

History of Microbiology

Chapter 2 The History of Microbiology

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Fermentation

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Microbiology and Society

History is the story of the achievements of men and women, but it records relatively few outstanding names and events. Many important contributions were made by people whose names have been forgotten and whose accomplishments have been lost in the longer and deeper shadows cast by those who caught the fancy of the chroniclers. It has been said that in science the credit goes to the one who convinces the world, not to the one who first had the idea. So, in the development of microbiology, the outstanding names are often of those who convinced the world—who developed a technique, a tool, or a concept that was generally adopted, or who explained their findings so clearly or dramatically that the science grew and prospered.

Antony van Leeuwenhoek's lurid reports on the ubiquity of microbes enabled Louis Pasteur 250 years later to discover the involvement of these creatures in fermentation reactions and allowed Robert Koch, Theobald Smith, Pasteur, and many others to discover the association of microbes with disease. Koch is remembered for his isolation of the bacteria that cause anthrax and tuberculosis and for the rigid criteria he demanded before a specific bacterium be held as the cause of a disease. His important contributions to the creation of the science of microbiology won him the 1905 Nobel prize.

The building of the Panama Canal dramatized Walter Reed's studies of the epidemiology of yellow fever, but historians remember that Theobald Smith's work on transmission of Texas fever pointed the way for Walter Reed's subsequent work.

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In 1845 Louis Pasteur presented those who follow of them with tools and ideas with which to work. Paul Ehrlich's discovery of a chemical compound that would destroy the syphilis spirochete in the human body without injury to tissue cells paved the way for his developments in the use of chemicals in treating disease. For this he shared the Nobel prize in 1908 with Elie Metchnikoff, who discovered a system in the human body that combated infection.

The age of relatively short duration, the history of microbiology is filled with thrilling achievements. We have won many battles with microorganisms and have learned not only to make them work for us but also to control some of those that work against us.

THE MICROSCOPE

Microbiology began when people learned to grind lenses from pieces of glass and combine them to produce magnifications great enough to enable microbes to be seen. During the thirteenth century Roger Bacon (1220–1292) postulated that disease is produced by invisible living creatures. This suggestion was made again by Girolamo Fracastoro of Verona (1483–1553) and Anton von Plenciz in 1782, but these people had no proof. As early as 1656, a monk named Athanasius Kircher (1601–1680) referred to “worms” invisible to the naked eye in decaying bodies, meat, milk, and diarrheal secretions. Although his description lacked accuracy, Kircher was the first person to recognize the significance of bacteria and other microbes in disease. In 1665 Robert Hooke's description of cells in a piece of cork established the fact that the bodies of “animals and plants, complex as they may appear, are yet composed of a few elementary parts frequently repeated”—a quotation not from Hooke but from Aristotle's description of the cellular structure of living things back in the fourth century B.C.

Although he was probably not the first to see bacteria and protozoa, Antony van Leeuwenhoek, who lived in Delft, Holland, from 1632 to 1723, was the first to report his observations with accurate descriptions and drawings (Fig. 2-1). Leeuwenhoek had the means and opportunity to pursue his hobby of lens grinding and microscope making. During his lifetime he made more than 250 microscopes consisting of home-ground lenses mounted in brass and silver, the most powerful of which would magnify about 200 to 300 times (Fig. 2-2). These microscopes bear little resemblance to the compound light microscope of today, which is capable of magnifications of 1,000 to 3,000 times. However, the lenses of Leeuwenhoek's microscopes were well made and Leeuwenhoek had the openness of mind that is so very important in an investigator. His descriptions of protozoa were so accurate that many of the forms he described are easily recognized today.

Leeuwenhoek carefully recorded his observations in a series of letters to the British Royal Society. In one of the first letters, dated September 7, 1674, addressed to Henry Oldenburg, Secretary of the Royal Society, he described the “very little animalcules” which we recognize as free-living protozoa. On October 9, 1676, he wrote:

In the year 1675, I discovered living creatures in rain water which had stood but a few days in a new earthen pot, glazed blue within. This invited me to view this

Figure 2-1. Antony van Leeuwenhoek (1632–1723), a Dutch student of natural history whose hobby was making microscopes, is shown here with one of the more than 250 microscopes that he made. His best lenses were capable of magnifications up to $\times 270$, and he was the first person to report descriptions of microorganisms in detail. (Courtesy of Lambert-Hudnut, Division Warner-Lambert Pharmaceutical Company.)



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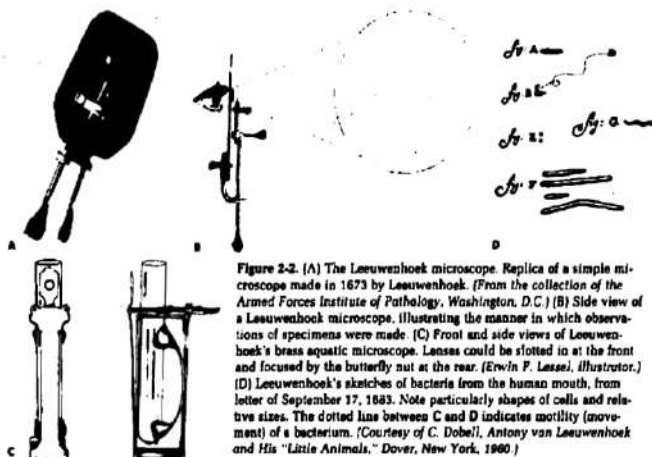


Figure 2-2. (A) The Leeuwenhoek microscope. Replica of a simple microscope made in 1673 by Leeuwenhoek. (From the collection of the Armed Forces Institute of Pathology, Washington, D.C.) (B) Side view of a Leeuwenhoek microscope, illustrating the manner in which observations of specimens were made. (C) Front and side views of Leeuwenhoek's brass aquatic microscope. Lenses could be slotted in at the front and focused by the butterfly nut at the rear. (Erwin F. Llesse, illustrator.) (D) Leeuwenhoek's sketches of bacteria from the human mouth, from a letter of September 17, 1683. Note particularly shapes of cells and relative sizes. The dotted line between C and D indicates motility (movement) of a bacterium. (Courtesy of C. Dobell, *Antony van Leeuwenhoek and His "Little Animals,"* Dover, New York, 1960.)

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water with great attention, especially those little animals appearing to me ten thousand times less than those . . . which may be perceived in the water with the naked eye.

He described his little animals in great detail, leaving little doubt that he saw bacteria, fungi, and many forms of protozoa. For example, he reported that on June 16, 1675, while examining well water into which he had put a whole pepper the day before:

I discovered, in a tiny drop of water, incredibly many very little animalcules, and these of divers sorts and sizes. They moved with bendings, as an eel always swims with its head in front, and never tail first, yet these animalcules swam as well backwards as forwards, though their motion was very slow.

His enthusiastic letters were read with interest by the British scientists, but the importance of his discoveries evidently went unappreciated. The talents and astuteness of this remarkable man can best be appreciated by reading Dobell's biography of Leeuwenhoek.

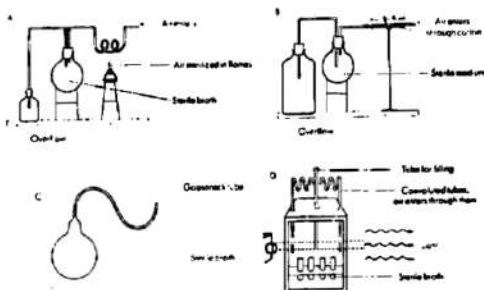
Before the time of Pasteur, microorganisms were studied mainly to satisfy curiosity concerning their characteristics and their relationships to higher living forms, without awareness of their importance in fermentation and disease.

SPONTANEOUS GENERATION VERSUS BIOGENESIS

The discovery of microbes spurred interest in the origin of living things, and argument and speculation grew. As far as human beings were concerned, the Greek explanation that the goddess Gaia was able to create people from stones and other inanimate objects had been largely discarded. But even the astute Aristotle (384–322 B.C.) taught that animals might originate spontaneously from the soil, plants, or other unlike animals, and his influence was still strongly felt in the seventeenth century. About 40 B.C., Virgil (70–19 B.C.) gave directions for the artificial propagation of bees. This was but one of many fanciful tales of a similar nature that persisted into the seventeenth century. For example, it was accepted as a fact that maggots could be produced by exposing meat to warmth and air, but Francesco Redi (1626–1697) doubted this. Proof that his skepticism was well founded came from an experiment in which he placed meat in a jar covered with gauze. Attracted by the odor of the meat, flies laid eggs on the covering, and from the eggs maggots developed. Hence the experiment established the fact that the origin of the maggots was the flies and not the meat. This experiment and others involving mice and scorpions appear to have settled the matter so far as these forms of life were concerned. But microbes were another matter; surely such minute creatures needed no parents!

There appeared champions for and challengers of the theory that living things can originate spontaneously, each with a new and sometimes fantastic explanation or bit of experimental evidence. In 1749, while experimenting with meat exposed to hot ashes, John Needham (1713–1781) observed the appearance of organisms not present at the start of the experiment and concluded that the bacteria originated from the meat. About the same time, Lazzaro Spallanzani (1729–1799) boiled beef broth for an hour and then sealed the flasks. No microbes appeared following incubation. But his results, confirmed in repeated

Figure 2-3. The theory of spontaneous generation was disproved with the devices illustrated here, all of which eliminated airborne bacteria. Schwann heat-sterilized the air which flowed through the glass tube to his culture flask (A). Schröder and von Dusch filtered the air entering the culture flask through cotton (B). Simple goose-necked flasks (C) were devised by Pasteur. Tyndall constructed a dust-free incubation chamber (D).



experiments, failed to convince Needham, who insisted that air was essential to the spontaneous production of microscopic beings and that it had been excluded from the flasks by sealing them. This argument was answered some 60 or 70 years later independently by two other investigators, Franz Schulze (1815–1873) and Theodor Schwann (1810–1882). Schulze passed air through strong acid solutions into boiled infusions, whereas Schwann passed air into his flasks through red-hot tubes (Fig. 2-3A). In neither case did microbes appear. But the die-hard advocates of spontaneous generation were still not convinced. Acid and heat altered the air so that it would not support growth, they said. About 1850, H. Schröder and T. von Dusch performed a more convincing experiment by passing air through cotton into flasks containing heated broth (Fig. 2-3B). Thus the microbes were filtered out of the air by the cotton fibers so that growth did not occur, and a basic technique of plugging bacterial culture tubes with cotton stoppers was initiated.

The concept of spontaneous generation was revived for the last time by Felix-Archimède Pouchet (1800–1872), who published in 1859 an extensive report “proving” its occurrence. But Pouchet reckoned without the ingenious, tireless, and stubborn Pasteur (1822–1895). Irritated by Pouchet’s logic and data, Pasteur performed experiments that ended the argument for all time. He prepared a flask with a long, narrow gooseneck opening (Fig. 2-3C). The nutrient solutions were heated in the flask, and air—untreated and unfiltered—could pass in or out; but the germs settled in the gooseneck, and no microbes appeared in the solution.

Pasteur reported his results with a great flourish at the Sorbonne in Paris on April 7, 1864. His flasks would yield no sign of life, he said:

For I have kept from them, and am still keeping from them, that one thing which is above the power of man to make; I have kept from them the germs that float in the air. I have kept from them life.

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In his exuberance, Pasteur sent a few darts at those he disagreed with:

There is no condition known today in which you can affirm that microscopic beings come into the world without germs, without parents like themselves. They who allege it have been the sport of illusions, of ill-made experiments, vitiated by errors which they have not been able to perceive and have not known how to avoid.

Finally, John Tyndall (1820–1893) conducted experiments in a specially designed box to prove that dust carried the germs (Fig. 2-3D). He demonstrated that if no dust was present, sterile broth remained free of microbial growth for indefinite periods.

FERMENTATION

Louis Pasteur (Fig. 2-4) began his brilliant career as professor of chemistry at the University of Lille, France. A principal industry of France being the manufacture of wines and beer, Pasteur studied the methods and processes involved in order to help his neighbors produce a consistently good product. He found that fermentation of fruits and grains, resulting in alcohol, was brought about by microbes. By examining many batches of “ferment,” he found microbes of different sorts. In good lots one type predominated, and in the poor products another kind was present. By proper selection of the microbe, the manufacturer might be assured of a consistently good and uniform product. Pasteur suggested that the undesirable types of microbes might be removed by heating—not enough to hurt the flavor of the fruit juice, but enough to destroy a very high percentage of the microbial population. He found that holding the juices at a temperature of 62.8°C (145°F) for half an hour did the job. Today pasteurization is widely used in fermentation industries, but we are most familiar with it in the dairy industry.

Figure 2-4. Louis Pasteur in his laboratory. (Courtesy of Institut Pasteur, Paris.)



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THE GERM THEORY OF DISEASE

Even before Pasteur had proved by experiment that bacteria are the cause of some diseases, many observant students had expressed strong arguments for the germ theory of disease. Fracastoro of Verona suggested that diseases might be due to invisible organisms transmitted from one person to another. In 1762 von Plenciz not only stated that living agents are the cause of disease but suspected that different germs were responsible for different diseases. That the concept of parasitism was becoming quite general is reflected in the following bit of doggerel written by Jonathan Swift (1667-1745) early in the eighteenth century:

So naturalists observe, a flea
Hath smaller fleas that on him prey;
And these have smaller fleas to bit 'em;
And so proceed ad infinitum.

This is better known in the colloquial version:

Big bugs have little bugs,
Upon their backs to bit 'em;
And little bugs have smaller ones,
And so ad infinitum.

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Oliver Wendell Holmes (1809-1894), a successful physician as well as a scholar, insisted that puerperal fever, a disease of childbirth, was contagious and that it was probably caused by a germ carried from one mother to another by midwives and physicians. He wrote *The Contagiousness of Puerperal Fever* in 1842. At approximately the same time, the Hungarian physician Ignaz Philipp Semmelweis (1818-1865) was pioneering in the use of antiseptics in obstetrical practice. Deaths due to infections associated with childbirth were reduced in the cases handled according to his instructions, which minimized chances for infection. As part of his crusade he published *The Cause, Concept and Prophylaxis of Childbed Fever* in 1861. Still, most physicians ignored his advice, and it was not until about 1890, when the work of Joseph Lister in England had become known, that the importance of antiseptics was fully appreciated by the medical profession.

Pasteur's success in solving the problem of fermentation led the French government to request that he investigate pebrine, a silkworm disease that was ruining an important French industry. For several years Pasteur struggled with this problem, heartaches and disappointments following one after another. Eventually he isolated the parasite causing the disease. He also showed that silkworm farmers could eliminate the disease by using only healthy, disease-free caterpillars for breeding stock.

Turning from silk to wool, Pasteur next tackled the problem of anthrax, a disease of cattle, sheep, and sometimes human beings. He grew the microbes in laboratory flasks after isolating them from the blood of animals that had died of the disease. Meanwhile Robert Koch (1843-1910) was busy with the anthrax problem in Germany. Koch, a quiet, meticulous physician, sometimes neglected his medical practice to play with the fascinating new science of bacteriology. It was he who discovered the typical bacilli with squarish ends in the blood of cattle that had died of anthrax. He grew these bacteria in cultures in his laboratory, examined them microscopically to be sure he had only one kind present,

and then injected them into other animals to see if these became infected and developed clinical symptoms of anthrax. From these experimentally infected animals he isolated microbes like those he had originally found in sheep that died of anthrax. This was the first time a bacterium had been proved to be the cause of an animal disease. (Pebrine is caused by a protozoan rather than by a bacterium.) This series of observations led to the establishment of Koch's postulates, which provided guidelines to identify the causative agent of an infectious disease. Koch's postulates are: (1) A specific organism can always be found in association with a given disease. (2) The organism can be isolated and grown in pure culture in the laboratory. (3) The pure culture will produce the disease when inoculated into a susceptible animal. (4) It is possible to recover the organism in pure culture from the experimentally infected animal.

LABORATORY TECHNIQUES AND PURE CULTURES

As we have previously stated, microorganisms occur in nature in extremely large populations made up of many different species. In order to study the characteristics of a particular species it is first necessary to separate it from all other species. Laboratory procedures have been developed that make it possible to isolate microorganisms representing each species and to grow (cultivate) each of the species separately. The growth of a mass of cells of the same species in a laboratory vessel (such as a test tube) is called a pure culture.

Pure cultures of bacteria were first obtained by Joseph Lister in 1878 using serial dilutions in liquid media. With a specially constructed syringe he diluted a fluid (probably milk) containing a mixture of bacteria until a single organism was delivered into a container of sterile milk. After incubation, bacteria in this container were of a single kind, identical to the parent cell. Lister named the organism *Bacterium Inctis*.

Meanwhile Koch was carefully refining methods for the study of bacteria. He found that by smearing bacteria on a glass slide and adding certain dyes to them, individual cells could be seen more clearly with the microscope. He added gelatin and other solidifying materials such as agar to media in order to obtain isolated growths of organisms known as colonies, each of which contained millions of individual bacterial cells packed tightly together. From these colonies, pure cultures could be transferred to other media. The development of a liquefiable solid-culture medium was of fundamental importance.

Using techniques he had devised, Koch studied with painstaking care material taken from patients with pulmonary tuberculosis. After performing a series of rigid tests, as he had done with the anthrax bacillus, he announced the discovery of the microorganism that causes tuberculosis.

The importance of pure cultures to the development of the science of microbiology cannot be overestimated, since by using pure-culture techniques the microorganisms responsible for many infections, certain fermentations, nitrogen fixation in soil, and other activities were isolated and identified. However, strict adherence to pure-culture techniques and Koch's postulates sometimes led investigators up dead-end streets. Early investigators did not know about viruses, nor did they know about the cooperation of two or more microorganisms in causing disease or in bringing about a desirable fermentation such as we find in the ripening of cheese. Today we are as much interested in mixed microbial

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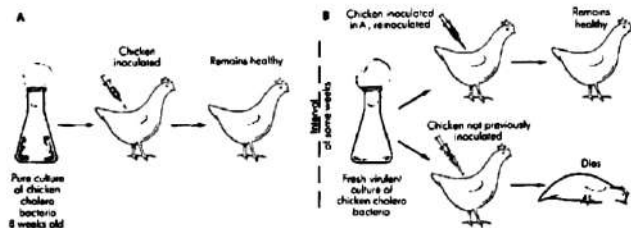
populations and the effects they produce as we are in pure cultures. Further advances in marine microbiology, rumen microbiology, microbiology of the intestinal tract, and many other systems will depend upon understanding first the physiology of individual microorganisms in pure culture and then upon the ecological relationships of the total microbial populations in a given environment.

PROTECTION AGAINST INFECTION: IMMUNITY

Figure 2-5. The principle of immunization was demonstrated by Pasteur when he inoculated chickens with cultures of chicken cholera bacteria several weeks old and the chickens remained healthy. They did not become sick when inoculated with a fresh culture several weeks later although this fresh culture killed chickens that had not received the attenuated (old) culture.

Pasteur continued to make discoveries concerning the cause and prevention of infectious diseases. About 1880 he isolated the bacterium responsible for chicken cholera and grew it in pure culture. Here again, the practical Pasteur made use of the fundamental techniques devised by the more theoretical Koch. To prove that he really had isolated the organisms responsible for chicken cholera, Pasteur arranged for a public demonstration where he repeated an experiment (Fig. 2-5) that had been successful in many previous trials. He inoculated healthy chickens with his pure cultures, but to his dismay, the chickens failed to get sick and die! Reviewing each step of the experiment, Pasteur found that he had accidentally used cultures several weeks old instead of the fresh ones grown especially for the demonstration. Some weeks later he repeated the experiment, using two groups of chickens. One of these groups had been inoculated at the first demonstration with the old cultures that had proved ineffective, and the second had not been previously exposed. Both groups received bacteria from fresh young cultures. This time the chickens in the second group got sick and died, but those in the first group remained hale and hearty. This puzzled Pasteur, but he soon found the explanation. In some way bacteria could lose their ability to produce disease, i.e., their virulence, after standing and growing old. But these attenuated (having decreased virulence) bacteria still retained their capacity for stimulating the host to produce substances, i.e., antibodies, that protect against subsequent exposure to virulent organisms.

This demonstration explained the principle involved in Edward Jenner's successful use of cowpox virus, in 1798, to immunize people against smallpox (Fig. 2-8). Pasteur next applied this principle to the prevention of anthrax, and again



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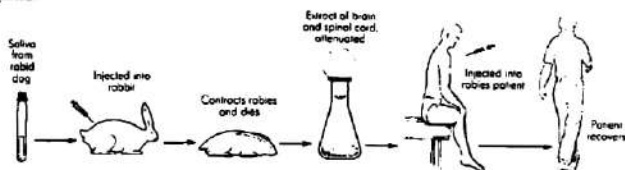
Figure 2-6. Edward Jenner vaccinating (inoculating) James Phipps with cowpox material, which resulted in development of resistance to smallpox infection. (Courtesy of Culver Pictures, New York.)



it worked. He called the attenuated cultures *vaccines*, a term derived from the Latin *vacca*, meaning "cow." Pasteur was honoring Jenner when he applied the term *vaccination* to immunization with attenuated cultures of bacteria that had no connection with cows.

Pasteur's fame was by now well established throughout France, and the belief became prevalent that he could work miracles with bacteria and the control of infections. It was not surprising, then, that he was given an even greater challenge: he was asked to work on a disease affecting human beings. As he was a chemist and not a physician, studying a human disease might prove risky. But Pasteur again accepted the challenge to be of service to humanity and set out to make a vaccine for hydrophobia, or rabies, a disease transmitted to people by bites of dogs, cats, and other animals. Because it was invariably fatal, when a boy named Joseph Meister was bitten by a mad wolf, his family did not hesitate to take the one chance in thousands that Pasteur could make a vaccine that would save him.

Figure 2-7. Rabies vaccine is made by inoculating a rabbit with saliva from a rabid dog. Virus in the extract of the rabbit's spinal cord is attenuated before injection into a patient.



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It had been established that the rabies virus was too minute to be seen even with a microscope: it had never been grown in laboratory culture, and it was not a bacterium. The disease could be produced in rabbits by inoculating them with saliva from mad dogs. Then the brain and spinal cord could be removed from the infected rabbits, dried for several days, pulverized, and mixed with glycerin. Injecting this mixture into dogs protected them against rabies (Fig. 2-7). But vaccinating dogs was quite different from treating a sick boy. Perhaps the worried Pasteur was as surprised as anyone else when after the crucial trial, which took several weeks, Joseph Meister did not die. As with Jenner's vaccination for smallpox, the principles of the preventive treatment of rabies have not changed.

WIDENING HORIZONS

Medical Microbiology

The success of Pasteur and Koch brought honors and accolades from their appreciative countrymen. Koch became Professor of Hygiene and Director of the Institute for Infective Diseases, which was founded for him at the University of Berlin. France showed its gratitude by establishing the Pasteur Institute in Paris in 1888. To each of these men came scholars from all over the world, and these students later carried the spirit and knowledge of Koch and Pasteur to America and throughout Europe.

New bacteria were being discovered with increasing frequency, and their disease-producing capacities were proved by Koch's postulates. After Edwin Klebs in 1883 and Frederick Loeffler in 1884 discovered the diphtheria bacillus and demonstrated that it produced its poisons in a laboratory flask, Emil von Behring and Shibasaburo Kitasato devised a method of producing immunity to infections caused by these organisms by injecting their toxins (poisons) into animals so that an antitoxin (a substance that neutralizes toxin) would develop. Similarly, Kitasato cultivated *Clostridium tetani*, the cause of tetanus (lockjaw), and with von Behring made antitoxin for the prevention and treatment of this disease. For his work on serum therapy, von Behring was awarded the Nobel prize in physiology or medicine in 1901. During this time, D. E. Salmon and Theobald Smith demonstrated that immunity to many infections could be produced by inoculation with killed cultures of microorganisms.

Working in Pasteur's laboratory, Elie Metchnikoff (Fig. 2-8A), a Russian, described how certain leukocytes (white blood cells) could ingest (eat) disease-producing bacteria in the body. He called these special defenders against infection phagocytes ("eating cells") and the process phagocytosis (Fig. 2-8B). Metchnikoff formulated the theory that the phagocytes were the body's first and most important line of defense against infection.

