

## CHAPTER 1

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# INTRODUCTION TO MICROBIAL PHYSIOLOGY

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### THE *ESCHERICHIA COLI* PARADIGM

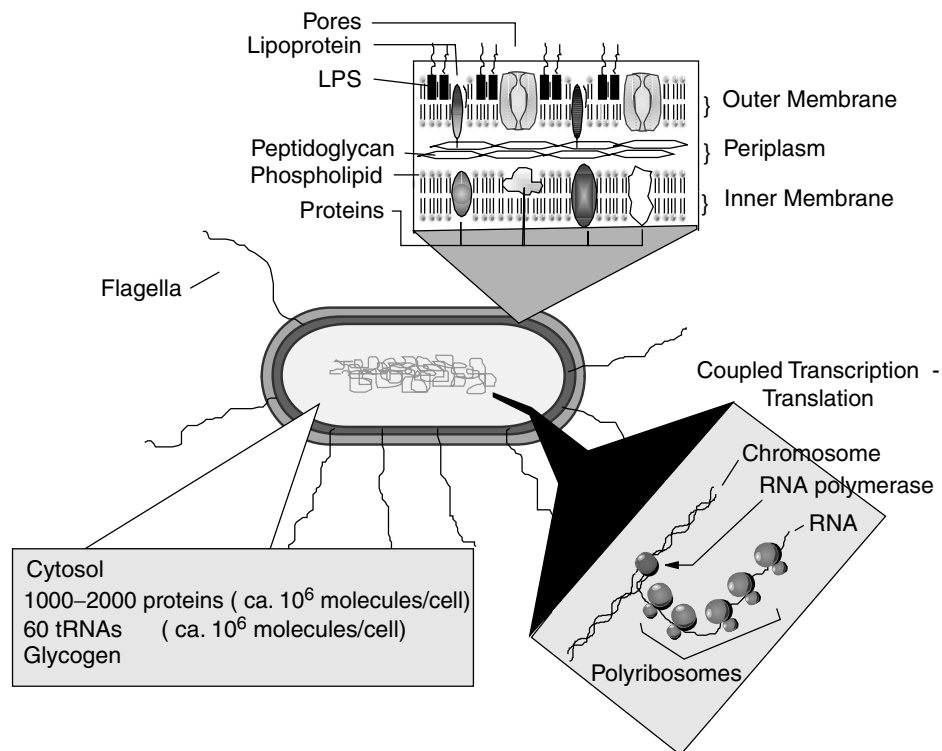
Microbial physiology is an enormous discipline encompassing the study of thousands of different microorganisms. It is, of course, foolhardy to try to convey all that is known on this topic within the confines of one book. However, a solid foundation can be built using a limited number of organisms to illustrate key concepts of the field. This text helps set the foundation for further inquiry into microbial physiology and genetics. The gram-negative organism *Escherichia coli* is used as the paradigm. Other organisms that provide significant counterexamples to the paradigm or alternative strategies to accomplish a similar biochemical goal are also included. In this chapter we paint a broad portrait of the microbial cell with special focus on *E. coli*. Our objective here is to offer a point of confluence where the student can return periodically to view how one aspect of physiology might relate to another. Detailed treatment of each topic is provided in later chapters.

### CELL STRUCTURE

As any beginning student of microbiology knows, bacteria come in three basic models: spherical (coccus), rod (bacillus), and spiral (spirillum). They do not possess a membrane-bound nucleus as do eukaryotic microorganisms; therefore, they are prokaryotic. In addition to these basic types of bacteria, there are other more specialized forms described as budding, sheathed, and mycelial. Figure 1-1 presents a schematic representation of a typical (meaning *E. coli*) bacterial cell.

#### The Cell Surface

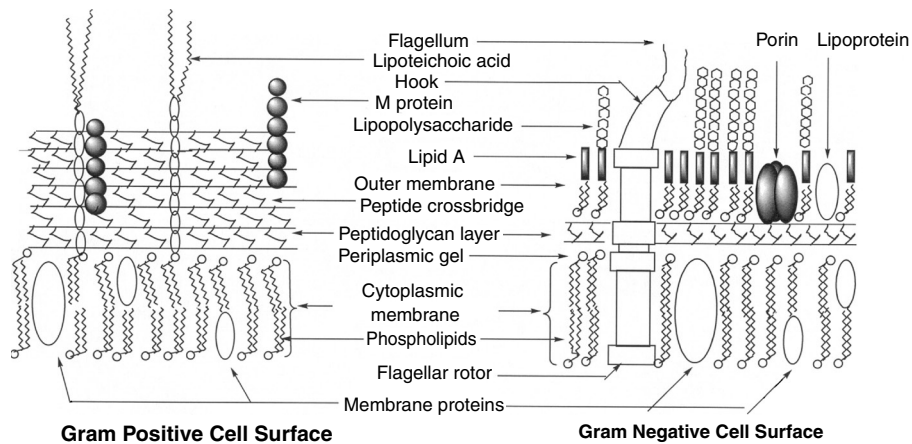
The interface between the microbial cell and its external environment is the cell surface. It protects the cell interior from external hazards and maintains the integrity of the cell



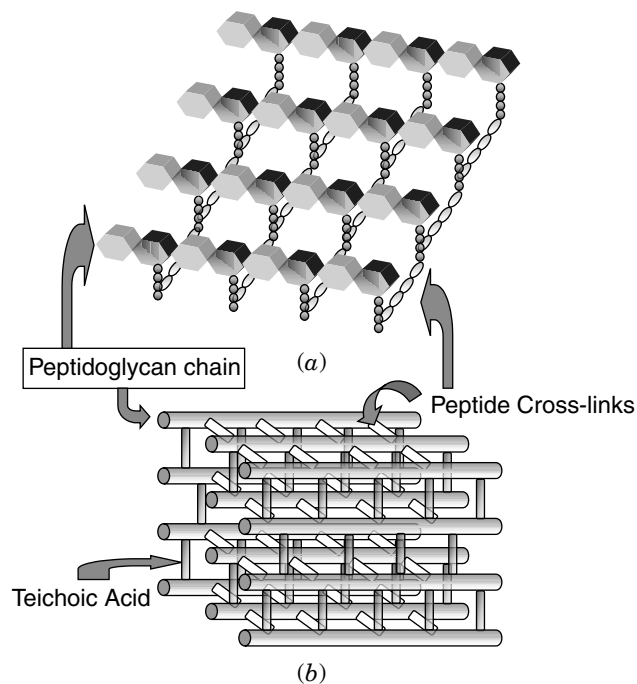
**Fig. 1-1. Diagrammatic representation of a “typical” bacterial cell (*Escherichia coli*).** Portions of the cell are enlarged to show further details.

as a discrete entity. Although it must be steadfast in fulfilling these functions, it must also enable transport of large molecules into and out of the cell. These large molecules include carbohydrates (e.g., glucose), vitamins (e.g., vitamin B<sub>12</sub>), amino acids, and nucleosides, as well as proteins exported to the exterior of the cell. The structure and composition of different cell surfaces can vary considerably depending on the organism.

**Cell Wall.** In 1884, the Danish investigator Christian Gram devised a differential stain based on the ability of certain bacterial cells to retain the dye crystal violet after decoloration with 95% ethanol. Cells that retained the stain were called gram positive. Subsequent studies have shown that this fortuitous discovery distinguished two fundamentally different types of bacterial cells. The surface of gram-negative cells is much more complex than that of gram-positive cells. As shown in the schematic drawings in Figure 1-2, the gram-positive cell surface has two major structures: the cell wall and the cell membrane. The cell wall of gram-positive cells is composed of multiple layers of peptidoglycan, which is a linear polymer of alternating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). A short peptide chain is attached to muramic acid. A common feature in bacterial cell walls is cross-bridging between the peptide chains. In a gram-positive organism such as *Staphylococcus aureus*, the cross-bridging between adjacent peptides may be close to 100%. By contrast, the frequency of cross-bridging in *Escherichia coli* (a gram-negative organism) may be as low as 30% (Fig. 1-3). Other components—for example, lipoteichoic acid (only



**Fig. 1-2. Composition of the cell surfaces of gram-positive and gram-negative bacteria.** Not all structures shown are found in all organisms. For example, M protein is only used to describe a structure in some of the streptococci. Also, not all organisms have flagella.



**Fig. 1-3. Diagrammatic views of bacterial peptidoglycan.** (a) Monolayer of peptidoglycan. Lightly shaded hexagons represent N-acetylglucosamine; darkly shaded hexagons represent N-acetylmuramic acid; vertically arranged spheres represent the peptide side chains; horizontal ovals represent the amino acid cross-bridges between peptide chains. (b) Diagrammatic representation of the multilayered peptidoglycan in the gram-positive cell wall. Long horizontal bars denote the chains of N-acetylglucosamine and N-acetylmuramic acid. Short horizontal bars indicate peptide cross-bridges and vertical bars represent teichoic acid.

present in gram-positive organisms)—are synthesized at the membrane surface and may extend through the peptidoglycan layer to the outer surface.

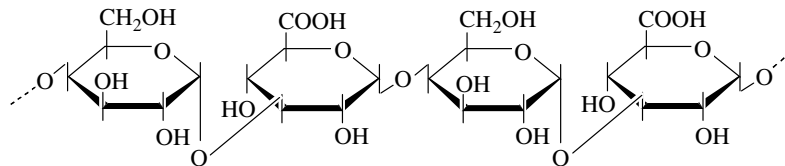
The peptidoglycan layer of a gram-negative cell is generally a single monolayer. An outer membrane surrounding the gram-negative cell is composed of phospholipids, lipopolysaccharides, enzymes, and other proteins, including lipoproteins. The space between this outer membrane and the inner membrane is referred to as the **periplasmic space**. It may be traversed at several points by various enzymes and other proteins (Fig. 1-2).

