms often have complex nutritional requirements.
8 The family occurs as parasites of vertebrates.

Some genera included in the family are described below:

Pasteurella

These organisms are parasitic on the mucous membranes of the upper respiratory tract of mammals (rarely humans) and birds. The major pathogen is *P. multocida*, which causes hemorrhagic septicemia in cattle and fowl cholera in domestic and wild birds.

Haemophilus

These bacteria are distinguished by unusual nutritional requirements: the *X factor* (heme, occurring in blood) and/or the *V factor* (the coenzyme nicotinamide adenine dinucleotide). *Haemophilus* species occur as parasites of the mucous membranes of humans and animals. Some are pathogenic for humans: for example, *H. influenzae* (see Fig. 13-13) is a leading cause of meningitis in children.

Actinobacillus

These bacteria are also parasitic on mammals and birds. The organisms are only occasionally pathogenic for humans, but several species are pathogenic for

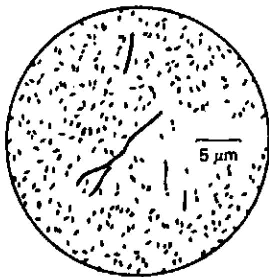


Figure 13-13. Drawing of cells of *Haemophilus influenzae*. (Erwin F. Lessel, illustrator.)

animals, e.g., *A. lignieresii*, which causes granulomatous lesions in cattle and sheep, and *A. suis*, which causes septicemia, pneumonia, and arthritis in pigs.

Other Genera of Facultatively Anaerobic Gram-Negative Rods Not Assigned to Any Family

Several genera of facultatively anaerobic rods are not assigned to any family but belong to this section of *Bergey's Manual*. A few examples are listed below.

Zymomonas

These are saprophytic rods that occur in honey and as spoilage organisms in beer and cider. They are unusual because they form large amounts of ethanol from glucose.

Chromobacterium

These motile, rod-shaped organisms have the unusual property of forming violet colonies, due to a pigment called violacein. The species *C. violaceum* occurs as a saprophyte in soil and water but can occasionally cause infections of humans and other mammals.

Gardnerella

These nonmotile pleomorphic rods stain Gram-negative to Gram-variable. Whether they should be classified with Gram-negative bacteria based on studies of cell-wall ultrastructure and chemical composition is still inconclusive. The only species included in the genus, *G. vaginalis*, occurs in the human genitourinary tract and is a major cause of bacterial "nonspecific" vaginitis.

Streptobacillus

During cultivation of these pleomorphic rods, *L-phase variants* may occur spontaneously; these have a defective cell wall, are more or less spherical in shape, and form tiny "fried-egg" colonies similar to those formed by mycoplasmas (discussed later in this chapter). The single species of the genus, *S. moniliformis*, is a parasite of rats and causes one form of rat-bite fever in humans.

ANAEROBIC, GRAM-NEGATIVE STRAIGHT, CURVED, AND HELICAL RODS

The organisms in this section are placed within a single family, which is described as follows.

The Family *BACTEROIDACEAE*

This family is a diverse assemblage of bacteria that exhibit the following features:

1 They are anaerobic organisms that do not form endospores.
2 They may be motile or nonmotile.
3 They do not respire anaerobically by using inorganic sulfur compounds as electron acceptors; some do respire anaerobically with nitrate or fumarate; most have only a fermentative type of metabolism.
4 Most species produce detectable amounts of organic acids as the result of their fermentative metabolism.

The genera are differentiated mainly on the basis of their morphology and the kinds of organic acid end products they produce, as indicated in Table 13-5. The organic acids are identified by means of a gas chromatograph. The organisms occur in the oral cavity and intestinal tract of humans and animals, in the rumen of cattle, sheep, and other ruminant animals, or in other anaerobic environments. Some genera contain species that are pathogenic for humans: e.g., *Bacteroides fragilis* (Fig. 13-14) is the most common anaerobic bacterium isolated from human soft tissue infections and anaerobic blood infections.

Table 13-5. Characteristics of Some Genera of Anaerobic, Gram-Negative Straight, Curved, and Helical Rods

Genus	Morphology	Major Organic Acid End Products of Fermentation
Bacteroides	Straight rods; nonmotile or motile by peritrichous flagella	Mixtures including succinate, acetate, formate, lactate, and propionate; butyrate is either not formed or, if produced, is accompanied by isobutyrate and isovalerate
Fusobacterium	Straight rods; nonmotile	Butyrate
Succinimonas	Short rods or coccobacilli; motile by a single polar flagellum	Succinate and acetate
Wolinella	Helical, curved, or straight rods; motile by a single polar flagellum	Do not have a fermentative type of metabolism; respire anaerobically with H_2 or formate as electron donors and fumarate or nitrate as electron acceptors; the formate is oxidized to CO_2, and the fumarate is reduced to succinate
Selenomonas	Crescent-shaped cells; motile by a tuft of flagella located at the middle of the concave side	Acetate, propionate, and sometimes lactate
Anaerovibrio	Slightly curved rods; motile by a single polar flagellum	Propionate and acetate

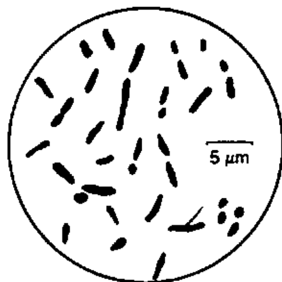


Figure 13-14. Drawing of cells of *Bacteroides fragilis*. (Erwin F. Lessel, illustrator.)

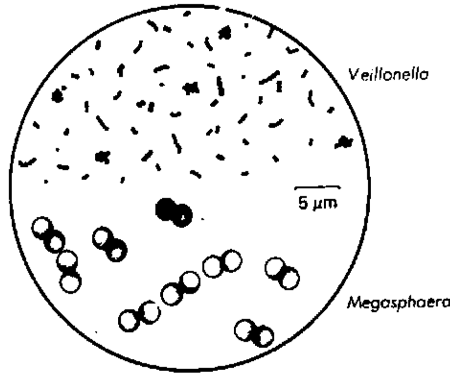


Figure 13-15. Drawing of the cells of two genera of anaerobic cocci, *Veillonella* and *Megasphaera*. (Erwin F. Lessel, illustrator.)

DISSIMILATORY SULFATE- OR SULFUR-REDUCING BACTERIA

The characteristic feature of this diverse group of anaerobic bacteria is that they all respire anaerobically by using inorganic sulfur compounds as electron acceptors with the consequent formation of large amounts of H_2S.

The various genera occur in mud from freshwater, marine, or brackish environments, and in the intestinal tract of humans and animals. One genus (*Desulfuromonas*) can respire with elemental sulfur as the electron acceptor; the other genera cannot use sulfur but can use sulfate, thiosulfate, or other oxidized sulfur compounds. The latter genera are differentiated mainly on the basis of morphology; e.g., *Desulfovibrio*—vibrioid or helical cells, *Desulfococcus* spherical cells, etc.

ANAEROBIC GRAM-NEGATIVE COCCI

The bacteria in this section constitute a single family, *VEILLONELLACEAE*, which consists of nonmotile cocci, typically occurring in pairs, often with the adjacent sides flattened. They are placed into three genera, *Veillonella*, *Acidaminococcus*, and *Megasphaera*, which differ with regard to their size (see Fig. 13-15) and their energy sources and fermentation products. They are inhabitants of the oral cavity, respiratory tract, or intestinal tract of humans, ruminants, rodents, and pigs.

THE RICKETTSIAS AND CHLAMYDIAS

Most of these tiny, nonmotile Gram-negative bacteria are obligate parasites, able to grow only within host cells. Although their size in some instances approaches that of some large viruses (which are also obligate parasites) they are classified as bacteria on the basis of characteristics indicated in Table 13-6.

The Order *RICKETTSIALES*

This order contains the rickettsias, which differ from the chlamydias (order CHLAMYDIALES) by (1) having a more complex metabolism that allows them to synthesize ATP, and (2) lacking a complex developmental cycle. Rickettsias are associated with various arthropods which may serve as major hosts or which can act as vectors for transmission of the rickettsias to vertebrates. In some instances there may be a mutualistic relationship in which the rickettsias provide factors essential for the growth and reproduction of their arthropod host.

Table 13-6. Characteristics of Typical Bacteria, Rickettsias, Chlamydias, and Viruses

Property	Typical Bacteria	Rickettsias	Chlamydias	Viruses
Nucleic acid, DNA or RNA	Both	Both	Both	One or the other
Multiplication by binary fission	Yes	Yes	Yes*	No
Cell walls contain muramic acid	Yes	Yes	No†	No
Ribosomes are present	Yes	Yes	Yes	No
Contain metabolically active enzymes	Yes	Yes	Yes	No
Inhibited by antibacterial drugs	Yes	Yes	Yes	No
Synthesize ATP as a source of energy	Yes	Yes	No	No

* Only the reticulate bodies undergo binary fission.
† However, inhibitors of bacterial cell-wall synthesis, such as penicillin and D-cycloserine, do prevent reorganization of reticulate bodies into elementary bodies, suggesting that some form of peptidoglycan may be present.

Those rickettsias which are capable of multiplying within vertebrates grow within the reticuloendothelial cells, vascular endothelial cells, or erythrocytes of these animals.

The Family *RICKETTSIACEAE*

This family consists of small rod-shaped or coccoid rickettsias which are often pleomorphic. The family is subdivided into three tribes on the following basis:

The tribe *RICKETTSIEAE*	Pathogenic for humans
The tribe *EHRLICHIEAE*	Pathogenic for vertebrate hosts other than humans (usually domestic animals)
The tribe *WOLBACHIEAE*	Not pathogenic for vertebrates; infect arthropods only

The tribe *RICKETTSIEAE* contains three genera, which are described as follows.

Rickettsia

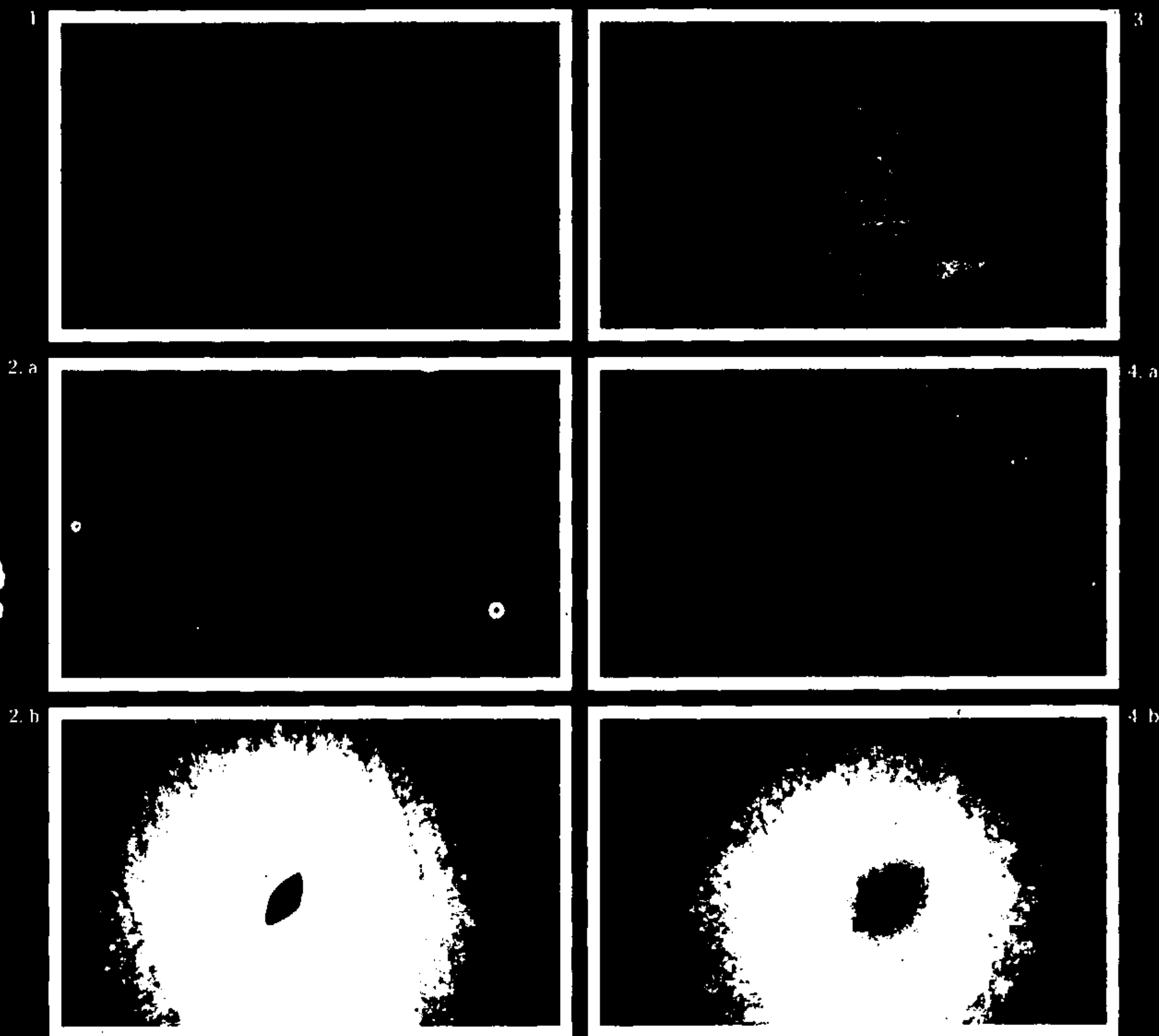
The features of this genus are:

1. Transmission to humans occurs via an arthropod vector (lice, fleas, ticks, or mites, depending on the species).
2. The organisms multiply within the cytoplasm, and sometimes within the nucleus, of host cells.
3. In the laboratory they are cultured in (*a*) host animals, such as guinea pigs or mice (see Fig. 13-16A and B), (*b*) embryonated chicken eggs, particularly within the cells of the yolk sac membrane (Fig. 13-16C and D); (*c*) tissue cell cultures, usually cells from 10-day-old chicken embryos.

Diseases caused by *Rickettsia* species, and the arthropod vectors which transmit them, include: Rocky Mountain spotted fever (ticks), classical typhus fever (lice), murine typhus fever (fleas), rickettsialpox (mites), and scrub typhus (mites). (See also Table 35-5).

Rochalimaea

This genus is similar to *Rickettsia* except for the following features:



Hemolytic reactions of streptococci:

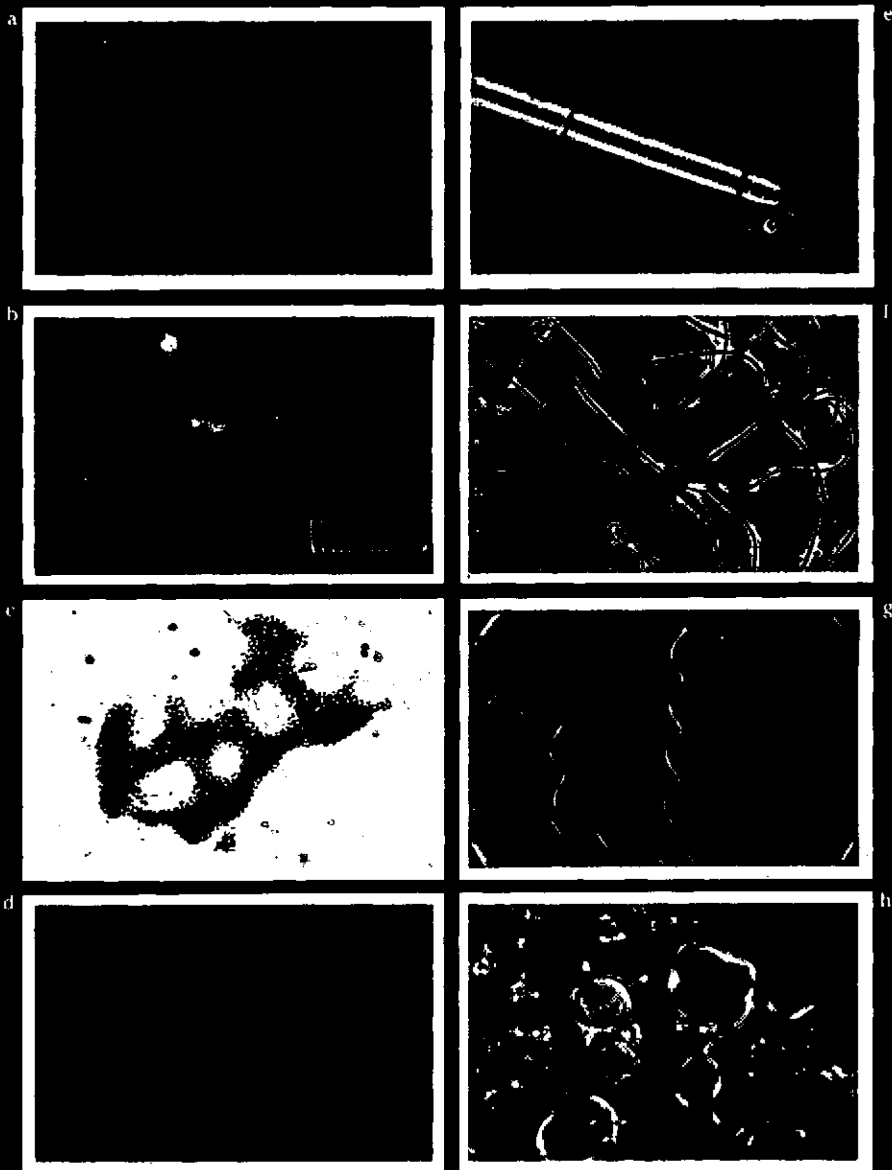
1. Alpha hemolysis (α): an indistinct zone of partial destruction of red blood cells around the colony. This illustration shows a typical colony of an alpha hemolytic streptococcus observed microscopically within a blood agar pour plate.