In Germany, one of Koch's students had a different concept of how the body destroys bacteria. Paul Ehrlich explained immunity on the basis of certain soluble substances in the blood. Today we know that there is much merit in both arguments and that both mechanisms play their parts. Ehrlich made another important discovery that opened the door to future developments in chemotherapy and antibiotics: he found that the 606th compound tested, an organic chemical compound containing arsenic, would destroy the syphilis microbe in the body. This was the first chemotherapeutic substance scientifically discovered and evaluated. The period from 1850 to 1900 was indeed a golden time for

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Figure 2-8. (A) Elie Metchnikoff was the first person to recognize the role of phagocytes in combating bacterial infections. (Courtesy of René Dubos.) (B) The process of phagocytosis, the ingestion of particulate matter by certain cells, shown in three steps. Phagocytosis is a natural defense mechanism against disease. (Erwin F. Lessel, illustrator.)

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microbiology because the infant science grew to adolescence during those years. As shown in Table 2-1, most of the causative agents of bacterial diseases that had plagued the world for centuries were isolated and identified.

The students of Koch and Pasteur continued to discover the causative agents of diseases and new methods for diagnosis; e.g., the Widal test for typhoid fever and the Wassermann test for syphilis made diagnosis of these diseases accurate and quick.

In the 1860s, while all these things were happening on the continent of Europe, an English surgeon, Joseph Lister, was trying to combat the microbes that caused postoperative and wound infections. Deaths from these infections were frequent in the nineteenth century. Disinfectants as such were unknown, but since carbolic acid would kill bacteria, Lister used a dilute solution of this acid to soak surgical dressings. Wounds protected in this way did not become infected, and healing took place rapidly. So remarkable was Lister's success that the technique was quickly accepted, and this antiseptic surgical practice established the principles of present-day aseptic ("without infection") techniques.

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Table 2-1. Discovery of Causative Agents of Microbial Diseases Following Establishment of Germ Theory of Disease

Date	Disease of Infection	Causative Agent*	Discoverer†
1876	Anthrax	<i>Bacillus anthracis</i>	Koch
1879	Gonorrhea	<i>Neisseria gonorrhoeae</i>	Neisser
1880	Typhoid fever	<i>Salmonella typhi</i>	Eberth
1880	Malaria	<i>Plasmodium</i> spp.	Laveran (C. Alphonse)
1881	Wound infections	<i>Staphylococcus aureus</i>	Ogston
1882	Tuberculosis	<i>Mycobacterium tuberculosis</i>	Koch
1882	Glanders	<i>Pseudomonas mallei</i>	Koch
1883	Cholera	<i>Vibrio cholerae</i>	Loeffler and Schütz
1883	Diphtheria	<i>Corynebacterium diphtheriae</i>	Klebs
1884	Diphtheria	<i>Corynebacterium diphtheriae</i>	Loeffler
1885	Swine erysipelas	<i>Erysipelothrix rhusiopathiae</i>	Loeffler
1885	Tetanus	<i>Clostridium tetani</i>	Nicolaier
1886	Bacterial pneumonia	<i>Streptococcus pneumoniae</i>	Franke
1887	Menigitis	<i>Neisseria meningitidis</i>	Wachsmann
1887	Malta fever	<i>Brucella</i> spp.	Bruce
1888	Equine strangles	<i>Streptococcus</i> spp.	Schütz
1889	Chancroid	<i>Haemophilus ducreyi</i>	Ducrey
1892	Gas gangrene	<i>Clostridium perfringens</i>	Weich and Nuttall
1894	Plague	<i>Yersinia pestis</i>	Kittasato and Yersin
1895	Fowl typhoid	<i>Salmonella gallinarum</i>	Moore
1896	Botulism (food poisoning)	<i>Clostridium botulinum</i>	Van Ermengem
1897	Bang's disease (bovine abortion)	<i>Brucella abortus</i>	Bang
1898	Dysentery	<i>Shigella dysenteriae</i>	Shiga
1898	Pleuropneumonia of cattle	<i>Mycoplasma mycoides</i>	Nocard and Roux
1905	Syphilis	<i>Treponema pallidum</i>	Schaudinn and Hoffmann
1906	Whooping cough	<i>Bordetella pertussis</i>	Bordet and Gengou
1906	Rocky Mountain spotted fever	<i>Rickettsia rickettsii</i>	Ricketts
1912	Tularemia	<i>Francisella tularensis</i>	McCoy and Chapin

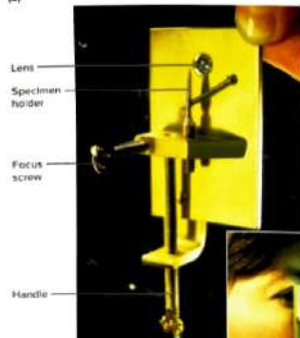
* Present name of causative agent; original name, in many instances, was different.

† In some instances the individual simply observed the causative agent; in other instances the investigator isolated the agent in pure culture.

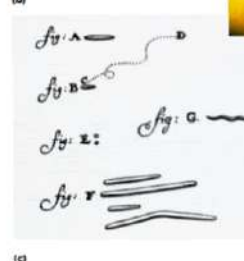
Microbiologists began to appear in America at the turn of the century. Some had studied under Koch, and others had visited the Pasteur Institute or watched Lister operate in a mist of carbolic acid vapors or a spray of bichloride of mercury. Whether they actually received their training in the "laboratories of the masters," first- and second-generation bacteriologists in Europe and America were greatly influenced by them. These Americans who actually studied under Pasteur and Koch—William Henry Welch at Johns Hopkins, Harold Clarence Ernst at Harvard, F. C. Novy at Michigan, and H. L. Russell at Wisconsin—were, as P. F. Clark, longtime professor at the University of Wisconsin, said, "responsible directly or indirectly for the development of bacteriology in the United States." Their students and their students' students constitute the who's who of those who built the science of microbiology in America.



(a)



(b)



(c)



Figure 1.10 Antony van Leeuwenhoek. (a) An oil painting of Leeuwenhoek. (b) A brass replica of the Leeuwenhoek microscope. Inset photo shows how it is held. (c) Leeuwenhoek's drawings of bacteria from the human mouth. (a) © Bettmann/Getty Images; (b) © Kathy Park Tasci/Alamy; (c) © Dr. J. van der Vliet/Alamy

these experiments, the French naturalist Felix Pouchet (1800–1872) claimed in 1859 to have carried out experiments conclusively proving that microbial growth could occur without contact with air.

Pouchet's claim provoked Louis Pasteur (1822–1895) to settle the matter of spontaneous generation. Pasteur (figure 1.11) first filtered air through cotton and found that objects resembling plant spores had been trapped. If a piece of the cotton was placed in sterile medium after air had been filtered through it, microbial growth occurred. Next he placed nutrient solutions in flasks, heated their necks in a flame, and pulled them into a variety of curves. The swan-neck flasks that he produced in this way had necks open to the atmosphere (figure 1.12). Pasteur then boiled the solutions for a few minutes and allowed them to cool. No growth took place even though the contents of the flasks were exposed to the air (figure 1.12). Pasteur inferred that growth did not occur because dust and germs had been trapped on the walls of the curved necks. If the necks were broken, growth commenced immediately. Pasteur had not only resolved the controversy by 1861 but also had shown how to keep solutions sterile.

The English physicist John Tyndall (1820–1893) and the German botanist Ferdinand Cohn (1828–1898) dealt a final blow to spontaneous generation. In 1877 Tyndall demonstrated that dust



Figure 1.11 Louis Pasteur.

(© Postage & Telecommunications)

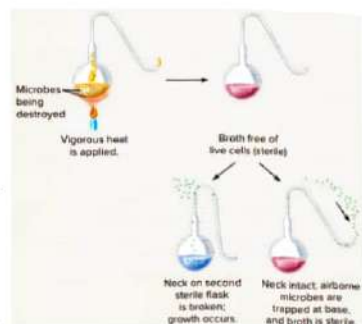


Figure 1.12 Pasteur's Experiments with Swan-Neck Flasks.

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did indeed carry germs and that if dust was absent, broth remained sterile even if directly exposed to air. During the course of his studies, Tyndall provided evidence for the existence of exceptionally heat-resistant forms of bacteria. Working independently, Cohn discovered that the heat-resistant bacteria recognized by Tyndall were species capable of producing bacterial endospores. Cohn later played an instrumental role in establishing a classification system for bacteria based on their morphology and physiology.

► **Bacterial endospores are a survival strategy (section 3.9)**

These early microbiologists not only disproved spontaneous generation but also contributed to the rebirth of microbiology. They developed liquid media and the methods for sterilizing it so that microbes could be cultured. These techniques were next applied to understanding the role of microorganisms in disease.

Microorganisms and Disease

For hundreds of years, most people believed that disease was caused by supernatural forces, poisonous vapors, and imbalances among the four humors thought to be present in the body. The role of the four humors (blood, phlegm, yellow bile [choleric], and black bile [melancholy]) in disease had been widely accepted since the time of the Greek physician Galen (129–199). Support for the idea that microorganisms cause disease—that is, the germ theory of disease—began to accumulate in the early nineteenth century from diverse fields. Agostino Bassi (1773–1856) demonstrated in 1835 that a silkworm disease was due to a fungal infection. In 1845 M. J. Berkeley (1803–1889) proved that the great potato blight of Ireland was caused by a water mold (then thought to be a fungus), and in 1853 Heinrich de Bary (1831–1888) showed that fungi caused cereal crop diseases.

Pasteur also contributed to this area of research in several ways. His contributions began in what may seem an unlikely way. Pasteur was trained as a chemist and spent many years studying the alcoholic fermentations that yield ethanol and are used in the production of wine and other alcoholic beverages. When he began his work, the leading chemists were convinced that fermentation was due to a chemical instability that degraded the sugars in grape juice and other substances to alcohol. Pasteur did not agree; he believed that fermentations were carried out by living organisms.

In 1856 M. Bigo, an industrialist in Lille, France, where Pasteur worked, requested Pasteur's assistance. His business produced ethanol from the fermentation of beet sugars, and the alcohol yields had recently declined and the product had become sour. Pasteur discovered that the fermentation was failing because the yeast normally responsible for alcohol formation had been replaced by bacteria that produced acid rather than ethanol. In solving this practical problem, Pasteur demonstrated that fermentations were due to the activities of specific yeasts and bacteria, and he published several papers on fermentation between 1857 and 1860.

Pasteur was also called upon by the wine industry in France for help. For several years, poor-quality wines had been produced. Pasteur referred to the wines as diseased and demonstrated that particular wine diseases were linked to particular microbes contaminating the wine. He eventually suggested a

method for heating the wines to destroy the undesirable microbes. The process is now called pasteurization.

Indirect evidence for the germ theory of disease came from the work of the English surgeon Joseph Lister (1827–1912) on the prevention of wound infections. Lister, impressed with Pasteur's studies on fermentation, developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds. Instruments were heat sterilized, and phenol was used on surgical dressings and at times sprayed over the surgical area. The approach was remarkably successful in reducing the rate of surgical infection. It also provided strong indirect evidence for the role of microorganisms in disease.

Koch's Postulates

The first direct demonstration that bacteria cause disease came from the study of anthrax by the German physician Robert Koch (1843–1910). Koch (figure 1.13) used the criteria proposed by his former teacher Jacob Henle (1809–1885) and others to establish the relationship between *Bacillus anthracis* and anthrax. In these studies, published in 1876, Koch used mice as his model or test organism. Following the steps outlined in figure 1.14, he next used guinea pigs to show that *Mycobacterium tuberculosis* causes tuberculosis (TB). The importance of this discovery cannot be understated as TB was a leading cause of death in Europe at the time. In 1905 Koch was awarded the Nobel Prize in Physiology or Medicine and his criteria for proving the causal relationship between a microorganism and a specific disease are known as Koch's postulates. Koch's postulates have since been used to discover the causative microorganisms for many infectious diseases.

While Koch's postulates are still widely used, their application is at times not feasible. For instance, viruses and organisms that must



Figure 1.13 Robert Koch. Koch examining a specimen in his laboratory.

(© Bettmann/Getty Images)

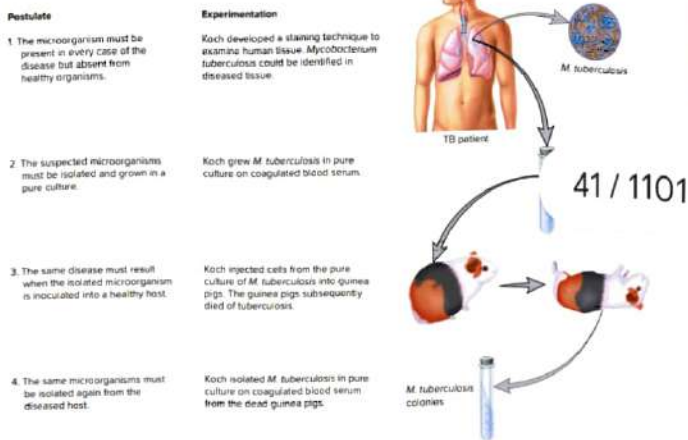


Figure 1.14 Koch's Postulates Applied to Tuberculosis.

MICRO INQUIRY Why is the fourth postulate necessary?

live within host cells such as *Mycobacterium leprae*, the causative agent of leprosy, cannot be isolated in pure culture. Some human diseases lack an appropriate animal model so the postulates cannot be fully met. To avoid some of these difficulties, microbiologists sometimes use molecular and genetic evidence. For instance, molecular methods might be used to detect the nucleic acid of a microorganism in body tissues, rather than isolating it, or the genes thought to be associated with the virulence of a disease-causing microbe (pathogen) might be mutated. In this case, the mutant organism should have decreased ability to cause disease. Introduction of the normal gene back into the mutant should restore the pathogen's virulence.

Pure Culture Methods

During Koch's studies on bacterial diseases, it became necessary to isolate suspected bacterial pathogens in pure culture. At first Koch cultured bacteria on the sterile surfaces of cut, boiled potatoes, but the bacteria did not always grow well. Eventually he developed culture media using meat extracts and protein digests, reasoning these were similar to body fluids. Initially he tried to solidify the media by adding gelatin. Separate bacterial colonies developed after the surface of the solidified medium had been

streaked with a bacterial sample. The sample could also be mixed with liquefied gelatin medium. When the medium hardened, individual bacteria produced separate colonies.

Despite its advantages, gelatin was not an ideal solidifying agent because it can be digested by many microbes and melts at temperatures above 28°C. A better alternative was provided by Fanny Eilshemius Hesse (1850–1934), the wife of Walther Hesse (1846–1911), one of Koch's students and a physician. She often assisted her husband with his experiments and suggested using agar, which she used to make jellies, as a solidifying agent. Agar was not broken down by most bacteria. Furthermore, it did not melt until reaching a temperature of 100°C and, once melted, did not solidify until reaching a temperature of 50°C; this eliminated the need to handle boiling liquid. Some of the media developed by Koch and his associates, such as nutrient broth and nutrient agar, are still widely used. Another important tool developed in Koch's laboratory was a container for holding solidified media—the Petri dish (plate), named after Richard Petri (1852–1921), who devised it. These developments directly stimulated progress in all areas of microbiology. ▶ Culture media (section 7.7); Enrichment and isolation of pure cultures (section 7.7)

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Our focus thus far has been on the development of methods for culturing bacteria. But viral pathogens were also being studied during this time, and methods for culturing them were also being developed. The discovery of viruses and their role in disease was made possible when Charles Chamberland (1851–1908), one of Pasteur's associates, constructed a porcelain filter to remove bacteria. Dimitri Ivanowski (1864–1920) and Martinus Beijerinck (pronounced “by-a-rink”, 1851–1931) used the filter to study tobacco mosaic disease. They found that plant extracts and sap from diseased plants were infectious, even after being filtered with Chamberland's filter. Because the infectious agent passed through a filter that was designed to trap bacterial cells, they reasoned that the agent must be something smaller than a bacterium. Beijerinck proposed that the agent was a “filterable virus.” Eventually viruses were shown to be tiny, acellular infectious agents.

Immunology

The ability to culture microbes also played an important role in early immunological studies. During studies on the bacterium that causes chicken cholera, Pasteur and Pierre Roux (1853–1933) discovered that bacteria incubated in cultures for long periods of time lost their ability to cause disease. These bacteria were said to be attenuated or weakened. When chickens were injected with attenuated bacteria, they remained healthy and were surprisingly able to resist the disease when exposed to virulent bacteria. Pasteur called the attenuated bacteria a vaccine (Latin *vacca*, cow) in honor of Edward Jenner (1749–1823) because, many years earlier, Jenner had used material from cowpox lesions to protect people against smallpox (see *Historical Highlights* 36.5). Shortly after this, Pasteur and Chamberland developed an attenuated anthrax vaccine. ▶ Vaccines immunize susceptible populations (section 36.6)

Pasteur also prepared a vaccine using an attenuated strain of rabies virus. During the course of these studies, a nine-year-old boy named Joseph Meister was bitten by a rabid dog and was brought to Pasteur. Since Joseph's death was certain in the absence of treatment, Pasteur agreed to try vaccination. Joseph was injected 13 times over the next 10 days with increasingly virulent preparations of the attenuated virus. His survival marked a huge advance in the use of vaccines. To thank Pasteur, people from around the world contributed to the construction of the Pasteur Institute in Paris, France. One of the initial tasks of the institute was vaccine production.

These early advances in immunology were made without any concrete knowledge about how the immune system works. Immunologists now know that white blood cells and the chemicals they produce play a central role in immunity. Among the chemicals are soluble proteins called antibodies, found in blood, lymph, and other body fluids. The role of soluble substances in preventing disease was recognized by Emil von Behring (1854–1917) and Shibasaburo Kitasato (1852–1931). After the discovery that diphtheria was caused by a toxin produced by bacteria, they injected inactivated diphtheria toxin into rabbits. The inactivated toxin induced rabbits to produce an antitoxin, which

protected against the disease. Antitoxins are now known to be antibodies that specifically bind and neutralize toxins. The first immune system cells were discovered when Elie Metchnikoff (1845–1916) found that some white blood cells could engulf disease-causing bacteria. He called these cells phagocytes and the process phagocytosis (Greek *phagēin*, eating).

Microbial Ecology

Culture-based techniques were also applied to the study of microbes in soil and aquatic habitats. Early microbial ecologists studied microbial involvement in the carbon, nitrogen, and sulfur cycles. The Russian microbiologist Sergei Winogradsky (1856–1953) made many contributions to soil microbiology, including the discovery that soil bacteria could oxidize iron, sulfur, and ammonia to obtain energy and that many of these bacteria could incorporate CO₂ into organic matter much as photosynthetic organisms do. Winogradsky also isolated anaerobic nitrogen-fixing soil bacteria and studied the decomposition of cellulose. Martinus Beijerinck made fundamental contributions to microbial ecology as well as virology. He isolated several kinds of nitrogen-fixing bacteria and sulfate-reducing bacteria. Beijerinck and Winogradsky also developed enrichment culture techniques and selective media, which have been of great importance in microbiology. ▶ Biogeochemical cycling sustains life on Earth (section 28.1); Culture media (section 7.7); Enrichment cultures (section 7.7)

Comprehension Check

1. What does the theory of spontaneous generation propose? What did Pasteur prove when he showed that a cotton plug that had filtered air would trigger microbial growth when transferred to a sterile medium? What argument made previously was he addressing?
2. Discuss the contributions of Lister, Pasteur, and Koch to the germ theory of disease and the treatment or prevention of diseases.
3. Describe Koch's postulates. What is a pure culture? Why are pure cultures important to Koch's postulates?
4. How did Jenner, Pasteur, von Behring, Kitasato, and Metchnikoff contribute to the development of immunology? How was the ability to culture microbes important to their studies?
5. How did Winogradsky and Beijerinck contribute to the study of microbial ecology? What new culturing techniques did they develop in their studies?

1.4 Microbiology Encompasses Many Subdisciplines

After reading this section, you should be able to:

- a. Construct a concept map, table, or drawing that illustrates the diverse nature of microbiology and how it has improved human conditions
- b. Discuss the belief held by many microbiologists that microbiology is experiencing its second golden age

Classification

Ecological Characteristics

For example, bacteria known as bdellovibrions are predatory on other bacteria, and viruses called bacteriophages can infect and destroy bacterial cells.

The habitat of a microorganism is important in characterizing that organism. For example, microorganisms normally found in marine environments generally differ from those in freshwater environments. The microbial population of the oral cavity differs from that of the intestinal tract. Some kinds of microorganisms are widely distributed in nature, but others may be restricted to a particular environment. The relation of an organism to its environment is often complex and may involve special characteristics of the organism that are not yet known.

MICROBIAL CLASSIFICATION, NOMENCLATURE, AND IDENTIFICATION

Once the characteristics of microorganisms have been determined and appropriately catalogued, the process of classification can begin.

Classification

In microbiology, taxa are initially constructed from strains. A strain is made up of all the descendants of a pure culture; it is usually a succession of cultures derived from an initial colony. Each strain has a specific history and designation.

Taxonomic Groups (Taxa)

For example, strain ATCC 19554 is a strain of spirilla isolated originally from pond water in Blacksburg, Virginia in 1965 by Wells and Krieg, and cultures of this strain are maintained at the American Type Culture Collection (ATCC), Rockville, Maryland. Cultures of the same species that were isolated from other sources would be considered different strains.

The basic taxonomic group (taxon) is the species, i.e., a collection of strains having similar characteristics. Bacterial species consist of a special strain called the type strain together with all other strains that are considered sufficiently similar to the type strain as to warrant inclusion in the species. The type strain is the strain that is designated to be the permanent reference specimen for the species. Unfortunately, it is not always the strain that is most typical of all the strains included in the species, but it is the strain to which all other strains must be compared to see if they resemble it closely enough to belong to the species. Therefore, type strains are particularly important and special attention is given to their maintenance and preservation, particularly by national reference collections such as the ATCC in the United States or the National Collection of Type Cultures in England. Many other culture collections are maintained throughout the world.

In the definition just given for a bacterial species, the phrase "considered sufficiently similar to the type strain" indicates that the definition contains an element of subjectivity. In other words, the criteria which one taxonomist believes to constitute "sufficient similarity" may be quite different from those used by another taxonomist. At present there are no specific criteria for a bacterial species that are universally accepted. However, certain criteria based on DNA homology experiments (described later in this chapter) are probably more widely accepted today than any others and eventually may lead to a unifying concept for defining a species.

Just as a bacterial species is composed of a collection of similar strains, a

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bacterial genus is composed of a collection of similar species. One of the species is designated the type species, and this serves as the permanent example of the genus; that is, other species must be judged to be sufficiently similar to the type species to be included with it in the genus. Unfortunately, there is even less agreement about the criteria for a bacterial genus than there is for a bacterial species.

Taxonomic groups of higher rank than genus are listed below, and the same considerations about subjectivity apply here as well:

Family	A group of similar genera
Order	A group of similar families
Class	A group of similar orders
Division	A group of similar classes
Kingdom	A group of similar divisions

The Goals of Classification

Taxonomists strive to make classifications that have the following two qualities:

- 1 **Stability.** Classifications that are subject to frequent, radical changes lead to confusion. Every attempt should be made to devise classifications that need only minor changes as new information becomes available.
- 2 **Predictability.** By knowing the characteristics of one member of a taxonomic group, it should be possible to assume that the other members of the same group probably have similar characteristics. If this cannot be done, the classification has little value.

General Methods of Classifying Bacteria

Three methods are used for arranging bacteria into taxa:

bacterial genus is composed of a collection of similar species. One of the species is designated the **type species**, and this serves as the permanent example of the genus; that is, other species must be judged to be sufficiently similar to the type species to be included with it in the genus. Unfortunately, there is even less agreement about the criteria for a bacterial genus than there is for a bacterial species.

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General Methods of Classifying Bacteria

Three methods are used for arranging bacteria into taxa:

The Intuitive Method. A microbiologist who is thoroughly familiar with the properties of the organisms he or she has been studying for several years may decide that the organisms represent one or more species or genera. The trouble with this method is that the characteristics of an organism that seem important to one person may not be so important to another, and different taxonomists may arrive at very different groupings. However, some classification schemes based on the intuitive method have proved to be quite useful.