**Membranes.** The cytoplasmic membrane of both gram-positive and gram-negative cells is a lipid bilayer composed of phospholipids, glycolipids, and a variety of proteins. The proteins in the cytoplasmic membrane may extend through its entire thickness. Some of these proteins provide structural support to the membrane while others function in the transport of sugars, amino acids, and other metabolites.

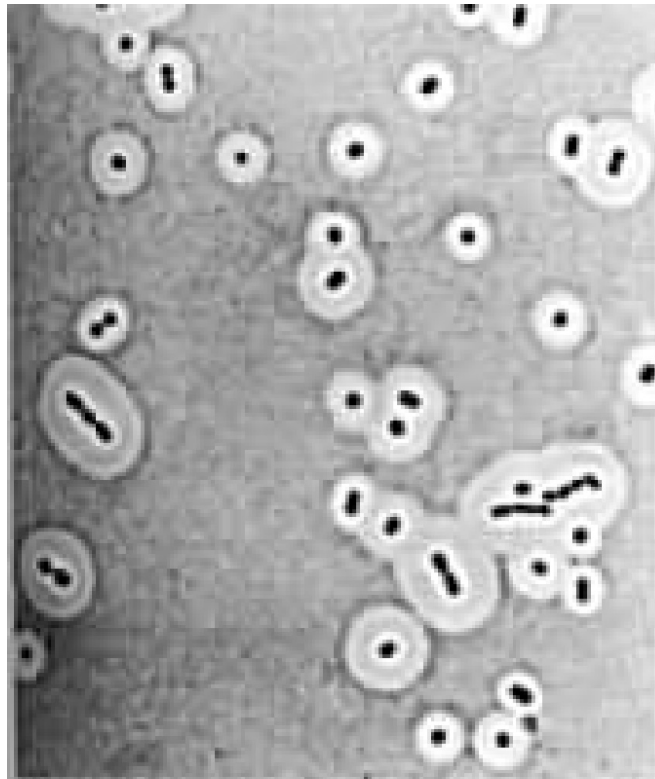
The outer membrane of gram-negative cells contains a relatively high content of **lipopolysaccharides**. These lipid-containing components represent one of the most important identifying features of gram-negative cells: the **O antigens**, which are formed by the external polysaccharide chains of the lipopolysaccharide. This lipid-containing component also displays **endotoxin** activity—that is, it is responsible for the shock observed in severe infections caused by gram-negative organisms. Bacterial cell surfaces also contain specific carbohydrate or protein receptor sites for the attachment of **bacteriophages**, which are viruses that infect bacteria. Once attached to these receptor sites, the bacteriophage can initiate invasion of the cell.

Gram-positive and gram-negative cells have somewhat different strategies for transporting materials across the membrane and into the cell. The cytoplasmic membrane of gram-positive organisms has immediate access to media components. However, chemicals and nutrients must first traverse the outer membrane of gram-negative organisms before encountering the cytoplasmic membrane. Gram-negative cells have **pores** formed by protein triplets in their outer membrane that will permit passage of fairly large molecules into the periplasmic space. Subsequent transport across the inner or cytoplasmic membrane is similar in both gram-positive and gram-negative cells.

**Capsules.** Some bacterial cells produce a capsule or a **slime layer** (Fig. 1-4) of material external to the cell. Capsules are composed of either polysaccharides (high-molecular-weight polymers of carbohydrates) or polymers of amino acids called polypeptides (often formed from the D- rather than the L-isomer of an amino acid). The capsule of *Streptococcus pneumoniae* type III is composed of glucose and glucuronic acid in alternating  $\beta$ -1, 3- and  $\beta$ -1, 4- linkages:



This capsular polysaccharide, sometimes referred to as pneumococcal polysaccharide, is responsible for the virulence of the pneumococcus. *Bacillus anthracis*, the anthrax



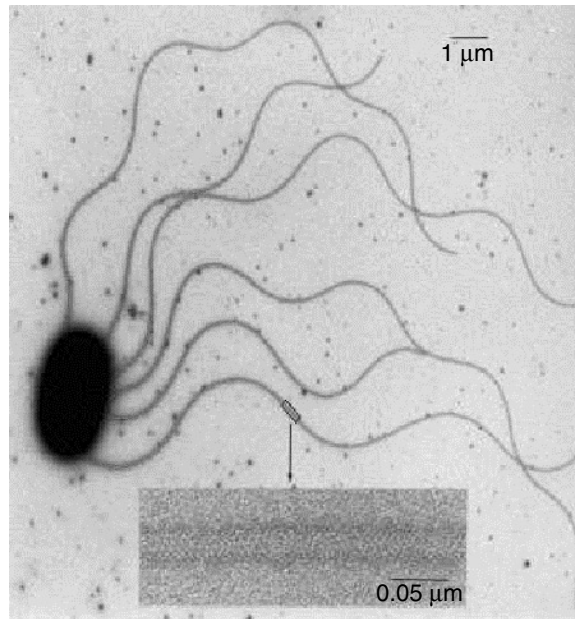
**Fig. 1-4. Capsules of *Streptococcus pneumoniae*.**

bacillus, produces a polypeptide capsule composed of D-glutamic acid subunits, which is a virulence factor for this organism.

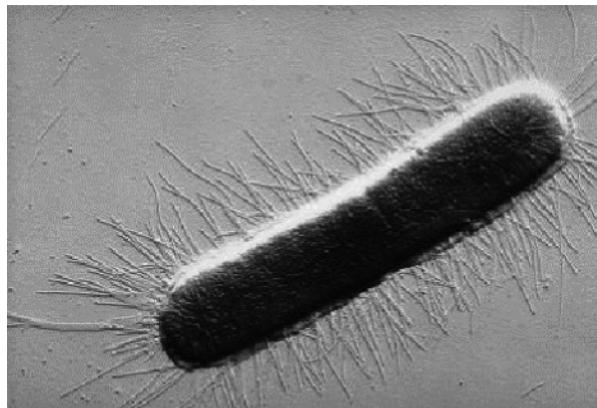
**Organs of Locomotion.** Many microorganisms are motile—that is, able to move from place to place in a concerted manner—especially in an aqueous environment. In the case of bacteria, this motility is accomplished by means of simple strands of protein (flagellin) woven into helical organelles called flagella. The bacterial flagellum is attached at the cell surface by means of a basal body (Fig. 1-5a). The basal body contains a motor that turns the flagellum, which propels the organism through the liquid environment.

**Pili or Fimbriae.** Many bacteria possess external structures that are shorter and more rigid than flagella. These structures have been termed pili (from Latin meaning “hair”) or fimbriae (from Latin meaning “fringe”). These appendages also appear to arise from a basal body or granule located either within the cytoplasmic membrane or in the cytoplasm immediately beneath the membrane (Fig. 1-5b). Generalized or common pili play a role in cellular adhesion to surfaces or to host cells.

**Ribosomes.** The cytoplasm of all cells has a fine granular appearance observed in many electron micrographs. Tiny particles called ribosomes are responsible for this look. Ribosomes contain approximately 65% RNA and 35% protein (see Fig. 1-1).



(a)



(b)

**Fig. 1-5. Microbial appendages.** (a) Flagella of *Salmonella typhimurium*. (b) Pili of *Escherichia coli*. (Source: Pili Image courtesy Indigo Instruments. Visit <http://www.indigo.com>.) Reprint permission is granted with this footer included.

The ribosome orchestrates the polymerization of amino acids into proteins (i.e., protein synthesis). At higher magnification under the electron microscope the ribosome particles are spherical. In properly prepared specimens the ribosomes are observed as collections or chains held together on a single messenger RNA (mRNA) molecule and are referred to as **polyribosomes** or simply polysomes.

The more or less spherical ribosome particle, when examined by sucrose gradient sedimentation, has been found to have a svedberg coefficient of 70S. (A svedberg unit denotes the rate of sedimentation of a macromolecule in a centrifugal field and

is related to the molecular size of that macromolecule.) The prokaryotic ribosome may be separated into two lower-molecular-weight components: one of 50S and another of 30S. Only the complete 70S particle functions in polypeptide synthesis. By comparison, the ribosomes of eukaryotic cells are associated with the endoplasmic reticulum, are larger (80S), and are composed of 40S and 60S subunits. The function of both 70S and 80S ribosomes in protein synthesis is identical. Curiously, eukaryotic mitochondria characteristically display 70S ribosomes—not the 80s particles that you would expect—because mitochondria probably evolved from endosymbiotic prokaryotic cells, a hypothesis supported by extensive analyses comparing bacterial and mitochondrial genomes.

### SYNTHESIS OF DNA, RNA, AND PROTEIN

The chromosome of *E. coli* is a single, circular, double-stranded DNA molecule whose nucleotide sequence encodes all the information required for cell growth and structure. The major molecular events required for propagating the species start with the chromosome and include DNA replication, transcription, and translation. In bacteria, replication involves the accurate duplication of chromosomal DNA and the formation of two daughter cells by **binary fission**. In binary fission the cell grows until a certain mass-to-DNA ratio is achieved, at which point new DNA is synthesized and a centrally located cross-wall is constructed that will ultimately separate the two daughter cells.