2. a, b Beta hemolysis (β): a clear, colorless zone around the streptococcus colonies in which the red blood cells have undergone complete dissolution. Figure 2b shows a closer view of subsurface colony.

3. Gamma hemolysis (γ): no apparent hemolytic activity or discoloration produced around the colony.

4. a, b Alpha prime (α') or wide-zone alpha (wzα): a small halo or envelope of intact or partially lysed red blood cells lying adjacent to the bacterial colony with a zone of complete hemolysis extending further out into the medium. When examined macroscopically α' hemolysis can be confused with β hemolysis. (The similarity of hemolysis in slides 2 and 4a is striking and accounts for confusion of α' and β hemolysis. However, the hemolysis revealed by macroscopic observation of the surface and subsurface colonies in slide 4a is not beta but a variation of alpha hemolysis. Figure 4b shows the true nature of the hemolytic activity as revealed by microscopic observation.)

(Courtesy of National Medical Audiovisual Center, Centers for Disease Control, Atlanta, Georgia.)



Cyanobacteria:

a *Merismopedia glauca*
b *Oscillatoria* sp
c *Microcystis aeruginosa*
d *Anabaena planktonica*
e *Lyngbya aestuarii*
f *Gloeotrichia echinulata*
g *Arthrospira jenneri*
h *Gloeocapsa rupestris*

(Courtesy of G. J. Schumacher, Harpur College, SUNY-Binghamton, New York.)

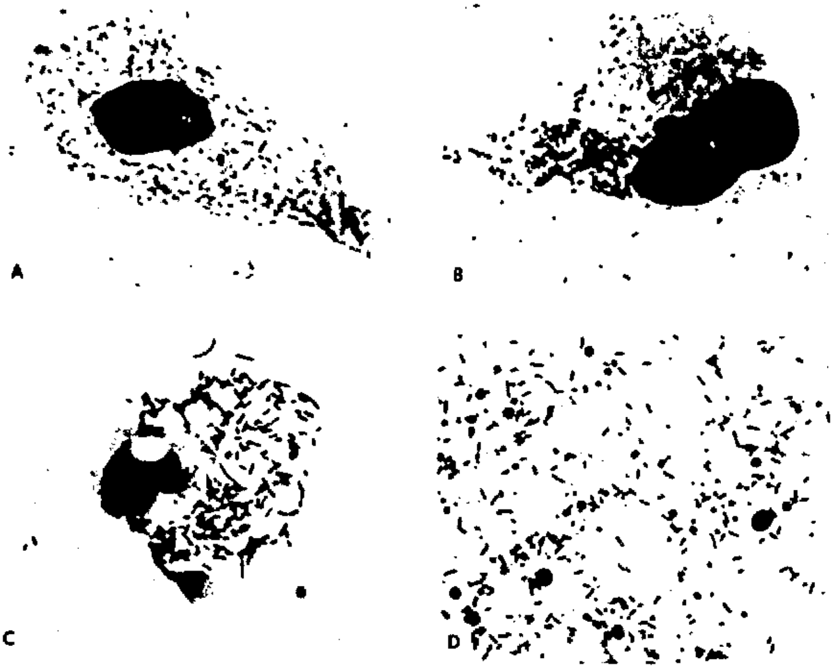


Figure 13-16. Photomicrographs of rickettsias grown under various conditions in the laboratory. (A) *Rickettsia akari* in smear of peritoneal scraping of infected laboratory mouse (X940). (B) *R. tsutsugamushi* in cytoplasm of infected cell (X940). (C) *R. prowazekii* in yolk-sac culture (X1,500). (D) *R. typhi* in yolk-sac culture (X1,000). *(Courtesy of N. J. Kramis and the Rocky Mountain Laboratory, U.S. Public Health Service.)*

1 Although the organisms are mainly parasites of humans and human body lice, they can be cultivated in vitro on laboratory media (e.g., a blood-based agar).
2 They grow *epicellularly* (i.e., on the surface of host cells) rather than in the cytoplasm or nucleus.

The single species of the genus, ***R. quintana***, causes a louseborne disease, trench fever, in humans.

Coxiella

This genus is distinguished by several unusual properties:

1 Growth occurs preferentially within membrane-bounded vacuoles of host cells rather than free in the cytoplasm or nucleus.
2 The organisms have an unusually high resistance to heat [may survive a temperature of 62°C (143°F) for 30 min], probably due to the occurrence of endospore-like structures in the cells.
3 Although transmission to vertebrates can occur via an arthropod vector, it occurs mainly by inhalation of airborne infectious dust. The organisms can also be acquired by drinking contaminated unpasteurized milk.

The single species of the genus, ***C. burnetii***, is the causative agent of Q fever, a type of pneumonia.

The Family *BARTONELLACEAE*

This family consists of parasites of the red blood cells of humans and other vertebrates. The organisms can be cultivated on nonliving laboratory media. The genus ***Bartonella*** causes Oroya fever in humans and is transmitted by biting

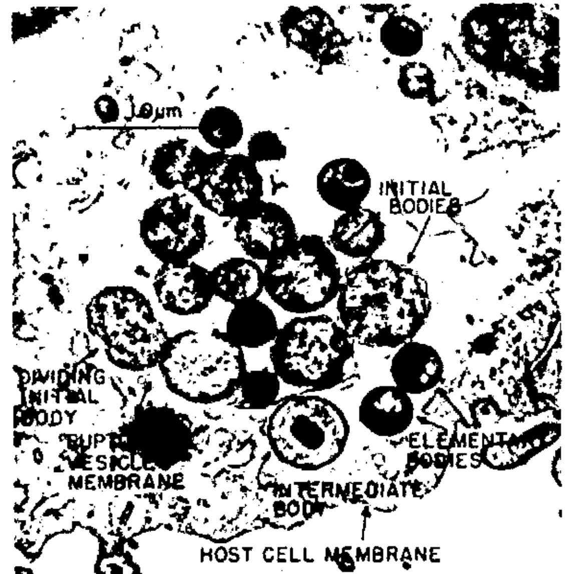


Figure 13-17. Electron micrograph of ultrathin section through a microcolony of *Chlamydia psittaci* in cytoplasm of a McCoy cell after 48-h incubation. The various developmental forms are labeled. The membrane of the vacuole containing the chlamydias has been ruptured, and the chlamydias are being released into the cytoplasm. The multilaminated nature of the wall of the elementary bodies and the double-unit membrane surrounding the reticulate (initial) bodies and the intermediate bodies (intermediate between the elementary and reticulate forms) are visible (X28,700). *(Courtesy of Randall C. Cutlip, Inf and Immun, 1:500, 1970.)*

flies that occur along the western slopes of the Andes mountains in South America.

The Family *ANAPLASMATACEAE*

These organisms grow within or on erythrocytes or occur free in the plasma of various wild and domestic animals. None have been cultivated on nonliving laboratory media.

The Order *CHLAMYDIALES*

This order contains the chlamydias, intracellular parasites that are distinguished from rickettsias by (1) an inability to make ATP (they have an absolute reliance on host cells for this compound and are sometimes termed "energy parasites"), and (2) occurrence of a characteristic developmental cycle. In the laboratory, chlamydias are cultivated in the yolk sac membrane of embryonated chicken eggs or in tissue cultures of mammalian cells, such as McCoy and HeLa cells.

Reproduction of chlamydias usually proceeds according to the following sequence.

1 An infectious small particle, or *elementary body* (see Fig. 13-17), having an electron-dense nucleoid, is taken into the host cell by phagocytosis.
2 The elementary body is enclosed within a membrane-bounded vacuole in the cytoplasm of the host cell.
3 Within the vacuole the elementary body is reorganized into a *reticulate body* (also termed *initial body*), which is two or three times the size of the elementary body and contains a less dense arrangement of nucleoid material (see Fig. 13-17). The reticulate body is not infectious (i.e., when cells are disrupted at this stage of chlamydial development the reticulate bodies that are liberated cannot infect other host cells).
4 The reticulate body undergoes *binary fission* until a number of reticulate bodies are formed, which then undergo reorganization into elementary bodies. This

aggregate of reticulate bodies and elementary bodies within the vacuole of the host cell forms a *host cell inclusion* which can be seen by light microscopy.

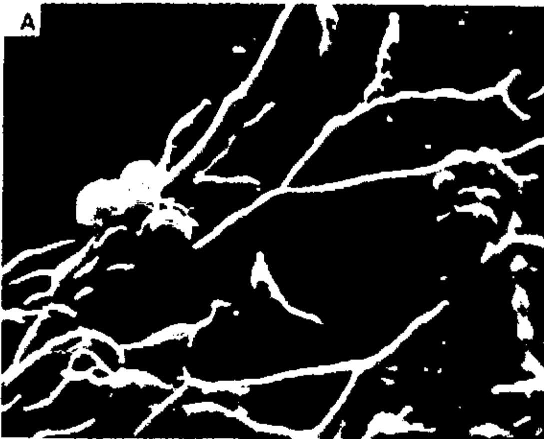
5 The progeny elementary bodies are then liberated from the host cell and proceed to infect other cells.

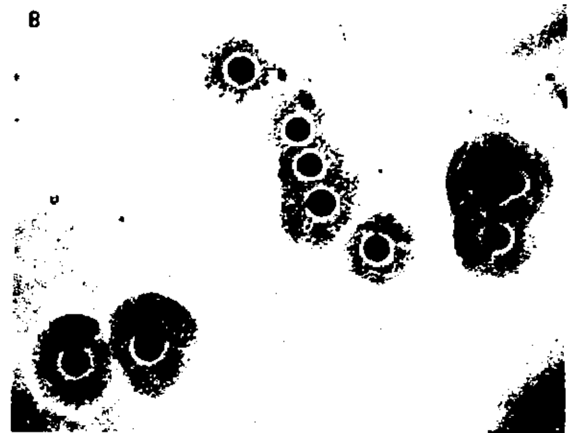
All chlamydias belong to a single family, *CHLAMYDIACEAE*, which contains a single genus, ***Chlamydia***. Some strains of ***C. trachomatis*** cause a type of keratoconjunctivitis, trachoma, that often results in blindness. Other strains cause nongonococcal urethritis, which is the most prevalent sexually transmitted disease in the United States today. Still other strains cause the sexually transmitted disease lymphogranuloma venereum. The species ***C. psittaci*** is mainly a pathogen of birds and domestic and wild mammals but can also cause psittacosis in humans.

THE MYCOPLASMAS

The mycoplasmas are distinguished by their lack of a cell wall, the outer boundary of the cells being the cytoplasmic membrane. As a result, the cells have plasticity and can assume many different shapes ranging from spheres to branched filaments (Fig. 13-18A). The plasticity allows many of the cells to pass through bacteriological filters even though the smallest cells are about 0.3 μm in diameter. They are susceptible to lysis by osmotic shock caused by sudden dilution of the medium with water. Because of the lack of a cell wall, mycoplasmas are not inhibited by even high levels of penicillin; however, they can be inhibited by antibiotics that affect protein synthesis, such as tetracyclines or chloramphenicol. Mycoplasmas can be cultivated in vitro on nonliving media (of rich composition) as facultative anaerobes or obligate anaerobes. They have

Figure 13-18. Mycoplasma cells and colonies. (A) Scanning electron micrograph of *Mycoplasma pneumoniae* from a 6-day culture showing irregular forms, crossing filaments, and piling up of spherical organisms probably representing an early stage of colony formation (X27,600). *(Courtesy of G. Biberfeld and P. Biberfeld, J Bacteriol,* **102**:*855, 1970.)* (B) Colonies of *M. molare* showing the typical "fried-egg" appearance (X35). *(Courtesy of S. Rosendal and Int J Syst Bacteriol* **24**:*125, 1974.)*





genomes that are about one-fifth to one-half the size of those of other bacteria capable of growth on nonliving media, which explains why these organisms have complex nutritional requirements and limited biosynthetic abilities. Colonies on agar plates are usually tiny and require observation by means of a low-power microscope. The colonies are embedded in the agar surface and usually have a characteristic fried-egg appearance (Fig. 13-18B).

Mycoplasmas differ from the "L-phase variants" that can develop from other bacteria. Such variants are osmotically fragile, cell-wall-defective forms that may occur spontaneously (as in the genus *Streptobacillus*) or as the result of continuous exposure to sublethal levels of penicillin. They form fried-egg colonies resembling those of mycoplasmas. However, L-phase variants are derived from walled bacteria and can usually revert to the normal walled bacterial form (e.g., when penicillin is removed), whereas mycoplasmas do not give rise to walled forms. Moreover, penicillin-binding proteins and peptidoglycan precursors can be demonstrated in the membranes of L-phase variants but not in the membranes of mycoplasmas.

At present, mycoplasmas are placed in the taxonomic class ***MOLLICUTES***, which contains the single order ***MYCOPLASMATALES***. Three families are included in this order.

The Family *MYCOPLASMATACEAE*

These mycoplasmas are parasites of the mucous membranes and joints of humans or animals and require cholesterol for growth. Many species of the genus ***Mycoplasma*** are pathogenic for animals; the species ***M. pneumoniae*** has the most significance for humans and is the causative agent of primary atypical pneumonia. Members of the genus ***Ureaplasma*** require urea for growth and cause urethritis in humans, pneumonia in cattle, and urogenital disease in cattle and other animal species.

The Family *ACHOLEPLASMATACEAE*

These mycoplasmas do not require cholesterol for growth. They are widely distributed in vertebrates, in sewage and soil, and possibly on plants. Their pathogenicity is unknown. Only a single genus, ***Acholeplasma***, occurs.

The Family *SPIROPLASMATACEAE*

These organisms are unusual in that they are helical and exhibit a swimming motility. How a helical shape is maintained in the absence of a cell wall and how the cells can swim while lacking flagella is unknown. The family contains a single genus, ***Spiroplasma***. The organisms are pathogenic for citrus and other plants. They can be isolated from plant fluids and plant surfaces and from arthropods that feed on plants.

ENDOSYMBIONTS

A great variety of bacteria-like forms have been observed within the cells of protozoa, insects, fungi, sponges, coelenterates, helminths, and annelids. Most of these bacteria-like forms have not been cultivated in the laboratory, and information about them has generally been restricted to observations made with the light microscope or electron microscope. Most endosymbionts do not harm their hosts; indeed, many appear to be beneficial or even necessary for the growth and development of their hosts. This is suggested, for example, by the inability of certain insects to develop without vitamin supplements after being deprived of their endosymbionts by treatment with antibiotics or other means.

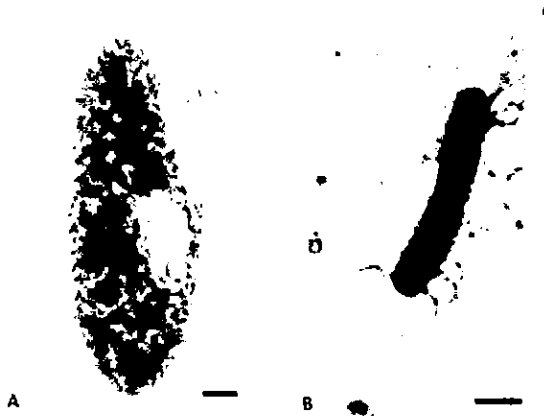


Figure 13-19. (A) Osmium-lacto-orcein preparation of the protozoan *Paramecium tetraurelia* bearing ensymbiont *Lyticum flagellatum*. The numerous black rods throughout the cytoplasm are the endosymbionts. Bar indicates 20 μm. (B) *L. flagellatum* separated from its protozoan host, showing the peritrichous flagella; negatively stained with phosphotungstic acid. Bar indicates 1 μm. *(Courtesy of J. R. Preer, Jr., L. B. Preer, and A. Jurand, Bacteriol Rev* **38**:*113, 1974.)*

The greatest amount of information has been obtained for endosymbionts of protozoa, even to the point of classifying many of them by separating their DNA from that of their host cells and performing base composition and homology experiments with the DNA. Indeed, many of the protozoan endosymbionts now bear formal genus and species names. For example, ***Lyticum flagellatum*** is an endosymbiont carried by certain strains of the protozoan *Paramecium tetraurelia* (see Fig. 13-19). One function of *L. flagellatum* is to synthesize the vitamin folic acid for its host; symbiont-free lines of the same strains of the protozoan need to be supplied with this vitamin. Another function of *L. flagellatum* is to produce a toxin that is liberated into the culture medium: when an endosymbiont-bearing strain of *P. tetraurelia* (called a *killer* strain) is mixed with certain strains lacking it (called *sensitive* strains), the latter protozoa are rapidly killed and lysed. The endosymbiont-bearing strains are resistant to this toxin.