Numerical Taxonomy. In an effort to be more objective about grouping bacteria, a scientist may determine many characteristics (usually 100 to 200) for each strain studied, giving each characteristic equal weight. Then using a computer he or she calculates the % similarity (%S) of each strain to every other strain. For any two strains, this is:

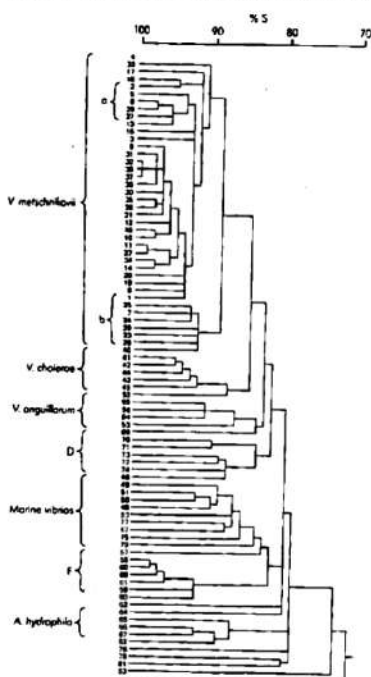
$$\%S = \frac{NS}{NS + ND}$$

where NS is the number of characteristics that are the same (positive or negative) for the two strains, and ND is the number of characteristics that are different. (The method is sometimes made more rigorous by making NS equal to the number of positive characteristics that are the same for the two strains, since what organisms can do may be more important than what they cannot do.) Those strains having a high %S to each other are placed into groups; those

groups having a high %S to each other are in turn placed into larger groups, and so on (see Fig. 3-1). The degree of similarity needed to rank a group as a species, genus, or other taxon is a matter of judgment on the part of the taxonomist. This method of classification has great practical usefulness as well as being relatively unbiased in its approach; it also yields classifications that have a high degree of stability and predictability.

Genetic Relatedness. The third and most reliable method of classification is

Figure 3-1. Diagram showing the arrangement of 83 strains of oxidase-positive, vibriolike bacteria according to a numerical taxonomic study. Some of the resulting groups represent species (e.g., *Vibrio metschnikovii*, *V. cholerae*, etc.); others are designated only by vernacular names (Marine vibrios, Group D, etc.). Courtesy of J. V. Lee, T. J. Donovan, and A. L. Furniss, *Int J Syst Bacteriol* 28:99-111, 1978.)



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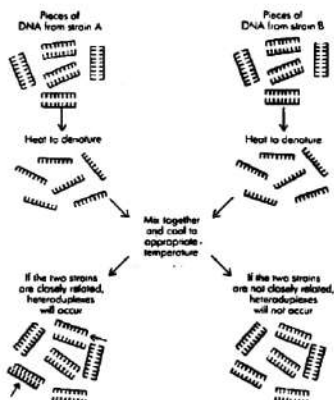
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based on the degree of genetic relatedness between organisms. This method is the most objective of all and is based on the most fundamental aspect of organisms, their hereditary material (DNA). In the 1960s the development of that branch of science known as molecular biology provided techniques by which the DNA of one organism could be compared with that of other organisms. At first only crude comparisons could be made, based on mol% G + C values. It is true that two organisms of the same or similar species that are very closely related will have very similar mol% G + C values, and it is also true that two organisms having quite different mol% G + C values are not very closely related. However, it is important to realize that organisms that are completely unrelated may have similar mol% G + C values. Therefore, much more precise methods of comparison were needed—namely, methods by which the DNA molecules from various organisms could be compared with respect to the sequence of their component nucleotides. This sequence is the most fundamental characteristic of an organism. Modern techniques have now made it possible to make such a comparison. The basic principles can be described briefly as follows:

1 DNA homology experiments. The double-stranded DNA molecules from two organisms are heated to convert them to single strands. The single strands from one organism are then mixed with those from the other organism and allowed to cool. If the two organisms are closely related, heteroduplexes will form. In other words, a strand from one organism will pair with a strand from the other organism (see Fig. 3-2). If the two organisms are not closely related, no heteroduplexes will form. This method is most useful at the species level of classification.

Figure 3-2. Schematic diagram illustrating the basic principle behind DNA homology experiments.



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2 Ribosomal RNA homology experiments and ribosomal RNA oligonucleotide cataloging. Two organisms may not be so closely related as to give a high level of DNA homology, yet they may still have some degree of relatedness. Ribosomes, the small granular-appearing structures within the cell which manufacture proteins, are composed of proteins and RNA. The ribosomal RNA (rRNA) is coded for by only a small fraction of the DNA molecule, the rRNA cistrons. In all bacteria so far studied, the nucleotide sequence of these rRNA genes has been found to be highly conserved; that is, during evolution, the nucleotide sequence has changed more slowly than that of the bulk of the DNA molecule. This means that even if two organisms are only distantly related and show no significant DNA homology, there still may be considerable similarity in the nucleotide sequences of their rRNA cistrons. The degree of similarity that exists can therefore be used as a measure of relatedness between organisms, but at a level beyond that of species (at the level of genus, family, order, etc.). RNA homology experiments and RNA oligonucleotide cataloging are two modern methods used to determine the degree of similarity between the rRNA cistrons of different organisms. The techniques are complex and are being used by only a few laboratories.

Classifications based on genetic relatedness come the closest to taxonomic goals of stability and predictability. Moreover, the development of such classifications allow microbiologists to infer the way in which organisms have evolved, so that the present-day bacterial genera and species can be arranged in a hierarchy that reflects their ancestral relationships, i.e., in a phylogenetic classification. Much of the work is still fragmentary, but some of the results, especially those obtained by Dr. C. R. Woese of the University of Illinois and his colleagues, have already revolutionized current thinking about how bacteria have evolved and how they are related to one another. In fact, it is now apparent that present-day bacteria evolved by at least two very different major routes from an early ancestral form and that they now comprise two very large groups: the eubacteria (which are the traditional, familiar ones that have received the most study) and the archaeobacteria (consisting of methane-producers, extreme halophiles, and thermoacidophiles). It has been proposed that these two groups be considered as two separate kingdoms of life, and, indeed, they do seem as distantly related to each other as they are to eucaryotic organisms. Although the kingdom question is still debatable, data obtained from rRNA oligonucleotide cataloging nevertheless make it clear that the archaeobacteria are separated from other bacteria by a great phylogenetic gulf (see Fig. 3-3).

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Nomenclature

Each species of microorganism has only one officially accepted name, by international agreement. This system provides for precise communication. If an organism were to be called *Escherichia coli* in one country and *Coproacterium intestinale* in another, chaos would result. It would be difficult to know that the same organism was being studied.

The name of a species is merely a convenient label. It is not necessarily even descriptive, although some names are. For example, *Micrococcus luteus* means "yellow berry" in Latin, and *Proteus vulgaris* is Latin for "common organism of many shapes." Some species are named after persons: for example, *Escherichia coli*—the organism of the colon, named after Theodor Escherich (a German bacteriologist); or *Clostridium barkeri*—the spindle-shaped organism,

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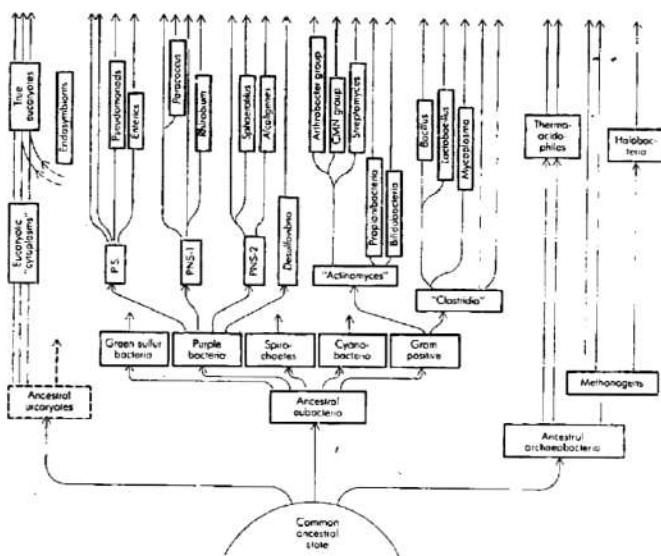


Figure 3-3. Schematic representation of the major lines of procaryotic descent, based on rRNA oligonucleotide cataloging. The archaeobacteria arose from an early ancestral form according to the pathway at the center. (A third line of descent which led to eucaryotic organisms is depicted at the left.) (Courtesy of G. E. Fox et al., *Science* 209:457-463, 1986.)

named after H. A. Barker (an American biochemist). Some names are even nonsensical [e.g., *Rumella slithyformis*—"the organism whose shape resembles runes (characters of an ancient alphabet) and which is slithy"; the latter term being taken from Lewis Carroll's poem "Jabberwocky" from *Alice in Wonderland*]. The important point is that names are only convenient designations. For example, instead of referring to "the rod-shaped, acid-fast bacterium that is slow-growing, is stimulated by glycerol, causes pulmonary tuberculosis in humans, is spread mainly by airborne droplets, forms buff-colored colonies, synthesizes niacin, reduces nitrate to nitrite, and is pathogenic for guinea pigs," it is much more convenient simply to say "*Mycobacterium tuberculosis*."

Although it might seem that microbial names could be constructed almost at random, the fact is that certain rules must be followed. Bacteria, for example, are named according to rules set down in the *International Code of Nomenclature of Bacteria*; other codes govern the naming of algae, fungi, and viruses.

One rule in bacteriological nomenclature is that a name must be written as a Latin or latinized binomial (two words) and must follow certain rules of Latin grammar. The first word in the binomial is the genus name and is always capitalized. The second word is the specific epithet and is never capitalized. Both the genus name and specific epithet are given in italics (or underlined, which means "italics" to a printer). Bacteria are sometimes referred to by common or colloquial names, which have no official standing in nomenclature and are never italicized (for example, the "colon bacillus," which is *E. coli*, or the "tubercle bacillus," which is *M. tuberculosis*). Such names do not lead to precise communication: for instance, many bacteria occur in the colon besides *E. coli*, and other organisms besides *M. tuberculosis* can cause tuberculosis.

Those bacterial names which have official standing in microbiology were published in the *Approved Lists of Bacterial Names* in January, 1980. Any new or additional names must be published in the *International Journal of Systematic Bacteriology* in order to achieve official recognition.

The International Code of Nomenclature of Bacteria was developed with reference to the much earlier established International Codes of Zoological and Botanical Nomenclature. All of these codes incorporate certain common principles as listed below.

- 1 Each distinct kind of organism is designated as a species.
- 2 The species is designated by a Latin binomial to provide a characteristic international label (binomial system of nomenclature).
- 3 Regulation is established for the application of names.
- 4 A law of priority ensures the use of the oldest available legitimate name.
- 5 Designation of categories is required for classification of organisms.
- 6 Requirements are given for effective publication of new specific names, as well as guidance in coining new names.

Identification

An organism must be classified before it can be identified—that is, given a name. This is true even if the classification is merely the recognition that the organism is different from any known organism. (For example, this occurred with the Legionnaires' disease agent, which caused the famous pneumonia epidemic in 1976 in Philadelphia; this organism was unlike bacteria of any established species; it has now been classified in a new bacterial genus, *Legionella*, and has been assigned the species name *L. pneumophila*.) Once an organism is classified, a few of its characteristics are selected by which it can be identified by other microbiologists. In order to be useful for identification, the combination of characteristics chosen must occur only in that particular kind of organism and in no other. The characteristics chosen should also be ones that are easy to determine, such as shape, staining reactions, and sugar fermentations. For example, DNA homology experiments, while very useful for classifying an organism, would be quite unsatisfactory for the routine identification of an organism because of the complexity of the procedure.

Many identification schemes are in the form of keys, which give identifying characteristics arranged in a logical fashion. Identification tables are also useful and generally contain more characteristics than do keys, with the information arranged in an easy-to-read, summarized form.

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48 INTRODUCTION TO MICROBIOLOGY

The Past and Present State of Bacterial Taxonomy

The first classification scheme for bacteria was published in 1773, and many more have appeared since. The early schemes were based only on morphological characteristics, but as the science of microbiology developed, other kinds of characteristics became increasingly important for classifications. Each successive classification scheme reflected the level of knowledge available at the time, and this continues to be true. Even present arrangements of bacteria are only provisional, subject to modification or replacement as new information appears.

Many classification schemes presently exist, but most cover only one or a few groups of bacteria. One classification scheme is unique, however, because of its broad scope and wide acceptance: *Bergey's Manual of Determinative Bacteriology*. This international reference work not only provides descriptions of all established genera and species of bacteria, but it also provides a practical arrangement of these taxa that is useful for their identification, together with appropriate keys and tables. Eight editions of *Bergey's Manual* have appeared since 1923, and a new edition is now in preparation, part of which has already been published. The title has been changed to *Bergey's Manual of Systematic Bacteriology* to reflect an increased coverage of bacterial characterization, classification, and taxonomic problems, in addition to the identification aspects. *Bergey's Manual* is written by hundreds of authors from around the world, each an authority on a particular bacterial group.

The arrangement of bacterial taxa in the new edition of *Bergey's Manual* is mainly along traditional, practical lines. Each volume is divided into a number of sections, each bearing a vernacular name such as "The Spirochetes" or "Gram-Negative Anaerobic Cocci" rather than a formal taxonomic name. The emphasis is largely on genera and species and, because of the present incomplete and fragmentary understanding of the real relationships that exist among bacteria, no attempt is made to adhere to any comprehensive, formal taxonomic hierarchy. The present classification scheme has considerable practical value, but the editorial board of *Bergey's Manual* regards it only as an interim arrangement that must eventually give way to a new, general, comprehensive classification scheme based on genetic relatedness. This is expected to provide greater stability and predictability, to lead to improved identification schemes, and to aid our understanding of the origin of present-day genera and species.

QUESTIONS

- 1 Define the following terms:

Classification	Taxon
Nomenclature	Taxa
Identification	Bacterial strain
Pure culture	Bacterial species
Mol% G + C	%S
Phylogenetic	<i>Bergey's Manual</i>
- 2 Why is it essential to classify microorganisms and what must be done before they can be classified?
- 3 List and describe briefly the major kinds of microbial characteristics and indicate those that must be determined by examination of individual cells.
- 4 Why is the type strain the most important strain in a bacterial species?
- 5 Explain the subjectivity that exists in the definition of a bacterial species.

not yet been grown in the lab, these microbes contribute to nutrient cycling and host-microbe physiology in ways that are defined in aggregate but not at the level of a single microbial taxon (e.g., genus or species). The undiscovered, however, are most intriguing. These are the microbes yet to be found—the real microbial dark matter. Do they exist? If so, how much bigger is the microbial universe than previously thought?

The only way to answer these questions is to explore. As discussed in chapter 18, metagenomic and single-cell genome sequencing harness the power of next-generation sequencing to probe genes and genomes that belong to uncultured microorganisms. The results have been startling: Hundreds of new archaeal and bacterial phyla have recently been proposed. Even more exciting is the fact that some newly discovered gene sequences don't match any previously identified archaeal, bacterial, or eukaryotic nucleotide sequences. For example, very large and unusual viruses have been found to possess genes whose evolutionary history places them somewhere between archaeal and eukaryotic domains. If cellular counterparts to these viruses exist, would they represent a new domain?

As we discuss in this chapter, phyla (s., phylum) are higher-level taxa to which a group of related organisms belong. The recent, sudden increase in the number of phyla has raised concern among taxonomists. While the criteria used to define a particular bacterial or archaeal species have long

- ✓ Outline in general terms how small subunit rRNA has been used to define the three domains of life (section 1.2)
- ✓ Summarize the mechanisms of horizontal gene transfer (chapter 16)
- ✓ Describe the rationale for the RNA world hypothesis of the origin of life (section 1.2)
- ✓ Contrast and compare the structural differences between bacterial, archaeal, and eukaryotic microbes (chapters 3–5)
- ✓ Explain the principles of next-generation sequencing and metagenomics (sections 18.1–18.3)

19.1 Microbial Taxonomy Is Based on the Comparison of Multiple Traits

After reading this section, you should be able to:

- a. Explain the utility of taxonomy and systematics
- b. Draw a concept map illustrating the differences between phenetic and genotypic classification

Microbiologists are faced with the daunting task of understanding the diversity of life forms that cannot be seen with

the naked eye but can live anywhere on Earth. Obviously a reliable classification system is paramount. The science of classifying living things is called **taxonomy** (Greek *taxis*, arrangement or order; *nomos*, law, or *nemein*, to distribute or govern). In a broader sense, taxonomy consists of three separate but interrelated parts: classification, nomenclature, and identification. A taxonomic scheme is used to arrange organisms into groups called **taxa** (s., **taxon**) based on mutual similarity. **Nomenclature** is the branch of taxonomy concerned with the assignment of names to taxonomic groups in agreement with published rules. Identification is the practical side of taxonomy—the process of determining if a particular isolate belongs to a recognized taxon and, if so, which one. The term **systematics** is often used for taxonomy, although it sometimes infers a more general scientific study of organisms with the ultimate objective of arranging them in an orderly manner. Thus systematics encompasses morphology, ecology, epidemiology, biochemistry, genetics, molecular biology, and physiology.

One of the oldest classification systems, called **natural classification**, arranges organisms into groups whose members share many characteristics. The Swedish botanist Carl von Linné, or Carolus Linnaeus as he often is called, developed the first natural classification in the eighteenth century. It was based largely on anatomical characteristics and was a great improvement over previously employed systems because natural classification provided information about many biological properties. For example, classification of humans as mammals denotes that they have hair, self-regulating body temperature, and milk-producing mammary glands in the female.

When natural classification is applied to higher organisms, evolutionary relationships become apparent simply because the morphology of a given structure (e.g., wings) in a variety of organisms (ducks, songbirds, hawks) suggests how that structure might have been modified to adapt to specific environments or behaviors. However, the traditional taxonomic assignment of microbes was not rooted in evolutionary relatedness. For instance, bacterial pathogens and microbes of industrial importance were historically given names that described the diseases they cause or the processes they perform (e.g., *Vibrio cholerae*, *Clostridium tetani*, and *Lactococcus lactis*). Although these labels are of practical use, they do little to guide

the taxonomist concerned with the vast majority of microbes that are neither pathogenic nor of industrial consequence. Our present understanding of the evolutionary relationships among microbes now serves as the theoretical underpinning for taxonomic classification.

In practice, determining the genus and species of a newly isolated microbe is based on **polyphasic taxonomy**. As the term “polyphasic” suggests, this encompasses many aspects that describe the microorganism. These include phenotypic and genotypic features. To understand how all of these data are incorporated into a coherent profile of taxonomic criteria, we must first consider the individual components.

Phenetic Classification

For a very long time, microbial taxonomists had to rely exclusively on a **phenetic system**, which classifies organisms according to their phenotypic similarity (Table 19.1). This system succeeded in bringing order to biological diversity and clarified the function of morphological structures. For example, because motility and flagella are always associated in particular microorganisms, it is reasonable to suppose that flagella are involved in at least some types of motility. Although phenetic studies can reveal possible evolutionary relationships, this is not always the case. For example, not all flagellated bacteria belong to the same phylum. This is why the best phenetic classification is one constructed by comparing as many attributes as possible.

Genotypic Classification

As the name suggests, **genotypic classification** seeks to compare the genetic similarity between organisms. Individual genes or whole genomes can be compared. Since the 1970s it has been widely accepted that bacteria and archaea whose genomes are at least 70% homologous belong to the same species. However, there is now a consensus building to replace this metric with a genomics-based assay that measures the average nucleotide identity between organisms. The means by which microbes are genotypically classified is discussed further in section 19.3.

Table 19.1 Components of Polyphasic Taxonomy

Classification System	Basis of Classification	Traits	Microbial Example
Phenetic	Phenotype	Morphology Physiology	<i>Clostridium</i> spp. (anaerobic, endospore-forming, Gram-positive rod) compared to <i>Bacillus</i> spp. (aerobic, endospore-forming, Gram-positive rod)
Genotypic	Genes and genomes	Average nucleotide identity rRNA genes	<i>E. coli</i> strain K12 compared to <i>E. coli</i> O157:H7 <i>E. coli</i> compared to <i>Salmonella enterica</i>

Phylogenetic Classification

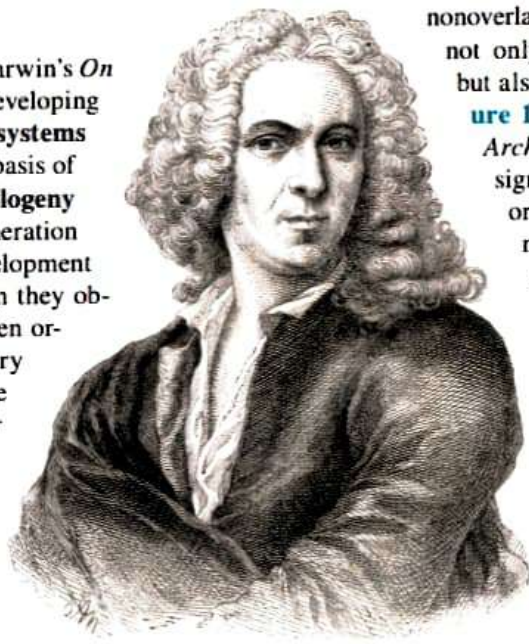
With the publication in 1859 of Charles Darwin's *On the Origin of Species*, biologists began developing **phylogenetic** or **phyletic classification systems** that sought to compare organisms on the basis of evolutionary relationships. The term **phylogeny** (Greek *phylon*, tribe or race; *genesis*, origin or development) refers to the evolutionary history of a group of organisms. Phylogenetic classification is based on a nonoverlapping hierarchy so that each level includes



not only the traits that define the rank above but also a new set of more restrictive traits (Figure 19.1). Thus within each domain—Bacteria, Archaea, or Eukarya—each organism is assigned (in descending order) to a phylum, class, order, family, genus, and species epithet.

Phylogenetic Classification

With the publication in 1859 of Charles Darwin's *On the Origin of Species*, biologists began developing **phylogenetic** or **phyletic classification systems** that sought to compare organisms on the basis of evolutionary relationships. The term **phylogeny** (Greek *phylon*, tribe or race; *genesis*, generation or origin) refers to the evolutionary development of a species. Scientists realized that when they observed differences and similarities between organisms as a result of evolutionary processes, they also gained insight into the history of life on Earth. However, for much of the twentieth century, microbiologists could not effectively employ phylogenetic classification, primarily because of the lack of a good fossil record. When Carl Woese and George Fox proposed using small subunit (SSU) rRNA nucleotide sequences to assess evolutionary relationships among microorganisms, the door opened to the resolution of long-standing inquiries regarding the origin and evolution of the majority of life forms on Earth—microbes. Whole genome sequencing is the most recent analytical tool to elucidate phylogenetic relationships among microbes.



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nonoverlapping hierarchy so that each level includes not only the traits that define the rank above it but also a new set of more restrictive traits (**figure 19.1**). Thus within each domain—*Bacteria*, *Archaea*, or *Eukarya*—each organism is assigned (in descending order) to a phylum, class, order, family, genus, and species epithet or name. Some microbes are also given a subspecies designation. Microbial groups at each level have a specific suffix that indicates rank or level.

The application of Linnaeus's classification system to bacteria began in Pasteur and Koch's time, about 1872. However, within 20 years microbiologists became dissatisfied, considered it haphazard, and called for more uniform criteria in organizing phyla, classes, orders, families, genera, and species. With the ongoing explosion in metagenomic analysis, some would argue that history is repeating itself. Within the last few decades, thousands of 16S rRNA genes and protein-coding genes have been sequenced that do not belong to any previously defined taxa. This data explosion has led to the recent development of the taxonomic classification **superphylum**, below domain and above phylum (e.g., in figure 19.1, superphylum would be placed between *Bacteria* and *Proteobacteria*). Ideally a superphylum includes organisms of several phyla that share a number of distinctive characteristics, such as unusual morphological or metabolic features. However, some feel that the term is being loosely applied based on insufficient data—for instance, to SSU rRNA sequences alone.

At the other end of the classification scheme is the most fundamental definition of a bacterial or archaeal **species**. A species is a collection of strains that share many stable properties and differ significantly from other groups of strains. A **strain** consists of the descendants of a single, pure microbial culture. Strains within a species may be described in a number of different ways. **Biovars** are variant strains characterized by biochemical or physiological differences, **morphovars** differ morphologically, and **serovars** have distinctive antigenic (immunologically reactive) properties. Because changes in species assignment are not uncommon, one strain is designated as the **type strain** for each species. The type strain is the holder of the species name. This ensures permanence of names when nomenclature revisions occur because the type species must remain within the original species. It is usually one of the first strains studied and often is more fully characterized than others; however, it does not have to be the most representative member. Only those strains very similar to the type strain or type species are included in a species. Each species is assigned to a **genus**, the next rank in the taxonomic hierarchy. A genus is a well-defined group of one or more species that is clearly separate from other genera. In practice,

Comprehension Check

1. What is a natural classification? What microbial features might have been considered when devising a natural classification scheme?
2. What is polyphasic taxonomy, and what types of data does it consider? Should each type of data be of equal weight? Why or why not?
3. Consider the finding that bacteria capable of anoxygenic photosynthesis belong to several different phyla. How do you think these bacteria might have been originally classified, and what types of data do you think were key in making the most recent taxonomic assignments?