A simplified view of **DNA replication** in *E. coli* is shown in the diagram in Figure 1-6. The double-stranded DNA molecule unwinds from a specific starting point (**origin**). The new DNA is synthesized opposite each strand. The enzyme involved in

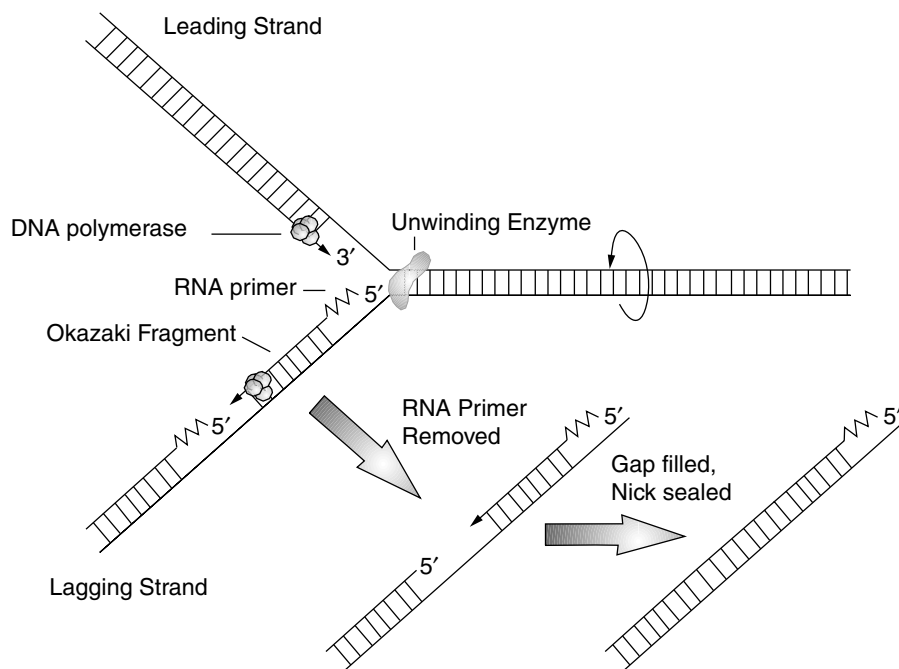
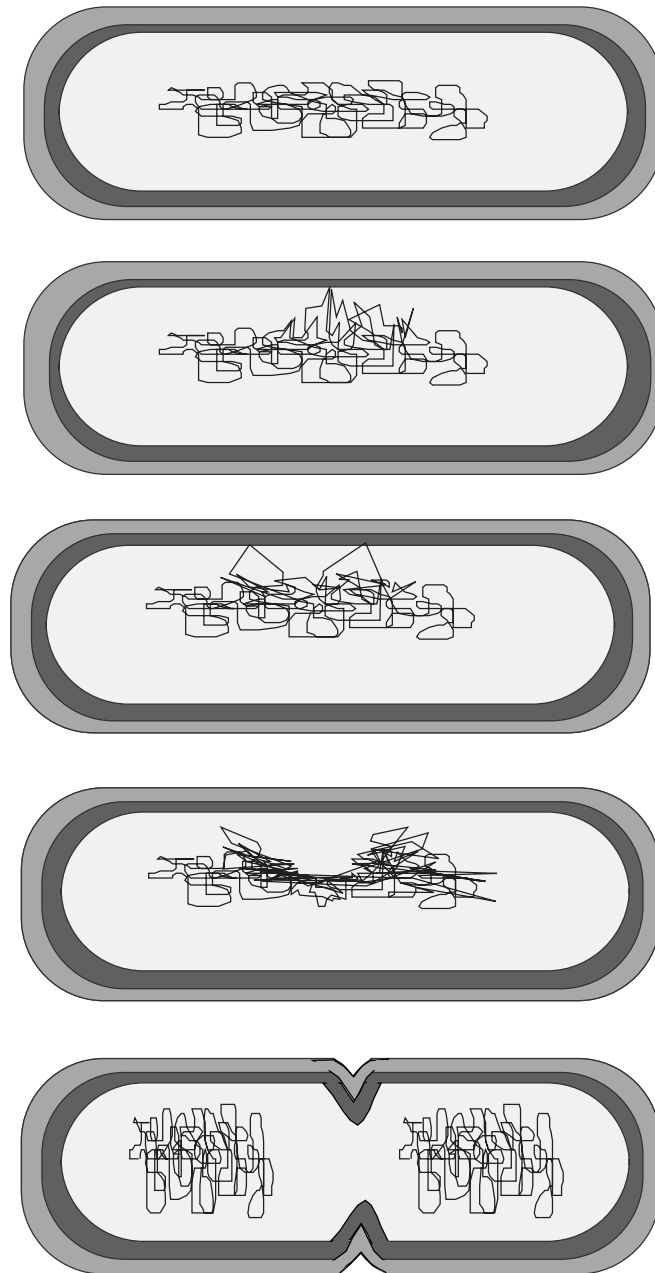


Fig. 1-6. Simplified depiction of DNA replication.



**Fig. 1-7. Segregation of the bacterial chromosome.**

replication (**DNA polymerase**) uses a parent strand as a template, placing adenine residues opposite thymine, and cytosine residues opposite guanine. New DNA is synthesized in both directions from the origin and continues until both replication forks meet at the **terminus** 180° from the origin. At this point, cell division proceeds with cross-wall formation occurring between the two newly synthesized chromosomes



(Fig. 1-7). Note that the chromosome appears attached to the cell membrane as the daughter chromosomes begin to separate. At some point about midway to the ends of the cell, the nascent chromosomes separate from the membrane but continue to move toward the cell poles by a still undefined mechanism. Chromosomal segregation into pre-daughter cells must occur before the cell completes construction of the cross-wall that will separate the two offspring (see “Termination of DNA Replication and Chromosome Partitioning” in Chapter 2).

The genetic information contained within DNA is processed in two steps to produce various proteins. Protein synthesis (**translation**) is depicted in Figure 1-8. The enzyme **RNA polymerase** (DNA-dependent RNA polymerase) first locates the beginning of a gene (**promoter**). This area of the chromosome then undergoes a localized unwinding, allowing RNA polymerase to transcribe RNA from the DNA template. Before the RNA—called messenger RNA (mRNA)—is completely transcribed, a ribosome will attach to the beginning of the message.

As already noted, the ribosome contains of two subunits, 30S and 50S, each composed of special ribosomal proteins and **ribosomal ribonucleic acids (rRNA)**. rRNA molecules do not, by themselves, code for any protein but form the architectural scaffolding that directs assembly of the proteins to form a ribosome. The ribosome translates mRNA into protein by reading three nucleotides (known as a **triplet codon**) as a specific amino acid. Each amino acid used by the ribosome must first be attached to an adaptor or **transfer RNA (tRNA)** molecule specific for that amino acid. tRNA containing an attached amino acid is referred to as a **charged tRNA molecule**. A part of the tRNA molecule called the **anticodon** will base-pair with the codon in mRNA.

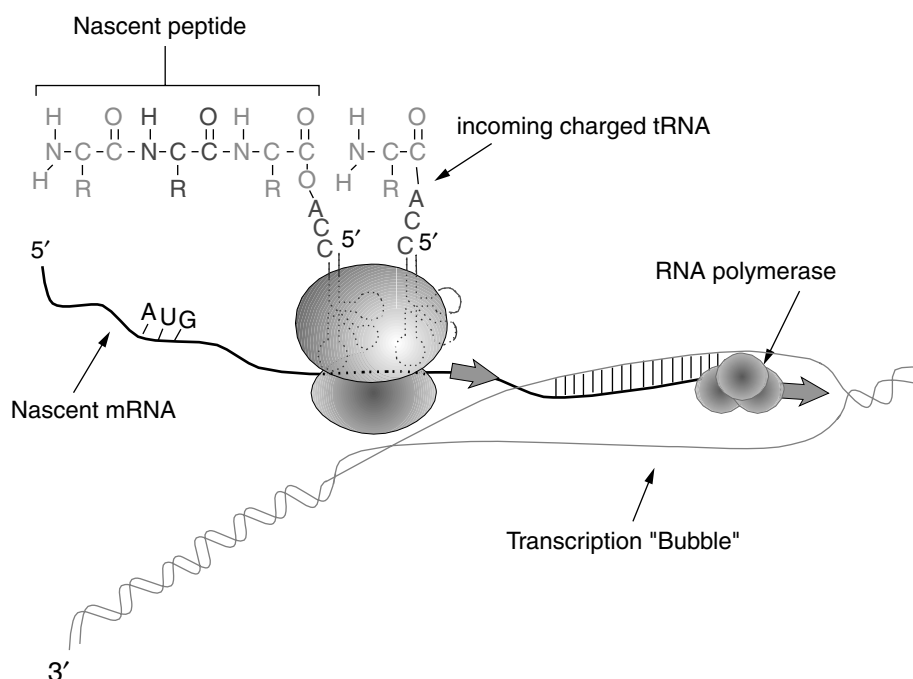


Fig. 1-8. Sequence of events involved in transcription and translation.

When two such charged tRNA molecules simultaneously occupy adjacent sites on the ribosome, the ribosome catalyzes the formation of a peptide bond between the two amino acids.

At this point, the two amino acids are attached to one tRNA while the other tRNA is uncharged and eventually released from the ribosome. The ribosome is then free to move along the message to the next codon. The process continues until the ribosome reaches the end of the message, at which point a complete protein has been formed. Notice that synthesis of the protein begins with the N-terminal amino acid and finishes with the C-terminal amino acid. Also note that the ribosome begins translating at the 5' end of the mRNA while the DNA strand encoding the mRNA is read by RNA polymerase starting at the 3' end. Although the beginning of a gene is usually called the 5' end, this doesn't refer to the strand that is actually serving as a template for RNA polymerase. It refers to the complementary DNA strand whose sequence is the same as the mRNA (except for containing T instead of U). The details of replication, transcription, and translation are discussed in Chapter 2.