QUESTIONS

1. In what ways do spirochetes differ from other bacteria? What combination of characteristics sets them apart?
2. Which genera of Gram-negative bacteria are associated with plants as nitrogen fixers? As plant pathogens?
3. What general kinds of Gram-negative bacteria (i.e., aerobes, facultative anaerobes, or anaerobes) are associated with the ability to grow autotrophically with H_2 as the energy source? With the ability to use methane gas as a carbon source?
4. List four genera of Gram-negative bacteria that produce distinctive pigments.
5. How are various sugars and other carbon sources used in the laboratory differentiation of *Pseudomonas* species? How does this differ from the way sugars are used to differentiate the genera of *Enterobacteriaceae*?
6. On what bases are the genera of the family *Bacteroidaceae* differentiated?
7. What is an opportunistic pathogen? List four Gram-negative bacteria that are opportunistic pathogens.
8. In what type of environment is one most likely to find (a) *Aquaspirillum*, (b) *Escherichia coli*, (c) *Thermus*, (d) *Erwinia*, (e) *Vibrio fischeri*, (f) *Zymomonas*?

9 List two anaerobic genera whose members obtain energy by respiration rather than by fermentation.

10 What are the major differences between rickettsias and chlamydias? Between mycoplasmas and other bacteria? Between *Mycoplasma* and *Acholeplasma*?

11 How are rickettsias generally transmitted to humans? How does this differ from the way in which Q fever is transmitted?

12 How are rickettsias and chlamydias cultured in the laboratory? In what way can *Rochalimaea quintana* be cultured that differs from the methods used for *Rickettsia* and *Chlamydia*?

13 What agent could you add to a growth medium for mycoplasmas that would make the medium selective for these organisms? Explain the basis for your answer.

14 What functions do endosymbionts serve for their hosts? How might endosymbionts have originated?

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Chapter 14 The World of Bacteria II: "Ordinary" Gram-Positive Bacteria

OUTLINE *Bergey's Manual of Systematic Bacteriology,* **Volume 2**

Gram-Positive Cocci
Aerobic/Facultatively Anaerobic Cocci • Aerotolerant Fermentative Cocci • Anaerobic Gram-Positive Cocci

Endospore-Forming Gram-Positive Bacteria
Aerobic/Facultatively Anaerobic Sporeforming Rods and Cocci • Anaerobic Sporeforming Rods

Nonsporeforming Gram-Positive Rods of Regular Shape

Nonsporeforming Gram-Positive Rods of Irregular Shape
Aerobic/Facultatively Anaerobic Nonfilamentous Rods • Aerobic/Facultatively Anaerobic Branched Filamentous Rods • Anaerobic Nonfilamentous or Filamentous Rods

Mycobacteria

Nocardioforms

Like Volume 1 of *Bergey's Manual*, Volume 2 includes many of the familiar or "ordinary" chemoheterotrophic eubacteria; however, the organisms are Gram-positive rather than Gram-negative. Most have a simple morphology and none form prosthecae, sheaths, or cysts. Some genera form heat-resistant endospores. Although most of the organisms have a regular, uniform shape, some are pleomorphic. Most of the organisms occur singly, in pairs, or in chains, but some are arranged in trichomes and others form branching hyphae. Motility, if present, is by means of flagella. In general, reproduction occurs mainly by transverse binary fission; however, multiplication by fragmentation or by conidiospore production can occur in several genera. Saprophytes and parasites are included; some of the parasitic organisms can be highly pathogenic for humans, animals, or plants. In this chapter we shall describe briefly some of the organisms included in the second volume of *Bergey's Manual*.

BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY, VOLUME 2

As in Chap. 13, the arrangement of the bacteria continues to be based primarily on practical considerations rather than on phylogenetic relationships. The organisms are divided into major sections, each bearing a descriptive common name. The major sections of Volume 2 are listed in Table 14-1. Formal taxonomic names are emphasized mainly at the family, genus, and species level of classification. In this chapter we shall indicate the great diversity of the organisms included in Volume 2 of *Bergey's Manual* by highlighting their important characteristics.

GRAM-POSITIVE COCCI

Aerobic/Facultatively Anaerobic Cocci

All the cocci in this group have the following features

1 They possess cytochromes.
2 They are able to respire with oxygen, i.e., have an oxidative type of metabolism.
3 Some can also obtain energy under anaerobic conditions by fermentation (have a fermentative type of metabolism).

The organisms have been placed in two families. Members of the family *DEINOCOCCACEAE* exhibit the following characteristics:

1 The cocci occur mainly in tetrads or cubical packets.

Table 14-1. Gram-Positive Bacteria Included in *Bergey's Manual*, Volume 2

Section	Other Major Characteristics
GRAM-POSITIVE COCCI	May have a strictly respiratory type of metabolism, a respiratory plus a fermentative metabolism, or a strictly fermentative metabolism; in the latter category they may be able to grow in air (aerotolerant), or they may be anaerobic
ENDOSPORE-FORMING GRAM-POSITIVE BACTERIA	Mainly rod-shaped, but some are cocci; range from aerobic to facultatively anaerobic to anaerobic; most of the anaerobes live by fermentation, but some respire anaerobically with sulfate
NONSPOREFORMING GRAM-POSITIVE RODS OF REGULAR SHAPE	The cells have a uniform appearance without swellings, branching, or other types of variation; some occur in characteristic trichomes; aerobes, facultative anaerobes, or aerotolerant anaerobes are included
NONSPOREFORMING GRAM-POSITIVE RODS OF IRREGULAR SHAPE	The cells may exhibit swellings, Y or V shapes, rod/coccus cycles, or other deviations from a uniform morphology; some are filamentous during at least some stage of their growth; aerobic, facultatively anaerobic, and anaerobic genera are included
MYCOBACTERIA	Aerobic, slightly curved or straight rods which sometimes show branching; stain acid-fast
NOCARDIOFORMS	Aerobic organisms that tend to form a substrate mycelium and sometimes an aerial mycelium; the hyphae fragment into rod-shaped or coccoid elements; conidiospores may develop from the aerial hyphae

2 The organisms have an unusually high resistance to gamma and ultraviolet radiation.

The family contains a single genus, ***Deinococcus***, which forms red colonies. The radiation resistance of the genus is reflected by the name of one of the species, ***D. radiodurans***. The organisms can often be isolated as spoilage agents from foods preserved by treatment with ionizing radiation.

The family ***MICROCOCCACEAE*** exhibits the following features:

1 The cocci occur mainly in clusters, tetrads, or cubical packets of eight cells.
2 The cells do not exhibit any unusual resistance to gamma and ultraviolet radiation.

Three of the genera included in the family are described below.

Micrococcus

These nonmotile cocci are aerobic, oxidative, and are catalase-positive. Their colonies may be red, orange, yellow, or nonpigmented. Micrococci are harmless saprophytes occurring in soil and freshwater, but they can also be found on the skin of humans and animals.

Planococcus

These organisms are also aerobic, oxidative, catalase-positive cocci; however, the cells are motile and possess one to three flagella. The colonies are yellow-brown. Planococci are harmless saprophytes that occur in marine environments.

Staphylococcus

See Fig. 14-1. Staphylococci are nonmotile cocci that are catalase-positive and facultatively anaerobic, having both an oxidative and a fermentative type of metabolism. They are parasites, occurring on the skin and mucous membranes of humans and warm-blooded animals. The major pathogenic species is ***S. aureus***, which can cause boils, abscesses, wound infections, postoperative infections, toxic shock syndrome, and food poisoning in humans, and infections in animals, such as mastitis in cattle. In the laboratory, *S. aureus* produces white to golden-colored colonies and is positive for the *coagulase test* (a test for the ability of bacteria to cause blood plasma to clot). ***S. epidermidis*** and ***S. saprophyticus***, which are coagulase-negative, can cause wound infections, endocarditis, and urinary tract infections.

Aerotolerant Fermentative Cocci

These cocci have the following characteristics:

1 They do not possess cytochromes.
2 They have only a fermentative type of metabolism and do not respire; yet they can grow anaerobically or aerobically.
3 The cells are arranged in pairs, chains, or tetrads.

Some representative genera are described below.

Streptococcus

This genus has the following features:

1 The cells are arranged in pairs or chains (see Fig. 14-1).
2 They are catalase-negative.
3 The organisms are *homofermentative*, i.e., the predominant end product of sugar fermentation is lactic acid. (In the case of Streptococcus, it is the L(+) optical isomer of lactic acid.)

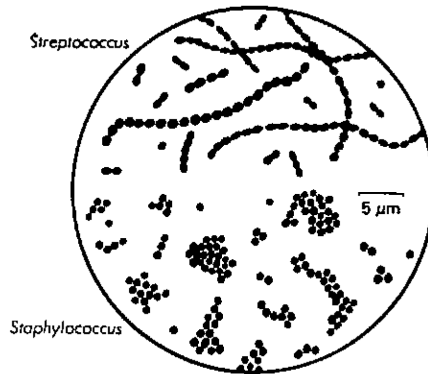

Figure 14-1. Drawing of the cells of *Staphylococcus* and *Streptococcus*. (*Erwin F. Lessel, illustrator.*)

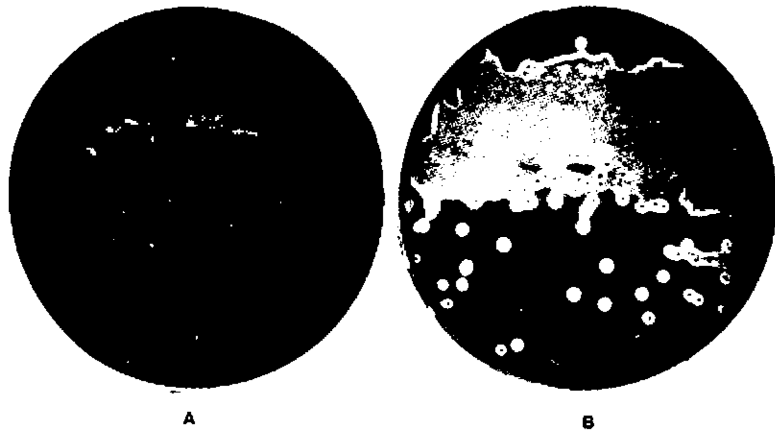

Figure 14-2. (A) α-hemolysis. Enzymes produced by some streptococci, such as *S. pneumoniae*, only partially hemolyze red blood cells of certain species of animals. Colonies on blood agar plates are surrounded by a greenish-colored zone which is due to the reduction of hemoglobin in the red blood cells to methemoglobin. (B) β-hemolysis. Enzymes produced by some streptococci, such as *S. pyogenes*, completely hemolyze red blood cells of certain species of animals. Colonies on blood agar plates are surrounded by a clear, colorless zone. (*Courtesy of Liliane Therrien and E. C. S. Chan, McGill University.*)

Although the genus is usually considered aerotolerant, some strains can tolerate only low levels of oxygen and some are anaerobic. Nutritional requirements are complex, including several amino acids and vitamins. The streptococci are divided into categories known as the *Lancefield groups* based on differences in their cell-wall polysaccharides.

Some streptococci are β-*hemolytic* on blood agar: the colonies are surrounded by a clear, colorless zone that indicates complete lysis of the erythrocytes (Fig. 14-2). Other streptococci are α-*hemolytic* (colonies surrounded by a cloudy, colorless or greenish zone of partially lysed erythrocytes; see Fig. 14-2) or are *nonhemolytic*.

Most streptococci are parasites of humans and animals, and several species are pathogenic. There are many species of streptococci; a few examples follow.

S. pyogenes (β-hemolytic; Lancefield group A) is the most clinically important species. It causes streptococcal sore throat, scarlet fever, erysipelas, acute glomerulonephritis, rheumatic fever, and other human infections.

S. mutans (nonhemolytic; not placed in any Lancefield group) inhabits the human oral cavity and is the major causative agent of dental caries.

S. faecalis (α-, β-, or nonhemolytic; Lancefield group D) occurs normally in

the intestinal tracts of humans and animals and is therefore called an "enterococcus"; it can be an opportunistic pathogen, causing urinary tract infections and endocarditis.

S. lactis and *S. cremoris* (Lancefield group N) are harmless contaminants of milk and dairy products; they cause rapid souring and curdling of milk, and because of this are widely used as "starter cultures" in the manufacture of buttermilk and cheeses.

S. pneumoniae (α-hemolytic; not placed in any Lancefield group) is colloquially called the "pneumococcus" and has great clinical significance, causing nearly 70 percent of all cases of lobar pneumonia in humans.

Leuconostoc

This genus has the following characteristics:

1 The cocci are arranged in pairs and chains.
2 They are catalase-negative.
3 The organisms are *heterofermentative:* they form CO_2 and ethanol or acetic acid in addition to lactic acid; moreover, the lactic acid is of the D(−) type.

Leuconostocs are harmless saprophytes and are isolated from diverse sources such as grass, silage, grape leaves, sauerkraut, and spoiled food. They are often used in "starter cultures" for the manufacture of butter, buttermilk, and cheese because of their formation of the flavor compound diacetyl (2,3-butanedione) from citrate.

Pediococcus

This genus has the following features:

1 Cocci occur in pairs and tetrads (see Fig. 14-3).
2 They are catalase-negative.
3 They exhibit a homolactic type of fermentation, forming optically inactive lactic acid, i.e., a mixture of the L(+) and D(−) types.

Pediococci are saprophytes and are particularly noted for their ability to form capsular material that causes beer to become ropy and viscous.

Anaerobic Gram-Positive Cocci

These cocci have a fermentative type of metabolism. Some genera must be supplied with a fermentable carbohydrate in order to grow; others can ferment amino acids and do not require carbohydrates. Lactic acid, if formed, is not a major fermentation product (unlike the genus *Streptococcus*). Most genera form CO_2, H_2, short-chain fatty acids, and in some cases ethanol or succinic acid.

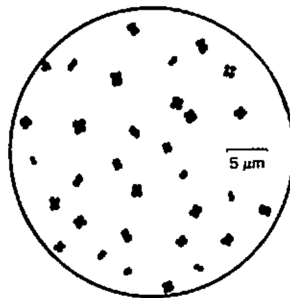

Figure 14-3. Drawing of the cells of *Pediococcus cerevisiae*. (Erwin F. Lessel, illustrator.)

Table 14-2. Characteristics of Anaerobic Gram-Positive Cocci

Genus	Arrangement of Cells	Main Sources of Carbon and Energy	Occurrence
Peptococcus	Pairs, clusters, tetrads, and short or long chains	Peptone or amino acids	Human intestine and respiratory tract; clinical specimens; tidal bay mud
Peptostreptococcus	Pairs and short or long chains	Peptone or amino acids	Human clinical specimens
Ruminococcus	Pairs and short or long chains	Carbohydrates	Bovine and ovine rumen; animal ceca
Coprococcus	Pairs and short or long chains	Carbohydrates	Human feces
Sarcina	Cubical packets of eight cells	Carbohydrates	Soil; mud; cereal grains; diseased human stomachs

Table 14-2 summarizes some of the characteristics for the various genera included in the group, and the morphological features of two of the genera are depicted in Fig. 14-4.

ENDOSPORE-FORMING GRAM-POSITIVE BACTERIA

Most of the endospore-forming bacteria are rod-shaped, but some are cocci. The majority stain Gram-positive but some species stain Gram-negative. Motility, if present, is by means of peritrichous flagella. Some of the genera included in the group are described briefly here.

Aerobic/Facultatively Anaerobic Sporeforming Rods and Cocci

Bacillus

This genus contains rod-shaped bacteria. Most species are harmless saprophytes occurring in soil, freshwater, or sea water. Many form exocellular enzymes that hydrolyze proteins or complex polysaccharides, activities that are often important causes of food spoilage. Moreover, because of the heat resistance of the endospores, *Bacillus* species may survive milk pasteurization or inadequate heat treatment during canning of foods. A few examples of the many species are listed below.

B. subtilis and ***B. cereus.*** (See Fig. 5-29.) These common mesophilic sapro-

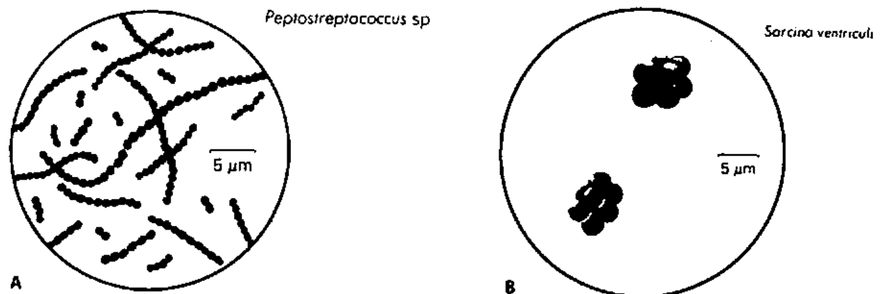

Figure 14-4. Anaerobic Gram-positive cocci. (A) Drawing of the cells of *Peptostreptococcus*. (B) Drawing of the cells of *Sarcina*. (Erwin F. Lessel, illustrator.)

phytes are widely distributed in nature. Both species produce exoenzymes that hydrolyze starch and casein. *B. cereus* can cause a type of food poisoning.

B. stearothermophilus. This is a thermophilic species having a minimum growth temperature of 30 to 45°C and a maximum of 65 to 75°C. The endospores are highly resistant to heat and, therefore, this species is one of those associated with spoilage of canned goods.

B. polymyxa. Unlike many other *Bacillus* species, *B. polymyxa* has the ability to form gas during sugar fermentation. Another unusual characteristic is its ability to fix N_2 under anaerobic conditions.

B. thuringiensis. This species is noted for its pathogenicity for insects. Ingestion of the sporulated cultures of *B. thuringiensis* by larvae of *Lepidoptera* results in a paralytic disease. Other *Bacillus* species that are pathogenic for insects include ***B. popilliae*** ("milky disease" of Japanese beetle grubs) and certain strains of ***B. sphaericus*** (lethal for mosquito larvae).