19.2 Taxonomic Ranks Provide an Organizational Framework

After reading this section, you should be able to:

- a. Outline the general scheme of taxonomic hierarchy
- b. Explain how the binomial system of Linnaeus is used in microbial taxonomy

The definition of a bacterial or archaeal species is widely debated, as discussed in section 19.5. Nonetheless, for practical reasons it is essential that the established rules of taxonomy are followed. Microbes are placed in taxonomic levels arranged in a

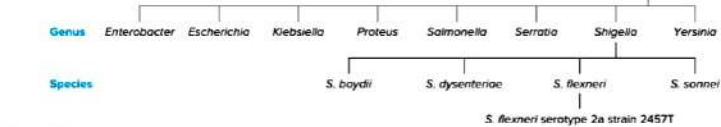


Figure 19.1 Hierarchical Arrangement in Taxonomy. In this example, members of the genus *Shigella* are placed within higher taxonomic ranks. Not all classification possibilities are given for each rank to simplify the diagram. Note that -oies denotes order and -ceae indicates family.

considerable subjectivity occurs in assigning species to a genus, and taxonomists may disagree about the composition of genera.

Microbiologists name microorganisms using the **binomial system** of Linnaeus. The Latinized, italicized name consists of two parts. The first part is the generic name (i.e., the genus), and the second is the species name (e.g., *Yersinia pestis*, the causative agent of plague). The species name is stable; the oldest epithet for a particular organism takes precedence and must be used. In contrast, a generic name can change if the organism is assigned to another genus. For example, some members of the genus *Streptococcus* were placed into two new genera, *Enterococcus* and *Lactococcus*, based on rRNA analysis and other characteristics. Thus *Streptococcus faecalis* is now *Enterococcus faecalis*. To be recognized as a new species, genomic, metabolic, morphological, reproductive, and ecological data must be accepted and published in the *International Journal of Systematic and Evolutionary Microbiology*; until that time, the new species name will appear in quotation marks. Microbes that have not been grown in pure culture but for which there is sufficient genetic characterization may be given a provisional genus and species name preceded by the term *Candidatus*, meaning candidate. For instance, *Candidatus Mycoplasma gireddii* is believed to be associated with preterm birth. It was first detected by metagenomic sequencing in the microbial community in a pre-term infant's mouth. Although complete genomes are known for several related strains, none have been successfully cultured. Note that its provisional genus and species names are not italicized. *Bergey's Manual of Systematics of Bacteria and Archaea* contains only recognized bacterial and archaeal species as discussed in section 19.6.

Comprehension Check

1. What is the difference between a microbial species and a strain?
2. Why is it important to have a type strain for each species?
3. The genus *Salmonella* was once thought to contain five species. Most scientists now consider only two species valid: *S. bongori* and *S. enterica*. The latter contains six subspecies, and *S. enterica* subspecies *enterica* is further subdivided into eight serovars. Three of these, Enteritidis, Typhi, and Typhimurium, were once considered *Salmonella* species. (a) Inspect figure 19.1 and construct a similar lineage for *Salmonella*. (b) What criteria do you think were used to make these taxonomic changes?

19.3 Microbial Taxonomy and Phylogeny Are Largely Based on Molecular Characterization

After reading this section, you should be able to:

- a. Construct a concept map describing the approaches commonly used to determine taxonomic classification
- b. Assess the impact molecular methods have had on the field of microbial taxonomy and phylogeny
- c. Compare and contrast nucleotide sequencing and nonsequencing-based molecular approaches used in microbial taxonomy and phylogeny
- d. Select an appropriate technique to identify a microbial genus, species, and strain
- e. Predict the basic biological as well as public health implications of microbial taxonomic identification

19.3 Microbial Taxonomy and Phylogeny Are Largely Based on Molecular Characterization 451

Many different approaches are used to classify and identify microorganisms that have been isolated and grown in pure culture. For clarity, we divide these approaches into two groups: classical and molecular. The most durable identifications are those that are based on a combination of approaches. Methods often employed in routine laboratory identification of pathogenic bacteria are covered in chapter 37, Clinical Microbiology and Immunology.

Classical Characteristics

Classical approaches to taxonomy make use of morphological, physiological, biochemical, and ecological characteristics. These characteristics have been employed in microbial taxonomy for many years and form the basis for phenetic (phenotypic) classification. When used in combination, they are quite useful in routine identification of well-characterized microbes.

Morphological Characteristics

Morphological features are important in microbial taxonomy for many reasons (table 19.2). Morphology is easy to study and analyze, particularly in eukaryotic microorganisms and more complex bacteria and archaea. In addition, morphological comparisons are valuable because structural features depend on the expression of many genes and are usually genetically stable. Thus morphological similarity often is a good indication of phylogenetic relatedness.

Physiological and Metabolic Characteristics

Physiological and metabolic characteristics are useful because they are directly related to the nature and activity of microbial proteins. For instance, the detection of specific end products of fermentation in a newly discovered microorganism reveals the

presence of specific catabolic enzymes and the genes that encode them. Therefore analysis of catabolic products, energy metabolism and nutrient transport, provides an indirect comparison of microbial genomes. Table 19.3 lists some of the most important of these properties.

Biochemical Characteristics

Among the more useful biochemical characteristics used in microbial taxonomy are bacterial fatty acids, which can be analyzed using a technique called fatty acid methyl ester (FAME) analysis. A fatty acid profile reveals differences in chain length, degree of saturation, branched chains, and hydroxyl groups. Microbes of the same species will have identical fatty acid profiles, provided they are grown under the same conditions; this limits FAME analysis to only those microbes that can be grown in pure culture. Finally, because the identification of a species is done by comparing the results of the unknown microbe in question with the FAME profile of other, known microbes, identification is only possible if the species in question has been previously analyzed. Nonetheless, FAME analysis is particularly important in public health, food, and water microbiology. In these applications, microbiologists seek to identify specific pathogens. **Microbiology of Food (chapter 41): Purification and sanitary analysis ensures safe drinking water (section 43.1)**

Advances in mass spectrometry (MS) have resulted in the fast and accurate identification of bacteria based on the presence of specific, highly abundant proteins. The specific type of MS used is called matrix-assisted laser desorption/ionization-time of flight (MALDI-ToF). MALDI-ToF enables the analysis of complex biomolecules that could not previously be studied by MS. The material to be analyzed is dried on a sample holder (called a target) and

Table 19.2 Some Morphological Features Used in Classification and Identification	
Feature	Microbial Groups
Cell shape	All major groups ¹
Cell size	All major groups
Colony morphology	All major groups
Staining behavior	Bacteria, some fungi
Cilia and flagella	All major groups
Mechanism of motility	Gliding bacteria, spirochetes, protists
Spore morphology and location	Bacteria, protists, fungi
Cellular inclusions	All major groups

¹ Used in classifying and identifying at least some bacteria, archaea, fungi, and protists.

Table 19.3 Some Physiological and Metabolic Characteristics Used in Classification and Identification	
Carbon, nitrogen, and energy sources	
Cell envelope constituents	
Fermentation products	
Motility	
Oxygen requirements and tolerance	
pH and temperature optimum and growth range	
Photosynthetic pigments	
Salt requirements and tolerance	
Secondary metabolites formed	
Storage inclusions	

Staining behavior	Bacteria, some fungi
Cilia and flagella	All major groups
Mechanism of motility	Gliding bacteria, spirochetes, protists
Spore morphology and location	Bacteria, protists, fungi
Cellular inclusions	All major groups

Used in classifying and identifying at least some bacteria, archaea, fungi, and protists.

pH and temperature optimum and growth range

Photosynthetic pigments

Salt requirements and tolerance

Secondary metabolites formed

Storage inclusions

then mixed with a molecular film called a matrix. When a UV laser beam strikes the sample target, the matrix helps stimulate release of the sample from the surface; this is matrix-assisted desorption. Once the matrix-sample is released (desorbed), the matrix transfers protons to the sample, which then becomes ionized; this is matrix-assisted ionization. Once ionized, biomolecules are “flown” across a space within the instrument; the time taken for a molecule to fly from one side to the other is used to determine the mass of each molecule; this is time of flight.

In its simplest form, MALDI-ToF identification of bacteria involves the transfer of whole cells from a single colony grown under specific conditions to a sample target. Once dried, a matrix is deposited and the bacterial samples are analyzed. MALDI-ToF yields the masses of many highly abundant bacterial proteins. Like FAME analysis, each experimentally derived protein profile must be matched to a protein profile from a known bacterium. MALDI-ToF is becoming increasingly important in medical microbiology laboratories where the same strains of organisms are regularly encountered. Like FAME, microbes must be grown under very specific conditions and MALDI-ToF cannot be used to identify newly discovered microbes. **Proteomics explores total cellular proteins (section 18.5)**

Ecological Characteristics

The ability of a microorganism to colonize a specific environment is of taxonomic value. Some microbes may be very similar in many other respects but inhabit different ecological niches, suggesting they may not be as closely related as first suspected. Some examples of taxonomically important ecological properties are life cycle patterns; the nature of symbiotic relationships; the ability to cause disease in a particular host; and habitat preferences such as requirements for temperature, pH, oxygen, and osmotic concentration. Many growth requirements are considered physiological characteristics as well (table 19.3). **Environmental factors affect microbial growth (section 7.5)** **Many types of microbial interactions exist (section 27.1)**

Molecular Characteristics

It is hard to overestimate how the study of DNA, RNA, and proteins has advanced our understanding of microbial evolution and taxonomy. Evolutionary biologists studying plants and animals draw from a rich fossil record to assemble a history of morphological changes; in these cases, molecular approaches supplement such data. In contrast, microorganisms have almost no fossil record, so molecular analysis is the only feasible means of collecting a large and accurate data set that explores microbial evolution. When scientists are careful to make only valid comparisons, phylogenetic inferences based on molecular approaches provide the most robust analysis of microbial evolution.

Microbial genomes can be directly compared and taxonomic similarity can be estimated in many ways. DNA sequence can be determined for one or a few genes, or for an entire genome, depending on the degree of identification required. The recent rapid drop in cost and time to generate a genome sequence has

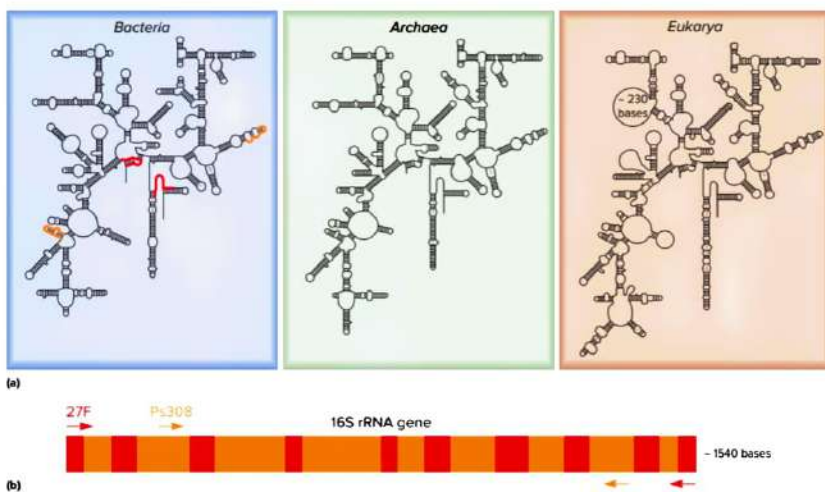
made this approach viable for many taxonomic applications. Molecular techniques developed in the late twentieth century are now in transition to techniques based on whole-genome sequencing (WGS). In many instances, the original nomenclature remains, although the technique is performed in silico rather than in vitro. As with phenetic approaches, comparison to standards and type strains forms the basis for identification and phylogenetic placement. Many classical properties used in strain identification are now inferred from genome sequences.

Signature Sequences

The rRNAs from small ribosomal subunits (16S from bacterial and archaeal cells and 18S from eukaryotes) have become the molecules of choice for inferring microbial phylogenies and making taxonomic assignments at the genus level. The **small subunit rRNAs (SSU rRNAs)** are widely applicable in studies of microbial evolution, relatedness, and genus identification for several important reasons (figure 19.2). First, they play the same role in all microorganisms. In addition, because ribosomes are absolutely necessary for survival and ribosomes cannot function without SSU rRNAs, the genes encoding these rRNAs cannot tolerate large mutations. Thus these genes change very slowly with time. Finally, rRNA genes are rarely subject to horizontal gene transfer, an important factor in comparing sequences for phylogenetic purposes. The utility of SSU rRNAs is extended by the presence of certain sequences within SSU rRNA genes that are variable among organisms as well as other regions that are quite similar. The variable regions enable comparison between closely related microbes, whereas the stable sequences allow the comparison of distantly related microorganisms.

Comparative analysis of SSU rRNA sequences from thousands of organisms has demonstrated the presence of **oligonucleotide signature sequences**. These are short, conserved nucleotide sequences that are specific for phylogenetically defined groups of organisms. Thus the signature sequences found in bacterial rRNAs are rarely or never found in archaeal rRNAs and vice versa. Likewise, the 18S rRNA of eukaryotes bears signature sequences that are specific to the domain *Eukarya*. In addition, signature sequences for a lower taxon, for example, the genus *Pseudomonas*, may be found in the variable regions of a higher taxon like *Bacteria* (figure 19.2b).

The ability to amplify regions of rRNA genes (rDNA) by the polymerase chain reaction (PCR) and sequence the DNA using next-generation techniques has greatly increased the efficiency by which SSU rRNA sequences can be obtained. PCR can amplify rDNA from the genomes of different organisms because conserved nucleotide sequences flank the nucleotides that can be used to reveal the microbe's identity (figure 19.2b). In practice, this means that PCR primers can be generated to amplify rDNA from both cultured and uncultured microbes. Any uncultivated microorganism that is identified solely on its nucleic acid sequence (or other observable, quantifiable phenotype) is termed a **phylotype**.



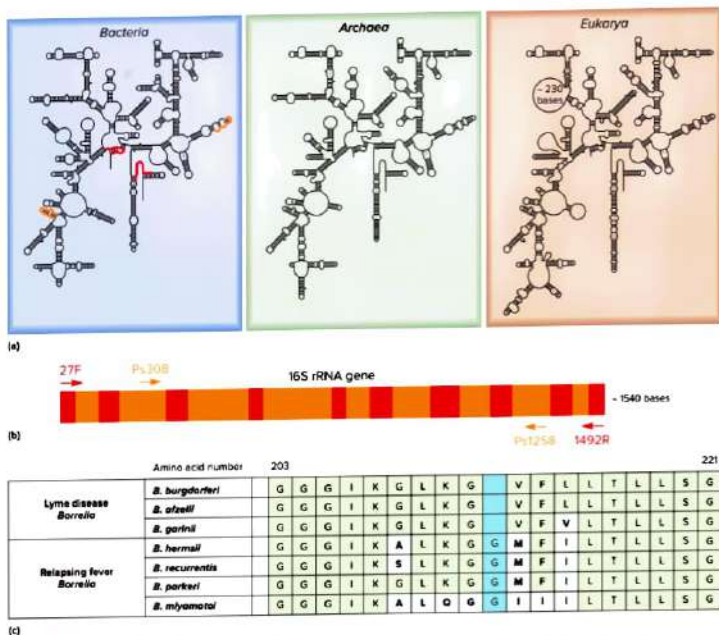


Figure 19.2 Signature rRNA and Amino Acid Sequences Help to Identify Microbes. (a) Representative examples of rRNA secondary structures from the three domains: Bacteria (*Escherichia coli*), Archaea (*Methanococcus vannielii*), and Eukarya (*Saccharomyces cerevisiae*). (b) Diagram of a bacterial 16S rRNA gene. Conserved regions (red) are present in all bacteria, and bacteria-specific PCR primers 27F and 1492R bind where indicated. These sites are highlighted in red in (a). Variable regions (gold) contain genus-specific sequence, and PCR primers Pst308 and Pst258 indicate the positions of signature sequences specific to *Pseudomonas*. These sites are highlighted in gold in (a). (c) Alignment of amino acids 203-221 in L-lactate permease from *Borrelia* species. Shaded boxes are amino acids identical to the *B. burgdorferi* sequence. An amino acid indel (shaded blue) distinguishes species that cause Lyme disease from those that cause the condition known as relapsing fever.

The use of SSU rRNA sequences as a taxonomic marker has been validated as a molecular measure of relatedness. Correlating SSU rRNA sequence data with traditional measures of assigning species, organisms of the same species show at least 98.65% sequence identity in their SSU rDNA sequences. This means that when comparing the SSU rRNA sequences of two organisms, differences at more than 20 positions of a ~1,540

base rRNA indicate that they probably derive from different species. **14 Polymerase chain reaction amplifies targeted DNA** (section 17.2)

Signature sequences are present in genes other than those encoding rRNA. Many genes have nucleotide insertions or deletions of specific lengths and sequences at fixed positions. A particular nucleotide sequence that is inserted or deleted may be found

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exclusively among all members of a phylum. These taxon-specific insertions and deletions are called conserved indels (for insertion/deletion). Indels are particularly useful in phylogenetic studies when they are flanked by conserved regions. In such cases, changes in the signature sequence cannot be due to sequence misalignments. When an indel occurs in the coding region of a gene, it must be a multiple of three nucleotides to preserve the reading frame. Alignment of the inferred amino acid sequences reveals indels useful for phylogenetic analysis (figure 19.2c).

Whole-Genome Comparison

As the field of taxonomy shifts to whole-genome sequencing (WGS) for strain identification and taxonomic classification, new quantitative measures of relatedness are being developed and evaluated. As with any metric, standardization of methods and interpretations are required. These processes are ongoing. An initial step to identify a newly determined sequence is SSU rRNA comparison, which provides confident identification to the genus level.

Identification to the species level requires detailed gene-by-gene comparison of the new isolate and type strains of its closest relatives. Average nucleotide identity (ANI) is now widely considered to be the standard for species identification. This technique uses pairwise alignments between all sequences shared between two genomes and calculates the fraction of identical nucleotides. ANI values for two genomes of the same species should be at least 95 to 96%.

ANI is poised to replace a biochemical technique called DNA-DNA hybridization (DDH). DDH is performed by mixing genomic DNA from two strains. The mixture is heated until denaturation occurs, and then slowly cooled to allow renaturation. Noncomplementary regions remain unpaired, and the degree of renaturation is calculated. This is a relatively complex assay and results depend on quality of the extracted DNA and other factors. By contrast, bioinformatics software quickly calculates digital DDH values using WGS data, although this technique is less commonly used than ANI.

Another biochemical technique gene digital is determination of G + C content. This metric is a simple percentage of the bases in DNA that are G + C, and it is readily determined computationally from WGS data. Organisms range from around 30% to 80% G + C. Despite the wide range of variation, the G + C content of strains within a species is constant and varies little within a genus.

Subspecies and Strain Identification

For many applications, identification to levels below species is required. Typically genes that evolve more quickly than those that encode rRNA must be analyzed. The technique called multilocus sequence analysis (MLSA) compares the sequences of several conserved housekeeping genes (figure 19.3). At least 5 genes are examined to avoid misleading results that can arise through horizontal gene transfer. Because many

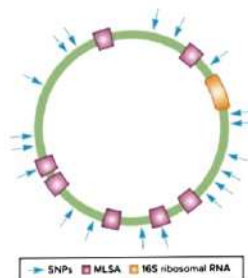


Figure 19.3 Genome Coverage of Genetic Taxonomic Approaches.

A bacterial or archaeal genome contains at least one locus encoding 16S rRNA. MLSA samples multiple genes throughout the genome. SNP analysis compares more, but shorter, nucleotide sequences that span the entire genome.

different versions, or alleles, of each gene can exist, the finding that two microbial isolates share the same alleles for multiple genes is strong evidence that the two strains are closely related, perhaps even the same strain. MLSA is now often performed from whole genome sequences, where it is termed wgMLSA. The availability of wgMLSA data enables extended gene-by-gene comparison.

To survey a large fraction of the genome, single nucleotide polymorphisms (SNPs, pronounced “snips”) are identified in specific genes, intergenic regions, or other noncoding regions (figure 19.3). Originally developed to analyze human DNA, SNP analysis targets specific regions because they are normally conserved, so single base pair differences reveal evolutionary change. SNP analysis shares features with analysis of restriction fragment length polymorphism (RFLP). This technique identifies differences in restriction endonuclease digestion patterns, which reflect individual base pair changes. When this analysis is applied to the gene encoding the SSU rRNA, it is termed **ribotyping**. With the advent of WGS, these techniques are increasingly performed in silico.

Comprehension Check

- What are the advantages of using each major group of characteristics (morphological, physiological and metabolic, biochemical, ecological, and molecular) in classification and identification? How is each group related to the nature and expression of the genome? Give examples of each type of characteristic.
- Why is rRNA suitable for determining relatedness?
- List the steps you would take to identify an organism just isolated in pure culture. What criteria would tell you if it is a new species?

19.4 Phylogenetic Trees Illustrate Evolutionary Relationships

After reading this section, you should be able to:

- Paraphrase the rationale underpinning the construction of phylogenetic trees
- Compare and contrast rooted and unrooted trees
- Outline the general considerations used in building a phylogenetic tree
- Characterize the challenges horizontal gene transfer introduces in the study of microbial evolution

Microbial taxa within *Bacteria* and *Archaea* form discrete, genealogically clustered groups that can be illustrated in phylogenetic trees. Phylogenetic trees show inferred evolutionary relationships in the form of multiple branching lineages connected by nodes (figure 19.4). Organisms whose nucleotide

sequences have been analyzed are identified at the tip of each branch. Each node (branchpoint) represents a divergence event, and the length of the branches correlates with the number of molecular changes that have taken place between the two nodes.

Often sequences are obtained from well-classified microbes that have been grown in pure culture; however, this is not always the case. SSU rRNA sequences have become particularly important in both identifying microbes and constructing phylogenetic trees to describe their evolutionary relationships. An inclusive term for the organism at each branch tip, regardless of whether or not it has been cultured, is the **operational taxonomic unit (OTU)**. The term *clade* refers to a group of organisms with a common ancestor. We now briefly describe how phylogenetic trees can be built with the intention of increasing an understanding of what they represent.

There are five steps in building a phylogenetic tree. First, the nucleotide or amino acid sequences must be aligned. Alignments are performed with software, although manual inspection of the alignment is also important (figure 19.4a). In addition to SSU rRNA gene sequences, protein-coding genes may be analyzed, in which case the alignment of amino acids is preferred. This is because the genetic code is degenerate, so even if a nucleotide sequence is not conserved, the amino acid sequence may be. For instance, if a protein-coding gene were mutated so that a codon changed from AGA to AGG, arginine would still be added to the growing polypeptide during translation. Next the alignment must be examined for a phylogenetic signal; this will determine if it is appropriate to continue with tree building (figure 19.4b). There are two extremes in this regard: At one end of the spectrum, the sequences align perfectly. The other extreme is 25% identity, or what would be expected if two random DNA sequences were aligned. (When there are four possible nucleotides, each position has a 1 in 4 chance of matching.) Phylogenetic analysis can only be performed on those sequences that fall in the middle, with a mixture of random and matched positions. The third step is the hardest: choosing which tree-building method to use. We briefly review some of the more popular methods. The last two steps involve the application of the selected method, performed by a computer, followed by manual examination of the resulting tree to confirm that it is logical. For example, a computer-generated tree

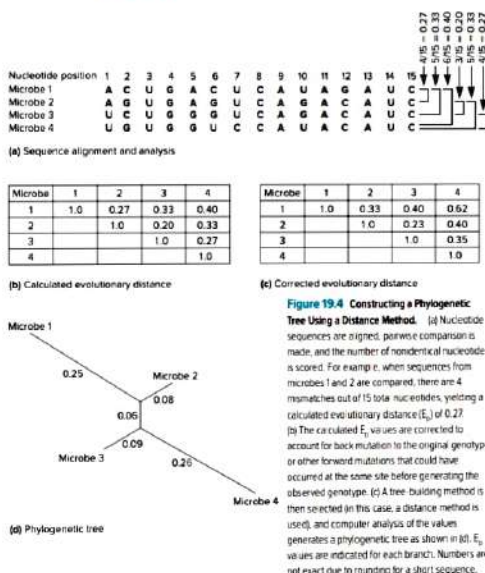


Figure 19.4 Constructing a Phylogenetic Tree Using a Distance Method. (a) Nucleotide sequences are aligned, pairwise comparison is made, and the number of nonidentical nucleotides is scored. For example, when sequences from microbes 1 and 2 are compared, there are 4 mismatches out of 15 total nucleotides, yielding a calculated evolutionary distance (E_0) of 0.27. (b) The calculated E_0 values are corrected to account for back mutation to the original genotype or other forward mutations that could have occurred at the same site before generating the observed genotype. (c) A tree-building method is then selected (in this case, a distance method is used), and computer analysis of the values generates a phylogenetic tree as shown in (d). E_0 values are indicated for each branch. Numbers are not exact due to rounding for a short sequence.