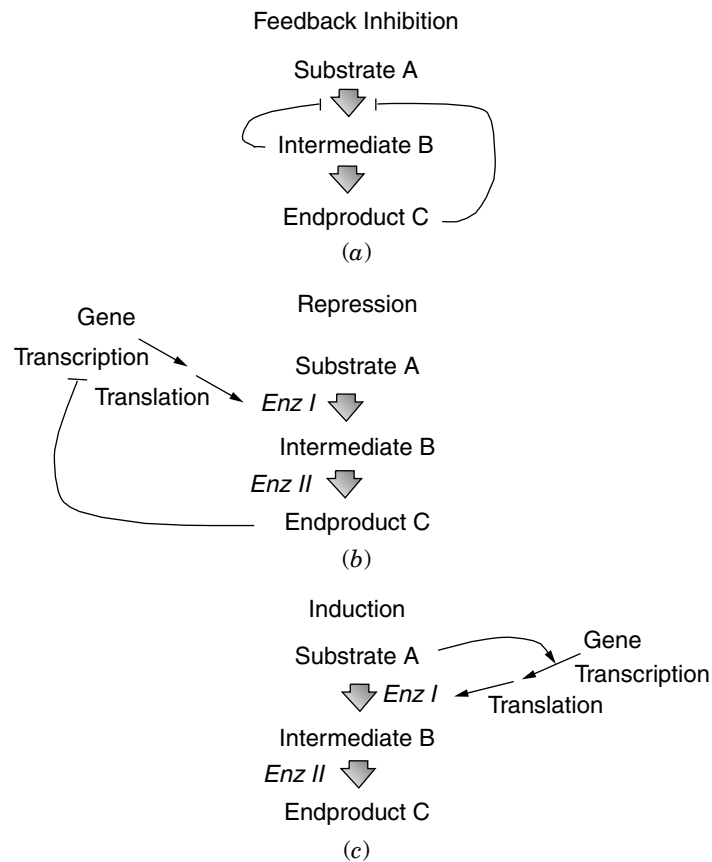
### Metabolic and Genetic Regulation

For a cell to grow efficiently, all the basic building blocks and all the macromolecules derived from them have to be produced in the correct proportions. With complex metabolic pathways, it is important to understand the manner by which a microbial cell regulates the production and concentration of each product. Two common mechanisms of metabolic and genetic regulation are

1. **Feedback inhibition** of enzyme activity (**metabolic regulation**)
2. **Repression** or **induction** of enzyme synthesis (**genetic regulation**)

In feedback inhibition, the *activity* of an enzyme already present in the cell is inhibited by the end product of the reaction. In genetic repression, the *synthesis* of an enzyme (see previous discussion of transcription and translation) is inhibited by the end product of the reaction. Induction is similar except the substrate of a pathway stimulates synthesis of the enzyme. Hypothetical pathways illustrating these concepts are presented in Figure 1-9. In Figure 1-9a, excessive production of intermediate B results in the inhibition of enzyme 1 activity, a phenomenon known as feedback or end-product inhibition. Likewise, an excess of end-product C may inhibit the activity of enzyme 1 by feedback inhibition.

In contrast to feedback inhibition, an excess intracellular concentration of end-product C may cause the cell to stop synthesizing enzyme 1, usually by inhibiting transcription of the genes encoding the biosynthetic enzymes (Fig. 1-9b). This action is referred to as **genetic repression**. The logic of this control is apparent when considering amino acid biosynthesis. If the cell has more than enough of a given amino acid, that amino acid will activate a repressor protein, which then blocks any further transcription of the biosynthesis genes. In contrast, substrates such as carbohydrates can stimulate the transcription of genes whose protein products consume that carbohydrate. This genetic process is called **induction** (Fig. 1-9c). Different organisms may employ quite different combinations of feedback inhibition, repression, and induction to regulate a metabolic pathway. In Chapter 5, these and other regulatory mechanisms are discussed in greater detail.



**Fig. 1-9. Diagrammatic presentation of feedback inhibition of enzyme activity and end-product repression of enzyme synthesis.** *a*, *b* and *c* are chemical intermediates in the hypothetical pathway. Arrow indicates activation, line with cross indicates inhibition.

## MICROBIAL GENETICS

Having just outlined the processes of transcription, translation, and replication, it is now possible to define several genetic terms. The gene may be defined as a heritable unit of function composed of a specific sequence of purine and pyrimidine bases, which in turn determines the base sequence in an RNA molecule, and, of course, the sequence of bases in an RNA molecule specifies the sequence of amino acids incorporated into a polypeptide chain. The **genotype** of an organism is the sum total of all of the hereditary units of genes. The observed expression of the genetic determinants—that is, the structural appearance and physiological properties of an organism—is referred to as its **phenotype**.

An individual gene can exist in different forms as a result of nucleotide sequence changes. These alternative gene forms are referred to as **alleles**. Genetic material is not absolutely stable but can change or mutate. The process of change is known as **mutagenesis**. Altered genes are referred to as mutant alleles in contrast to the normal or **wild-type** alleles. **Spontaneous mutations** are thought to arise during replication,

repair, and recombination of DNA as a result of errors made by the enzymes involved in DNA metabolism. Mutations may be increased by the activity of a number of environmental influences. Radiation in the form of X rays, ultraviolet (UV) rays, or cosmic rays may affect the chemical structure of the gene. A variety of chemicals may also give rise to mutations. Physical, chemical, or physicochemical agents capable of increasing the frequency with which mutations occur are referred to as **mutagens**. The resulting alterations are **induced mutations** in contrast to *spontaneous mutations*, which appear to occur at some constant frequency in the absence of intentionally applied external influences. Since bacterial cells are haploid, mutants are usually easier to recognize because the altered character is more likely to be expressed, particularly if the environment is favorable to mutant development.

The use of mutants has been a tremendous tool in the study of most, if not all, biochemical processes. Genes are usually designated by a three-letter code based on their function. For example, genes involved in the biosynthesis of the amino acid arginine are called *arg* followed by an uppercase letter to indicate different *arg* genes (e.g., *argA*, *argB*). A gene is always indicated by lowercase italic letters (e.g., *arg*), whereas an uppercase letter in the first position (e.g., *ArgA*) indicates the gene product. At this point, we need to expose a common mistake made by many aspiring microbial geneticists concerning the interpretation of mutant phenotypes. Organisms such as *E. coli* can grow on basic minimal media containing only salts, ammonia as a nitrogen source, and a carbon source such as glucose or lactose because they can use the carbon skeleton of glucose to synthesize all the building blocks necessary for macromolecular synthesis. The building blocks include amino acids, purines, pyrimidines, cofactors, and so forth. A mutant defective in one of the genes necessary to synthesize a building block will require that building block as a supplement in the minimal medium (e.g., an *arg* mutant will require arginine in order to grow). Microorganisms also have an amazing capacity to catabolically use many different compounds as carbon sources. However, a mutation in a carbon source utilization gene (e.g., *lac*) does not mean it requires that carbon source. It means the mutant will *not grow* on medium containing that carbon source if it is the only carbon source available (e.g., a *lac* mutant will not grow on lactose).

The chromosome of our reference cell, *E. coli*, is 4,639,221 base pairs long. Gene positions on this map can be given in base pairs starting from the gene *thrL*, or in minutes based on the period of time required to transfer the chromosome from one cell to another by conjugation (100-minute map with *thrL* at 0).

## CHEMICAL SYNTHESIS

### Chemical Composition

Our paradigm cell (the gram-negative cell *E. coli*) can reproduce in a minimal glucose medium once every 40 minutes. As we proceed through a detailed examination of all the processes involved, the amazing nature of this feat will become increasingly obvious. It is useful to discuss the basic chemical composition of our model cell. The total weight of an average cell is  $9.5 \times 10^{-13}$  g, with water (at 70% of the cell) contributing  $6.7 \times 10^{-13}$  g. The total dry weight is thus  $2.8 \times 10^{-13}$  g. The components that form the dry weight include protein (55%), ribosomal RNA (16.7%), transfer RNA (3%), messenger RNA (0.8%), DNA (3.1%), lipids (9.1%),

lipopolysaccharides (3.4%), peptidoglycans (2.5%), building block metabolites, vitamins (2.9%), and inorganic ions (1.0%). It is interesting to note that the periplasmic space forms a full 30% of the cell volume, with total cell volume being approximately  $9 \times 10^{-13}$  ml (0.9 femtoliters). An appreciation for the dimensions of the cell follows this simple example. One teaspoon of packed *E. coli* weighs approximately 1 gram (wet weight). This comprises about one trillion cells—more than 100 times the human population of the planet. When calculating the concentration of a compound within the cell, it is useful to remember that there are 3 to 4 microliters of water per 1 milligram of dry weight. Our reference cell, although considered haploid, will contain two copies of the chromosome when growing rapidly. It will also contain 18,700 ribosomes and a little over 2 million total molecules of protein, of which there are between 1000 and 2000 different varieties. As you might gather from these figures, the bacterial cell is extremely complex. However, the cell has developed an elegant strategy for molecular economy that we still struggle to understand. Some of what we have learned is discussed throughout the remaining chapters.