B. anthracis. This is the only *Bacillus* species that is highly pathogenic for animals and humans; it is the causative agent of anthrax.

Sporosarcina

This genus contains cocci that are arranged in tetrads or cubical packets of eight cells (see Fig. 14-5). Sporosarcinae are widely distributed in fertile soil, where they play an active role in the decomposition of urea.

Anaerobic Spore-forming Rods

Clostridium

The members of this genus have a fermentative type of metabolism. They are widely distributed in soil, in marine and freshwater anaerobic sediments, and in the intestinal tract of humans and animals. The species are differentiated on the basis of their proteolytic activity, various enzyme activities, acid production from carbohydrates, and the kinds and amounts of organic acid end products of fermentation (the latter being determined by gas chromatography). A few of the many species in the genus are listed below:

C. botulinum causes a severe and often fatal type of food poisoning known as botulism.

C. tetani is the causative agent of tetanus. The characteristic terminal spores formed by this species are illustrated in Fig. 5-29.

C. perfringens is the major causative agent of the wound infection known as gas gangrene. Some strains of *C. perfringens* (enterotoxigenic strains) can cause a type of food poisoning.

C. difficile causes pseudomembranous colitis, a severe disease of the bowel.

C. thermosaccharolyticum is thermophilic, growing optimally at 55°C (minimum temperature 45°C; maximum, 67°C). The spores are extremely heat-resistant, and this species is often able to survive inadequate heat treatment during canning of foods and subsequently can cause spoilage of canned goods.

C. pasteurianum is a mesophilic soil clostridium that is particularly noted for its ability to fix N_2.

Desulfotomaculum

Unlike clostridia, members of this genus obtain energy by anaerobic respiration, with sulfate serving as the terminal electron acceptor and organic substrates such as lactic or pyruvic acid serving as the electron donors. Large amounts of H_2S are formed during growth. The organisms occur in soil, freshwater, intestines of insects, and the rumen of animals. See Fig. 14-5.

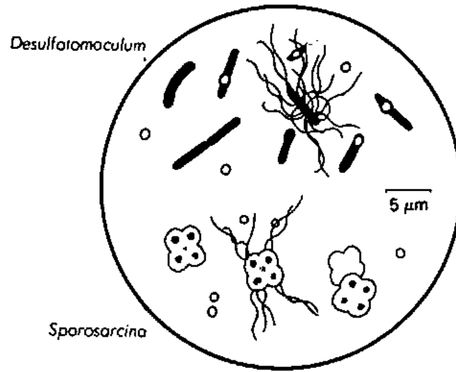

Figure 14-5. Drawing of the cells of *Sporosarcina* and *Desulfotomaculum*, showing the endospores and flagella. The flagella are not visible by ordinary staining. *(Erwin F. Lessel, illustrator.)*

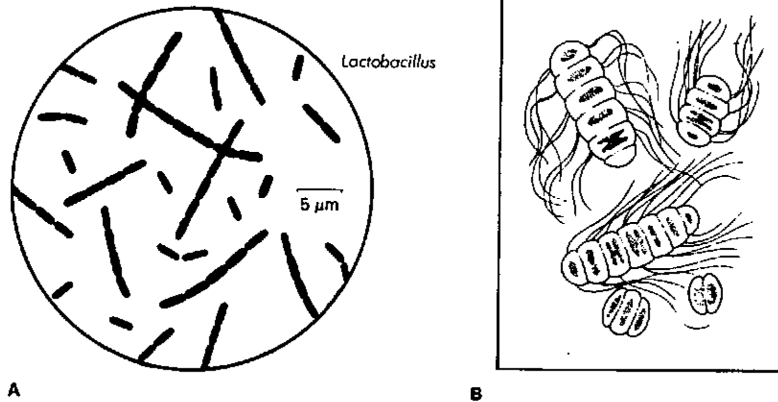

Figure 14-6. (A) Drawing of the cells of *Lactobacillus*. *(Erwin F. Lessel, illustrator.)* (B) Sketch of *Caryophanon* showing trichomes 3 μm in diameter composed of disk-shaped cells, together with numerous peritrichous flagella. *(Redrawn from K. A. Bisset, Bacteria, E. and S. Livingstone, Ltd., Edinburgh, 1952.)*

NONSPOREFORMING GRAM-POSITIVE RODS OF REGULAR SHAPE

This heterogenous group is composed of harmless saprophytes as well as parasitic and pathogenic organisms. The cells range from long rods to very short rods as, for example, in the genus ***Lactobacillus*** (Fig. 14-6A). One genus, ***Caryophanon***, is unusual in that it is composed of large, disk-shaped cells arranged in trichomes (Fig. 14-6B). Some characteristics of the various genera included in the group are indicated in Table 14-3.

NONSPOREFORMING GRAM-POSITIVE RODS OF IRREGULAR SHAPE

This group contains a heterogenous variety of bacteria, the few common features being:

Aerobic/Facultatively Anaerobic Nonfilamentous Rods

1 Straight to slightly curved rods that exhibit swellings, club shapes, or other deviations from a uniform rod shape
2 An aerobic or facultatively anaerobic nature, being capable of a respiratory type of metabolism and in some instances also of a fermentative type of metabolism

Some examples of the genera included in this group follow.

Corynebacterium

This genus contains rod-shaped cells which are pleomorphic and frequently

Table 14-3. Genera of Nonsporeforming Gram-Positive Rods of Regular Shape

Genus	Morphology and Motility	Oxygen Relationships	Catalase Test	Other Characteristics
Lactobacillus	Long to very short rods, often in chains; usually nonmotile	Str ctly fermentative organisms, but can usually tolerate air; some strains are anaerobic	−	Large amounts of lactic acid formed; homo- or heterofermentative; occur as saprophytes in fermenting animal or plant products or as parasites in the mouth, vagina, and intestinal tract of humans and warm-blooded animals
Listeria	Very short rods, often in chains; motile by peritrichous flagella when grown at 25°C; few flagella are formed at 37°C	Aerobic to microaerophilic	+	The species *L. monocytogenes* is a parasite and pathogen of a wide variety of animals; in humans it causes meningitis in adults and prenatal or postnatal disease in infants
Erysipelothrix	Filament-forming rods; nonmotile	Aerobic	−	Parasitic on mammals, birds, and fish; causes erysipelas in swine and erysipeloid in humans
Brocothrix	Rods, often occurring in long, kinked filaments; nonmotile	Facultatively anaerobic	+	Best growth occurs at 20 to 22°C; none at 37°C; saprophytes, found in meat and meat products
Renibacterium	Short rods; nonmotile	Aerobic	+	Best growth occurs at 15 to 18°C; parasites of salmonid fishes, causing a kidney disease
Kurthia	Rods in chains; motile by peritrichous flagella	Aerobic	+	Harmless saprophytes occurring in meat and meat products and in animal dung
Caryophanon	Large disk-shaped cells arranged in trichomes; motile by peritrichous flagella	Aerobic	Not reported	The morphology is unusual and distinctive; saprophytic, occurring in ruminant dung

exhibit club-shaped swellings and a palisade arrangement (see Fig. 14-7). The cells accumulate intracellular volutin granules (metachromatic granules) which

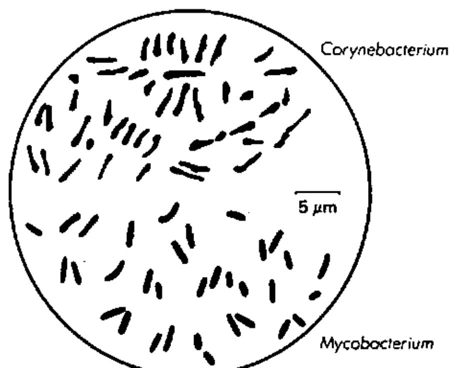

Figure 14-7. Drawing of the cells of *Corynebacterium* and *Mycobacterium*. (Erwin F. Lessel, illustrator.)

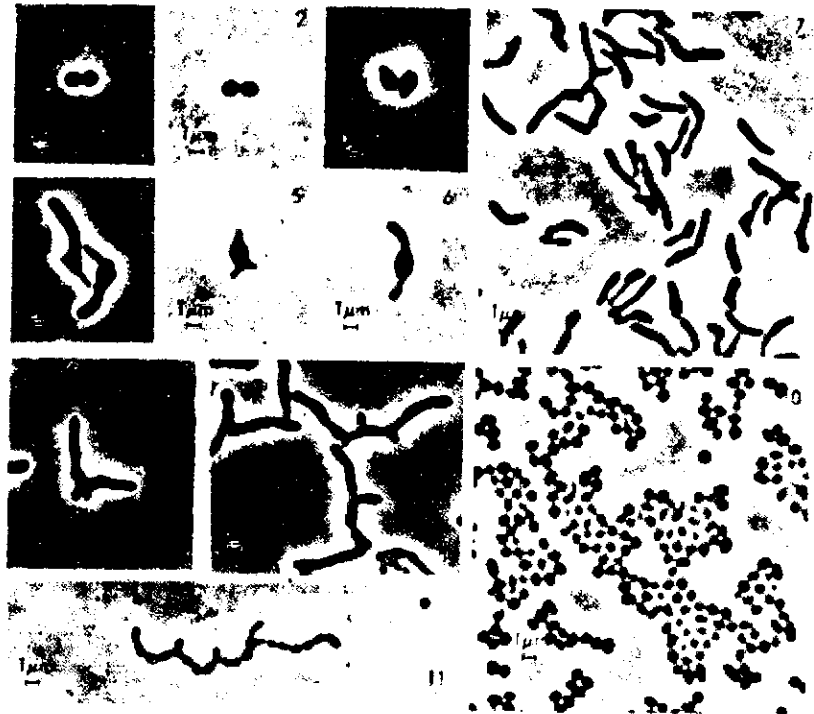

Figure 14-8. Morphology of *Arthrobacter globiformis* at different times of growth and on different media. Insets 1, 2, 3, and 4, slide cultures grown on yeast extract–soil extract medium for 1.5, 4.25, 6, and 9.75 h, respectively; insets 7 and 8, after growth for 24 h. Insets 5 and 6, cultures grown on yeast extract–mineral salts medium for 5 h; inset 11, grown for 3 days. Insets 9 and 10, grown on yeast extract–peptone–soil extract medium for 11.5 h and 3 days. *(Courtesy R. M. Keddie and Bergey's Manual of Determinative Bacteriology, 8th ed., Williams & Wilkins, Baltimore, 1974.)*

stain reddish purple with dilute methylene blue. The cell walls contain mycolic acids (corynemycolic acids) containing 32 to 36 carbon atoms.

The corynebacteria are divided into three large groups: saprophytes occurring in soil and water, the animal or human parasites and pathogens, and the plant pathogens. Of the animal or human pathogens, the major species is ***C. diphtheriae***, the causative agent of diphtheria in humans.

Arthrobacter

This genus of saprophytic soil organisms is characterized by an unusual "rod-coccus" cycle. Cells in the log phase of growth are irregularly shaped rods that may show a tendency toward rudimentary branching. In contrast, cells in the stationary phase of growth are distinctly coccoid; when these are inoculated into fresh media, they give rise to rod-shaped cells (see Fig. 14-8).

Brevibacterium

Like arthrobacters, brevibacteria exhibit a rod-coccus cycle. The only recognized species, ***B. linens***, forms orange colonies and is salt-tolerant; its usual habitat is on the surface of certain cheeses such as brick and Limburger, where it produces proteolytic enzymes that aid in the cheese-ripening process.

Microbacterium

These bacteria are small, slender, irregularly shaped rods that do not exhibit a rod-coccus cycle (although the rods become shorter in the stationary phase of growth). Microbacteria are saprophytes that occur in milk, in dairy products, and on dairy equipment.

Cellulomonas

This genus contains irregularly shaped rods that may be slightly filamentous and show rudimentary branching. No *Arthrobacter*-like rod-coccus cycle occurs, although a few cells in old cultures may be coccoid. The outstanding characteristic of the genus is the ability to degrade cellulose and to use it as a major carbon and energy source.

Aerobic/Facultatively Anaerobic Branched Filamentous Rods

The bacteria of this group form colonies which at first are microscopic in size (microcolonies)and contain branched filamentous cells. As the colonies develop to macroscopic size, many of the cells become diphtheroid (i.e., resemble corynebacteria) or coccoid in shape. One genus, *Agromyces,* is microaerophilic to aerobic, catalase-negative, and is a saprophyte that occurs in soil. The genus *Arachnia* is facultatively anaerobic, catalase-negative, and parasitic and pathogenic for humans and animals, being one of the causative agents of actinomycosis. The genus *Rothia* is aerobic, catalase-positive, and a normal inhabitant of the human mouth.

Anaerobic Nonfilamentous or Filamentous Rods

The organisms of this group are either anaerobes or, if facultatively anaerobic, are preferentially anaerobic. They are differentiated by their morphology and by their fermentation end products as determined by gas chromatography. Table 14-4 indicates the characteristics of several genera included in the group, and the morphological features of two genera, *Propionibacterium* and *Actinomyces,* are depicted in Figs. 14-9 and 14-10.

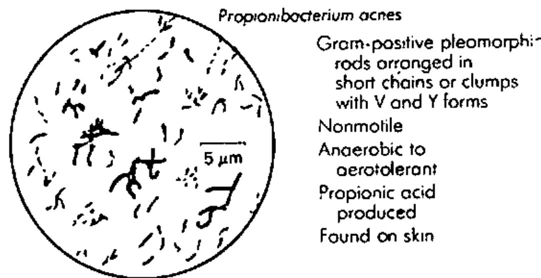

Figure 14-9. (Right) Drawing of the cells of *Propionibacterium acnes.* (*Erwin F. Lessel, illustrator.*)

Figure 14-10. (Below) *Actinomyces israelii.* (A) Dark-field preparation showing V and Y forms. (B) Gram-stain preparation showing elongated filaments, branching, and irregular staining. (C) Gram-stain preparation showing mass of intertwined filaments. Some filaments are irregularly stained, and some have bulbous ends. (Approximately X800.) *From J. M. Slack, S. Landfried, and M. A. Gerencser, J Bacteriol, 97:873, 1969. By permission.)*

MYCOBACTERIA

This group of aerobic bacteria contains a single genus, *Mycobacterium*, which consists of slightly curved or straight rods (see Fig. 14-7) that may show branching. Mycolic acids having about 90 carbon atoms occur in the cell walls. A major characteristic of mycobacteria is that they are acid-fast; that is, once stained with aniline dyes, they are difficult to decolorize, even when treated with a mixture of acid and alcohol. Some species such as *M. phlei* and *M. smegmatis* are harmless saprophytes. Many species are pathogenic, for example, *M. tuberculosis*, the causative agent of tuberculosis in humans; *M. kansasii* and *M. intracellulare*, which cause noncontagious tuberculosis-like infections; *M. scrofulaceum*, which causes lymphadenitis in children; and *M. leprae*, the causative agent of leprosy.

NOCARDIOFORMS

This group contains aerobic bacteria that produce a substrate mycelium, i.e., a mat of branching hyphae formed under the surface of the agar medium. This mycelium may range from rudimentary to extensively developed. The substrate mycelium usually *fragments* into rod-shaped or coccoid cells. Some genera also form an aerial mycelium that may give rise to conidiospores. Because the various genera of the group resemble the genus *Nocardia* with respect to their morphology and cell-wall composition, they are referred to as the nocardioforms. Several genera possess mycolic acids (nocardomycolic acids) in the cell walls; however, only the genus *Nocardia* contains acid-fast species.

The taxonomic placement of mycelium-forming bacteria, including the nocardioforms, is confused and controversial. Among the methods that have been used to establish various groupings are studies of the chemical composition of the cell walls. The walls of the nocardioform bacteria have the following characteristics, which define the type IV chemotype:

Table 14-4. Some Anaerobic, Irregularly Shaped, Nonsporeforming Gram-Positive Bacteria

Genus	Morphology	Organic Acids from Fermentation	Occurrence
Propionibacterium	Pleomorphic, nonmotile	Mainly propionic + acetic acids	Some species occur in dairy products; others are normal flora of human skin and of the intestines of humans and animals; *P. acnes* may be related to the skin disease acne vulgaris
Eubacterium	Pleomorphic, motile or nonmotile	Either butyric + other acids; acetic + formic; or no major acids	Human oral cavity, intestinal tract of humans and animals, infected tissues, soil, water, spoiled food; usually not pathogenic
Actinomyces	Initially, cells are filamentous with branching; eventually, diphtheroid cells predominate	Moderate amounts of acetic and sometimes formic, together with large amounts of succinic or lactic, or both	Oral cavity of humans and animals and human female genital tract; *A. israe* and other species can cause human actinomycosis; *A. bovis* causes actinomycosis (lumpy jaw) in cattle
Bifidobacterium	Pleomorphic, nonmotile	Acetic and lactic acids	Intestinal tract of humans and animals; not known to be pathogenic

1 The peptidoglycan contains meso-diaminopimelic acid.
2 No glycine interpeptide bridges occur between the peptidoglycan chains.
3 The walls contain the sugars arabinose and galactose.

Other groups of mycelium-forming bacteria differ in these respects; for example, the walls of members of the genus *Streptomyces* (see Chap. 16) contain LL-diaminopimelic acid, glycine interpeptide bridges, and no distinctive sugars (type I chemotype).