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that places a mammal and an archaeon on the same branch would certainly require correction.

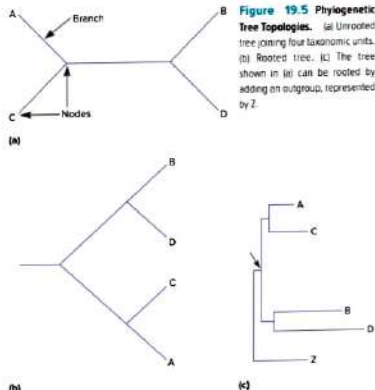
Approaches to building a phylogenetic tree can be divided into two broad categories: a distance-based (phenetic) approach and a character-based (cladistic) approach. Distance-based approaches are the most intuitive. Here the differences between the aligned sequences are counted for each pair and summarized in a single statistic, which is roughly the percent difference between the two sequences (figure 19.4b,c). A tree is generated by serially linking pairs that are ever more distantly related (i.e., start with those with the least number of sequence differences and move to those with the most). This is called cluster analysis and should be carefully applied as it has the unattractive capability of generating trees even in the absence of evolutionary relationships. Neighbor joining is another distance-based method that uses a different matrix that attempts to avoid this problem by modifying the distance between each pair of nodes based on the average divergence from all other nodes.

Character-based methods for phylogenetic tree building are more complicated but generate more robust trees. These methods start with assumptions about the pathway of evolution, infer the ancestor at each node, and choose the best tree according to a specific model of evolutionary change. These methods include maximum parsimony, which assumes that the fewest number of changes occurred between ancestor and extant (living) organisms. Another approach is called maximum likelihood. This requires a large data set because for each possible tree that can be built, its probability (i.e., the likelihood) based on certain evolutionary and molecular information is determined so that the tree with the greatest probability based on these criteria is selected. All tree building methods have their advantages and disadvantages, so it is advisable to use several methods to analyze the same data set. Similar trees generated by different approaches is the desired outcome.

Importantly, a tree may be unrooted or rooted. An unrooted tree (figure 19.5a) represents phylogenetic relationships but does not indicate which organisms are more primitive relative to the others. Figure 19.5a shows that A is more closely related to C than it is to either B or D, but it does not indicate which of the four species might be the oldest. In contrast, the rooted tree (figure 19.5b) includes a node that serves as the common ancestor and shows the development of the four species from this root. It is much more difficult to develop a rooted tree. For example, there are 15 possible rooted trees that connect four species but only three possible unrooted trees.

An unrooted tree can be rooted by adding data from an outgroup—a species known to be very distantly related to all the species in the tree (figure 19.5c). The root is determined by the point of the tree where the outgroup joins. This provides a point of reference to identify the oldest node on the tree, which is the node closest to the outgroup. So, for example, in figure 19.5c, organism Z is the outgroup and the oldest node on the tree is marked with an arrow.

Once a tree is constructed, it is important to get a sense of whether the placement of its branches and nodes is legitimate.



There are a variety of methods to assess the “strength” of a tree, but the most common is bootstrapping. Bootstrapping involves phylogenetic analysis of a randomly selected subset of the data presented on the tree. A bootstrap value is the percent of analyses in which that particular branch was found. Typically bootstrap values of 70% or greater are thought to support a tree. Another approach, called Bayesian inference, may also be used. Rather than looking at a single tree, Bayesian inference analyzes multiple potential trees and calculates the probability that each branch would appear based on this comparison. Although these values are also reported as percentages, they are not directly comparable to bootstrap values. Only values greater than 95% are acceptable when Bayesian inference is used.

An important feature in phylogenetic trees is the scale. Just as a scale bar on a road map indicates distance, the scale bar on a phylogenetic tree illustrates the evolutionary distance. This is usually measured in number of mutations per 100 nucleotides or amino acid substitutions per 100 amino acid residues. This may be expressed as a number without units, for example, 0.02 (2 per 100). To continue the map analogy, just as a map does not reveal how long it takes to get from one point to another (due to traffic and weather), the branches on a phylogenetic tree do not indicate the length of time it took for an ancestral microbe to give rise to an extant form. As discussed in section 19.5, the theory of punctuated equilibria is one important reason evolutionary distance, as measured by the similarity of genes or proteins in living organisms, provides little or no information regarding how long ago evolutionary divergence occurred.

One of the biggest challenges in constructing a satisfactory tree is widespread, frequent horizontal gene transfer (HGT). Although microbiologists are careful to exclude from their analysis genes and

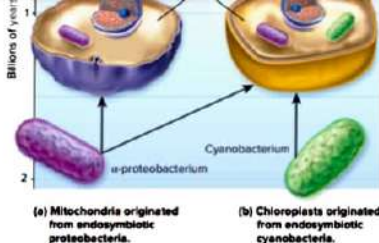


Figure 19.7 The Endosymbiotic Theory. (a) According to this hypothesis, mitochondria derived from a proteobacterium. (b) A similar phenomenon occurred for chloroplasts, which derived from cyanobacteria.

MICRO INQUIRY On what evidence is this hypothesis based?

► **Phylum Cyanobacteria:** oxygenic photosynthetic bacteria (section 21.4); **Protists** (chapter 24)

What Is a Microbial Species?

The term “species concept” describes a theoretical framework used to understand how and why certain organisms can be sorted into discrete taxonomic groups. We discussed “species definition” in sections 19.2 and 19.3, when we reviewed the criteria used to identify a microbial genus, species, or strain. From this, we can see that species definition is the application of the species concept.

Both species concept and definition have changed over time and continue to be difficult for microbiologists to agree upon. Because bacteria and archaea lack sexual reproduction, extensive morphological features, and a fossil record, microbiologists are at a distinct disadvantage when defining species, as compared to biologists studying other forms of life.

Historically, the application of different criteria in making species assignments has led to taxonomic confusion. In some

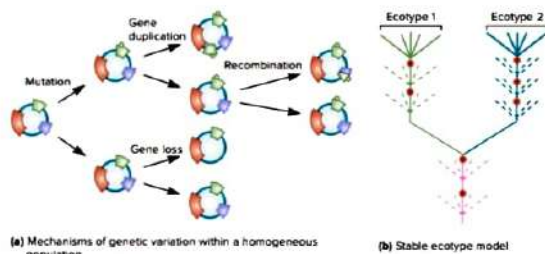
to others in the group, whole genome similarity as determined by DNA-DNA hybridization must be at least 70%, the melting temperature of the DNA (a reflection of the G + C content) within 5°C, and less than 3% divergence in rRNA gene sequence. As noted previously, these techniques may soon be replaced with a genomic metric, such as average nucleotide identity. Even with these proposed updates, some remain uncomfortable with these criteria. They point out that two microorganisms with, for instance, only 75% similarity in DNA and 98% rRNA gene sequence identity can be considered the same species, but if these criteria were applied to eukaryotes, all primates (monkey, apes, you) would be lumped together as a single species! Indeed, it remains unresolved whether the species concept can be applied to microbes.

Microbial Evolutionary Processes

While the debate regarding the operational definition of a microbial species continues, microbiologists agree that the microbial species concept is grounded in natural selection and evolution. As the most ancient life forms on Earth, bacteria and archaea have evolved and adapted to virtually every habitat. While their diverse metabolic strategies and ability to tolerate extreme conditions explain why microbes display such enormous diversity, natural selection explains how this diversity came to be.

Recall that genetic diversity in members of *Archaea* and *Bacteria* must occur asexually. Thus heritable genetic changes in these organisms are introduced principally by two mechanisms: mutation and HGT, both of which are subject to natural selection. Generally speaking, it is thought that mutation drives initial speciation events, and HGT permits more rapid radiation thereafter. In other words, a new species must arise from its single, ancestral species. This makes sense if one assumes that the ancestral population of microbes was genetically homogeneous. Genetic variation can only arise within a population possessing identical (or nearly identical) genomes by mutation, gene loss and gain, and intragenomic recombination (figure 19.8a). By definition, there is not enough genetic diversity to drive speciation among such a population by HGT.

Anagenesis, also known as **genetic drift**, refers to small, random genetic changes that occur over generations. It might



(a) Mechanisms of genetic variation within a homogeneous population

(b) Stable ecotype model

Figure 19.8 Evolution of Microbial Diversity. (a) Mechanisms by which a single, genetically homogeneous population of microbes can develop genetic variation include mutation, gene loss and duplication, and recombination. (b) Genetic changes within a population can lead to the development of new ecotypes. Each ecotype is subject to periodic selection events (indicated by stars) that enable cells with adaptive mutations to outcompete other lineages, which are eventually driven to extinction (dotted lines). The solid lines represent successful populations or lineages; those at the top are extant.

MICRO INQUIRY Construct a scenario in which each of the following factors leads to the establishment of two ecotypes from a single common ancestor, as shown in (b): the availability of carbon and nitrogen sources; terminal electron acceptor; and mean local temperature.

seem that very small genetic differences within a microbial population would be of little evolutionary significance. However, model studies designed to assess competition between microbial populations has led to some surprising observations. When selection is applied, very small genetic differences can result in one population overtaking another. How does this happen when individuals within a population have similar mutation rates and most of these mutations are neutral and have no phenotypic effect? Only those rare mutations that confer a growth advantage, called **adaptive mutations**, are retained and passed from one generation to the next, in which case we say the mutation is fixed. The descendants of that individual continue to evolve through mutation and other intraspecific mechanisms.

Adaptive mutation is key to the ecotype model of microbial evolution. An **ecotype** is a population of microbes that is genetically very similar but ecologically distinct. Ecotypes arise when members of a microbial population living in a specific ecosystem undergo a genetic event (or series of events) that enables them to outcompete the remainder of the population. According to the ecotype model, the acquisition of adaptive mutations ultimately drives the remaining members of the population into extinction and reduces the amount of genetic diversity within the surviving population (figure 19.8b). The fossil record shows that evolution does not always occur at a constant rate but is periodically interrupted by rapid bursts of speciation driven by abrupt environmental changes. Niles

Eldredge and Steven Jay Gould coined the term **punctuated equilibria** to describe this phenomenon. Certainly, the 3.5-billion-year history of microbial life on Earth affords the accumulation of many, many mutations; that in turn has resulted in vast speciation.

► **Mechanisms of genetic variation** (chapter 16); **Comparative genomics** (section 18.7)

Unlike variation introduced in the ecotype model, HGT-driven genetic variation requires genetically diverse groups of microbes. This is because HGT does not rely on replication but rather on the exchange of genetic material between microbes. The rate of HGT is extremely variable. Some microbes have very reduced genomes with no evidence of HGT. These microbes are generally highly adapted to a specific, stable ecological niche. The most extreme examples are obligate intracellular symbionts that can only grow within their host cells, where no other microbes exist with

which to exchange genes. By contrast, some microbes appear to have high rates of HGT with more than half of their genome acquired from other organisms, as is the case for the members of the ancient phylum *Thermotogae*. In such microbes, genes acquired by HGT frequently expand metabolic capabilities, thereby enabling rapid adaptation to new environmental challenges. ► **Aquificae** and **Thermotogae** are ancient bacterial lineages (section 21.1)

Finally, we pose the question: How many bacterial and archaeal species are there? There are two major obstacles to formulating an answer. First, most microbial species resist growth in the laboratory, so they can only be detected by metagenomic or other culture-independent approaches. Second, as we have seen, microbiologists cannot agree on a biological species concept. So we must resort to the operational definition of a species in terms of nucleic acid homologies, as well as similarities in physiology, morphology, and ecology. Based on these criteria, estimates range from 100,000 to 1,000,000 species in nature, with about 10^{11} individual cells. These estimates reveal that there are probably about a billion more microbes on Earth than stars in the universe.

Comprehension Check

1. Define ecotype. Do you think it is necessary to obtain microbes in pure culture before assigning different ecotypes? Explain.
2. What is the difference between the core genome and pan-genome? What might you infer if you compare two genera, one

Cell Organisation

Bacterial Cell Structure

Hooking Up

Each year over 100 million people around the world become infected with *Neisseria gonorrhoeae*, the bacterium that causes gonorrhea. This troubling statistic is made even more disturbing by the increasing resistance of the bacterium to the antibiotics used to treat the disease. In men, infection is usually readily detected, but for women, infection is often asymptomatic and can lead to serious consequences such as pelvic inflammatory disease (PID) and sterility. These concerns have led scientists to consider methods for preventing infection. One method is to block transmission. Unfortunately, relatively little is known about the transmission process except that it occurs during sexual intercourse and that numerous hairlike structures (called pili) covering the surface of the bacterium play a role in establishing infection. The bacterium uses pili for a type of movement called twitching motility and to adhere to surfaces such as the sperm and epithelial cells of its human host. It has long been thought that by attaching to sperm cells the bacterium could hitch a ride during sexual intercourse. This explained transmission between partners. However, it did not clarify how transmission from women to men occurs.

It turns out that exposure of *N. gonorrhoeae* to seminal fluid increases its twitching motility and enhances formation of small clumps of bacteria. In addition, seminal fluid proteins appear to alter the morphology and function of pili. In particular these proteins cause bundles of pili to separate into single filaments, enhancing the interaction of bacterial cells with each other and with host surfaces. These changes evolved to promote infection of host epithelial cells and increase the likelihood of transmission during sexual intercourse from females to their male partner.

As this story illustrates, even small, seemingly simple organisms such as bacteria can exhibit complex behaviors. To understand these amazing microbes, we must first examine their cell structure and begin to relate it to the functions they carry out. As we consider bacterial cell structure, it is important to remember that only about 1% of bacterial species have been cultured. Of the cultivated species, only a few have been studied in great detail. From this small sample, many generalizations are made, and it is presumed that most other bacteria are like the well-studied model organisms. However, part of the wonder and fun of science is that nature is full of surprises. As the biology of more and more

bacteria is explored, our understanding of them may change in interesting and exciting ways.

Readiness Check:

Based on what you have learned previously, you should be able to:

- ✓ Describe the application of small subunit (SSU) rRNA analysis to the establishment of the three domain classification system proposed by Carl Woese (section 1.2)
- ✓ Identify the following structures or regions of a plant or animal cell and describe their functions: cell wall, plasma membrane, cytoplasm, mitochondria, chloroplasts, and ribosomes
- ✓ Define and give examples of essential nutrients; describe how they are used by cells

3.1 Use of the Term "Prokaryote" Is Controversial

After reading this section, you should be able to:

- a. List the characteristics originally used to describe prokaryotic cells
- b. Debate the "prokaryote" controversy using current evidence about bacterial cells

Bacteria and archaea have long been lumped together and referred to as prokaryotes. Although the term was first introduced early in the twentieth century, the concept of a prokaryote was not fully outlined until 1962, when R. Stanier and C. B. van Niel described prokaryotes in terms of what they lacked in comparison to eukaryotic cells. For instance, Stanier and van Niel pointed out that prokaryotes lack a membrane-bound nucleus, a cytoskeleton, membrane-bound organelles, and internal membranous structures such as the endoplasmic reticulum and Golgi apparatus. Since the 1960s, biochemical, genetic, and genomic analyses have shown that *Bacteria* and *Archaea* are distinct taxa. Because of these discoveries, Norman Pace proposed in 2006 that the term *prokaryote* should be abandoned and most microbiologists are in agreement.

This controversy illustrates that microbiology is an exciting, dynamic, and rapidly changing field of study. Throughout this text, we avoid the term *prokaryote* and are as explicit as

possible about which characteristics are associated with members of *Bacteria*, which with members of *Archaea*, and which with members of both taxa.

3.2 Bacteria Are Diverse but Share Some Common Features

After reading this section, you should be able to:

- a. Distinguish a typical bacterial cell from a typical plant or animal cell in terms of cell shapes and arrangements, size, and cell structures
- b. Discuss the factors that determine the size and shape of a bacterial cell

Much of this chapter is devoted to a discussion of individual cell components. Therefore a preliminary overview of the features common to many bacterial cells is in order. We begin by considering overall cell morphology and then move to cell structures.

Shape, Arrangement, and Size

It might be expected that bacterial cells, being small and relatively simple, would be uniform in shape and size. This is not the case, as the microbial world offers considerable variety in terms of morphology. However, the two most common shapes are cocci and rods (figure 3.1). Cocci (s., *coccus*) are roughly spherical cells. They can exist singly or can be associated in characteristic arrangements that can be useful in their identification. Diplococci (s., *diplococcus*) arise when cocci divide and remain together to form pairs. Long chains of cocci result when cells adhere after repeated divisions in one plane; this pattern is seen in the genera *Streptococcus*, *Enterococcus*, and *Lactococcus* (figure 3.1a). Members of the

genus *Staphylococcus* divide in random planes to generate irregular, grapelike clusters (figure 3.1b). Divisions in two or three planes can produce symmetrical groupings of cocci. Bacteria in the genus *Micrococcus* often divide in two planes to form square groups of four cells called tetrads. In the genus *Sarcina*, cocci divide in three planes, producing cubical packets of eight cells.

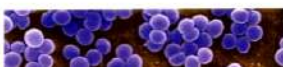
Legionella pneumophila is an example of a bacterium with a rod shape (figure 3.1c). Rods, sometimes called bacilli (s., *bacillus*), differ considerably in their length-to-width ratio, the coccobacilli being so short and wide that they resemble cocci. The shape of the rod's end often varies between species and may be flat, rounded, football-shaped, or bifurcated. Although many rods occur singly, some remain together after division to form pairs or chains (e.g., *Bacillus megaterium* is found in long chains).

There are several less common cell shapes and arrangements. *Vibrios* are comma-shaped (figure 3.2a). *Spirilla* are rigid, spiral-shaped cells (figure 3.2b). Many have tufts of flagella at one or both ends. *Spirochetes* are flexible, spiral-shaped bacteria that have a unique, internal flagellar arrangement (figure 3.2c). These bacteria are distinctive in other ways, and all belong to a single phylum, *Spirochaetes*. Some bacteria form stalks (e.g., *Caulobacter crescentus*) (figure 3.2d). Other bacteria are pleomorphic, being variable in shape and lacking a single, characteristic form. **Phylum Spirochaetes** (section 21.6); *Caulobacteraceae* and *Hyphomicrobiaceae* bacteria reproduce in unusual ways (section 22.1); *Order Vibrionales* includes aquatic bioluminescent bacteria and pathogens (section 22.3).

Some bacteria can be thought of as multicellular. Many actinobacteria form long filaments called hyphae. The hyphae form a network called a mycelium (figure 3.2e), and in this sense, they are similar to eukaryotic filamentous fungi. Many cyanobacteria, a group of photosynthetic bacteria, are also filamentous. Being filamentous allows some degree of



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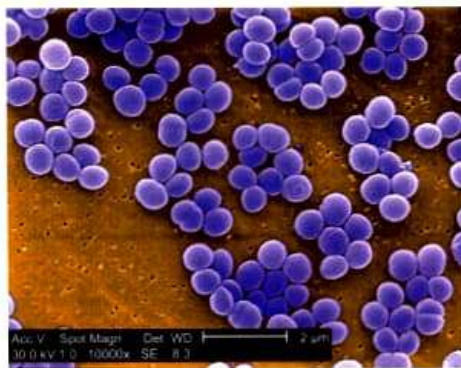
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(a) *S. agalactiae*—cocci in chains



(b) *S. aureus*—cocci in clusters



(c) *L. pneumophila*—rods in chains

Figure 3.1 Cocci and Rods Are the Most Common Bacterial Shapes. These images are color-enhanced scanning electron micrographs.

(a) *Streptococcus agalactiae*, the cause of group B streptococcal infections (×4,800). (b) *Staphylococcus aureus*. (c) *Legionella pneumophila*, the cause of Legionnaires' disease.

(a) © Science Source, (b, c) Source: CDC/Janice Haney Carr



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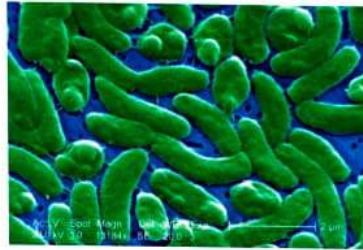
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(d) © Science Source. (b, c) Source: CDC, Janice Haney Carr

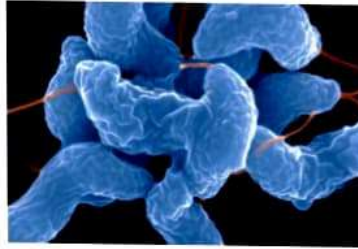
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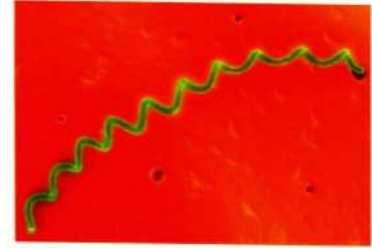
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(a) *V. vulnificus*—comma-shaped vibrios



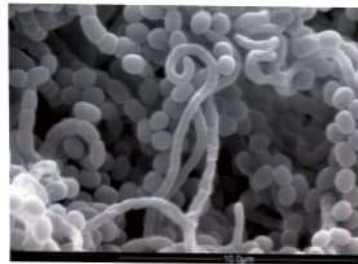
(b) *C. jejuni*—spiral-shaped



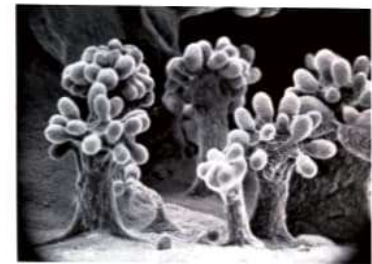
(c) *L. interrogans*—a spirochete



(d) *C. crescentus*—a stalked bacterium



(e) *Streptomyces*—a filamentous bacterium



(f) *C. crocatus* fruiting body

Figure 3.2 Other Cell Shapes and Aggregations. (a) *Vibrio vulnificus*, scanning electron micrograph (SEM, X13,184). (b) *Campylobacter jejuni*, SEM. (c) *Leptospira interrogans*, the spirochete that causes the disease leptospirosis. (d) *Caulobacter crescentus*, SEM. (e) *Streptomyces* sp., SEM. (f) Fruiting body of the myxobacterium *Chondromyces crocatus*. The fruiting body is composed of thousands of cells.

(a) © Media for Medical/Getty Images. (b) Source: Photo by DeWood, digital colorization by Stephen Ausmus/USDA ARS. (c) © Sebastian Kaulitzky/Getty Images. (d) © Biology Pics/Science Source. (e) © Dr. Amy Gehring. (f) © Yoav Levy/DIOMEDIA

differentiation among cells in the filament. For instance, some filamentous cyanobacteria form specialized cells within the filament, heterocysts, that carry out nitrogen fixation (see figure 21.10c). Myxobacteria are of particular note. These bacteria sometimes aggregate to form complex structures called fruiting bodies (figure 3.2f). **► Order Streptomycetales: an important source of antibiotics (section 23.1); Phylum Cyanobacteria: oxygenic photosynthetic bacteria (section 21.4); Order Myxococcales: bacteria with morphological complexity and multicellularity (section 22.4)**

Escherichia coli is an excellent representative of an average-sized bacterium. This rod-shaped bacterium is 1.1 to 1.5 μm wide by 2.0 to 6.0 μm long. However, the size range of bacterial cells extends far beyond this average (figure 3.3). Near the small end of the size continuum are members of the genus *Mycoplasma* (0.3 μm in diameter). At the other end of the continuum are bacteria such as some spirochetes, which can reach 500 μm in length, and the cyanobacterium *Oscillatoria*, which is about 7 μm in diameter (the same diameter as a red blood cell). Some bacteria are huge by "bacterial standards." For instance, *Epulopiscium fishelsoni* grows as large as 600 by 80 μm , a little smaller than a printed hyphen and clearly larger than the well-known eukaryote *Paramecium* (figure 3.4). An even larger bacterium, *Thiomargarita*

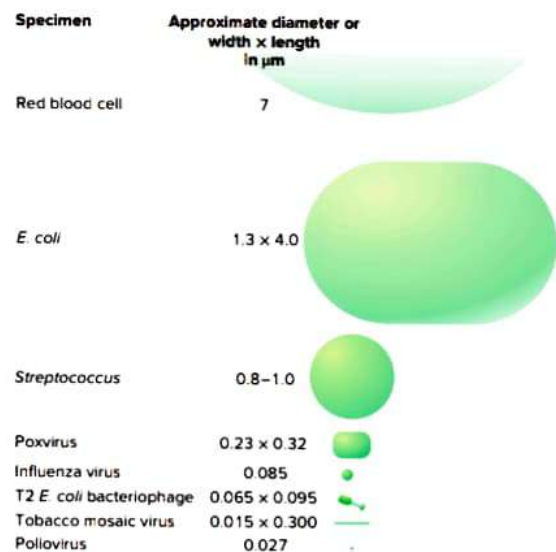


Figure 3.3 Sizes of Bacteria Relative to a Red Blood Cell and Viruses.