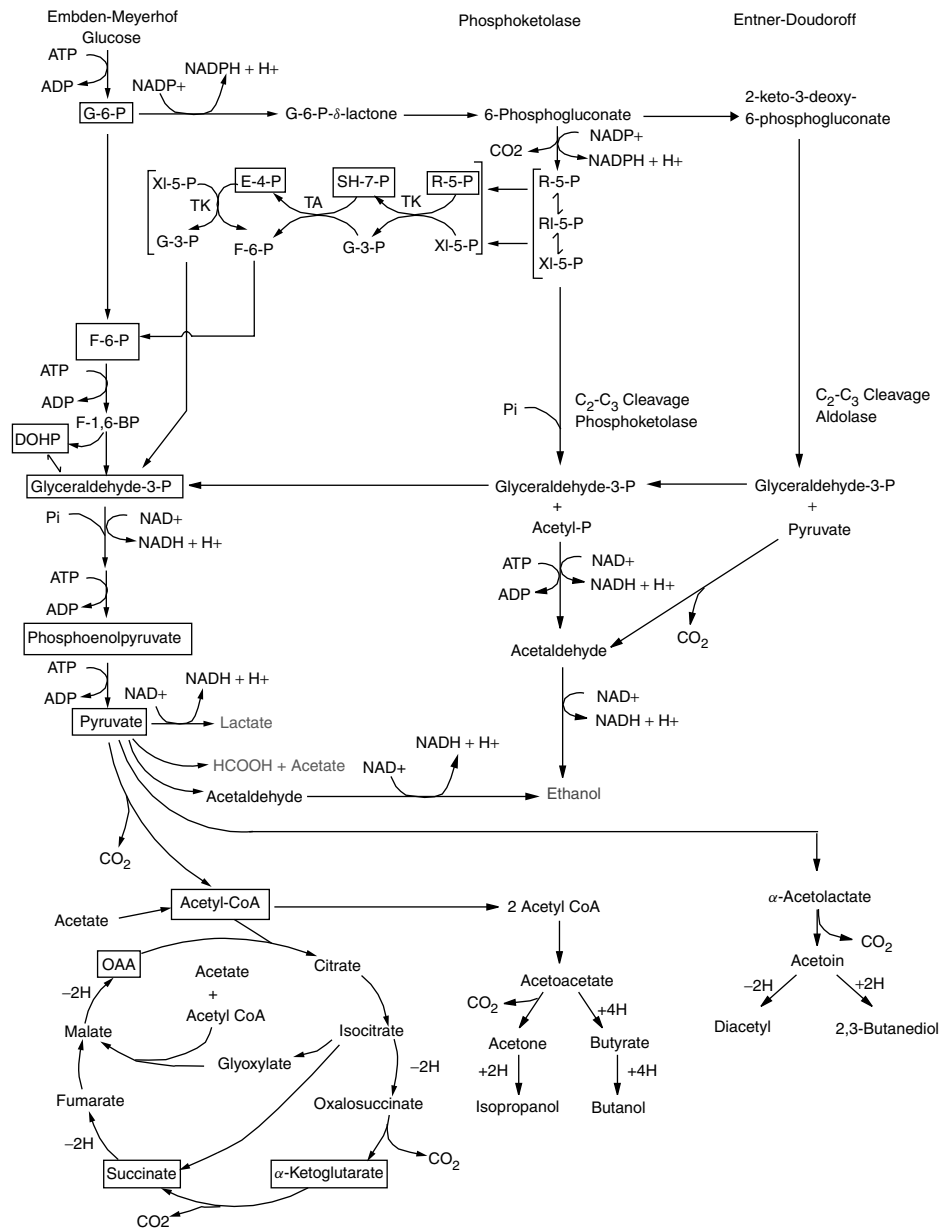
In just 40 minutes an *E. coli* cell can make a perfect copy of itself growing on nothing more than glucose, ammonia, and some salts. How this is accomplished seems almost miraculous! All of the biochemical pathways needed to copy a cell originate from just 13 precursor metabolites. To understand microbial physiology, you must first discover what the 13 metabolites are and where they come from.

The metabolites come from glucose or some other carbohydrate. The catabolic dissimilation of glucose not only produces them but also generates the energy needed for all the work carried out by the cell. This work includes biosynthetic reactions as well as movement, transport, and so on. Figure 1-10 is a composite diagram of major pathways for carbohydrate metabolism with the 13 metabolites highlighted. Most of them are produced by the Embden-Meyerhof route and the tricarboxylic acid cycle (see Fig. 1-10). Three are produced by the pentose phosphate pathway. Figure 1-11 illustrates how these compounds are siphoned off from the catabolic pathways and used as starting material for the many amino acids, nucleic acid bases, and cofactors that must be produced. Subsequent chapters deal with the specifics of each pathway, but this figure presents an integrated picture of cell metabolism.

## Energy

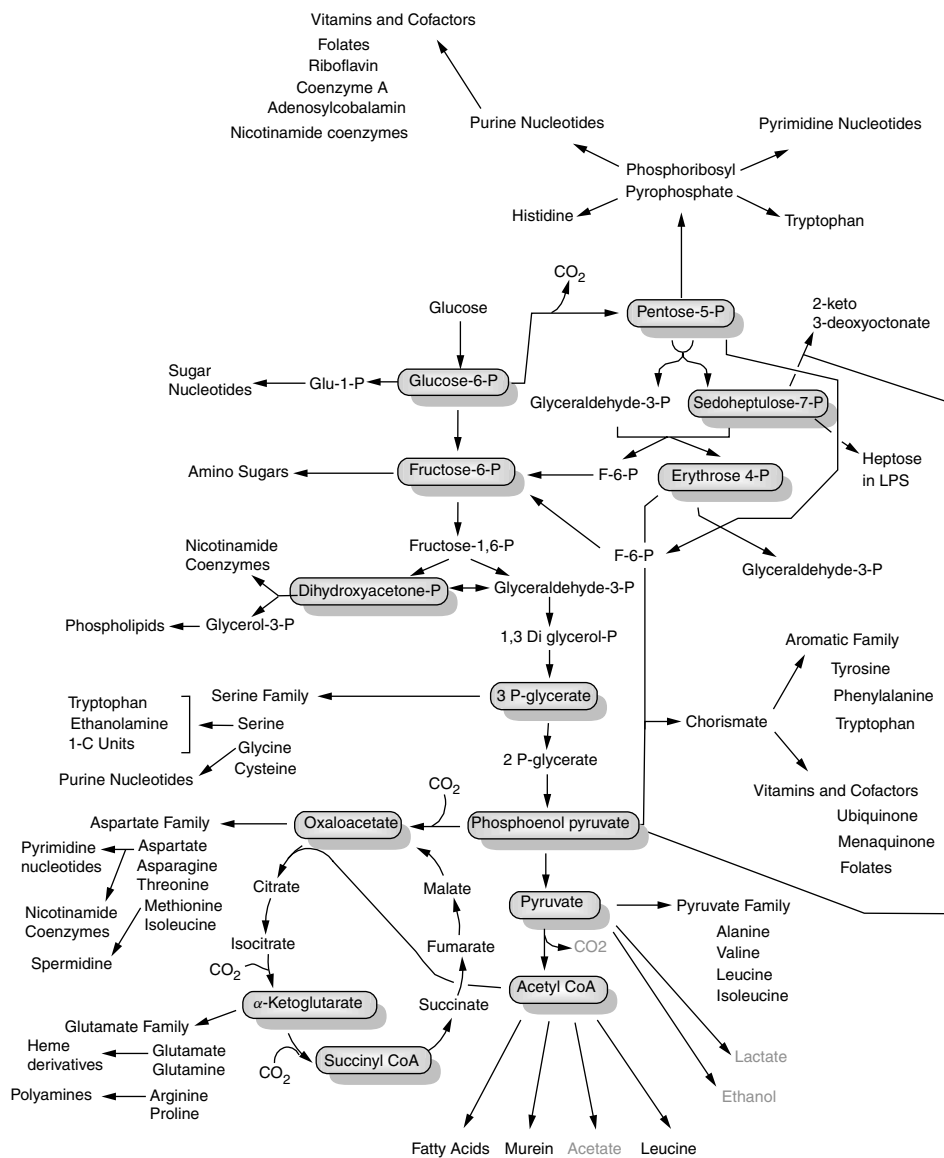
Another mission of carbohydrate metabolism is the production of energy. The most universal energy transfer compound found in living cells is adenosine triphosphate (ATP) (Fig. 1-12). The cell can generate ATP in two ways: (1) by **substrate-level phosphorylation** in which a high-energy phosphate is transferred from a chemical compound (e.g., phosphoenol pyruvate) to adenosine diphosphate (ADP) during the course of carbohydrate catabolism; or (2) by **oxidative phosphorylation** in which the energy from an electrical and chemical gradient formed across the cell membrane is used to drive a membrane-bound ATP hydrolase complex to produce ATP from ADP and inorganic phosphate.

The generation of an electrical and chemical gradient (collectively called the **proton motive force**) across the cell membrane requires a complex set of reactions in which  $H^+$  and  $e^-$  are transferred from chemical intermediates of the Embden-Meyerhof and TCA cycles to a series of membrane-associated proteins called **cytochromes**. As the  $e^-$  is passed from one member of the cytochrome chain to another, the energy released



**Fig. 1-10. Composite diagram of major pathways of carbohydrate metabolism.** The 13 key metabolites are boxed. G, glucose; E, erythrose; R, ribose; XI, xylulose; SH, sedoheptulose; DOHP, dihydroxyacetone phosphate; OAA, oxaloacetate; TK, transketolase; TA, transaldolase.

is used to pump  $H^+$  out of the cell. The resulting difference between the inside and outside of the cell in terms of charge (**electrical potential**) and pH (**chemical potential**) can be harnessed by the cell to generate ATP. Of course, in order for the cytochrome system to work, there must be a terminal electron acceptor molecule. Under aerobic conditions, oxygen will serve that function, but under anaerobic conditions, *E. coli*