A few examples of the nocardioform group are described below.

Nocardia

The morphological features of this genus are illustrated in Figs. 14-11 and 14-12A. Some nocardias form only a limited mycelium because the center of the colony undergoes *early fragmentation* into rod-shaped or coccoid cells. Other

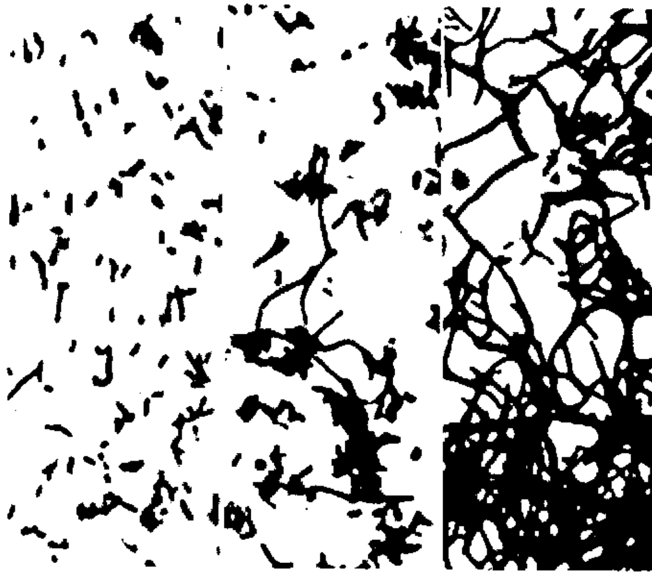

Figure 14-11. *Nocardia asteroides.* Three different strains of the same species, showing variations in morphology. (X700) *(Courtesy of Ruth E. Gordon.)*

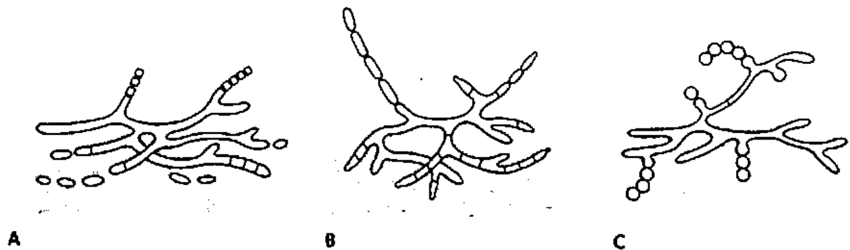

Figure 14-12. Schematic representation of the morphology of some nocardioform bacteria. Both substrate and aerial mycelia are illustrated. (A) *Nocardia*, showing fragmentation and aerial chains of spores. (B) *Pseudonocardia*, showing budding of the hyphae and aerial chains of cylindrical spores. (C) *Micropolyspora*, showing chains of round spores on both aerial and substrate mycelium.

nocardias have *delayed fragmentation* and are thus able to establish an extensive substrate mycelium, and sometimes an aerial mycelium that gives the surface of the colonies a dull, fuzzy appearance. The aerial hyphae of some species may bear chains of spores. Nocardias are saprophytes that are widely distributed in soil and water, but they can be opportunistic pathogens, causing nocardiosis and actinomycetoma in humans and animals.

Pseudonocardia

This genus does not contain nocardomycolic acids, and, in contrast to the hyphae of nocardias, the hyphae of pseudonocardias grow by a distinctive *budding process*. A constriction occurs behind the hyphal tip; the tip elongates to form a new hyphal segment; this segment develops a constriction behind the tip, and in turn undergoes budding, etc. The aerial mycelium bears long chains of cylindrical conidiospores, which arise terminally or laterally from the hyphae (see Fig. 14-12B). Pseudonocardias occur in soil and in fresh or rotten manure.

This genus forms an extensive aerial mycelium. The hyphae of both the substrate and aerial mycelia bear short chains of 1 to 20 round conidiospores (see Fig. 14-12C). The spores occur in moldy hay or silage or in the air of farm buildings and can be inhaled into the lungs; however, they are apparently not pathogenic. Nocardomycolic acids occur in the walls of some *Micropolyspora* species.

QUESTIONS

1 How is *Micrococcus* distinguished from *Staphylococcus*? From *Deinococcus*? From *Planococcus*?
2 How is *Leuconostoc* distinguished from *Streptococcus*? From *Pediococcus*?
3 How is *Clostridium* distinguished from *Bacillus*? From *Desulfotomaculum*?
4 What is the outstanding morphological feature of each of the following? *Caryophanon. Arthrobacter. Nocardia. Sarcina. Corynebacterium.*
5 Name three genera that contain mycolic acids in their cell walls. Which contain acid-fast organisms?
6 How can *Staphylococcus* be distinguished from *Streptococcus*? Give at least two characteristics.
7 What is the outstanding biochemical feature of *Propionibacterium*? Of *Cellulomonas*?
8 How has cell-wall chemistry been used in the classification of nocardioform bacteria?
9 What kinds of bacteria described in this chapter are noted for the following characteristics?
   (*a*) Insect pathogenicity
   (*b*) Radiation resistance
   (*c*) Growth on the surface of cheeses
   (*d*) Pathogens of fish
   (*e*) Occurrence of metachromatic granules
   (*f*) Cellulose degradation
10 Most strains of *Streptococcus* can grow in air, but some strains are anaerobic. The genus *Peptostreptococcus* cannot grow in air, and all strains are anaer-

obic. How could you differentiate an anaerobic strain of *Streptococcus* from a strain of *Peptostreptococcus*?

11 Some strains of *Actinomyces* produce large amounts of lactic acid. All strains of *Lactobacillus* produce large amounts of lactic acid. How could you differentiate a strain of *Lactobacillus* from *Actinomyces*?

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Chapter 15 The World of Bacteria III: Bacteria with Unusual Properties

OUTLINE ***Bergey's Manual of Systematic Bacteriology*, Volume 3**

Anoxygenic Phototrophic Bacteria
Purple Phototrophic Bacteria • Green Phototrophic Bacteria

Oxygenic Phototrophic Bacteria
Cyanobacteria (Blue-Green Algae) • Prochlorophytes

Gliding, Fruiting Bacteria

Gliding, Nonfruiting Bacteria

The Sheathed Bacteria

Budding and/or Appendaged Bacteria
Prosthecate Budding Bacteria • Prosthecate Nonbudding Bacteria • Nonprosthecate Budding Bacteria • Nonprosthecate Nonbudding Bacteria

Chemolithotrophic Bacteria
The Family ***NITROBACTERACEAE*** • Sulfur- and Sulfur-Compound-Metabolizing Bacteria • The Family ***SIDEROCAPSACEAE***

Archaeobacteria
Methanogenic Bacteria • Extreme Halophiles • Thermoacidophiles

The organisms in Volume 3 of *Bergey's Manual* have properties that are quite different from those of the bacteria described in Volumes 1 and 2. Some are distinguished by their unusual type of metabolism. For instance, some of the organisms are **phototrophic**, able to use light as an energy source. Others are **chemolithotrophic**, able to obtain energy by oxidizing inorganic compounds such as ammonia, nitrite, hydrogen sulfide, or ferrous iron. Still others are not distinguished by an unusual metabolism but rather by other features such as the occurrence of **gliding motility** rather than motility by the action of flagella, reproduction by **budding** rather than by binary fission, or special morphological structures such as **sheaths**, **prosthecae**, and **stalks**. Most of the organisms are Gram-negative **eubacteria**, but some, such as those that form methane gas, belong to the major bacterial group known as the **archaeobacteria**, which may stain Gram-negative or Gram-positive. In the present chapter we will describe the

properties of several groups of unusual eubacteria, and also the major features of various archaeobacteria.

BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY, VOLUME 3

The bacteria included in Volume 3 of *Bergey's Manual* are extremely diverse in their properties. As in Chaps. 14 and 15, they are grouped largely on the basis of practical considerations. The major sections of Volume 3 are listed in Table 15-1. The archaeobacteria are considered together as a group despite the diversity of their characteristics because they are all phylogenetically distinct from eubacteria.

ANOXYGENIC PHOTOTROPHIC BACTERIA

The bacteria of this group belong to the order *RHODOSPIRILLALES*. They are Gram-negative and are all capable of carrying out a photolithotrophic and/or photoorganotrophic type of metabolism, and they contain bacteriochlorophyll (which differs in structure from chlorophyll such as occurs in cyanobacteria and eucaryotic algae). Also present are various water-insoluble carotenoid pigments, which can also trap or absorb light energy and transmit it to the bacteriochlorophyll. The anoxygenic bacteria grow phototrophically only under anaerobic conditions and are incapable of forming O_2 (i.e., are *anoxygenic*) because they possess only photosystem I (see Chap. 10). They are believed to be more

Table 15-1. Bacteria Included in *Bergey's Manual*, Volume 3

Section	Some Major Characteristics
ANOXYGENIC PHOTOTROPHIC BACTERIA	Gram-negative bacteria that contain bacteriochlorophyll and can use light as an energy source; the organisms are anaerobic and do not evolve O_2 during photosynthesis
OXYGENIC PHOTOTROPHIC BACTERIA	Bacteria that contain chlorophyll, can use light as an energy source, and evolve O_2 in a manner similar to that of green plants; the group includes the cyanobacteria ("blue-green algae")
GLIDING, FRUITING BACTERIA	Gram-negative nonphototrophic bacteria that lack flagella, yet can glide across solid surfaces; they have a complex life cycle in which the cells swarm together in masses and form fruiting bodies
GLIDING, NONFRUITING BACTERIA	Gram-negative nonphototrophic rods, filaments, or multicellular trichomes that glide across solid surfaces; fruiting bodies are not produced
THE SHEATHED BACTERIA	Gram-negative nonphototrophic bacteria that form an external sheath that covers the chains or trichomes
BUDDING AND/OR APPENDAGED BACTERIA	Gram-negative nonphototrophic bacteria that reproduce asymmetrically by budding and/or form prosthecae or stalks
CHEMOLITHOTROPHIC BACTERIA	Gram-negative nonphototrophic bacteria that obtain energy for carbon dioxide fixation from the oxidation of ammonia, nitrite, reduced sulfur compounds, or ferrous iron
ARCHAEOBACTERIA	Gram-positive or Gram-negative bacteria that are phylogenetically distinct from eubacteria; some produce methane gas; some require unusually high levels of NaCl for growth; others are distinguished by their ability to grow at a low pH and a high temperature

primitive than oxygenic (O_2-evolving) phototrophic organisms: geological studies have provided evidence that the atmosphere of our planet was anaerobic at the time life began to develop and that oxygen did not appear in appreciable quantities in the atmosphere until oxygenic bacteria evolved (i.e., bacteria having both photosystem I and photosystem II).

Anoxygenic phototrophic bacteria occur in anaerobic freshwater or marine environments. They may occur beneath the surface of shallow aquatic environments rich in organic matter, such as stagnant ponds, ditches, and salt marsh pools, or, in some instances, they may have a much deeper habitat, as at the bottom of a lake. The bacteriochlorophyll absorbs light most strongly when the light is of long wavelength—about 725 to 745 nm (far red light, at the extreme end of the visible spectrum) to 1035 nm (infrared light, invisible to the human eye). This light is of longer wavelength than that absorbed by the chlorophyll of oxygenic bacteria or oxygenic eucaryotic algae (about 680 nm). Although oxygenic organisms may grow at the surface of a shallow, stagnant pond, they do not absorb far red or infrared light and thus do not prevent it from reaching the anoxygenic phototrophs below. The bacteriochlorophyll and the carotenoid pigments of anoxygenic bacteria can also absorb some light in the blue to blue-green range (between 400 and 550 nm). This becomes important when anoxygenic bacteria occur in the depths of a lake, because blue light can penetrate water to greater distances than red light can.

The color of anoxygenic phototrophic bacteria is determined mainly by the carotenoid pigments rather than by the bacteriochlorophyll, and the anoxygenic phototrophs can be divided into two major groups on the basis of their pigmentation: *purple bacteria* and *green bacteria*. Motility, if present in these two groups, is by means of polar flagella, except for the family *Chloroflexaceae* which exhibits a gliding type of motility. *Nitrogen can be fixed* by purple or green bacteria, but usually only under anaerobic conditions and illumination.

Purple Phototrophic Bacteria

These organisms contain bacteriochlorophyll types *a* or *b*. The pigments that harvest the energy of light (i.e., bacteriochlorophyll and auxiliary carotenoid pigments) are located in the cytoplasmic membrane, which may be greatly

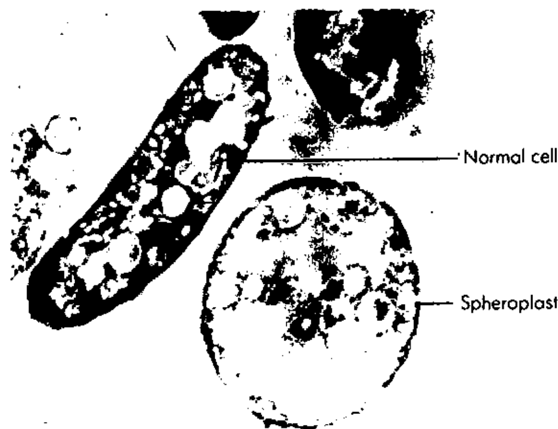


Figure 15-1. Electron micrograph of a purple nonsulfur bacterium, *Rhodospirillum rubrum*, showing the small intracellular vesicles that contain the photosynthetic apparatus. The large clear areas are poly-β-hydroxybutyrate granules (X14,000). [*From E. S. Boatman and H. C. Douglas, Electron Microscopy, vol. 2, Fifth International Congress of Electron Microscopy (Philadelphia), Academic, New York, 1962.*]

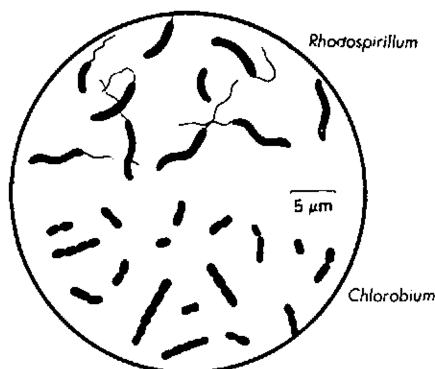


Figure 15-2. Drawing of the cells of *Rhodospirillum* (family *Rhodospirillaceae*) and *Chlorobium* (family *Chlorobiaceae*). The flagella of *Rhodospirillum* cannot be seen by ordinary staining. *(Erwin F. Lessel, illustrator.)*

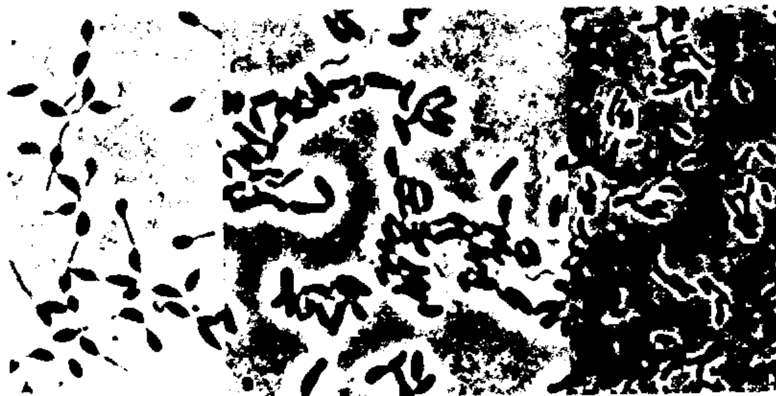


Figure 15-3. Some species of the family *Rhodospirillaceae*. (A) *Rhodomicrobium vannielii*, a prosthecate budding species. The bud forms at the tip of a prostheca and eventually reaches the size of the mother cell. (B) *Rhodopseudomonas acidophila*, a nonprosthecate budding species. The bud is sessile at the pole of the mother cell and separates by constriction when the bud reaches the size of the mother cell. Some bundles of polar flagella can be seen in the field. (C) *Rhodopseudomonas palustris*, a nonprosthecate budding species. The cells are narrower than those in (B). Phase-contrast (X1,464). *(From N. Pfennig, J Bacteriol,* **99**:*597, 1969.)*

invaginated to form vesicles (see Fig. 15-1), folded layers (lamellae), or tubules. Two families are recognized, as follows.

The family *RHODOSPIRILLACEAE* contains the purple nonsulfur bacteria. Cultures appear orange-brown to purple-red under aerobic conditions. Some may be similarly pigmented under anaerobic conditions, but others may be greenish-yellow. The purple nonsulfur bacteria exhibit a diversity of shapes: helical (e.g., ***Rhodospirillum***; see Fig. 15-2), nonprosthecate rod-shaped, ovoid, or spherical cells that multiply by binary fission or budding (e.g., ***Rhodopseudomonas***; see Figs. 15-3B and C), or ovoid cells that multiply by the formation of buds at the end of prosthecae (e.g., ***Rhodomicrobium***; see Figs. 15-3A and 15-4).

The purple nonsulfur bacteria are *photoorganotrophs*: organic substances serve both as carbon sources and as electron donors for the reduction of carbon dioxide. Some species can grow autotrophically by using H_2S as the electron donor, but only if very low concentrations are provided; none can use elemental sulfur as an electron donor. Photosynthesis occurs only under anaerobic conditions in the light. Some species can also grow under aerobic or microaerophilic conditions in the dark by respiration with organic compounds.