The larger viruses are comparable in size to the smaller bacteria.

Filamentous cyanobacteria form specialized cells within the filament, heterocysts, that carry out nitrogen fixation (see figure 21.10e). Myxobacteria are of particular note. These bacteria sometimes aggregate to form complex structures called fruiting bodies (figure 3.2f). ► **Order Streptomyces**: an important source of antibiotics (section 23.1); **Phylum Cyanobacteria**: oxygenic photosynthetic bacteria (section 21.4); **Order Myxococcales**: bacteria with morphological complexity and multicellularity (section 22.4)

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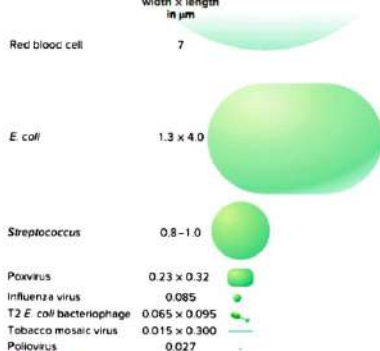


Figure 3.3 Sizes of Bacteria Relative to a Red Blood Cell and Viruses. The larger viruses are comparable in size to the smaller bacteria.



Figure 3.4 A Giant Bacterium. This phase-contrast micrograph shows *Epulopiscium fishelsoni* dwarfing the paramecia, which are protozoa. *E. fishelsoni* cells are about 530 μm long. ©Ethan Aronoff/Medical Images/DiMEDA

nambiensis, lives in ocean sediment (see figure 22.20). Thus a few bacteria are much larger than the average eukaryotic cell (typical plant and animal cells are around 10 to 50 μm in diameter).

The variety of sizes and shapes exhibited by bacteria raises a fundamental question: What causes a bacterial species to have a particular size and shape? Although far from being answered, recent discoveries have fueled a renewed interest in this question, and it is clear that size and shape determination are related and have been selected for during the evolutionary history of each bacterial species. For many years it was thought that microbes had to be small to increase the surface area-to-volume ratio (S/V ratio; figure 3.5). As this ratio increases, the uptake of nutrients and the diffusion of these and other molecules within the cell become more efficient, which in turn facilitates a rapid growth rate. Shape affects the S/V ratio. A rod with the same volume as a coccus has a higher S/V ratio than does the coccus. This means that a rod can have greater nutrient flux across its plasma membrane. However, the discovery of *E. fishelsoni* demonstrates that bacteria can be very large. For bacteria to be large, they must have other characteristics that maximize their S/V ratio, or their size must be beneficial in some way. For instance, *E. fishelsoni* has a highly convoluted plasma membrane, which increases its S/V ratio. In addition, large cells are less likely to be eaten by predatory protists. Cells that are filamentous, have stalks, or are oddly shaped are also less susceptible to predation.

Cell Organization

Structures often observed in bacterial cells are summarized and illustrated in table 3.1 and figure 3.6. Note that no single

3.2 Bacteria Are Diverse but Share Some Common Features 43

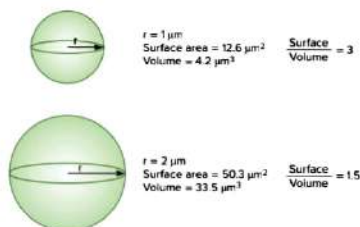


Figure 3.5 The Surface-to-Volume Ratio Is an Important Determinant of Cell Size. Surface area is calculated by the formula $4\pi r^2$. Volume is calculated by the formula $4/3\pi r^3$. Shape also affects the S/V ratio; rods with the same volume as a coccus have a greater S/V ratio.

bacterium possesses all of these structures at all times. Some are found only in certain cells in certain conditions or in certain phases of the life cycle.

There are several common features of bacterial cell structure. Bacterial cells are surrounded by several layers, which are collectively called the cell envelope. The most common cell envelope layers are the plasma membrane, cell wall, and capsule or slime layer. The innermost layer of the cell envelope is the plasma membrane, which surrounds the cytoplasm. Most bacteria have a chemically complex cell wall, which covers the plasma membrane. Many bacteria surround the cell wall with a capsule or slime layer. Because most bacteria do not contain internal, membrane-bound organelles, their interior appears morphologically simple. The genetic material is localized in a discrete region called the nucleoid and is not separated from the surrounding cytoplasm by membranes. Ribosomes and larger masses called inclusions are scattered about the cytoplasm. Finally, many bacteria use flagella for locomotion. In the remaining sections of this chapter, we describe these major structures observed in bacterial cells in more detail.

Comprehension Check

1. Why is the term *prokaryote* considered an inadequate descriptor by some microbiologists?
2. What characteristic shapes can bacteria assume? Describe the ways in which bacterial cells cluster together.
3. What advantages might a bacterial species that forms multicellular arrangements (e.g., clusters or chains) have that are not afforded unicellular bacteria?
4. What is the relevance of the surface area-to-volume ratio?

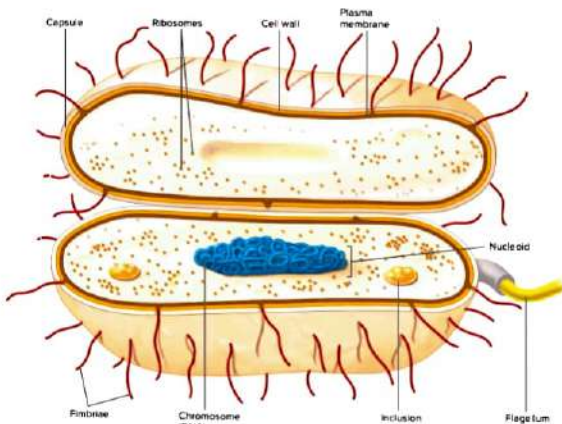
Table 3.1 Common Bacterial Structures and Their Functions

Plasma membrane	Selectively permeable barrier, mechanical boundary of cell, nutrient and waste transport, location of many metabolic processes (respiration, photosynthesis), detection of environmental cues for chemotaxis
Gas vacuole	An inclusion that provides buoyancy for floating in aquatic environments
Ribosomes	Protein synthesis
Inclusions	Storage of carbon, phosphate, and other substances; site of chemical reactions (microcompartments); movement
Nucleoid	Localization of genetic material (DNA)
Periplasmic space	In typical Gram-negative bacteria, contains hydrolytic enzymes and binding proteins for nutrient processing and uptake; in typical Gram-positive bacteria, may be smaller or absent
Cell wall	Protection from osmotic stress, helps maintain cell shape
Capsules and slime layers	Resistance to phagocytosis, adherence to surfaces

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Capsules and slime layers	Resistance to phagocytosis; adherence to surfaces
Fimbriae and pili	Attachment to surfaces, bacterial conjugation and transformation, twitching
Flagella	Swimming and swarming motility
Endospore	Survival under harsh environmental conditions

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**Figure 3.6** Structure of a Bacterial Cell.

3.3 Bacterial Plasma Membranes Control What Enters and Leaves the Cell

After reading this section, you should be able to:

- Describe the fluid mosaic model of membrane structure and identify the types of lipids typically found in bacterial membranes.
- Distinguish macroelements (macromolecules) from trace elements (micronutrients) and provide examples of each.
- Provide examples of growth factors needed by some microorganisms.
- Compare and contrast passive diffusion, facilitated diffusion, active transport, and group translocation, and provide examples of each.
- Discuss the challenge of iron uptake and describe how bacteria overcome this difficulty.

The **cell envelope** is defined as the plasma membrane and all the surrounding layers external to it. The cell envelopes of many bacteria consist of the plasma membrane, cell wall, and at least one additional layer (e.g., capsule or slime layer). Of all these layers, the **plasma membrane** is the most important because it encompasses the cytoplasm and defines the cell. If it is removed or compromised, the cell's contents spill into the environment and the cell dies. Furthermore, despite being the innermost layer of the cell envelope, the plasma membrane is responsible for much of the cell's relationship with the outside world. Thus we begin our consideration of bacterial cell structure by describing the plasma membrane.

First, let's consider what cells do to survive. Cells must interact in a selective fashion with their environment, acquire nutrients, and eliminate waste. They also have to maintain their interior in a constant, highly organized state in the face of external changes. Plasma membranes are an absolute requirement for all living organisms because they are involved in carrying out these cellular tasks.

A primary role of all plasma membranes is that they are selectively permeable barriers. They allow particular ions and molecules to pass either into or out of the cell, while preventing the movement of others. Thus the plasma membrane prevents the loss of essential components through leakage while allowing the movement of other molecules. Bacterial plasma membranes play additional critical roles. They are the location of several crucial metabolic processes: respiration, photosynthesis, and the synthesis of lipids and cell wall constituents.

In addition to the plasma membrane, some bacteria have extensive

intracytoplasmic membrane systems. These internal membranes and the plasma membrane share a basic design. However, they can differ significantly in the lipids and proteins they contain. To understand these chemical differences and the many functions of the plasma membrane and other membranes, it is necessary to become familiar with membrane structure.

Fluid Mosaic Model of Membrane Structure

The most widely accepted model for membrane structure is the **fluid mosaic model** of Singer and Nicholson, which proposes that membranes are lipid bilayers within which proteins float (figure 3.7). Bacterial membranes have roughly equal amounts of lipids and proteins. Cell membranes are very thin structures, about 2 to 3 nm thick, that look like two dark lines on either side of a light interior when imaged by TEM. This characteristic appearance is evidence that the membrane is composed of two sheets of lipid molecules arranged end-to-end (figure 3.7). Cleavage of membranes by freeze-etching, a technique that reveals fine detail, exposes the proteins lying within the membrane lipid bilayer. **Electron microscopes use beams of electrons to create highly magnified images (section 2.4). Scanning probe microscopy can visualize molecules and atoms (section 2.5).**

The chemical nature of membrane lipids is critical to their ability to form bilayers. Most membrane-associated lipids (e.g., the phospholipids shown in figure 3.7) are **amphipathic**. They are structurally asymmetric, with polar and nonpolar ends

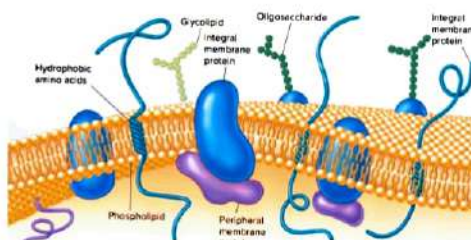


Figure 3.7 The Fluid Mosaic Model of Bacterial Membrane Structure. This diagram shows the integral membrane proteins (blue) floating in a lipid bilayer. Peripheral membrane proteins (purple) are associated loosely with the inner membrane surface and/or integral membrane proteins. Small tan spheres represent the hydrophilic ends of membrane phospholipids, and wiggly tails are the hydrophobic fatty acid chains. Phospholipids are drawn much larger than their actual size. Oligosaccharides (a chain of carbohydrates) protrude into the environment and may be attached to proteins or to membrane phospholipids (glycolipids).

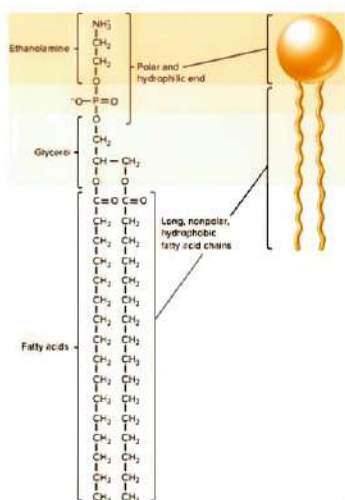


Figure 3.8 The Structure of a Phospholipid.
Phosphatidylethanolamine, a phospholipid often found in bacterial membranes.

(figure 3.8). The polar ends interact with water and are **hydrophilic**; the nonpolar **hydrophobic** ends are insoluble in water, and tend to associate with one another. In aqueous environments, amphipathic lipids can interact to form a bilayer. The outer surfaces of the bilayer are hydrophilic, whereas hydrophobic ends are buried in the interior away from the surrounding water (figure 3.7). **▶ Lipids (appendix 1)**

Two types of membrane proteins have been identified based on their ability to be separated from the membrane. **Peripheral membrane proteins** are loosely connected to the membrane and can be easily removed (figure 3.7). They are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. The remaining proteins are **integral membrane proteins**. These are not easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Integral membrane proteins, like membrane lipids, are amphipathic; their hydrophobic regions are buried in membrane lipids while the hydrophilic portions project from the membrane surface (figure 3.7).

Integral membrane proteins carry out some of the most important functions of the membrane. Many are transport proteins used to move materials either into or out of the cell. Others are involved in energy-conserving processes, such as the proteins found in electron transport chains. Those integral membrane proteins with regions exposed to the outside of the cell enable the cell to interact with its environment. **▶ Proteins (appendix 1)**

Bacterial Plasma Membranes Are Dynamic

Bacterial membranes are lipid bilayers and many of their amphipathic lipids are phospholipids (figure 3.8). The plasma membrane is dynamic: The lipid composition varies with environmental temperature in such a way that the membrane remains fluid during growth. For example, bacteria growing at lower temperatures have more unsaturated fatty acids in their membrane phospholipids; that is, there are one or more double covalent bonds in the long hydrocarbon chains. At higher temperatures, their phospholipids have more saturated fatty acids—those in which the carbon atoms are connected only with single covalent bonds. **▶ Environmental factors affect microbial growth (section 7.5)**

Although most aspects of the fluid mosaic model are well supported by experimentation, the suggestion that membrane lipids and integral proteins are homogeneously distributed has been challenged by the discovery of **microdomains**. These regions of the membrane are formed by phospholipids in conjunction with other lipids like farnesol, hopanoids, and carotenoids (see figure 11.31). **Hopanoids** are similar in structure to cholesterol found in eukaryotic membranes (figure 3.9), and their rigid planar structure makes them more hydrophobic than phospholipids. The specific lipids found in microdomains vary among organisms, but they all regulate membrane rigidity and mark microdomain boundaries.

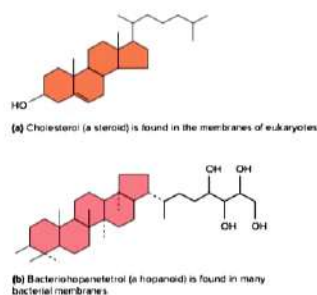


Figure 3.9 Membrane Stereols and Hopanoids.

3.3 Bacterial Plasma Membranes Control What Enters and Leaves the Cell 47

The integral membrane proteins in microdomains differ from those in the bulk membrane, and are organized by proteins called **flotillins**. Flotillins are themselves integral membrane proteins, and they function to assemble large protein complexes like secretion systems for transporting molecules out of the cell and complexes that transmit signals from the environment to molecules in the cytoplasm.

Bacteria Use Many Mechanisms to Bring Nutrients into the Cell

All plasma membranes function as barriers. Yet they must also allow movement of nutrients into the cell. If a microbe does not obtain nutrients from its environment, it will quickly exhaust its supply of amino acids, nucleotides, and other molecules needed to survive. In addition, if a microbe is to thrive and reproduce, it must have a source of energy. The energy source is used to generate the cell's major energy currency: the high-energy molecule ATP. Clearly, obtaining energy and nutrient sources is one of the most important jobs an organism has, and it is primarily a function of the bacterial plasma membrane. Here we discuss nutrient uptake, but first let's define some terms used to describe the nutrients needed by cells.

Microbiologists refer to carbon, oxygen, hydrogen, nitrogen, sulfur, and phosphorus as **macroelements** or **macronutrients** because they are required in relatively large amounts. They are found in organic molecules such as proteins, lipids, nucleic acids, and carbohydrates. Other macroelements are potassium, calcium, magnesium, and iron. They exist as cations and generally are associated with and contribute to the activity and stability of molecules and cell structures such as enzymes and ribosomes. Thus they are important in many cellular processes, including protein synthesis and energy conservation. **▶ Enzymes and ribosomes speed up cellular chemical reactions (section 10.6); Translation in bacteria (section 13.7); Electron transport chains: sets of sequential redox reactions (section 10.4)**

Other elements are required in small amounts—amounts so small that in the lab they are often obtained as contaminants in water, glassware, and growth media. Likewise in nature, they are ubiquitous and usually present in adequate amounts to support the growth of microbes. Microbiologists call these elements **micronutrients** or **trace elements**. The micronutrients—manganese, zinc, cobalt, molybdenum, nickel, and copper—are needed by most cells. Micronutrients are part of certain enzymes, and they aid in catalysis of reactions and maintenance of protein structure.

Some microbes are able to synthesize all the organic molecules they need from macroelements. However, some microbes are unable to synthesize certain molecules needed for survival. These molecules are called **growth factors**, and they must be obtained from the environment. There are three types of growth factors: amino acids, purines and pyrimidines, and vitamins.

What are the common features of nutrient uptake by bacteria? Bacteria can only take in dissolved molecules. Uptake mechanisms are specific; that is, the necessary substances, and not others, are acquired. It does a cell no good to take in a substance

that it cannot use. Bacteria are able to transport nutrients into the cell even when the concentration of a nutrient inside the cell is higher than the concentration outside. Thus they are able to move nutrients up a concentration gradient. This is important because bacteria often live in nutrient-poor habitats. In view of the enormous variety of nutrients and the complexity of the task, it is not surprising that bacteria use several different transport mechanisms: passive diffusion, facilitated diffusion, primary and secondary active transport, and group translocation.

Passive Diffusion

Passive diffusion, often called diffusion or simple diffusion, is the process by which molecules move from a region of higher concentration to one of lower concentration; that is, the molecules move down the concentration gradient. The rate of passive diffusion depends on the size of the concentration gradient between a cell's exterior and its interior (figure 3.10). A large concentration gradient is required for adequate nutrient uptake by passive diffusion (i.e., the external nutrient concentration must be high while the internal concentration is low). The rate of diffusion decreases as more nutrient accumulates in the cell. This occurs if the nutrient is not used immediately upon entry.

Most substances cannot freely diffuse into a cell. However, water and some gases, including O_2 and CO_2 , easily cross the

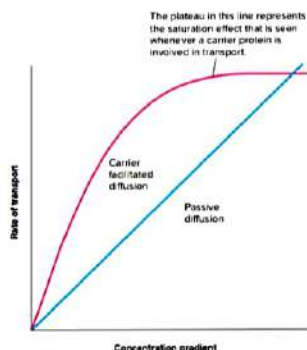


Figure 3.10 Passive and Facilitated Diffusion. The rate of diffusion depends on the size of the solute's concentration gradient (the ratio of the extracellular concentration to the intracellular concentration). This simple type of facilitated diffusion involves a carrier protein that can be saturated. Sometimes facilitated diffusion is mediated by a channel. Channels often do not exhibit a saturation effect.

plasma membrane by passive diffusion. H_2O also moves across membranes by passive diffusion. Larger molecules, ions, and polar substances must enter the cell by other mechanisms, all of which involve specialized proteins that are referred to as transport proteins. **How Diffusion Works**

Facilitated Diffusion

During **facilitated diffusion**, substances move across the plasma membrane with the assistance of transport proteins that are either channels or carriers. Channels, as their name indicates, are proteins that form pores in membranes through which substances can pass; they are often involved in facilitated diffusion. Channels show some specificity for the substances that pass through them, but this is considerably less than that shown by carriers, which are far more substrate specific. The rate of facilitated diffusion increases with the concentration gradient much more rapidly and at lower concentrations of the diffusing molecule than that of passive diffusion (figure 3.10). When the transporter is a carrier, the diffusion rate reaches a plateau above a specific gradient value because the carrier protein is saturated; that is, it is transporting as many solute molecules as possible. The resulting curve resembles an enzyme-substrate curve (see figure 10.16) and is different from the linear response seen with passive diffusion. An example of channel-mediated facilitated diffusion is that involving aquaporins (see figure 2.11), which transport water. Aquaporins are members of the major intrinsic protein (MIP) family of proteins. MIPs facilitate diffusion of small polar molecules, and they are observed in all organisms.

Although facilitated diffusion relies on transport proteins, it is truly diffusion because a concentration gradient spanning the membrane drives the movement of molecules, and no energy is used. If the concentration gradient disappears, net inward movement ceases. The gradient can be maintained by converting the transported nutrient to another compound, as occurs when a nutrient is metabolized.

Considerable work has been done on the mechanism of carrier-mediated facilitated diffusion. When the solute molecule binds to the outside of the carrier, it changes conformation and releases the molecule on the cell interior (figure 3.11). The carrier subsequently changes back to its original shape and is ready to pick up another molecule. The net effect is that a hydrophilic molecule can enter the cell in response to its concentration gradient.

Facilitated diffusion has been documented in some bacteria but it does not seem to be the major uptake mechanism for these microbes. Recall that many bacteria live in environments where nutrient concentrations are low, and facilitated diffusion cannot concentrate nutrients inside cells. Therefore

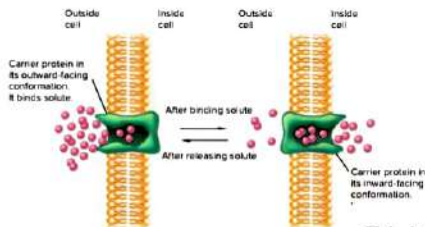


Figure 3.11 A Model of Facilitated Diffusion. Because there is no energy, solute continues to enter only as long as its concentration is greater on the outside.

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energy-dependent transport mechanisms capable of concentrating nutrients are significantly more important uptake mechanisms for bacterial cells.

Primary and Secondary Active Transport

Active transport is the transport of solute molecules to higher concentrations (i.e., against a concentration gradient) with the input of metabolic energy. Three types of active transport are observed in bacteria: primary active transport, secondary active transport, and group translocation. They differ in terms of the energy used to drive transport and whether or not the transported molecule is modified as it enters.

Active transport resembles facilitated diffusion in that it involves carrier proteins. Recall that carrier proteins bind particular solutes with great specificity. Active transport is also characterized by the carrier saturation effect at high solute concentrations (figure 3.10). Nevertheless, active transport differs from facilitated diffusion because it uses metabolic energy and can concentrate substances within the cell.

Primary active transport is mediated by carriers called primary active transporters. They use energy provided by ATP hydrolysis to move substances against a concentration gradient without modifying them. Primary active transporters are **uniporters**; that is, they move a single molecule across the membrane (figure 3.12). **ATP-binding cassette transporters (ABC transporters)** are important primary active transporters. Our focus here is on those ABC transporters that are used for import of substances. Other ABC transporters are used for export of substances, in particular proteins; these exporters are described in chapter 13. **Protein maturation and secretion (section 13.8)**

Most ABC transporters consist of two hydrophobic membrane-spanning regions (domains) with two ATP-binding

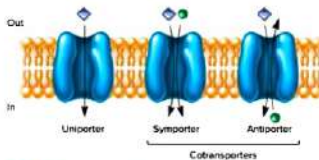


Figure 3.12 Carrier Proteins Can Be Uniporters or Cotransporters. Uniporters move a single substance into the cell. Cotransporters simultaneously move two substances across the membrane. When both substances move in the same direction, the carrier is a symporter. When the two substances move in opposite directions, the carrier is an antiporter.

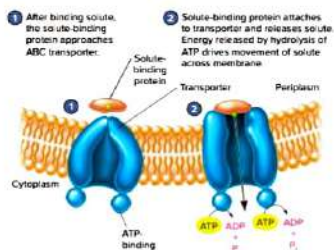


Figure 3.13 ABC Transporter Function. Shown here is a transporter that works with a solute-binding protein free in the periplasm. Other solute-binding proteins are associated with the plasma membrane, always associated with the transporter, or even fused to the transporter.

domains facing the cytoplasm (figure 3.13). The membrane-spanning domains form a pore in the membrane, and the ATP-binding domains bind and hydrolyze ATP to drive uptake. Most ABC transporters employ solute-binding proteins to deliver the molecule to be transported to the transporter.