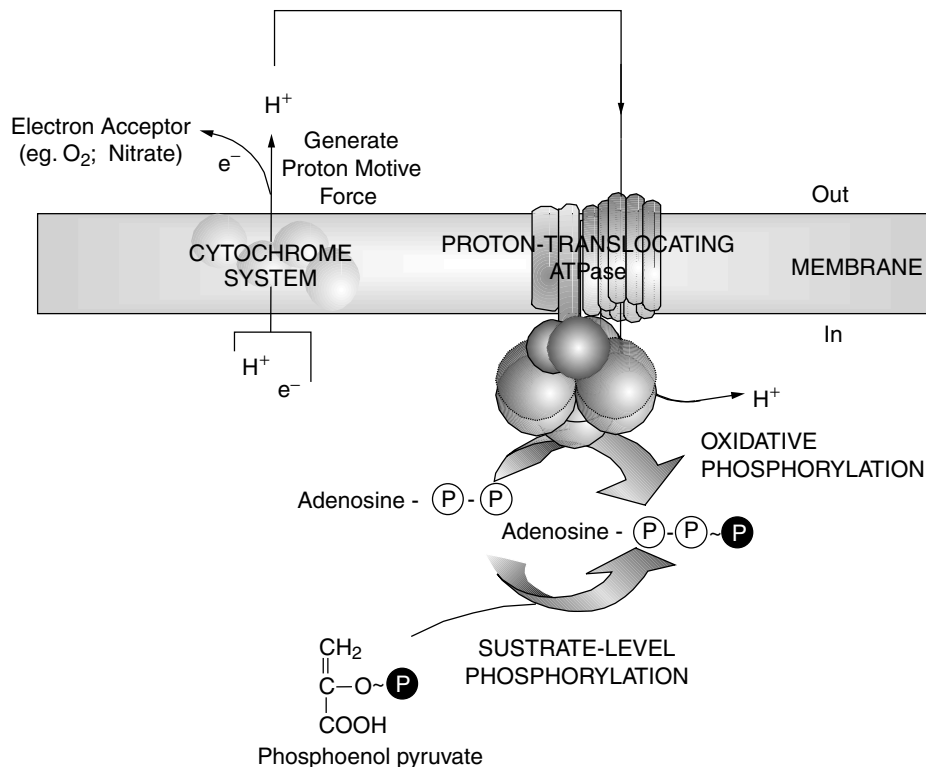


**Fig. 1-11. Biosynthetic pathways leading to the amino acids and related compounds.** The oblong-circled intermediates are the 13 key compounds that serve as biosynthetic precursors for a variety of essential end products.

has a menu of alternate electron acceptors from which it can choose depending on availability (e.g., nitrate). A more detailed accounting of this process is discussed in Chapter 9.

## Oxidation–Reduction Versus Fermentation

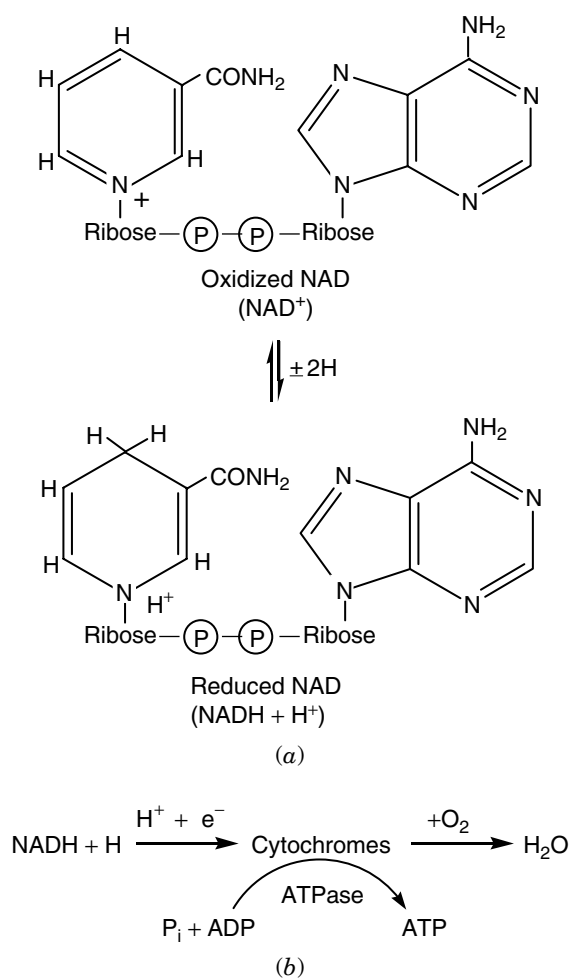
Carbohydrate metabolism is the progressive oxidation of a sugar in which hydrogens are transferred from intermediates in the pathway to hydrogen-accepting molecules.



**Fig. 1-12. Reactions essential to energy production.** Oxidative phosphorylation. The energy that comprises the proton motive force can be harnessed and used to generate ATP when protons from outside the cell pass through the membrane-associated proton-translocating ATPase. The energy released will run the ATPase in reverse. It is estimated that passage of three  $H^+$  through the ATPase is required to generate one ATP. Substrate-level phosphorylation. Energy contained within high-energy phosphate bonds of certain glycolytic intermediates can be transferred to ADP, forming ATP. The example shows phosphoenolpyruvate.

The most commonly used hydrogen acceptor compound is **nicotinamide adenine dinucleotide (NAD)** (Fig. 1-13). It is the reduced form of NAD (NADH) that passes the  $H^+$  and  $e^-$  to the cytochrome system. However, a problem can develop when a cell is forced to grow in an anaerobic environment without any alternate electron acceptors. This situation could lead to a complete depletion of  $NAD^+$ , with all of the NAD pool converted to NADH. NADH, produced during the early part of glycolysis, would not be able to pass its H along and therefore the cell could not regenerate  $NAD^+$ . If this situation were allowed to develop, the cell would stop growing because there would be no  $NAD^+$  to continue glycolysis! To avoid this problem, many microorganisms, including *E. coli*, can regenerate  $NAD^+$  by allowing NADH to transfer H to what would otherwise be dead-end intermediates in the glycolytic pathway (e.g., pyruvate or acetyl CoA). The process, known as **fermentation**, produces lactic acid, isopropanol, butanol, ethanol, and so on, depending on the organism. *E. coli* does not perform all of these fermentation reactions. It is limited to lactate, acetate, formate, ethanol,  $CO_2$ , and  $H_2$  production (Fig. 1-10). Table 1-1 lists the fermentation patterns for some other common organisms.





**Fig. 1-13. Nicotinamide adenine dinucleotide (NAD).** Function of NAD in oxidation–reduction reactions. (a) Hydrogen atoms removed from a hydrogen donor are transferred to the nicotinamide portion of NAD. (b) The hydrogen atoms can be transferred from NAD to an acceptor such as cytochrome pigments.

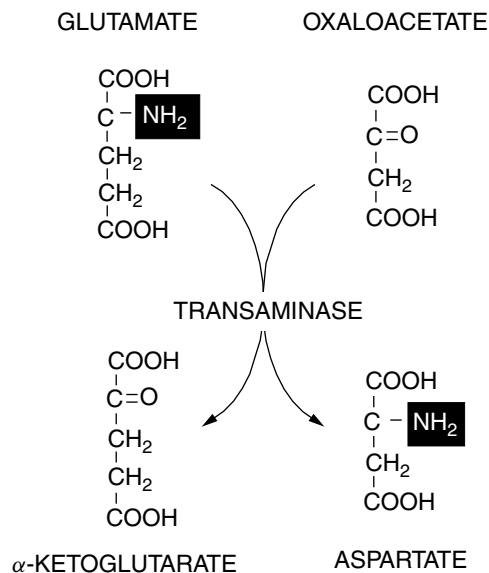
**TABLE 1-1. Variation in Fermentation Products Formed from Pyruvate**

Organism	Product(s)
<i>Saccharomyces</i> (yeast)	Carbon dioxide, ethanol
<i>Streptococcus</i> (bacteria)	Lactic acid
<i>Lactobacillus</i> (bacteria)	Lactic acid
<i>Clostridium</i> (bacteria)	Acetone, butyric acid, isopropanol, butanol
<i>Enterobacter</i> (bacteria)	Acetone, carbon dioxide, ethanol, lactic acid
<i>E. coli</i> (bacteria)	Lactic acid, acetic acid, $\text{H}_2$ , ethanol, formic acid, carbon dioxide

The cell does not only catabolize glucose via glycolysis. There are alternate metabolic routes available for the dissimilation of glucose. One use for alternate pathways of carbohydrate metabolism (e.g., the phosphoketolase pathway; see Fig. 1-10) is the generation of biosynthetic **reducing power**. The cofactor NAD is actually divided functionally into two separate pools. NAD(H) is used primarily for catabolic reactions, whereas a derivative, NAD phosphate (NADP), and its reduced form, NADPH, are involved in biosynthetic (anabolic) reactions. The phosphoketolase pathway is necessary for the generation of the NADPH that is essential for biosynthetic reactions.

### Nitrogen Assimilation

A major omission in our discussion to this point involves the considerable amount of nitrogen (N) needed by microorganisms. Every amino acid, purine, pyrimidine, and many other chemicals in the cell include nitrogen in their structures. Since glucose does not contain any nitrogen, how do cells acquire it? Some microorganisms can fix atmospheric nitrogen via nitrogenase to form ammonia ( $\text{NH}_4^+$ ) and then assimilate the ammonia into amino acids (e.g., *Rhizobium*). Other organisms such as *E. coli* must start with  $\text{NH}_4^+$ . The assimilation of N involves the amidation of one of the 13 key metabolites,  $\alpha$ -ketoglutarate, to form glutamic acid (Fig. 1-14). After assimilation into glutamate, the amino nitrogen is passed on to other compounds by transamination reactions. For example, glutamate can pass its amino group to oxaloacetate to form aspartate. From Figure 1-11, it can be seen that aspartate, like glutamate, is a precursor for several other amino acids. The subject of nitrogen assimilation is covered in depth in Chapter 14.



**Fig. 1-14. Transamination.** In this example, the amine group from glutamic acid is transferred to oxaloacetate, forming aspartic acid.