The family *CHROMATIACEAE* contains the **purple sulfur bacteria**. Cultures appear orange-brown to purple-violet. Purple sulfur bacteria may be ovoid to

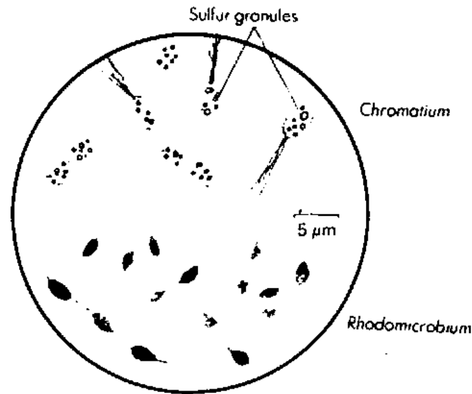


Figure 15-4. Drawing of the cells of *Chromatium* (family *Chromatiaceae*) and *Rhodomicrobium* (family *Rhodospirillaceae*). The intracellular sulfur granules of *Chromatium* are indicated. *(Erwin F. Lessel, illustrator.)*

rod-shaped (e.g., ***Chromatium;*** see Fig. 15-4), coccoid (e.g., ***Thiocystis***), or helical (e.g., ***Thiospirillum***). Coccal species may be arranged as diplococci (e.g., ***Lamprocystis***), in cubical packets (e.g., ***Thiosarcina***), or in flat sheets (e.g., ***Thiopedia***). Some species contain gas vacuoles.

All genera are capable of *photolithotrophic* growth, using H_2S or elemental sulfur as the electron donor for CO_2 fixation. When H_2S is the electron donor, globules of elemental sulfur are formed, usually within the cells. The sulfur is eventually oxidized to sulfate. Some strains can also grow photoorganotrophically. Most species are anaerobic and cannot grow in the dark even under microaerophilic conditions.

Green Phototrophic Bacteria

In contrast to the purple bacteria, these organisms contain bacteriochlorophyll types *c* or *d* and minor amounts of *a*. Moreover, cultures are green or brown. The pigments involved in photosynthesis are located in membrane-bound vesicles within the cell; some of these may be attached to the cytoplasmic membrane. Two families are recognized, as follows.

The family ***CHLOROBIACEAE*** contains the **green sulfur bacteria.** The cells are ovoid, bean-shaped, or rod-shaped (e.g., see the genus ***Chlorobium,*** Fig. 15-2) and multiply only by binary fission. One genus, ***Prosthecochloris,*** consists of star-shaped cells, this shape being caused by the production of about 20 prosthecae per cell. Gas vacuoles may occur in some genera.

Green sulfur bacteria live as *photolithotrophs,* using H_2S as the electron donor for CO_2 fixation. Granules of elemental sulfur are deposited *outside* the cells, never within the cells; the sulfur can eventually be oxidized to SO_4^{2-} (soluble sulfur). The organisms are anaerobic, being incapable of growing in the dark even under microaerophilic conditions.

The family ***Chloroflexaceae*** contains the **green nonsulfur bacteria.** The main genus, ***Chloroflexus,*** is thermophilic (optimum temperature 52 to 60°C) and occurs in hot springs where it forms green or orange mats. *Chloroflexus* cells occur as filaments or trichomes and exhibit gliding motility. The organisms are mainly *photoorganotrophic,* as the purple nonsulfur bacteria, but they can also grow as photolithotrophs with H_2S as the electron donor. In the dark they can grow aerobically as chemoheterotrophs.

OXYGENIC PHOTOTROPHIC BACTERIA

Cyanobacteria (Blue-Green Algae)

These organisms exhibit an enormous diversity of shapes and arrangements, from unicellular cocci (Fig. 15-5) or rods to long trichomes (Fig. 15-6). Gas vacuoles may be formed by many species (Fig. 15-6B). Some cyanobacteria are surrounded by a sheath that surrounds the aggregates (Fig. 15-5B) or trichomes. Unicellular cyanobacteria are usually nonmotile, but trichome-formers generally possess gliding motility. Flagella are absent. Cyanobacteria are widespread in soil, freshwater, and marine habitats. Some are thermophilic, growing in hot springs. Cyanobacteria can grow as mats on the surface of bare soil as primary colonizers. They are important in adding organic matter to the soil and in preventing incipient erosion. Some cyanobacteria grow in symbiosis with other organisms. For example, they may occur as algal symbionts of lichens (see Chap. 18). Some live within the plant bodies of certain liverworts, water ferns, cycads (a class of naked-seed plants), and angiosperms (plants whose seeds are borne within a fruit) where they fix nitrogen. Cyanobacteria have also been associated with certain protozoa, where they are called *cyanellae*.

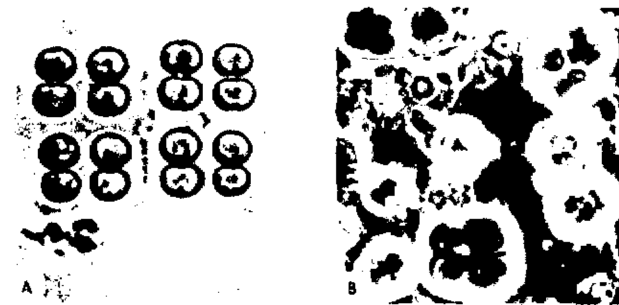


Figure 15-5. Examples of coccoid cyanobacteria. (A) *Merismopedia glauca*. The cells are 3 to 5 μm in diameter, and the colony is a flat plate. This species is a common member of the plankton of soft water lakes. (B) *Gloeocapsa rupestris*. The cells are 6 to 9 μm in diameter and are surrounded by a sheath. This species occurs on moist rocks, in soil, or on submerged objects and forms gelatinous masses that are often colored yellow, red, or brown. *(Courtesy of George J. Schumacher, State University of New York at Binghamton.)*

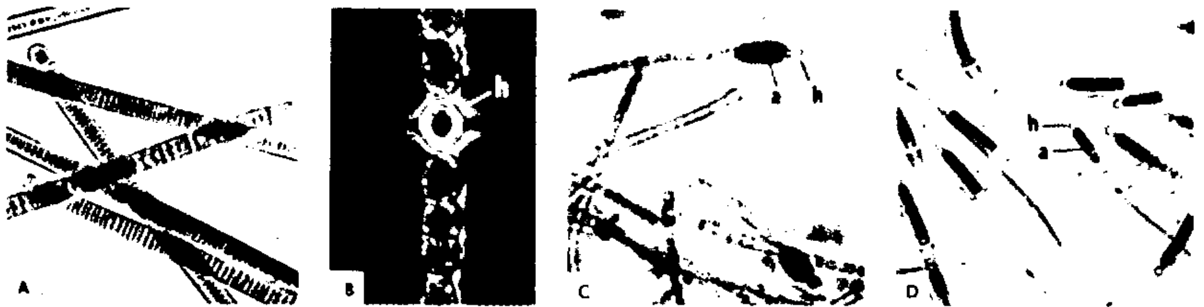


Figure 15-6. Examples of trichome-forming cyanobacteria. (A) *Oscillatoria limosa*. The trichomes consist only of vegetative cells, which are 12 to 18 μm wide. (B) *Anabaena planktonica*. The vegetative cells are 10 to 15 μm wide and contain gas vacuoles (bright areas). A heterocyst (h) is also shown. The heterocysts of this species are unique in that they possess lateral mucilaginous winglike structures, as shown in this photograph. (C) *Cylindrospermum majus*. The vegetative cells are 3 to 5 μm wide and the heterocysts (h), which are always terminal in location, are slightly larger. The akinetes (a) are much larger and are 25 to 30 μm in length. (D) *Gloeotrichia echinulata*. The vegetative cells are 8 to 10 μm wide but decrease in width along the long, tapering trichome. The terminal heterocysts (h) are 8 to 10 μm in diameter, and the akinetes (a) are 10 to 20 μm wide by 45 to 50 μm long. *(Courtesy of George J. Schumacher, State University of New York at Binghamton.)*

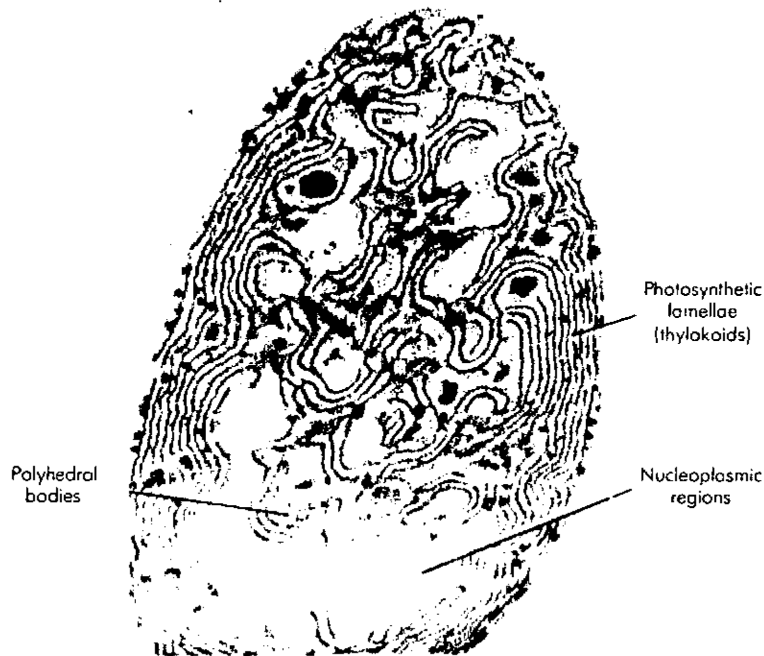


Figure 15-7. Section of a vegetative cell of the cyanobacterium *Anabaena azollae.* Most of the photosynthetic lamellae (thylakoids) are peripheral, but some extend into the midportions of the cell. *(Courtesy of Norma J. Lang and J Phycol, 1:127–134, 1965.)*

Cyanobacteria contain chlorophyll *a* rather than bacteriochlorophyll; because of this chlorophyll the cells absorb red light of 680 to 683 nm. Other pigments include water-insoluble carotenoids and also water-soluble *phycobilins*, which are the major light-absorbing pigments in cyanobacteria and which can transmit the energy of absorbed light to the chlorophyll. Blue phycobilins (phycocyanin and allophycocyanin) occur in all cyanobacteria and absorb light at wavelengths between 500 and 650 nm. A red phycobilin, phycoerythrin, occurs in some but not all species and absorbs shorter wavelengths between 470 to 600 nm. Cyanobacteria possessing phycoerythrin have a red or brown color instead of the usual bluish-green hue.

Cyanobacteria are *photolithotrophs*, and because of photosystem II (see Chap. 10) they can use H_2O as an electron donor for CO_2 fixation, in contrast to anoxygenic phototrophic bacteria. However, some cyanobacteria can also use H_2S as an electron donor in a manner similar to that used by the green sulfur bacteria. Many cyanobacteria are obligately photolithotrophic, but some can also grow as chemoorganotrophs at a slow rate in the dark.

The photosynthetic apparatus (i.e., chlorophyll *a*, carotenoid pigments, photochemical reaction centers, and the photosynthetic electron transport chain) is contained in the *thylakoids*—flattened membranous sacs located within the cell (see Fig. 15-7). The surface of the thylakoids is studded with granules called *phycobilisomes*, which contain the phycobilin pigments.

Many trichome-forming cyanobacteria can fix N_2. It seems strange that oxygen-evolving organisms can do this, since nitrogenase is highly oxygen-sensitive; however, in most instances it is not the vegetative cyanobacterial cells that carry

out nitrogen fixation but rather specialized cells called *heterocysts* (see Fig. 15-6), which occur periodically along or at the ends of the trichome. Nitrogen fixation is possible in heterocysts because they lack photosystem II and therefore do not evolve oxygen; moreover, their walls are much thicker than those of the vegetative cells, which may prevent rapid diffusion of oxygen into the heterocyst. Some cyanobacteria that form heterocysts also form large, thick-walled cystlike cells called *akinetes* (Fig. 15-6), which are resistant to desiccation.

The Prochlorophytes

Unlike cyanobacteria, these unicellular spherical organisms contain chlorophyll *b* in addition to chlorophyll *a*. Moreover, they lack phycobilin pigments; consequently, the cells appear grass-green rather than blue-green. Prochlorophytes live in association with certain marine invertebrates (ascidians) and have not yet been cultured; information about them has been obtained only by studying them in their natural symbiotic state or by analysis of cells mechanically removed from their hosts. Only a single genus is recognized, ***Prochloron***.

GLIDING, FRUITING BACTERIA

The organisms of this group are Gram-negative, nonphototrophic, and nonflagellated. They exhibit a creeping or gliding motility on solid surfaces. The mechanism of gliding is not known. The organisms are not filamentous; instead, the vegetative cells are unicellular short rods resembling typical bacteria except the walls are flexible. This flexibility may be due to the fact that the peptidoglycan layer is thin and occurs in patches that are connected by nonpeptidoglycan material. When the organisms are placed on a solid culture medium, growth, which is frequently of a slimy consistency, spreads rapidly over the surface. As the cells glide across the surface of the medium, they leave a layer of slime behind them. Indeed, the prefix *myxo*, which occurs in the name of the order to which these bacteria belong, *Myxobacterales*, reflects this property, being derived from the Greek noun *myxa*, meaning "mucus, slime."

Although there are some exceptions, a remarkable and striking feature of the myxobacters is that the vegetative cells at some stage of growth *swarm* together in masses and form *fruiting bodies*. [This behavior bears some resemblance to that of slime molds; however, the latter are eucaryotic organisms (see Chap. 17).] The fruiting bodies contain *myxospores*, which are shorter and thicker than the vegetative cells and are resistant to desiccation and ultraviolet radiation but not to heat. The fruiting bodies range from simple to complex. The simplest fruiting bodies are merely heaps of myxospores embedded in a mass of slime. The most elaborate fruiting bodies may have a *stalk* composed of slime, and walled containers (*sporangioles*, sometimes termed *cysts*) which enclose the myxospores. The life cycle of one species that forms complex fruiting bodies, ***Stigmatella aurantiaca***, is shown schematically in Fig. 15-8; electron micrographs of various stages in the growth cycle of another species, ***Chondromyces crocatus***, are shown in Fig. 15-9.

Myxobacters are strictly aerobic organisms found in surface layers of soil, compost, manure, rotting wood, and animal dung. Many myxobacters develop very colorful pigments in their normal environment. Some species produce exocellular enzymes that degrade complex substrates such as cellulose, agar, chitin, and even the cell walls of other bacteria.

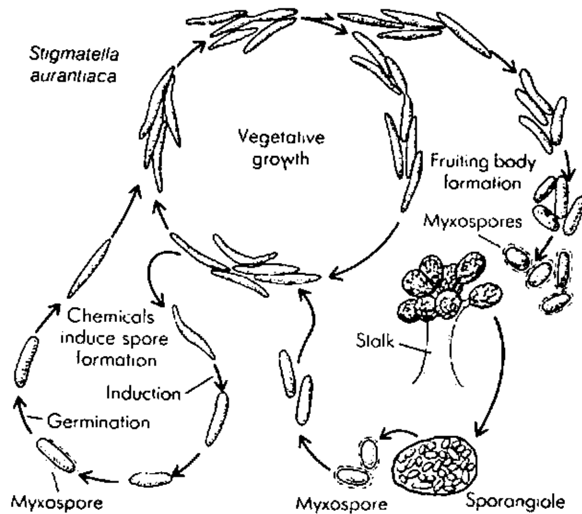


Figure 15-8. Life cycle of *Stigmatella aurantiaca* showing vegetative cells, myxospores, and fruiting body. [*After H. Reichenbach, from Martin Dworkin, "The Myxobacterales," in A. I. Laskin and H. A. Lechevalier (eds.), Handbook of Microbiology, CRC Press, Inc., Boca Raton, Fla., 1974.*]

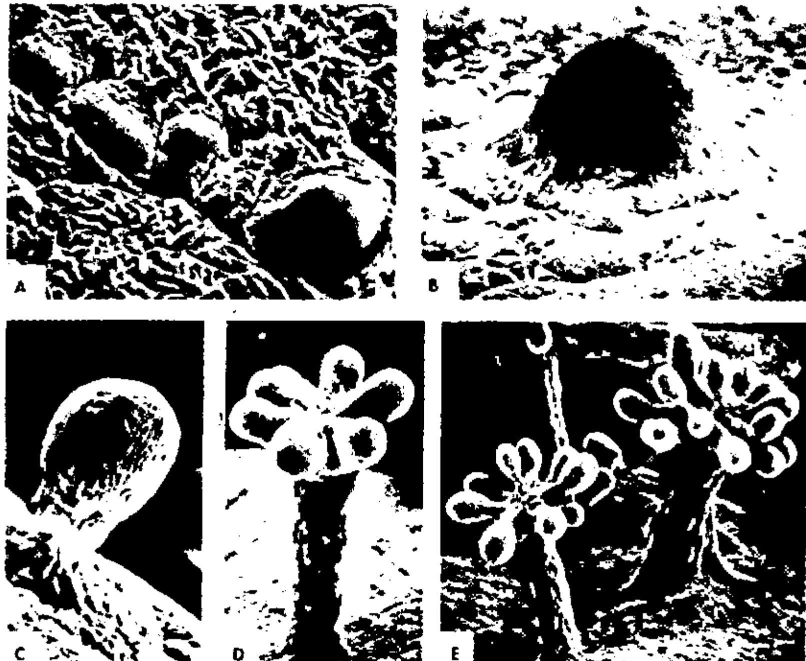


Figure 15-9. Stages in the fruiting body formation of the myxobacter *Chondromyces crocatus*. Early stages: (A) Initial stages of vegetative cell aggregation; (B) "fried-egg" stage showing orientation of peripheral cells; (C) bulb formation and development of stalk. Late stages: (D) Initial stages of sporangiole formation; (E) sporangiole formation after elongation of stalk to maximum length. Structures range in size from approximately 10 to 60 μm. *(From P. L. Grilione and J. Pangborn and J Bacteriol,* **124**:*1558, 1975.)*

GLIDING, NONFRUITING BACTERIA

These bacteria resemble myxobacters in their gliding motility; however, fruiting bodies are not formed, and only one genus, ***Sporocytophaga,*** forms myxospores. Most of the genera in the order are aerobic or microaerophilic organisms that live in soil or water. Many species can degrade natural polymers such as cel-

lulose, chitin, pectin, keratin, or even agar. One genus, ***Capnocytophaga***, is unusual because of its occurrence in the oral cavity of humans—in the gingival crevice (the space between the surface of the enamel of a tooth and the gum)—and may be involved in periodontal disease. Some aquatic genera such as ***Beggiatoa*** occur mainly in microaerophilic environments containing H_2S, which is oxidized by the cells to elemental sulfur; the sulfur accumulates as granules within the cells. Thus, *Beggiatoa* may possibly be an autotrophic organism.