Secondary active transport couples the potential energy of ion gradients to transport of substances without modifying them. Secondary active transporters are **cotransporters** (figure 3.12). They move two substances simultaneously; the ion whose gradient powers transport and the substance being moved across the membrane. When the ion and other substance both move in the same direction, it is called **symport**. When they move in opposite directions, it is called **antiport**. **Cotransport (Symport and Antiport)**

The ion gradients used by secondary active transporters arise primarily in three ways. The first results from bacterial metabolic activity. During energy-conserving processes, electron transport generates a proton gradient in which protons are at a higher concentration outside the cell than inside. The proton gradient is used to do cellular work, including secondary active transport. Some bacteria use the second method, in which an enzyme called a V_{ATPase} hydrolyzes ATP and uses the energy released to create either a proton gradient or a sodium gradient across the plasma membrane. Finally, a proton gradient can be used to create another ion gradient such as a sodium gradient. This is accomplished by an antiporter that brings protons in as sodium ions are moved out of the cell. The sodium gradient can then be used to drive uptake of nutrients by a symport mechanism. **Electron transport and oxidative phosphorylation (step 3) generate the most ATP (section 11.6)**

The lactose permease of *E. coli* is a well-studied symport secondary active transporter. It is a single protein that transports a lactose molecule inward as a proton simultaneously enters the cell. The proton is moving down a proton gradient, and the energy released drives solute transport. X-ray diffraction studies show that the carrier protein exists in outward- and inward-facing conformations. When lactose and a proton bind to separate sites on the outward-facing conformation, the protein changes to its inward-facing conformation, and the sugar and proton are released into the cytoplasm.

Bacteria often have more than one transport system for a nutrient, as can be seen with *E. coli*. This bacterium has at least five transport systems for the sugar galactose, three systems each for the amino acids glutamate and leucine, and two potassium transport complexes. When several transport systems exist for the same substance, the systems differ in such properties as their energy source, their affinity for the solute transported, and the nature of their regulation. This diversity gives the bacterium an added competitive advantage in a variable environment.

Group Translocation

The distinguishing characteristic of **group translocation** is that a molecule is chemically modified as it is brought into the cell. The best-known group translocation system is the **phosphoenolpyruvate: sugar phosphotransferase system (PTS)**, which is observed in many bacteria. The PTS transports a variety of sugars while phosphorylating them, using phosphoenolpyruvate (PEP) as the phosphate donor. PEP is a high-energy molecule that can be used to synthesize ATP, the cell's energy currency. However, when it is used in PTS reactions, the energy present in PEP is used to energize sugar uptake rather than ATP synthesis. **ATP: The major energy currency of cells (section 10.2)**

The transfer of phosphate from PEP to the incoming molecule involves several proteins and is an example of a **phosphorelay system**. In *E. coli* and *Salmonella*, the PTS consists of two enzymes and a low molecular weight heat-stable protein (HPr). A phosphate is transferred from PEP to enzyme II with the aid of

They move two substrates simultaneously: the ion whose gradient powers transport and the substance being moved across the membrane. When the ion and other substance both move in the same direction, it is called **symport**. When they move in opposite directions, it is called **antiport**. **❖ Cotransport (Symport and Antiport)**

❖ ATP: The major energy currency of cells (section 10.2)
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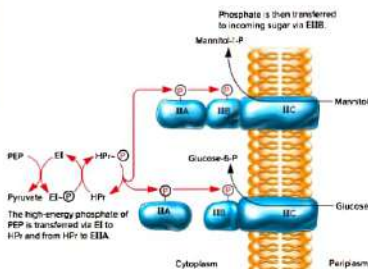


Figure 3.14 Group Translocation: Bacterial PTS Transport. Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated. The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system.

enzyme I and HPr (figure 3.14). Enzyme II then phosphorylates the sugar molecule as it is carried across the membrane. Many different PTSs exist, and they vary in terms of the sugars they transport. The specificity lies with the type of Enzyme II used in the PTS. Enzyme I and HPr are the same in all PTSs used by a bacterium. **❖ Enzymes and ribozymes speed up cellular chemical reactions (section 10.6)**

PTSs are widely distributed in bacteria, primarily among facultatively anaerobic bacteria (bacteria that grow in either the presence or absence of O_2); some obligately anaerobic bacteria (e.g., *Clostridium* spp.) also have PTSs. However, most aerobic bacteria lack PTSs. Many carbohydrates are transported by PTSs. *E. coli* takes up glucose, fructose, mannitol, sucrose, *N*-acetylglucosamine, cellobiose, and other carbohydrates by group translocation. **❖ Active Transport by Group Translocation**

Iron Uptake

Almost all microorganisms require iron for building molecules important in energy-conserving processes (e.g., cytochromes), as well as for the function of many enzymes. Iron uptake is made difficult by the extreme insolubility of ferric iron (Fe^{3+}) and its derivatives, which leaves little free iron available for transport. Many bacteria overcome this difficulty by secreting **siderophores** (Greek for iron bearers). Siderophores are low molecular weight organic molecules that bind ferric iron and supply it to the cell (figure 3.15). **❖ Electron transport chains: sets of sequential redox reactions (section 10.4)**

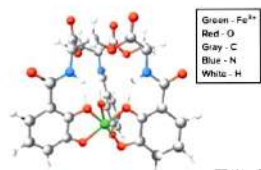


Figure 3.15 Enterobactin: A Siderophore Produced by *E. coli*. Ball and stick model of enterobactin complexed with Fe^{3+} .

Microorganisms secrete siderophores when iron is scarce in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a siderophore-receptor protein. Then either the iron is released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter. Iron is so crucial to microorganisms that more than one route of iron uptake may be used to ensure an adequate supply.

Comprehension Check

1. List the functions of bacterial plasma membranes. Why must their plasma membranes carry out more functions than the plasma membranes of eukaryotic cells?
2. Describe in words and with a labeled diagram the fluid mosaic model for cell membranes.
3. On what basis are elements divided into macroelements and trace elements?
4. Describe facilitated diffusion, primary and secondary active transport, and group translocation in terms of their distinctive characteristics and mechanisms. What advantage does a bacterium gain by using active transport rather than facilitated diffusion?
5. What are uniport, symport, and antiport?
6. What are siderophores? Why are they important?

3.4 There Are Two Main Types of Bacterial Cell Walls

After reading this section, you should be able to:

- a. Describe peptidoglycan structure
- b. Compare and contrast the cell walls of typical Gram-positive and Gram-negative bacteria
- c. Relate bacterial cell wall structure to the Gram-staining reaction

The cell wall is the layer that lies just outside the plasma membrane. It is one of the most important structures for several reasons:

It helps maintain cell shape and protect the cell from osmotic lysis; it can protect the cell from toxic substances; and in pathogens, it can contribute to pathogenicity. Cell walls are so important that most bacteria have them. Those that lack them have other features that fulfill cell wall function. Bacterial cell wall synthesis is an important target for many antibiotics. **❖ Antibacterial drugs (section 9.4)**

Overview of Bacterial Cell Wall Structure

After Christian Gram developed the Gram stain in 1884, it soon became evident that most bacteria could be divided into two major groups based on their response to the Gram-staining procedure. Gram-positive bacteria stained purple, whereas Gram-negative bacteria were pink or red. The true structural difference between these two groups did not become clear until the advent of the transmission electron microscope. Here we describe the long-held models of Gram-positive and Gram-negative cell walls developed from these studies. More recent studies of diverse groups of bacteria have shown that these models do not hold true for all bacteria (*Microbial Diversity & Ecology* 3.1). Because of ongoing discussions related to these new studies, we will refer to bacteria that fit the models as being typical Gram-positive or typical Gram-negative bacteria. **❖ Differential staining (section 2.3)**

The cell walls of *Bacillus subtilis* and many other typical Gram-positive bacteria consist of a single, 20- to 80-nm-thick homogeneous layer of **peptidoglycan** (murein) lying outside the plasma membrane (figure 3.16). In contrast, the cell walls of *E. coli* and many other typical Gram-negative bacteria have two distinct layers: a 2- to 7-nm-thick peptidoglycan layer covered by a 7- to 8-nm-thick **outer membrane**.

One important feature seen in typical Gram-negative bacteria is a space between the plasma membrane and the outer membrane. It also is sometimes observed between the plasma membrane and cell wall in typical Gram-positive bacteria. This space is called the **periplasmic space**. The substance that occupies the periplasmic space is the **periplasm**.

Peptidoglycan Structure

The feature common to nearly all bacterial cell walls is the presence of peptidoglycan, which forms an enormous meshlike structure often referred to as the peptidoglycan sacculus. Peptidoglycan is composed of many identical subunits. Each subunit within the sacculus contains two sugar derivatives, *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM), and several different amino acids. The amino acids form a short peptide, sometimes called the stem peptide, consisting of four alternating D- and L-amino acids; the peptide is connected to the carboxyl group of NAM (figure 3.17). Three of the amino acids are not found in proteins: D-glutamic acid, D-alanine, and *meso*-diaminopimelic acid. The presence of D-amino acids in the stem peptide protects against degradation by most peptidases, which recognize only the L-isomers of amino acid residues. The peptidoglycan subunit of many bacteria is shown in figure 3.17. **❖ Carbohydrates**

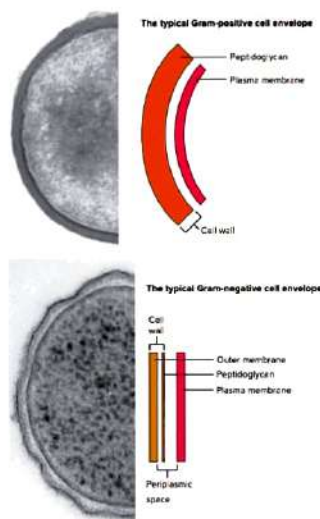


Figure 3.16

Figure 3.16 Cell Envelopes of Typical Gram-Positive and Gram-Negative Bacteria. Cell envelopes consist of the plasma membrane and any layers (e.g., cell wall) exterior to it. For simplicity, we show only the plasma membrane and cell wall. *Staphylococcus aureus* (top) has a typical Gram-positive cell wall that consists primarily of peptidoglycan. *Mycobacterium xenopus* (bottom) has a typical Gram-negative cell wall consisting of a thin layer of peptidoglycan, an outer membrane, and the periplasmic space.

(appendix 1); **Proteins (appendix 1); Proteins are polymers of amino acids (section 13.2)**

The peptidoglycan sacculus is formed by linking the sugars of the peptidoglycan subunits together to form a strand; the strands are then cross-linked to each other by covalent bonds formed between the stem peptides extending from each strand. As seen in figure 3.18, the backbone of each strand is composed of alternating NAG and NAM residues. The strand is helical, and the stem peptides extend out from the backbone in different directions. There are two types of cross-links: direct and indirect

identifying bacteria in clinical settings. The initial studies done to differentiate bacteria that stained Gram positive from those that stain Gram negative were done using model organisms such as *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative). At the time, it was thought that all other bacteria would have similar cell wall structures. However, the long-held models of Gram-positive and Gram-negative cell walls do not hold true for all bacteria. Iain Sutcliffe has proposed that microbiologists stop referring to bacteria as either Gram positive or Gram negative. He suggests that instead we should more precisely describe bacterial cell envelope architectures by focusing on the observation that some bacteria have envelopes with a single membrane—the plasma membrane as seen in typical Gram-positive bacteria—while others have envelopes with two membranes—the plasma membrane and an outer membrane as seen in typical Gram-negative bacteria. He proposed calling the former monoderms and the latter diderms.

But why make this change? Sutcliffe begins by pointing out that some bacteria staining Gram positive are actually diderms and some staining Gram negative are actually monoderms. By referring to Gram-positive-staining diderms as Gram-positive bacteria, it is too easy to mislead scientists and many a budding microbiologist into thinking that the bacterium

is Gram-positive, whereas almost all other bacterial phyla consist of diderms.

There are interesting exceptions to the relationship of phylogeny and cell envelope structure. For instance, members of the genus *Mycobacterium* (e.g., *M. tuberculosis*) belong to the predominantly monoderm phylum Actinobacteria. Mycobacteria have cell walls that consist of peptidoglycan and an outer membrane. The outer membrane is composed of mycolic acids rather than the phospholipids and lipopolysaccharides (LPSs) found in the typical Gram-negative cells' outer membrane. **Order Corynebacteriales includes important human pathogens (section 23.1)**

Members of the genus *Deinococcus* are another interesting exception. These bacteria stain Gram positive but are diderms. Their cell envelopes consist of the plasma membrane, what appears to be a typical Gram-negative cell wall, and an S-layer. Their outer membrane is distinctive because it lacks LPS. *Deinococci* are not unique in this respect, however. It is now known that members of several taxa have outer membranes that lack LPS.

Source: Sutcliffe, I. C. 2010. A phylum level perspective on bacterial cell envelope architecture. *Trends Microbiol.* 18(10):464–70.

via a peptide interbridge. A direct cross-link is characterized by connecting the carboxyl group of an amino acid in one stem peptide to the amino group of an amino acid in another stem peptide. For instance, many bacteria cross-link the strands by connecting the carboxyl group of the D-alanine at position 4 of the stem peptide directly to the amino group of diaminopimelic acid (position 3) of the other peptidoglycan strand's stem peptide (the position 5 D-alanine is removed as the cross-link is formed). Bacteria that have indirect linkage use a **peptide interbridge** (also called an interpeptide bridge), a short chain of amino acids that links the stem peptide of one peptidoglycan strand to that of another (figure 3.19). Cross-linking results in one dense, interconnected network of peptidoglycan strands. **Synthesis of peptidoglycan occurs in the cytoplasm, at the plasma membrane, and in the periplasmic space (section 12.4)**

The peptidoglycan sacculus is strong but elastic. It is able to stretch and contract in response to osmotic pressure. This is due to the rigidity of the backbone coupled with the flexibility of the cross-links. Peptidoglycan sacculi are also rather porous,

allowing globular proteins having a molecular mass of 50,000 to pass through, depending on whether they are relaxed or stretched; thus only extremely small molecules can pass through peptidoglycan.

Variants of peptidoglycan are found, particularly among typical Gram-positive bacteria. For example, some substitute the diamino acid lysine for meso-diaminopimelic acid (figure 3.20) and cross-link chains via interpeptide bridges. These interpeptide bridges can vary considerably (figure 3.21). Peptidoglycan can also vary in terms of the length of the peptidoglycan strands and the amount of cross-linking. Bacteria that stain Gram positive tend to have much more cross-linking, whereas those that stain Gram negative have considerably less.

Typical Gram-Positive Cell Walls Consist Primarily of Peptidoglycan

Most cultured bacteria that stain Gram positive belong to the phyla *Firmicutes* and *Actinobacteria*, and most of these bacteria have

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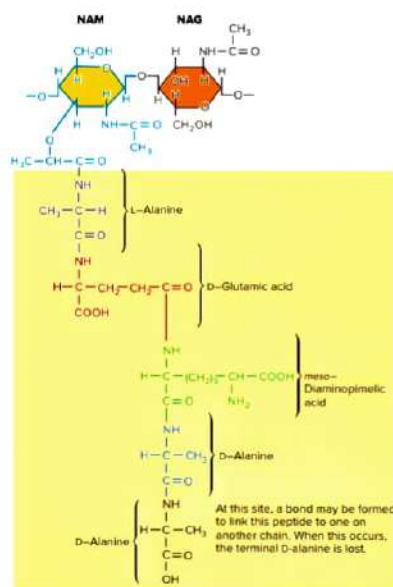


Figure 3.17 Peptidoglycan Subunit Composition. Shown is the peptidoglycan subunit of *E. coli*, many other typical Gram-negative bacteria, and many typical Gram-positive bacteria. This illustration shows the subunit before it has been inserted into the existing peptidoglycan polymer. NAG is N-acetylglucosamine. NAM is N-acetylmuramic acid. The stem peptide is composed of alternating D- and L-amino acids; it terminates with two D-alanines. The amino acids are shown in different colors for clarity.

thick cell walls composed of peptidoglycan and large amounts of other polymers such as teichoic acids (figure 3.22). **Teichoic acids** are polymers of glycerol or ribitol joined by phosphate groups (figure 3.23). Some teichoic acids are covalently linked to peptidoglycan and are referred to as wall teichoic acids. Others are covalently connected to the plasma membrane: they are called lipoteichoic acids. Wall teichoic acids extend beyond the surface of the peptidoglycan. They are negatively charged and help give the cell wall its negative charge. Teichoic acids are not present in other bacteria.

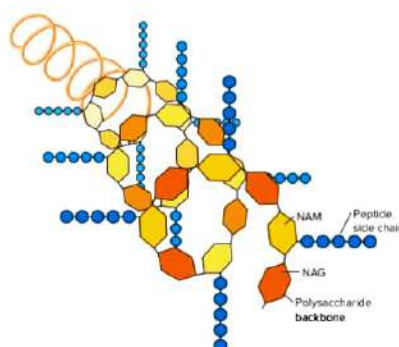


Figure 3.18 A Helical Peptidoglycan Strand. Because of the strand's helical nature, the stem peptides project out in different directions from the NAM-NAG backbone. Here the stem peptides are shown projecting out at 90-degree angles. Some studies suggest that the angle is actually 120 degrees.

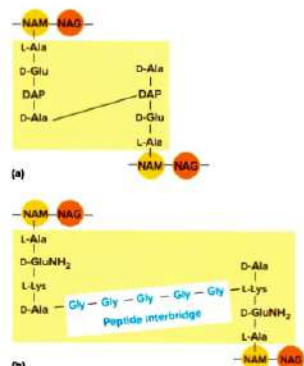


Figure 3.19 Peptidoglycan Cross-Links Can Be Direct or Indirect via a Peptide Interbridge. (a) *E. coli* peptidoglycan with direct cross-linking, typical of many Gram-negative bacteria. (b) *Staphylococcus aureus* peptidoglycan with an interbridge. *S. aureus* stains Gram positive. Gly is glycine. D-GluNH₂ is D-glutamic acid with an NH₂ group attached to the α carbon (the carbon next to the carboxyl group).

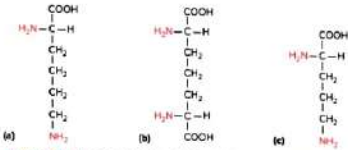


Figure 3.20 Diamino Acids Present in Peptidoglycan. (a) L-lysine, (b) meso-diaminopimelic acid, (c) D-ornithine.

Teichoic acids have several important functions. They help create and maintain the structure of the cell envelope by anchoring the wall to the plasma membrane. They are important during cell division, and they protect the cell from harmful substances in the environment (e.g., antibiotics and host defense molecules). In addition, they function in ion uptake and are involved in binding pathogenic species to host tissues, thus initiating the infectious disease process.

The periplasmic space lies between the plasma membrane and the cell wall and is so narrow that it is often not visible by electron microscopy. The periplasm has relatively few proteins; this is probably because the peptidoglycan sacculus is so porous that many proteins translocated across the plasma membrane pass through the sacculus. Some secreted proteins are enzymes called **exoenzymes**. Exoenzymes often serve to degrade polymers such as proteins and polysaccharides that would otherwise be too large for transport across the plasma membrane; the degradation products, the monomer building blocks, are then taken up by the cell. Those proteins that remain in the periplasmic space are usually attached to the plasma membrane.

Not all secreted proteins pass through the peptidoglycan sacculus; some become bound to it instead. These proteins are involved in interactions of the cell with its environment. Some are

noncovalently bound to teichoic acids or other cell wall polymers, while others are covalently attached to the peptidoglycan. Membrane-bound enzymes called **sortases** catalyze the formation of covalent bonds that join these proteins to the peptidoglycan. **▶ Protein maturation and secretion (section 13.8)**

Typical Gram-Negative Cell Walls Include Additional Layers Besides Peptidoglycan

As just noted, most cultured bacteria that stain Gram positive belong to the phyla *Firmicutes* and *Actinobacteria*. With a few exceptions, bacteria belonging to the remaining phyla stain Gram negative (Microbial Diversity & Ecology 3.1). Even a brief inspection of figure 3.16 shows that typical Gram-negative cell walls are more complex than typical Gram-positive walls. One of the most striking differences is the paucity of peptidoglycan. The peptidoglycan layer is very thin (2 to 7 nm, depending on the bacterium) and sits within the periplasmic space.

The periplasmic space is much larger than that of a typical Gram-positive cell, ranging from about 30 to 70 nm wide (figure 3.24). Some studies indicate that it may constitute about 20 to 40% of the total cell volume. The periplasmic space is home to a variety of proteins. Some periplasmic proteins participate in nutrient acquisition—for example, hydrolytic enzymes and transport proteins. Some periplasmic proteins are involved in energy conservation. For instance, some bacteria have electron transport proteins in their periplasm (e.g., denitrifying bacteria, which convert nitrate to nitrogen gas). Other periplasmic proteins are involved in peptidoglycan synthesis and modification of toxic compounds that could harm the cell. **▶ Anaerobic respiration uses the same three steps as aerobic respiration (section 11.7); Nitrogen cycle (section 28.1)**

The outer membrane lies outside the thin peptidoglycan layer. It is linked to the cell by **Braun's lipoprotein**, the most abundant protein in the outer membrane (figure 3.24). This small

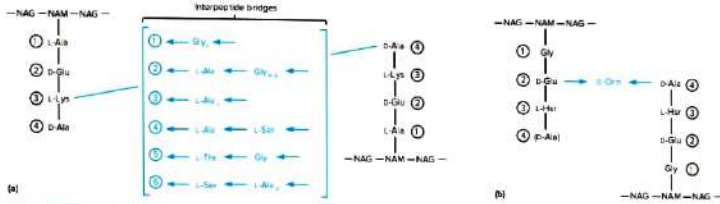


Figure 3.21 Examples of Peptidoglycan Cross-Links. Most variation in peptidoglycan structure occurs in the composition of the stem peptide and the method by which peptidoglycan strands are cross-linked. (a) Examples of interpeptide bridges that link D-alanine in position 4 with L-lysine in position 3. The bracket contains six typical bridges. (b) *Staphylococcus aureus*, (2) *S. epidermidis*, (3) *Micrococcus roseus* and *Streptococcus thermophilus*, (4) *Lactobacillus viridescens*, (5) *Streptococcus salivarius*, and (6) *Leuconostoc cremoris*. The arrows indicate the polarity of peptide bonds running in the C to N direction. (b) An interpeptide bridge observed in *Corynebacterium parvum*. The bridge extends between positions 2 and 4 and consists of the D-diamino acid ornithine (figure 3.20c). Note that L-homoserine (L-Hsr) is in position 3 rather than meso-diaminopimelic acid or L-lysine.

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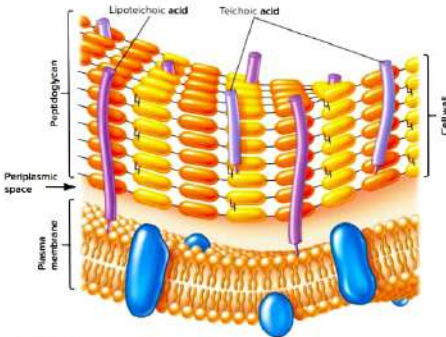


Figure 3.22 Typical Gram-Positive Cell Wall. This component of the cell envelope lies just outside the plasma membrane.

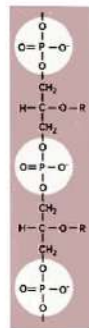


Figure 3.23 Teichoic Acid Structure. The segment of a teichoic acid made of phosphate, glycerol, and a side chain, R. R may represent D-alanine, glucose, or other molecules.

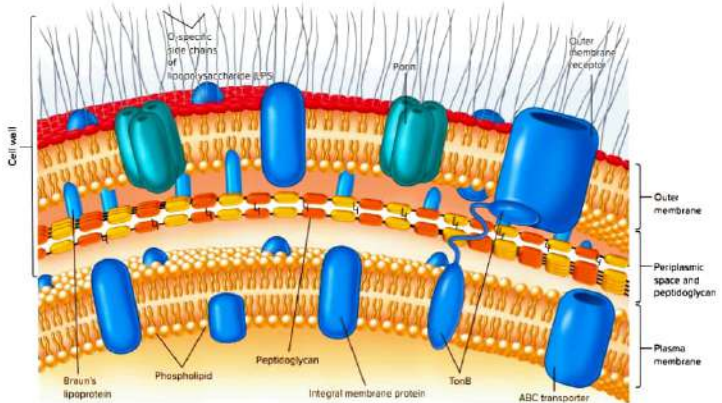


Figure 3.24 Typical Gram-Negative Cell Wall. Notice that these bacteria are bounded by two membranes, the plasma membrane and the outer membrane of the cell wall.