## SPECIAL TOPICS

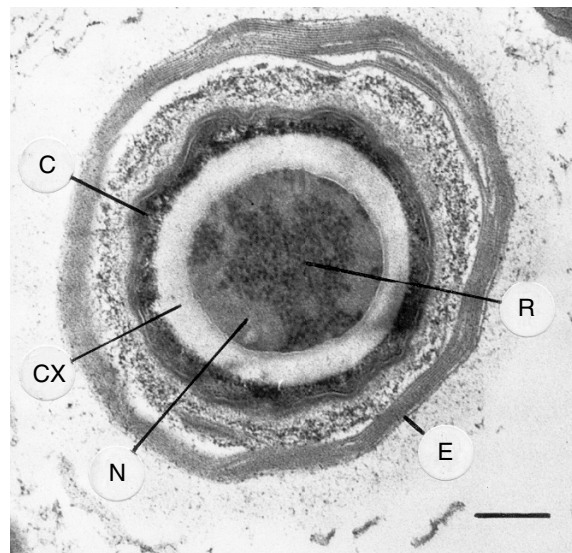
### Endospores

A few bacteria such as *Bacillus* and *Clostridium* produce specialized structures called endospores. Endospores are bodies that do not stain with ordinary dyes and appear as unstained highly refractile areas when seen under the light microscope. They provide resistance to heat, desiccation, radiation, and other environmental factors that may threaten the existence of the organism. Endospores also provide a selective advantage for survival and dissemination of the species that produce them. Under the electron microscope, spores show a well-defined multilayered exosporium, an electron-dense outer coat observed as a much darker area, and a thick inner coat. In the spore interior, the darkly stained ribosomes and the nuclear material may also be visible (Fig. 1-15).

### Growth

Growth of a cell is the culmination of an ordered interplay among all of the physiological activities of the cell. It is a complex process involving

1. Entrance of basic nutrients into the cell
2. Conversion of these compounds into energy and vital cell constituents
3. Replication of the chromosome
4. Increase in size and mass of the cell
5. Division of the cell into two daughter cells, each containing a copy of the genome and other vital components



**Fig. 1-15. Mature spore of *Clostridium botulinum*.** Shown is a well-defined, multilayered exosporium (E), an electron-dense outer coat layer, a thick inner coat (C) and a less dense cortex (CX). The darkly stained ribosomes (R) and nucleoid areas (N) are clearly differentiated in the spore interior. Bar equals 0.2  $\mu\text{m}$ . (Source: From Stevenson, K. E., R. H. Vaughn, and E. V. Crisan, 1972. *J. Bacteriol.* **109**:1295.)

Microbiologists usually consider the phenomenon of growth from the viewpoint of population increase, since most current techniques do not allow the detailed study of individual cells. A study of the increase in population implies that each cell, as it is produced, is capable of producing new progeny.

**Growth Cycle.** Under ideal circumstances in which cell division commences immediately and proceeds in unhampered fashion for a protracted period of time, prokaryotic cell division follows a geometric progression:

$$\begin{array}{ccccccccccccccc} 2^0 & \longrightarrow & 2^1 & \longrightarrow & 2^2 & \longrightarrow & 2^3 & \longrightarrow & 2^4 & \longrightarrow & 2^5 & \longrightarrow & 2^6 & \longrightarrow & 2^7 \\ & & & & & & & & & & & & & & \\ & \longrightarrow & 2^8 & \longrightarrow & 2^9 & \longrightarrow & \text{etc.} & & & & & & & & \end{array}$$

This progression may be expressed as a function of 2 as shown in the line above. The number of cells ( $b$ ) present at a given time may be expressed as

$$b = 1 \times 2^n$$

The total number of cells ( $b$ ) is dependent on the number of generations ( $n$  = number of divisions) occurring during a given time period. Starting with an inoculum containing more than one cell, the number of cells in the population can be expressed as

$$b = a \times 2^n$$

where  $a$  is the number of organisms present in the original inoculum. Since the number of organisms present in the population ( $b$ ) is a function of the number 2, it becomes convenient to plot the logarithmic values rather than the actual numbers. Plotting the number of organisms present as a function of time generates a curvilinear function. Plotting the logarithm of the number, a linear function is obtained as shown in Figure 1-16. For convenience, logarithms to the base 10 are used. This is possible because the logarithm to the base 10 of a number is equal to 0.3010 times the logarithm to the base 2 of a number.

Up to this point it has been assumed that the individual generation time (i.e., the time required for a single cell to divide) is the same for all cells in the population. However, in a given population, the generation times for individual cells vary, so the term **doubling time** encompasses the doubling time for the total population. As shown in Figure 1-16, the cells initially experience a period of adjustment to the new environment, and there is a lag in the time required for all of the cells to divide. Actually, some of the cells in the initial inoculum may not survive this **lag phase** and there may be a drop in the number of viable cells. The surviving cells eventually adjust to the new environment and begin to divide at a more rapid rate. This rate will remain constant until conditions in the medium begin to deteriorate (e.g., nutrients are exhausted). Since plotting the cell number logarithm during this period results in a linear function, this phase of growth is referred to as the **logarithmic (log) phase** or, more correctly, the **exponential phase**.

All cultures of microorganisms eventually reach a maximum population density in the **stationary phase**. Entry into this phase can result from several events. Exhaustion of essential nutrients, accumulation of toxic waste products, depletion of oxygen, or development of an unfavorable pH are the factors responsible for the decline in the

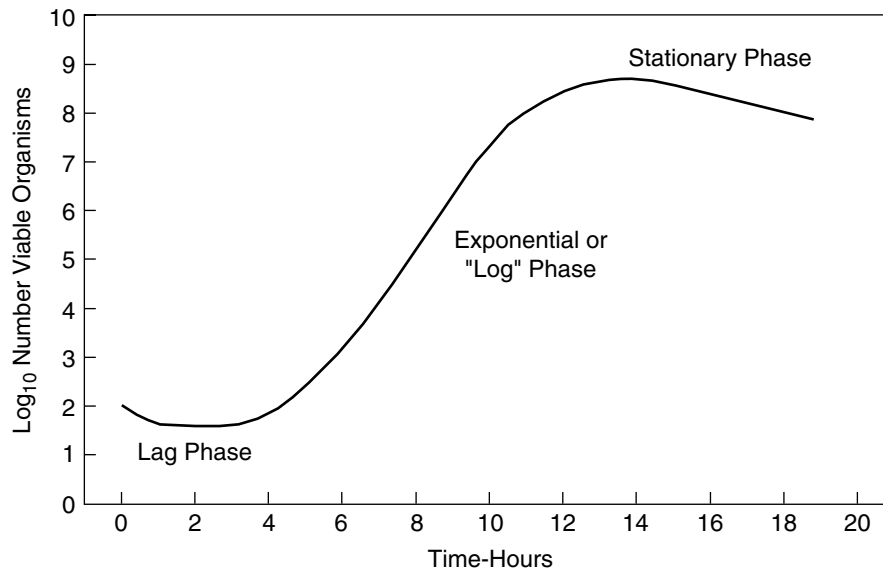


Fig. 1-16. A typical growth curve for a bacterial culture.

growth rate. Although cell division continues during the stationary phase, the number of cells that are able to divide (viable cells) are approximately equal to the number that are unable to divide (nonviable cells). Thus, the stationary phase represents an equilibrium between the number of cells able to divide and the number that are unable to divide.

Eventually, the death of organisms in the population results in a decline in the viable population and the **death phase** ensues. The exact shape of the curve during the death phase will depend on the nature of the organism under observation and the many factors that contribute to cell death. The death phase may assume a linear function such as during heat-induced death where viable cell numbers decline logarithmically.

Some additional considerations of the growth curve are important in assessing the effect of various internal as well as external factors on growth. Since the number of cells in a population ( $b$ ) is equal to the number of cells in the initial inoculum ( $a$ )  $\times 2^n$ ,

$$b = a \times 2^n$$

Then

$$\log_2 b = \log_2 a + n$$

$$\log_{10} b = \log_{10} a + n \log_{10} 2$$

$$\log_{10} b = \log_{10} a + (n \times 0.3010)$$

Solving the equation for  $n$ , the number of generations that occurred between the time of inoculation and the time of sampling is

$$n = \frac{\log_{10} b - \log_{10} a}{0.3010}$$

The generation time ( $t_g$ ) or doubling time may be determined by dividing the time elapsed ( $t$ ) by the number of generations ( $n$ ):

$$t_g = t/n$$

### Continuous Culture

Usually bacteria are grown in “batch” culture in which a flask containing media is inoculated and growth is allowed to occur. This is a closed system where it is actually very difficult to manipulate growth rate. In batch cultures, growth rate is determined internally by properties of the bacteria themselves. A batch culture can be used to grow bacteria at different rates as long as the nutrient added is at a concentration that does not support maximal growth. But, to accomplish this, the cell density, and thus the cell number, will be too low for certain analyses. To grow bacteria at slow growth rates and at high cell density, a chemostat is used. In this apparatus, fresh medium containing a limiting nutrient is added from a reservoir to the culture vessel at a set rate. The volume in the culture vessel is kept constant by an overflow device that removes medium and cells at the same rate as fresh medium is added. In a chemostat, growth rate is determined externally by altering the rate-limiting nutrient added to the culture vessel. The faster the limiting nutrient is added, the faster the growth rate.

## FACTORS AFFECTING GROWTH

### Nutrition

All living organisms have certain basic nutritional requirements: sources of carbon, nitrogen, energy, and essential growth factors (minerals and vitamins) are needed to support growth. Microorganisms vary widely in their nutritional requirements. Two main groups of organisms are classified on the basis of their ability to gain energy from certain sources and the manner in which they satisfy their carbon and nitrogen requirements for growth:

1. **Lithotrophs** utilize carbon dioxide as the sole source of carbon and gain energy through the oxidation of inorganic compounds (**chemolithotrophs** or “rock eaters”) or light (**photolithotrophs**). Inorganic nitrogen is utilized for the synthesis of organic compounds.