Gliding, nonfruiting bacteria may appear as individual rods or filaments (e.g., ***Cytophaga, Flexibacter***, or ***Vitreoscilla***), or as multicellular trichomes (e.g., ***Beggiatoa, Simonsiella, Saprospira***, or ***Thiothrix***). Some of the cells are very long; for example, *Flexibacter* cells may reach 50 μm in length (see Fig. 15-10). Some genera such as ***Herpetosiphon*** and ***Flexithrix*** produce a sheath which

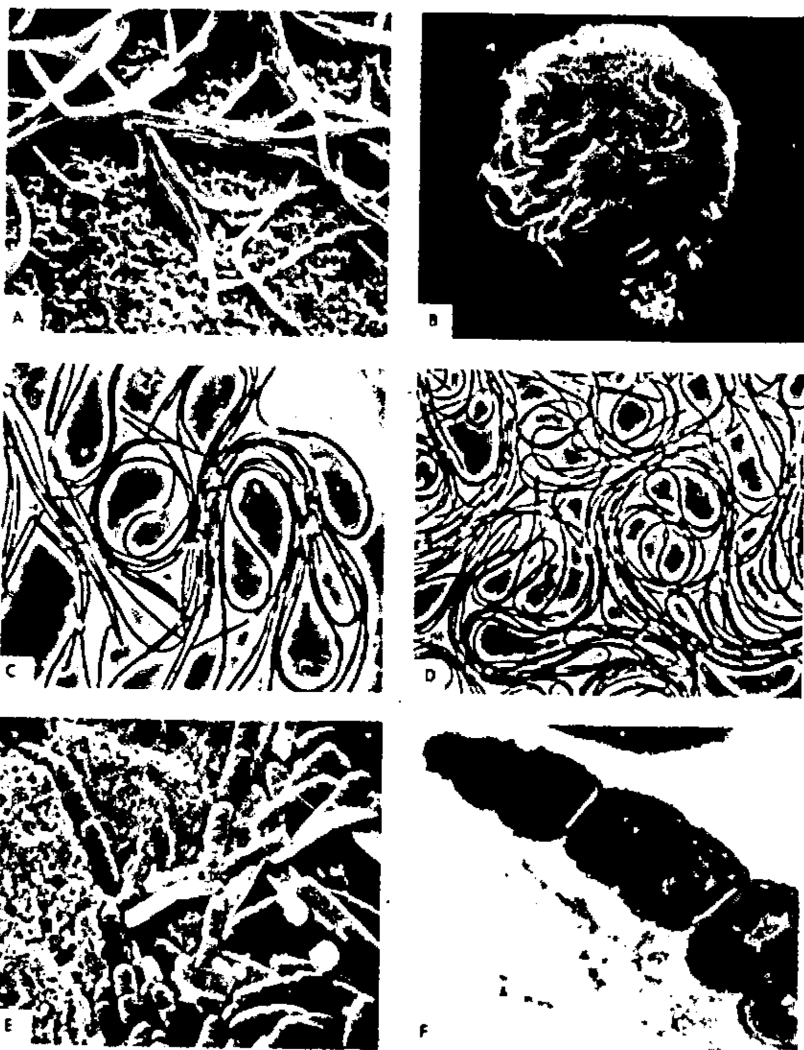


Figure 15-10. Species of gliding, nonfruiting bacteria. (A) *Flexibacter polymorphus*. Cells collected on the surface of a Nucleopore membrane filter (X730). (B) Colony of *F. polymorphus* growing on surface of Nucleopore membrane filter layered over a nutrient agar surface (X100). The holes in the filter are 5.0 μm in diameter. *(Courtesy of H. F. Ridgeway, Jr., Scripps Institution of Oceanography.)* (C) Filaments of the gliding bacterium *Herpetosiphon giganteus* on agar showing "bulbs" (bright spherical enlarged regions) (X500). (D) Same as (C) but at lower magnification (X330). *(Courtesy Hans Reichenbach.)* (E, F) *Simonsiella* sp. showing cells arranged in apposition to form trichomes with free faces of terminal cells rounded. (E) Scanning electron micrograph (X2,200); (F) transmission electron micrograph of thin section (X20,000). *(Courtesy J. Pangborn and Daisy Kuhn.)*

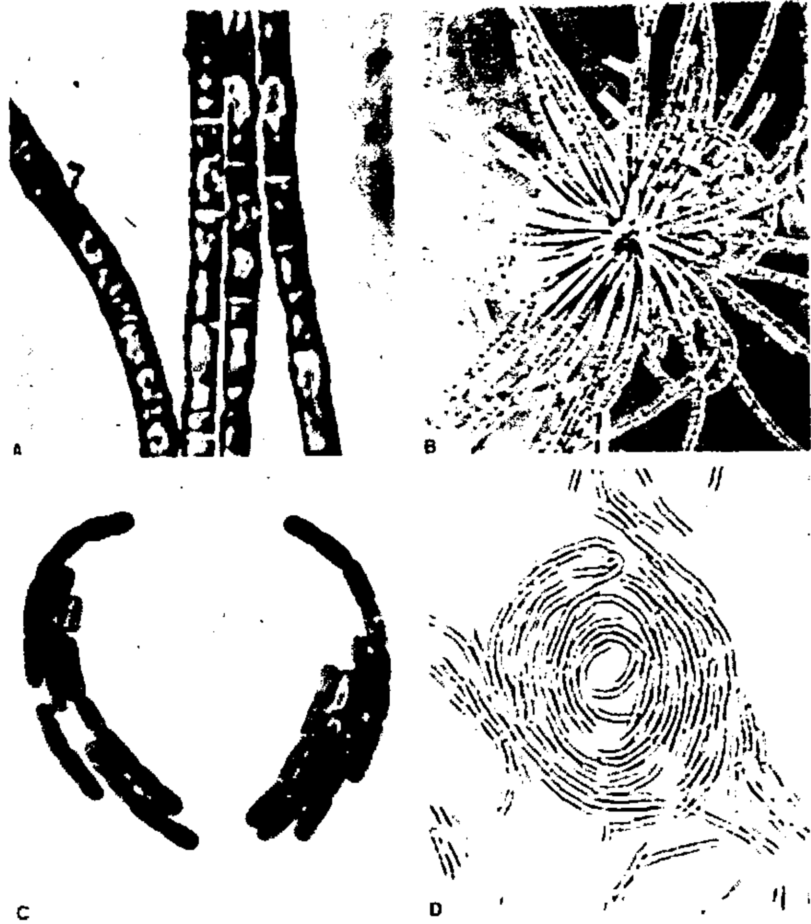


Figure 15-11. Gliding, nonfruiting bacteria. (A) Trichomes of *Beggiatoa* stained to demonstrate presence of a cell membrane (X2,250). *(From H. L. Scotten and J. L. Stokes, Arch Mikrobiol,* **42**:*353, 1962.)* (B) Trichomes attached to a common object are illustrated in this photomicrograph of *Thiothrix* sp.. (X420). *(Courtesy of F. E. Palmer and E. J. Ordal.)* (C) *Vitreoscilla* cell morphology. *(Courtesy of G. J. Hageage, Jr.)* (D) Pattern of cell arrangement of *Vitreoscilla*. *(Courtesy of V. B. D. Skerman.)*

encloses the individual cells. Examples of the morphological features characterisic of gliding, nonfruiting bacteria are shown in Figs. 15-10 and 15-11 (see also Fig. 5-4).

THE SHEATHED BACTERIA

Bacteria in this group are Gram-negative, aerobic, and nonphototrophic, and are characterized by the formation of a sheath surrounding a chain of cells or a trichome. The sheaths of some genera are encrusted with ferric and manganic oxides. Sheathed bacteria inhabit freshwater and marine environments. Among the genera included in the group are: ***Sphaerotilus, Leptothrix*** (Fig. 15-12), ***Haliscomenobacter, Streptothrix, Lieskeella, Phragmidiothrix, Crenothrix*** (Fig. 15-12), and ***Clonothrix***. Only the first three genera have been isolated; the others are characterized solely on the basis of their distinctive morphology as observed in samples from natural sources. One of the cultivatable genera, *Sphaerotilus*, is discussed here.

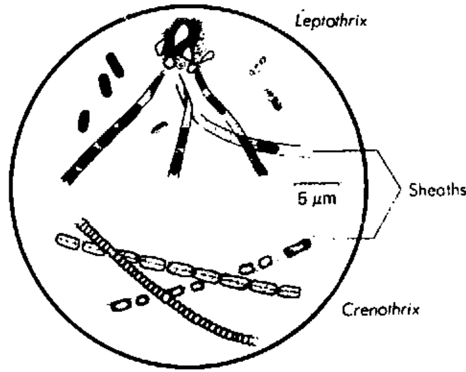


Figure 15-12. Drawing of sheathed bacteria of the genus *Leptothrix* and *Crenothrix*. (*Erwin F. Lessel, illustrator.*)

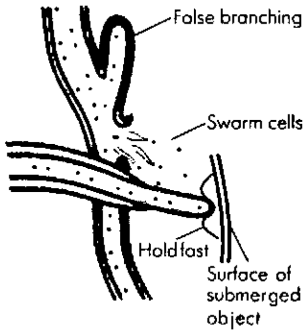


Figure 15-13. Sheathed bacteria. Drawing of *Sphaerotilus* showing sheath, false branching, and motile swarmers. When a swarmer encounters a solid object, it can become attached by a holdfast, formed by secreting a sticky substance that hardens. Sheath formation begins at the holdfast. (*Redrawn from K. A. Bisset, Bacteria, E. and S. Livingstone, Ltd., Edinburgh, 1952.*)

Sphaerotilus

The cells typically occur as chains of rods enclosed within a sheath, as shown in Fig. 5-17. The sheath may branch, giving the impression that the cells are branched; however, this is recognized as "false branching" (Fig. 15-13). The cells that emerge from an open end or a break in the sheath are called *swarm cells*. The hollow sheaths accumulate. The swarm cells are rod-shaped and possess polar or subpolar flagella; they thus resemble pseudomonads in appearance. *S. natans* is a common species that normally occurs in polluted waters, and its sheaths, of organic composition, are thin and colorless. In unpolluted water containing iron, iron hydroxide may be deposited in or on the sheaths, which turn yellow-brown and may become encrusted with ferric iron. Hence, these organisms are sometimes referred to as "iron bacteria."

BUDDING AND/OR APPENDAGED BACTERIA

This group of nonphototrophic Gram-negative bacteria is characterized by the formation of *prosthecae* (extensions of the cell wall and cytoplasmic membrane) or *stalks* (nonliving ribbonlike or tubular appendages that are excreted by the cell) and/or by the asymmetric mode of reproduction called budding. The organisms range from aerobic to microaerophilic to facultatively anaerobic. Although nonphototrophic, some genera have morphologically similar counterparts in the phototrophic group of bacteria. A few examples of budding and/or appendaged bacteria are described below.

Prosthecate Budding Bacteria

Hyphomicrobium

Individual cells are initially coccoid and flagellated but mature into oval or bean-shaped cells. Prosthecae are produced at either one or both ends of a cell. Buds develop at the tips of the prosthecae (see Fig. 15-14A and also Fig. 7-1E), and as they mature they separate from the prosthecae. Hyphomicrobia are aerobic and chemoorganotrophic; however, their morphology is similar in many ways to that of the phototrophic genus *Rhodomicrobium* (see Fig. 15-3). Hy-

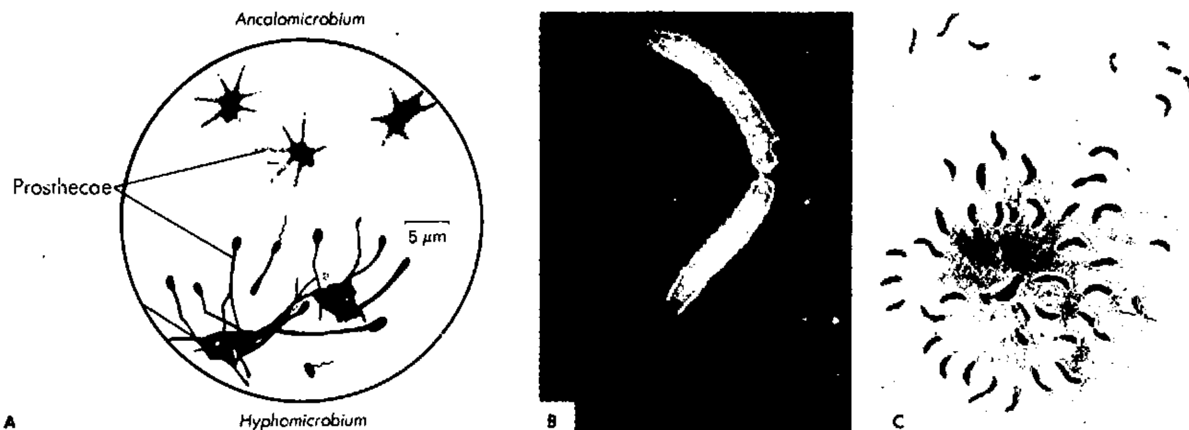


Figure 15-14. Budding and/or appendaged bacteria. (A) Drawing of cells of *Hyphomicrobium* and *Ancalomicrobium*. *(Erwin F. Lessel, illustrator.)* (B) *Caulobacter*, undergoing binary fission. The upper cell possesses a polar flagellum; the lower cell has a prostheca with terminal holdfast (X13,000). *(Courtesy of A. L. Houwink and W. van Iterson, Biochem Biophys Acta, 5:10, 1950.)* (C) *Caulobacter* cells attached to a common holdfast and exhibiting a rosette pattern. *(Courtesy of V. B. D. Skerman.)*

phomicrobia have been found widely in soils of all continents, as well as in numerous aquatic environments.

Ancalomicrobium

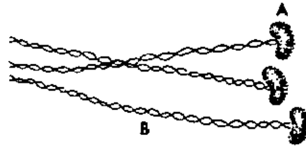
These facultatively anaerobic aquatic bacteria have three to eight, long, tapering prosthecae per cell (see Fig. 15-14A and also Fig. 5-18). Buds are formed directly from one position on the mother cell, never from the prosthecae. A genus of phototrophic green sulfur bacteria, ***Ancalochloris***, bears a morphological resemblance to *Ancalomicrobium*.

Prosthecate Nonbudding Bacteria

Caulobacter

In this genus of aerobic organisms, an individual cell is either a straight or curved rod with a single prostheca. The daughter cell arises by binary fission; it possesses a single polar flagellum (Fig. 15-14B) and is termed a *swarm cell*. The free motile swarm cell secretes an adhesive material (*holdfast*) at the end of the cell where the flagellum is located; eventually, a prostheca is produced at the same pole and the holdfast becomes located at the end of the prostheca. The flagellum is usually lost during formation of the prostheca. By means of the holdfast, cells may become attached to each other to form rosettes (Fig. 15-14C) or they may become attached to some other substance. Caulobacters are normally found in salt water and freshwater and have the ability to grow in environments with very low concentrations of nutrients.

Figure 15-15. Electron micrograph of stalked cells of a member of the *Blastocaulis-Planctomyces* group, morphotype II. A bud developing from a mother cell can be seen at the lower right (X4,400). *(Courtesy of Jean M. Schmidt, Arizona State University.)*



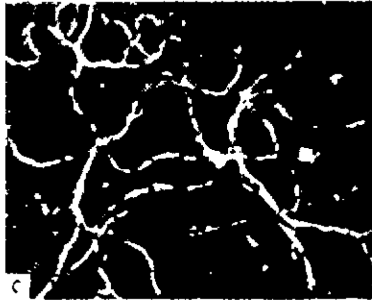


Figure 15-16. *Gallionella*, showing the kidney-shaped cells (A) bearing twisted stalks (B). (C) *Gallionella* sp. from salt water showing long entangled stalks (dark-phase microscopic preparation; X147). (*From J. M. Sharpley, Appl Petrol Microbiol, 9:380, 1961.*)

Nonprosthecate Budding Bacteria

Bacteria of the *Blastocaulis-Planctomyces* group occur in freshwater, brackish, or marine environments. The cells are spherical or ovoid and possess a stalk with a holdfast at the distal end. Budding occurs directly from a mother cell, as shown in Fig. 15-15.