2. **Organotrophs** generally prefer organic substrates as a source of energy and carbon. **Photoorganotrophs** utilize light as a source of energy for the assimilation of carbon dioxide as well as organic compounds. **Chemoorganotrophs** utilize organic compounds for growth.

Although their nutritional requirements are remarkably simple, chemolithotrophic bacteria must be metabolically complex since they synthesize all of their cellular components and provide the energy for this activity through the oxidation of inorganic compounds. One fundamental characteristic of strict chemolithotrophs is that they are unable to grow on or assimilate exogenous organic compounds. Facultative chemolithotrophs can utilize exogenous organic carbon sources. Chemolithotrophs possess unique mechanisms for carbon dioxide fixation such as the ribulose biphosphate (Calvin-Benson) cycle and the reductive carboxylic acid (Campbell-Evans) cycle (see Chapter 9).

Some organotrophic organisms utilize carbon dioxide as a source of carbon, but most prefer organic carbon sources and generally cannot subsist on carbon dioxide as the sole carbon source. Organotrophs may use inorganic nitrogen, but most members of the group grow better when supplied with organic nitrogen compounds. For example, *E. coli*, *Enterobacter aerogenes*, yeasts, and molds grow luxuriantly on glucose as the only organic nutrient. Other organotrophs such as streptococci and staphylococci also exhibit specific requirements for one or more nitrogen sources as amino acids, purines, or pyrimidines (see Table 1-2).

Fatty acids are required by some organisms, particularly in the absence of certain B vitamins. Replacement of a growth factor requirement by the addition of the end product of a biosynthetic pathway in which the vitamin normally functions is referred to as a **sparing action**. This type of activity has been reported for many growth factors, including amino acids, purines, pyrimidines, and other organic constituents. If a vitamin can completely replace a particular organic nutrient in a defined medium, that nutrient cannot be regarded as a true growth requirement since it can be synthesized in the presence of the requisite vitamin.

TABLE 1-2. Nutritional Requirements of Some Organotrophs

	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i> <sup>a</sup>	<i>Leuconostoc paramesenteroides</i> <sup>b</sup>
<b>Basic Nutrients</b>				
Glucose	} Required by all for maximum growth in defined medium			
NH <sub>4</sub> <sup>+</sup>				
Mn <sup>2+</sup>				
Mg <sup>2+</sup>				
Fe <sup>2+</sup>				
K <sup>+</sup>				
Cl <sup>-</sup>				
SO <sub>4</sub> <sup>2-</sup>				
PO <sub>4</sub> <sup>3-</sup>				
<b>Additional Requirements</b>				
	None	Tryptophan	Nicotinic acid Thiamine 10 amino acids	Nicotinic acid Thiamine Pantothenate Pyridoxal Riboflavin Cobalamin Biotin <i>p</i> -Aminobenzoate Folate Guanine Uracil 16 Amino acids Sodium acetate Tween 80

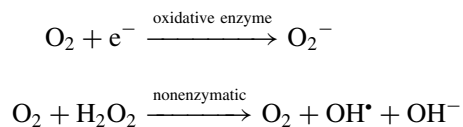
<sup>a</sup>From Gladstone, G. P. 1937. *Br. J. Exp. Pathol.* **18**:322.

<sup>b</sup>From Garvie, E. I. 1967. *J. Gen. Microbiol.* **48**:429.

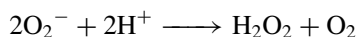
Although most bacterial membranes do not contain sterols, they are required in the membranes of some members of the Mycoplasmataceae. (These organisms do not possess a cell wall.) *Mycoplasma* require sterols for growth. *Acholeplasma* do not require sterols; however, they produce terpenoid compounds that function in the same capacity as sterols. Fungi (yeasts and molds) contain sterols in their cell membranes but in most cases appear to be capable of synthesizing them.

## Oxygen

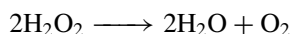
Microorganisms that require oxygen for their energy-yielding metabolic processes are called **aerobes**, while those that cannot utilize oxygen for this purpose are called **anaerobes**. **Facultative organisms** are capable of using either respiratory or fermentation processes, depending on the availability of oxygen in the cultural environment. Aerobic organisms possess cytochromes and cytochrome oxidase, which are involved in the process of oxidative phosphorylation. Oxygen serves as the terminal electron acceptor in the sequence and water is one of the resultant products of respiration. Some of the oxidation–reduction enzymes interact with molecular oxygen to give rise to superoxide ( $\text{O}_2^-$ ), hydroxyl radicals ( $\text{OH}^\bullet$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), all of which are extremely toxic:



The enzyme **superoxide dismutase** dissipates superoxide:



Superoxide dismutase is present in aerobic organisms and those that are aerotolerant, but not in strict anaerobes. Many, but not all, aerobes also produce catalase, which can eliminate the hydrogen peroxide formed:



Aerotolerant organisms generally do not produce catalase. Hence, growth of these organisms is frequently enhanced by culture on media containing blood or other natural materials that contain catalase or peroxidase activity. Organisms that do not utilize oxygen may tolerate it because they do not interact in any way with molecular oxygen and do not generate superoxide or peroxide.

Anaerobic bacteria from a variety of genera are present in the normal flora of the animal and human body as well as in a number of natural habitats such as the soil, marshes, and deep lakes. A number of the more widely known genera of anaerobic organisms are listed in Table 1-3.

## Carbon Dioxide

Many organisms are dependent on the fixation of carbon dioxide. Certain organisms thrive better if they are grown in an atmosphere containing increased carbon dioxide.



TABLE 1-3. Genera of Anaerobic Bacteria

Bacilli		Cocci	
Gram Positive	Gram Negative	Gram Positive	Gram Negative
<i>Clostridium</i>	<i>Bacteroides</i>	<i>Peptococcus</i>	<i>Veillonella</i>
<i>Actinomyces</i>	<i>Fusobacterium</i>	<i>Peptostreptococcus</i>	
<i>Bifidobacterium</i>	<i>Vibrio</i>	<i>Ruminococcus</i>	
<i>Eubacterium</i>	<i>Desulfovibrio</i>		
<i>Lactobacillus</i>			
<i>Propionibacterium</i>			

*Haemophilus*, *Neisseria*, *Brucella*, *Campylobacter*, and many other bacteria require at least 5 to 10% carbon dioxide in the atmosphere to initiate growth, particularly on solid media. Even organisms such as *E. coli* use carbon dioxide to replenish intermediates in the TCA (tricarboxylic acid) cycle that have been siphoned off as precursors for amino acid synthesis. These anapleurotic reactions include pyruvate carboxylase, phosphoenolpyruvate carboxylase, or malic enzyme (see Chapter 8).

### Extremophiles

Microorganisms vary widely in their ability to initiate growth over certain ranges of temperature (Table 1-4), hydrogen ion concentration (Table 1-5), and salt concentration. Organisms that function best under extreme environmental conditions are called **extremophiles**. Examples include bacteria found in hot springs and in the thermal vents on the ocean floor. These organisms prefer to grow at extremely high temperatures. Some microorganisms prefer to live in an acidic environment (**acidophilic** organisms) while others prefer an alkaline pH (**alkaliphilic** organisms). *E. coli* prefers a neutral pH environment and thus is classified as **neutrophilic**. (The older term, neutrophilic,

TABLE 1-4. Temperature Ranges of Bacterial Growth

Type of Organism	Growth Temperature (°C)		
	Minimum	Optimum	Maximum
Psychrophilic	−5–0	5–15	15–20
Mesophilic	10–20	20–40	40–45
Thermophilic	25–45	45–60	>80

TABLE 1-5. pH Limits for Growth of Various Microorganisms

Organism	Minimum	Optimum	Maximum
Bacteria	2–5	6.5–7.5	8–11
Yeasts	2–3	4.5–5.5	7–8
Molds	1–2	4.5–5.5	7–8

is not consistent with the nomenclature of the other two groups and can be confused with neutrophils, a form of white blood cell, and thus should not be used.) The ability of certain organisms to grow in extreme environments can be linked to the possession of unique membrane compositions and/or enzymes with unusual temperature or pH optima that are more suitable to their environment.

### **Microbial Stress Responses**

For **normalophiles**, meaning organisms that prefer to grow under conditions of 37°C, pH 7, and 0.9% saline, variations in pH and temperature have a marked impact on enzyme activity and, ultimately, viability. Outside their optimal parameters, enzymes function poorly or not at all, membranes become leaky, and the cell produces compounds (e.g., superoxides) that damage DNA and other macromolecular structures. All of these factors contribute to cell death when the cell is exposed to suboptimal environments. However, many, if not all, microorganisms have built-in stress response systems that sense when their environment is deteriorating, such as when medium acidifies to dangerous levels. At this point, signal transduction systems perceive the stress and transmit instructions to the transcription/translation machineries to increase expression of specific proteins whose job is to protect the cell from stress. The various genetic regulatory systems and protection strategies used by the cell to survive stress are discussed in Chapters 5 and 18.

### **SUMMARY**

This chapter is a highly condensed version of the remainder of this book, provided to build a coherent picture of microbial physiology from the start. Too often textbooks present a student with excruciatingly detailed treatments of one specific topic after another without ever conveying the “big picture.” As a result, the information overload is so great that the student, lost in the details, never develops an integrated view of the cell and what makes it work. Our hope is that the framework in this chapter will be used to build a detailed understanding of microbial physiology and an appreciation of its future promise.