Nonprosthecate, Nonbudding Bacteria

Gallionella

A twisted ribbonlike stalk extends from the middle of the curved or kidney-shaped cells (Fig. 15-16). The cells reproduce by binary fission. The organisms grow under microaerophilic conditions where both O_2 and a supply of ferrous iron are being continuously supplied. Since ferrous iron spontaneously oxidizes in the presence of O_2, these environmental conditions are unusual but can be met in aerated, iron-removal water-treatment plants, drainage from certain coal mines, or in various thermal springs. It is likely that *Gallionella* is autotrophic, obtaining energy by oxidizing ferrous iron to the ferric form. Because of its ability to form insoluble oxidized iron compounds, *Gallionella* may cause problems, such as clogging, in pipelines of water systems.

CHEMOLITHOTROPHIC BACTERIA

Three distinct metabolic types constitute this category of Gram-negative autotrophic bacteria, namely:

1 Obtain energy by oxidizing ammonia or nitrite (family *Nitrobacteraceae*)
2 Obtain energy by oxidizing sulfur or sulfur compounds; not assigned to any family
3 Deposit iron and/or manganese oxides (family *Siderocapsaceae*)

The Family *NITROBACTERACEAE*

The "nitrifying bacteria," as these organisms are called, include species of diverse morphological types—rods, cocci, and helical cells. They are nonmotile or motile by subpolar or peritrichous flagella. They are aerobic autotrophs, incapable of chemoheterotrophic growth with the exception of one species, *Nitrobacter winogradskyi.* The nitrifying bacteria comprise two distinct metabolic groups in terms of reactions that provide energy: (1) those which oxidize

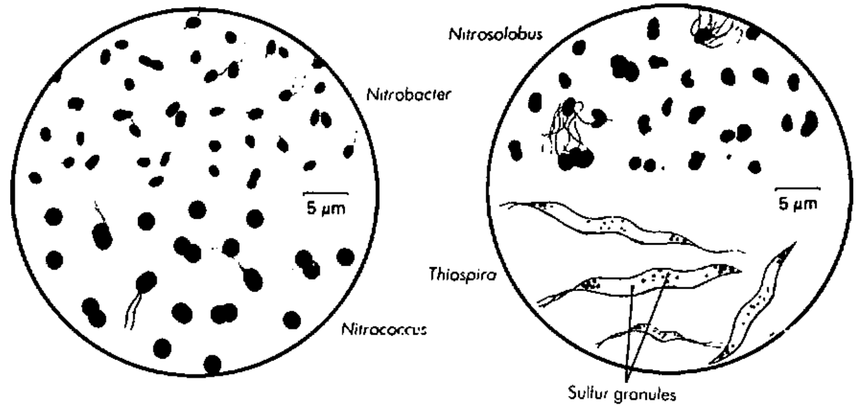


Figure 15-17. Drawing of chemolithoautotrophic bacteria. Nitrifying bacteria of the genera *Nitrobacter*, *Nitrococcus*, and *Nitrosolobus*, and sulfide-oxidizing bacteria of the genus *Thiospira*. (*Erwin F. Lessel, illustrator.*)

Figure 15-18. Electron micrograph of a longitudinal section of *Nitrobacter winogradskyi* showing intracellular membranes derived from invagination of the cytoplasmic membrane. This species reproduces by budding. The bar indicates 0.25 μm. (*From S. W. Watson, Int J Syst Bacteriol*, **21**:254–270, 1971.)

nitrite to nitrate, the generic names beginning with the prefix *nitro*, e.g., *Nitrobacter* (Fig. 15-17), *Nitrococcus* (Fig. 15-17), and *Nitrospina;* and (2) those which oxidize ammonia to nitrite, the generic names beginning with the prefix nitroso, e.g., *Nitrosolobus* (Fig. 15-17), *Nitrosomonas, Nitrosovibrio, Nitrosococcus,* and *Nitrosospira*. Some species have extensive invaginations of the cytoplasmic membrane [e.g., see *N. winogradskyi* (Fig. 15-18) and *Nitrosococcus oceanus* (Fig. 5-27)]. Nitrifying bacteria are commonly found in soils, where they play an important role in the nitrogen cycle and in maintaining the fertility of soil. They are discussed in more detail in Chap. 25.

Sulfur- and Sulfur-Compound-Metabolizing Bacteria

The bacteria of this group can be divided into those genera which have been isolated into pure culture and those which have not yet been isolated (noncultivatable). The cultivatable genera contain Gram-negative short rods (*Thiobacillus*) or helical cells (*Thiomicrospira*); most species are motile by means of polar flagella. Both genera are widely distributed in soil, freshwaters, coal-mine

drainage waters, and marine environments. They derive energy from the oxidation of reduced sulfur compounds, including sulfides, elemental sulfur, thiosulfate, polythionates, and sulfite. The final oxidation product is sulfate. One species, *Thiobacillus ferrooxidans*, can also derive energy by oxidizing ferrous iron to the ferric form. Some species are obligate autotrophs (e.g., *Thiobacillus thioparus*, *Thiobacillus thiooxidans*, and *Thiomicrospira pelophila*), and others are facultative autotrophs (e.g., *Thiobacillus novellus*). Most species are strictly aerobic, but a few can also grow anaerobically with nitrate as the terminal electron acceptor (e.g., *Thiobacillus denitrificans* and *Thiomicrospira denitrificans*). Some species are acidophilic. For instance, *T. thiooxidans* grows best at pH values of 2 to 5; moreover, it produces so much sulfuric acid that the pH of the medium may decrease to 0 or lower.

The noncultivatable genera include *Thiobacterium*, *Macromonas*, *Thiovulum*, *Achromatium*, and *Thiospira*. These genera are recognized by their distinctive morphological features as observed in samples from natural sources. Because these organisms have not been isolated, their metabolic nature cannot yet be described with certainty; however, they are probably chemolithotrophic because (1) they occur only in environments having a continuous supply of O_2 and H_2S, and (2) they accumulate sulfur granules intracellularly (e.g., see *Thiospira*, Fig. 15-17). H_2S is unstable (oxidized nonbiologically) in the presence of O_2; consequently, the environments that support growth are those located between the aerobic and anaerobic zones of freshwater or marine environments or in H_2S-bearing springs.

The Family *SIDEROCAPSACEAE*

This group includes unicellular, aerobic to microaerophilic organisms which do not form prosthecae or stalks and which deposit iron or manganese oxides on or in capsules or exocellular slime. The group includes such genera as *Siderocapsa*, *Siderococcus*, *Siderocystis*, and *Naumanniella*. (The prefix *sidero* which occurs in some of these names is derived from the Greek noun *siderus* meaning "iron.") There is some question as to whether they are indeed chemolithotrophs, since they generally occur in environments containing not only iron and manganese but also organic matter (stagnant waters, swamp ditches, mud, etc.). Only three species, *Siderocapsa eusphaera*, *Siderocapsa geminata*, and *Naumanniella polymorpha* have apparently been isolated (although their morphology differs from that seen in nature), but only *N. polymorpha* seems to be capable of autotrophic growth. The other species have not been isolated and are recognized solely by their morphological features as observed in samples from natural sources.

ARCHAEOBACTERIA

Evidence based on studies of ribosomal RNA indicates that archaeobacteria and eubacteria diverged at a very early stage in the evolution of life on earth (see Chap. 3). The phylogenetic gap that exists between the two groups is reflected by certain phenotypic differences, some of which are summarized in Table 15-2. One should recognize, however, that archaeobacteria do not comprise a homogeneous group. Just as great heterogeneity occurs among the eubacteria, so do the various kinds of archaeobacteria differ from each other in terms of morphology, chemical composition, metabolism, and habitat. At present, three

Table 15-2. Some Differences between Archaeobacteria and Eubacteria

Characteristic	Archaeobacteria	Eubacteria
Cell Walls		
Peptidoglycan containing muramic acid and D-amino acids is present	–	+
Lipids of Cytoplasmic Membrane		
Long-chain fatty acids bound to glycerol by ester linkages	–	+
Long-chain branched alcohols (phytanols) bound to glycerol by ether linkages	+	–
Properties Related to Protein Synthesis		
First amino acid to initiate a new polypeptide chain is		
Methionine	+	–
N-Formylmethionine	–	+
Translation process sensitive to action of		
Diphtheria toxin*	+	–
Chloramphenicol†	–	+

* For the action of diphtheria toxin, see Chap. 31.
† For the action of the antibiotic chloramphenicol, see Chap. 24.

main categories of archaeobacteria are recognized: the methane-producers (methanogens), the red extreme halophiles, and the thermoacidophiles.

Methanogenic Bacteria

These archaeobacteria are stringent anaerobes that share an ability to obtain energy for growth by oxidizing compounds such as H_2 or formate, and utilizing the electrons thus generated to reduce CO_2 with the formation of methane gas (CH_4). Some genera can grow as autotrophs, using H_2 and CO_2 as sole sources of carbon and energy; others require additional substances such as vitamins, acetate, amino acids, or organic sulfur compounds. Most species grow better in complex media (e.g., containing yeast extract) than in inorganic media.

At least two unusual coenzymes occur in almost all methanogens that have not been found in other bacteria: *Coenzyme M*, involved in methyl transfer reactions, and *Coenzyme* F_{420}, a flavin-like compound involved in the anaerobic electron transport system of these bacteria. The latter coenzyme fluoresces under ultraviolet light. Its presence can be detected by observing the organisms with a fluorescence microscope; this provides a convenient means to identify methanogens.

The genera of methane-producing bacteria are differentiated on the basis of morphology and Gram reaction (see Table 15-3 and Fig. 15-19). Differences in cell wall composition have been found to correlate with these genera (Table 15-3). The cell walls of two genera consist of *pseudomurein*, which differs from eubacterial peptidoglycan by (a) substitution of N-acetyltalosaminuronic acid for N-acetylmuramic acid, and (b) by a tetrapeptide composed entirely of L-amino acids, with glutamic acid at the C-terminal end.

Methanogens occur in various anaerobic habitats rich in organic matter which nonmethanogenic bacteria ferment to produce H_2 and CO_2. Such habitats include marshes, swamps, pond and lake mud, marine sediments, the intestinal tract of humans and animals, the rumen of cattle, and anaerobic sludge digesters in sewage-treatment plants.

Table 15-3. Methanogenic Bacteria

Genus	Morphology	Motility	Wall Composition
Methanobacterium	Gram-positive to Gram-variable long rods	–	Pseudomurein
Methanobrevibacter	Gram-positive lancet-shaped cocci or short rods	–	Pseudomurein
Methanomicrobium	Gram-negative short rods	+, single polar flagellum	Protein
Methanogenium	Gram-negative pleomorphic cocci	+, peritrichous flagella	Protein
Methanospirillum	Gram-negative curved rods or long wavy filaments	+, polar flagella	Protein; an external sheath is present
Methanosarcina	Gram-positive cocci in clusters	–	Heteropolysaccharide
Methanococcus	Gram-negative pleomorphic cocci	+, one flagellar tuft	Protein with trace of glucosamine

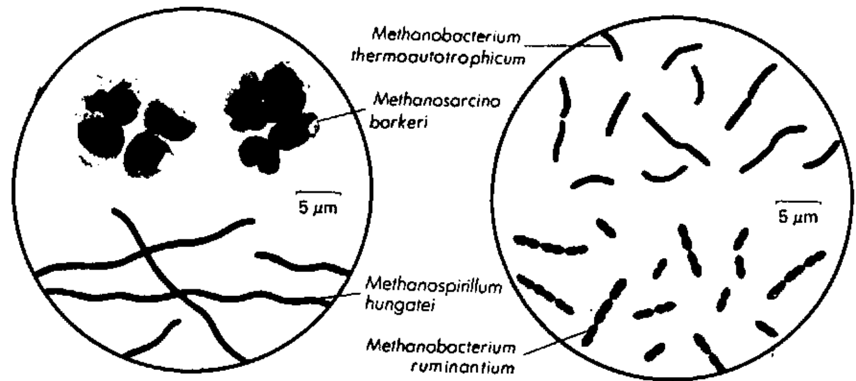


Figure 15-19. Drawing of the cells of various methane-producing bacteria. *(Erwin F. Lessel, illustrator.)*

Extreme Halophiles

These chemoorganotrophic, aerobic bacteria require approximately 17 to 23 percent NaCl for good growth. They stain Gram-negative and range from rod- or disk-shaped cells (the genus *Halobacterium*) to cocci (the genus *Halococcus*). They occur in salt lakes (e.g., the Dead Sea and the Great Salt Lake), industrial plants that produce salt by solar evaporation of sea water, and salted proteinaceous materials such as salted fish (in which they may cause spoilage). The colonies are a red to orange color due to carotenoids which seem to protect the cells against the damaging effect of sunlight.

At high NaCl concentrations the cells resist dehydration by maintaining a high intracellular osmotic concentration of KCl. The cytoplasmic membrane and ribosomes are stable only at high concentrations of KCl, and the enzymes are active only at high levels of either KCl or NaCl. *Halobacterium* cell walls are

composed of protein subunits that are held together only in the presence of salt; thus if the level of NaCl falls below about 10 percent, the cells lyse. On the other hand, the walls of *Halococcus* cells are composed of a complex heteropolysaccharide that is stable even at low salt concentrations.

ATP Synthesis

Halobacteria are mainly aerobic. As in other aerobic organisms, an electron transport chain generates a protonmotive force which in turn drives ATP synthesis (see Chap. 10). Halobacteria can also generate ATP by fermenting the amino acid arginine; this allows them to grow anaerobically. However, a third method of ATP generation is unique to halobacteria. At low oxygen levels, patches of a purple pigment called *bacteriorhodopsin* are formed in the cell membrane. (This pigment is so named because of its similarity to the photosensitive pigment rhodopsin that occurs in the retinal rods of higher vertebrates.) When cells containing the bacteriorhodopsin are exposed to light, the pigment bleaches. During this bleaching, protons (hydrogen ions, H^+) are extruded to the outside of the membrane, thus creating a protonmotive force which in turn drives ATP synthesis. Thus, like the phototrophic bacteria considered earlier in this chapter, halobacteria possess a mechanism for light-driven synthesis of ATP; however, *they possess no bacteriochlorophyll.*

Thermoacidophiles

These aerobic Gram-negative archaeobacteria are characterized by a remarkable ability to grow under *highly acidic conditions at high temperatures.* Two genera included in this group are described below.

Thermoplasma

These chemoorganotrophic organisms resemble mycoplasmas (see Chap. 13) by *lacking a cell wall and forming tiny "fried-egg" colonies.* Like mycoplasmas, the cells are pleomorphic, ranging from spherical to filamentous. The optimum temperature for growth is 55 to 59°C (maximum, 62°C; minimum, 40°C), and the optimum pH is 2 (maximum, 4; minimum, 1). Cells undergo lysis at a neutral pH. Thermoplasmas have been isolated from piles of burning coal refuse.

Sulfolobus

Cells of this genus are spherical or lobe-shaped. Unlike thermoplasmas, a cell wall is present (composed mainly of protein). Various species have temperature optima ranging from 70 to 87°C. The optimum pH is 2 (maximum, 4; minimum, 1). *Sulfolobus* species are facultatively autotrophic. They can grow as chemolithotrophs when supplied with elemental sulfur as an electron donor. Alternatively, they can grow as chemoorganotrophs in media containing organic substrates. In nature, the organisms are predominant in acidic hot springs.

QUESTIONS

1. List the major differences between the families of anoxygenic phototrophic bacteria.
2. In what ways do cyanobacteria differ from other phototrophic bacteria?
3. If cyanobacteria evolve oxygen, and if nitrogenase is oxygen-labile, how can cyanobacteria fix nitrogen?
4. How do the members of the order *Myxobacterales* differ from other gliding bacteria?
5. Besides the organisms listed under Gliding, Nonfruiting Bacteria, what other kinds of bacteria may exhibit gliding motility?

6 Prosthecae occur in bacteria other than those described in the section entitled Budding and/or Appendaged Bacteria. Give two examples.
7 Give an example of a genus of budding bacteria in which (a) the bud forms on a prostheca, (b) the bud forms directly on the mother cell.
8 How does "false branching" differ from "true branching"?
9 In what environments would one expect to find the following bacteria?
(a) *Halobacterium* (d) *Sulfolobus*
(b) *Chromatium* (e) *Gallionella*
(c) *Capnocytophaga* (f) *Sphaerotilus*
10 What is the most significant difference between the following?
(a) *Methylococcus* (see Chap. 13) and *Methanococcus*
(b) *Rhodomicrobium* and *Hyphomicrobium*
(c) *Nitrococcus* and *Nitrosococcus*
(d) *Aquaspirillum* (see Chap. 13), *Rhodospirillum*, *Thiospirillum*, and *Methanospirillum*
11 List the features of archaeobacteria that distinguish them from eubacteria.
12 What property of *Halobacterium* might allow one to consider this genus as facultatively phototrophic? What differences exist between halobacteria and the phototrophic bacteria described in the first section of this chapter?
13 Define the following terms:

Bacteriorhodopsin	Akinete	Fruiting body
Phycobilins	Coenzyme F_{420}	Swarm Cells
Thylakoids	Carotenoids	Thermoacidophile
Sporangiole	Heterocyst	Pseudomurein

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