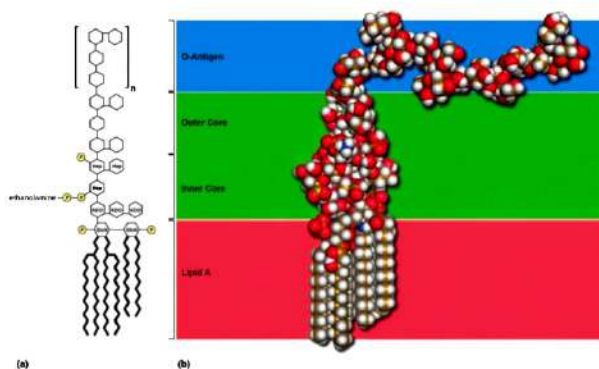


Figure 3.25 Lipopolysaccharide Structure. (a) A simplified diagram of LPS. Abbreviations: GlcN, glucosamine; Hep, heptulose; KDO, P, phosphate. Lipid A is buried in the outer membrane. (b) Molecular model of a lipopolysaccharide. The lipid A and core polysaccharide chain is bent at an angle in this model.

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lipoprotein is covalently joined to both the outer membrane and the peptide chain of peptidoglycan.

In contrast to the symmetry of the plasma membrane, the most distinctive structural feature of the outer membrane is the difference between the inner leaflet (facing the periplasm) and the outer leaflet (facing the environment). This external layer of the Gram-negative cell is comprised of **lipopolysaccharides (LPSs)**. These large, complex molecules contain both lipid and carbohydrate, and consist of three parts: (1) lipid A, (2) the core polysaccharide, and (3) the O side chain. The LPS from *Salmonella* spp. has been studied most, and its general structure is described here (figure 3.25). **Lipid A** contains two glucosamine sugar derivatives, each with fatty acids and phosphate attached. The fatty acids of lipid A are embedded in the outer membrane, while the remainder of the LPS molecule projects from the surface. The **core polysaccharide** is joined to lipid A and is constructed of 10 sugars, many of them unusual in structure. The **O side chain** or **O antigen** is a polysaccharide chain extending outward from the core. It has several peculiar sugars and varies in composition between bacterial strains.

LPS has many important functions. (1) It contributes to the negative charge on the bacterial surface because the core polysaccharide usually contains charged sugars and phosphate (figure 3.25). (2) It helps stabilize outer membrane structure because lipid A is a major constituent of the exterior leaflet of the outer membrane. (3) It helps create a permeability barrier. The geometry of LPS (figure 3.25b) and interactions between neighboring LPS molecules

are thought to restrict the entry of bile salts, antibiotics, detergents, and other toxic substances that might kill or injure the bacterium. (4) LPS helps protect pathogenic bacteria from host defenses. The O side chain of LPS is also called the O antigen because it elicits an immune response by an infected host. This response involves the production of antibodies that bind the strain-specific form of LPS that elicited the response. For example, microbiologists refer to specific strains of Gram-negative bacteria using the O antigen, such as *E. coli* O157; here the O side chain is the antigenic type, number 157. Unfortunately, many bacteria can rapidly change the antigenic nature of their O side chains, thus thwarting host defenses. (5) Importantly, the lipid A portion of LPS can act as a toxin and is called endotoxin. If LPS or lipid A enters the bloodstream, a form of septic shock develops for which there is no direct treatment. **▶ Endotoxins (section 35.4); Antibodies bind specific 3-D antigens (section 33.7)**

As the outermost layer of the cell envelope, the LPS presents an effective barrier for the cell. Within the LPS, the negatively charged phosphate groups on the core polysaccharide interact with calcium ions to stabilize and tightly pack the LPS molecules. This results in an impermeable barrier that excludes small molecules, including many antibiotics and toxins. Transport across the outer membrane must therefore be regulated by integral membrane proteins, including one class of proteins termed **porins**. Most porin proteins cluster together to form a trimer in the outer membrane (figure 3.24 and figure 3.26). Each porin protein spans the outer membrane and is more or less

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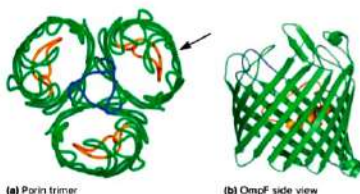


Figure 3.26 Porin Proteins. Two views of the OmpF porin of *E. coli*. (a) Structure of a trimeric porin observed when looking down at the outer surface of the outer membrane (i.e., top view). The center of each porin monomer is a water-filled channel. Each OmpF monomer can be divided into three parts: the green barrel forms the channel, the blue segment interacts with other porin proteins to help form the trimer, and the orange loop narrows the channel and dictates the chemical selectivity of the porin. The arrow indicates the area of a porin molecule viewed from the side in panel (b). (b) Side view of a porin monomer showing the β -barrel structure characteristic of porin proteins.

MICRO INQUIRY Are these transporter proteins categorized as channels or carriers?

tube-shaped; its narrow, water-filled channel allows passage of nutrients and hydrophilic molecules smaller than about 600 daltons. Larger molecules such as vitamin B₁₂, carbohydrates, and iron complexes are too large for porins and cross the outer membrane through the action of specific outer membrane receptors (figure 3.24). The receptor-solute complex in the outer membrane interacts with the TonB complex of proteins spanning the periplasm. TonB transfers the energy for the ligand transport from the plasma membrane across the periplasm to the receptor. Upon entering the periplasm, a periplasmic transport protein shuttles the ligand to an ABC-transporter in the plasma membrane for transfer to the cytoplasm.

Mechanism of Gram Staining

The difference between typical Gram-positive bacteria and typical Gram-negative bacteria is due to the physical nature of their cell walls. If the cell wall is removed, typical Gram-positive bacteria stain Gram negative. Furthermore, bacteria that never make cell walls, such as mycoplasmas, also stain Gram negative.

▶ Differential staining (section 2.3)

During the Gram-staining procedure, bacteria are first stained with crystal violet, a dye with a positive charge that is attracted to the bacterial cell's net negative charge. They are next treated with iodine, a mordant that interacts with the crystal violet, forming an insoluble complex and thus promoting dye retention. When bacteria are then treated with ethanol in the decolorization step, the pores of the thick peptidoglycan found in

the cell walls of typical Gram-positive bacteria appear to shrink, causing the peptidoglycan to act as a permeability barrier that prevents loss of crystal violet. Thus the dye-iodine complex is retained during the decolorization step and the bacteria remain purple, even after the addition of a second dye. In contrast, the peptidoglycan in typical Gram-negative cell walls is very thin, not as highly cross-linked, and has larger pores. Alcohol treatment also may extract enough lipid from the outer membrane to increase the cell wall's porosity further. For these reasons, alcohol more readily removes the crystal violet-iodine complex, decolorizing the cells. The counterstain safranin, also a dye with a net negative charge, easily stains the decolorized cells so that they appear red or pink.

Cell Walls and Osmotic Protection

Microbes have several mechanisms for responding to changes in osmotic pressure. Osmotic stress arises when the concentration of solutes inside the cell differs from that outside, and the responses work to equalize the solute concentrations. However, in certain situations, osmotic pressure can exceed the cell's ability to acclimate. In these cases, additional protection is provided by the cell wall. When cells are in hypotonic solutions—in which the solute concentration is less than that in the cytoplasm—water diffuses into the cell to dilute the solutes, causing it to swell. Without the peptidoglycan layer of the cell wall, the pressure on the plasma membrane would become so great that the membrane would be disrupted and the cell would burst—a process called **lysis**. Conversely, in hypertonic solutions, water flows out and the cytoplasm shrivels up—a process called **plasmolysis**.

The protective nature of peptidoglycan is most clearly demonstrated when bacterial cells are treated with lysozyme or penicillin. The enzyme **lysozyme** attacks peptidoglycan by hydrolyzing the bond that connects *N*-acetylmuramic acid with *N*-acetylglucosamine (figure 3.17; see also figure 32.4). Penicillin works by a different mechanism. It inhibits the enzyme transpeptidase, which is responsible for making the cross-links between peptidoglycan chains. If bacteria are treated with either of these substances while in a hypotonic solution, they lyse. However, if they are in an isotonic solution, they can survive and grow normally. Treatment of typical Gram-positive bacteria with lysozyme or penicillin results in the complete loss of the cell wall, and the cell becomes a protoplast. When typical Gram-negative bacteria are exposed to lysozyme or penicillin, the peptidoglycan sacculus is destroyed, but the outer membrane remains. These cells are called **spheroplasts**. Both protoplasts and spheroplasts are osmotically sensitive. If they are transferred to a hypotonic solution, they lyse due to uncontrolled water influx (figure 3.27). **▶ Antibacterial drugs (section 9.4)**

Bacteria That Lack Cell Walls

Most bacteria are defined by their cell wall—they are either Gram positive or Gram negative. A few bacteria, most notably a group called the mycoplasmas, are defined by their lack of a cell wall. Without a cell wall, they are pleomorphic and osmotically sensitive

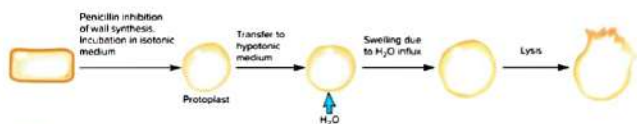


Figure 3.27 Protoplast Formation and Lysis. Protoplast formation induced by incubation with penicillin in an isotonic medium. Transfer to a hypotonic medium will result in lysis.

(see figure 21.3). Despite this, they often grow in dilute media or terrestrial environments because their plasma membranes are more resistant to osmotic pressure than those of walled bacteria. The precise reason for this is not clear, although the presence of sterols in the membranes of many species may provide added strength.

Comprehension Check

- Describe in detail the composition and structure of peptidoglycan. Why does peptidoglycan contain the unusual D-isomers of alanine and glutamic acid rather than the L-isomers observed in proteins?
- Draw a Venn diagram to classify the major molecules that make up typical Gram-positive and Gram-negative cell walls and those common to both types of cell wall. How do these molecules contribute to the functions of the cell wall?
- When protoplasts and spheroplasts are made, the shape of the cell becomes spherical regardless of the original cell shape. Why does this occur?
- The cell walls of most members of the phyla *Firmicutes* and *Actinobacteria* lack porins. Why is this the case?
- What two mechanisms allow the passage of nutrients across the outer membrane of typical Gram-negative bacteria?

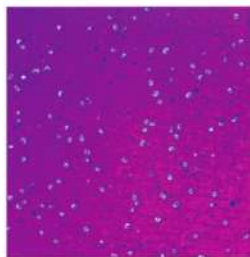


Figure 3.28 Bacterial Capsules. Capsule stain (a negative stain) of *Klebsiella pneumoniae*; bright-field light microscopy (X1,000).

Bacillus anthracis (the anthrax bacterium) has a proteinaceous capsule composed of poly-D-glutamic acid. Capsules are clearly visible in the light microscope when negative stains or specific capsule stains are employed; they also can be studied with the electron microscope.

Capsules are not required for growth and reproduction in laboratory cultures. However, they confer several advantages when bacteria grow in their normal habitats. They help pathogenic bacteria resist phagocytosis by host phagocytes. *Streptococcus pneumoniae*, which causes ear infections, pneumonia, and other diseases, provides a dramatic example. When it lacks a capsule, it is phagocytosed easily and does not cause disease. On the other hand, the capsulated variant commonly causes disease. Capsules can also protect against desiccation because they contain a great deal of water. They exclude viruses and most hydrophobic toxic materials such as detergents.

A **slime layer** is a zone of diffuse, unorganized material that is removed easily. It is usually composed of polysaccharides but is not as easily observed by light microscopy. Gliding bacteria often produce slime, which in some cases has been shown to facilitate motility (section 3.8).

3.5 The Cell Envelope Often Includes Layers Outside the Cell Wall

After reading this section, you should be able to:

- List the structures found in all the layers of bacterial cell envelopes.
- Identify the functions and major component molecules in cell envelope structures.

Figures 3.22 and 3.24 show the bacterial envelope as consisting solely of the plasma membrane and cell wall. However, many bacteria have another layer in their cell envelopes that lies outside the cell wall. This layer is given different names depending on its makeup and how it is organized.

Capsules and Slime Layers

Capsules are layers that are well organized and not easily washed off (figure 3.28). They are most often composed of polysaccharides, but some are constructed of other materials. For example,

A bacterial **glycocalyx** is a layer consisting of a network of polysaccharides extending from the surface of the cell. The term can encompass both capsules and slime layers because they usually are composed of polysaccharides. The glycocalyx aids in attachment to solid surfaces, including tissue surfaces in plant and animal hosts. ▶ *Pathogenicity islands encode virulence factors* (section 35.4)

S-Layers

Many bacteria have an ordered covering called an **S-layer** on their surface. The S-layer has a pattern something like floor tiles and is composed of protein or glycoprotein (figure 3.29). In typical Gram-negative bacteria, the S-layer adheres noncovalently to the outer membrane; it is associated with the peptidoglycan surface of typical Gram-positive cell walls.

S-layers are of considerable interest not only for their biological roles but also in the growing field of nanotechnology. Their biological roles include protecting the cell against ion and pH fluctuations, osmotic stress, enzymes, or predatory bacteria. The S-layer also helps maintain the shape and envelope rigidity of some cells, and it can promote cell adhesion to surfaces. Finally, the S-layer seems to protect some bacterial pathogens against host defenses, thus contributing to their virulence. The potential use of S-layers in nanotechnology is due to the ability of S-layer proteins to self-assemble; that is, S-layer proteins contain the information required to spontaneously associate and form the S-layer without the aid of any additional enzymes or other factors. Thus S-layer proteins could be used as building blocks for the creation of technologies such as drug-delivery systems and novel detection systems for toxic chemicals or bioterrorism agents.

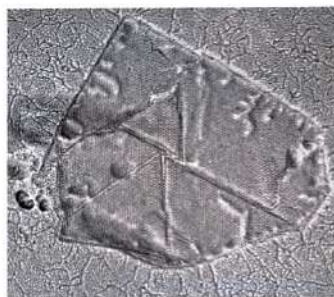


Figure 3.29 The S-Layer. An electron micrograph of the S-layer of the bacterium *Desulfococcus rediflavus* after shadowing.

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Comprehension Check

- What is the difference between a capsule and a slime layer? Why does the term *glycocalyx* usually encompass both?
- S-layers and some capsules are composed of proteins. How does an S-layer differ from a proteinaceous capsule?

3.6 The Bacterial Cytoplasm Is More Complex than Once Thought

After reading this section, you should be able to:

- Describe the function of three types of bacterial cytoskeletal proteins and compare their structure with those of eukaryotes, citing specific examples.
- Compare and contrast storage inclusions and microcompartments, citing specific examples.
- List the composition of bacterial ribosomes and their spatial organization within the cell.
- Differentiate the structure and function of bacterial chromosomes and plasmids.

The plasma membrane and everything within is called the **protoplast**. The **cytoplasm** is the material bounded by the plasma membrane; thus the cytoplasm is a major part of the protoplast. The cytoplasm is further divided into the liquid component, called the **cytosol**; those structures such as inclusions, ribosomes, and plasmids that float in the cytosol; and the many molecules dissolved in the cytosol. The high concentration of these macromolecules and their precursors and metabolites creates a phenomenon known as **macromolecular crowding**. Abundant solutes result in less available space around each molecule, a familiar experience to anyone who travels around a city at rush hour. The bacterial cytoplasm is estimated to be about 10 times more viscous than water, and physical and chemical processes like diffusion are affected.

For many years, bacterial cells were thought of as bags of biochemicals, but the exciting discovery of cytoskeletal proteins in bacterial cells has forever changed that view. Although the bacterial cytoskeleton is less complex than that of eukaryotes, it clearly helps organize the cytoplasm. In this section, we consider the bacterial cytoskeleton and then a variety of other structures in the cytoplasm.

Bacterial Cytoskeleton

Eukaryotes possess three major cytoskeletal elements: actin filaments, microtubules, and intermediate filaments. Actin filaments are made from actin, and microtubules are made from tubulin. Intermediate filaments are composed of a mixture of one or more members of different classes of proteins. Homologues of all three types of eukaryotic proteins have been identified in bacteria (table 3.2). Bacterial cytoskeletal proteins are structurally similar to their eukaryotic counterparts and carry out similar functions: They participate in cell division, localize proteins to certain sites in the cell, and determine cell shape. In addition, some bacterial cytoskeletal proteins appear to be unique. Thus it is likely that the evolution of the cytoskeleton was an early event in the history of

Table 3.2 Some Bacterial Cytoskeletal Proteins

Type	Function	Comments
Tubulin Homologues		
FtsZ	Cell division	Widely observed in bacteria and archaea
TubZ	Bacteriophage replication	Observed in multiple bacteriophages
Actin Homologues		
FtsA	Cell division	Attaches the FtsZ ring to the cell envelope
MamK	Positioning magnetosomes	Observed only in magnetotactic species
MreB/Mri	Helps determine cell shape; may be involved in chromosome segregation, localizing proteins, motility, and establishing cell polarity	Observed in most rod-shaped bacteria
ParM	Plasmid segregation	Plasmid encoded
Intermediate Filament Homologues		
CreS (crescentin)	Induces curvature in curved rods	<i>Caulobacter crescentus</i>
Unique Bacterial Cytoskeletal Proteins		
MinD	Prevents polymerization of FtsZ at cell poles	Observed in many rod-shaped bacteria
ParA	Segregates chromosomes and plasmids; helps localize chemotaxis proteins and type IV pil to one pole of certain rod-shaped bacteria	Observed in many species, including <i>Vibrio cholerae</i> , <i>C. crescentus</i> , and <i>Thermus thermophilus</i>
Bactofilin	Scaffold for protein and chromosome positioning	Widely observed in bacteria

life on Earth. ► *The eukaryotic cytoplasm contains a cytoskeleton and organelles (section 5.3). Bacterial cell cycles can be divided into three phases (section 7.2)*

The cytoskeletons of *Escherichia coli*, *Bacillus subtilis*, and *Caulobacter crescentus* are the most important bacterial model systems and are the focus of our discussion. *E. coli* is a Gram-negative rod that has been extensively studied and can be easily manipulated. *B. subtilis* is a Gram-positive rod found in soil. It is an endospore-forming bacterium, making it a good model for cellular differentiation (section 3.9). Both were models for cell wall structure as described in section 3.4. *C. crescentus* is a curved rod found in aquatic habitats. It is interesting in part because it exhibits a complex life cycle that includes two different stages: a motile swarmer cell and a sessile, stalked cell that attaches to surfaces by a holdfast (see figure 22.8). ► *Caulobacteraceae* and *Hyphomicrobiaceae* reproduce in unusual ways (section 22.1)

The best studied bacterial cytoskeletal proteins are FtsZ, MreB, and CreS (also known as crescentin). FtsZ was one of the first bacterial cytoskeletal proteins identified and has since been found in most bacteria as well as mitochondria and chloroplasts. FtsZ is a homologue of the eukaryotic protein tubulin. It forms a ring at the center of a dividing cell and is required for the formation of the septum that will separate the daughter cells (figure 3.30a). MreB and its relative Mbl are actin homologues. Their major function is to determine cell shape in rod-shaped

cells so it is not found in cocci. MreB and Mbl determine cell shape by properly positioning the machinery needed for peptidoglycan synthesis (figure 3.30b,c). CreS (crescentin) was discovered in *C. crescentus* and is responsible for its curved shape. CreS is a homologue of lamin and keratin, two intermediate filament proteins. ► *Cellular growth and determination of cell shape (section 7.2)*

The movement of newly replicated DNA to daughter cells occurs in eukaryotes by DNA attachment to the mitotic spindle. Although it is not a general phenomenon in bacteria, there are a few examples of cytoskeletal filaments contributing to chromosome segregation (see figure 7.4). Certain plasmids, in particular, rely on partition (Par) proteins to ensure equal distribution in daughter cells following cell division. ► *Chromosome replication and partitioning (section 7.2)*

Intracytoplasmic Membranes

Although members of *Bacteria* do not contain complex membranous organelles like mitochondria or chloroplasts, internal membranous structures are observed in some bacteria (figure 3.31). These can be extensive and complex in photosynthetic bacteria and in bacteria with very high respiratory activity, such as nitrifying bacteria. The internal membranes of the photosynthetic cyanobacteria are called thylakoids and are analogous to the thylakoids of chloroplasts. ► *Photosynthetic bacteria are diverse (section 21.4). Nitrifying*

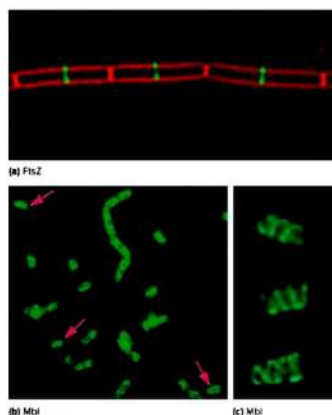


Figure 3.30 The Bacterial Cytoskeleton. (a) FtsZ protein in a chain of *Bacillus subtilis* cells. FtsZ gives fluorescent (GFP) fusion protein viewed by fluorescence microscopy. (b) The MreB-like cytoskeletal protein (Mbl) of *B. subtilis*. Mbl-GFP in live cells was examined by fluorescence microscopy. With this method, Mbl seems to form helices (arrows). More sensitive methods show that Mbl forms patches that move perpendicular to the long axis of the cell. (c) Three of the cells from (b) are shown at a higher magnification.

(a) C. D. Joseph-Piquero. (b) C. Jeff. (c) Joseph-Piquero. © 2011 Sinauer Associates, Inc. and W. H. Freeman & Co.

bacteria oxidize ammonium or nitrite to gain energy and electrons (section 22.1). Mitochondria, related organelles, and chloroplasts are involved in energy conservation (section 5.6).

The internal membranous structures observed in bacteria may be aggregates of spherical vesicles, flattened vesicles, or tubular membranes (see figure 22.3). They are often connected to the plasma membrane and arise from it by invagination. However, these internal membranes differ from the plasma membrane by being enriched for proteins and other molecules involved in energy conservation. For instance, the thylakoids of cyanobacteria contain the chlorophyll and photosynthetic reaction centers responsible for converting light energy into ATP. Thus the function of internal membranes may be to provide a larger membrane surface for greater metabolic activity.

Inclusions

Inclusions are common in all cells. They are formed by the aggregation of substances that may be either organic or inorganic. Inclusions



Figure 3.31 Internal Bacterial Membranes. The nitrifying bacterium *Nitrososphaera oceanus* has parallel membranes traversing the whole cell. Note the nucleoid (n) with fibrillar structure.

(C) D. Robert G. Murray/University of Western Ontario

can take the form of granules, crystals, or globules; some are amorphous. Some inclusions lie free in the cytoplasm. Other inclusions are enclosed by a shell that is single-layered and may consist of proteins or of both proteins and phospholipids. Some inclusions are surrounded by invaginations of the plasma membrane. Many inclusions are used for storage (e.g., of carbon compounds, inorganic substances, and energy) or to reduce osmotic pressure by tying up molecules in particulate form. The quantity of inclusions used for storage varies with the nutritional status of the cell. Some inclusions are so distinctive that they are increasingly being referred to as macrocompartments. A brief description of several important inclusions follows.

Storage Inclusions

Many storage inclusions form when one nutrient is in ready supply but another nutrient is not. Others store end products of metabolic processes. In some cases, these end products are used by the microbe when it is in different environmental conditions. The most common storage inclusions are glycogen inclusions, polyhydroxyalkanoate granules, sulfur globules, and polyphosphate granules. Some storage inclusions are observed only in certain organisms, such as the cyanophycin granules in cyanobacteria. ► *Phylum Cyanobacteria: oxygenic photosynthetic bacteria (section 21.4)*

Carbon is often stored as polyhydroxyalkanoate (PHA) granules, also termed carbonosomes. Several types of PHA granules have been identified, but the most common contain poly-β-hydroxybutyrate (PHB) (figure 3.32). The structure of PHB inclusions has been well studied, and PHB granules are surrounded by a single-layered shell composed of proteins. Much of the interest in PHB and other PHA granules is due to their industrial use in making biodegradable plastics. ► *Biopolymers (section 42.1)*

Polyphosphate granules and sulfur globules are inorganic inclusions observed in many organisms. **Polyphosphate granules** store the phosphate needed for synthesis of important cell constituents such as nucleic acids. In some cells, they act as an energy reserve, and polyphosphate also can serve as an energy source in some reactions, when the bond linking the final phosphate in the polyphosphate chain is hydrolyzed. Sulfur globules

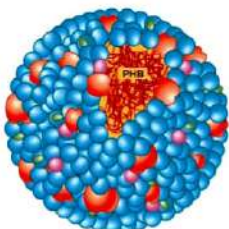


Figure 3.32 PHB inclusions in bacteria. PHB is a polymer of β -hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. PHB inclusions are around 0.2 to 0.7 μm in diameter. PHB (red lines) is enclosed by a shell composed of several different proteins, including the PHB synthesizing enzyme (red) and the PHB-degrading enzyme (green).

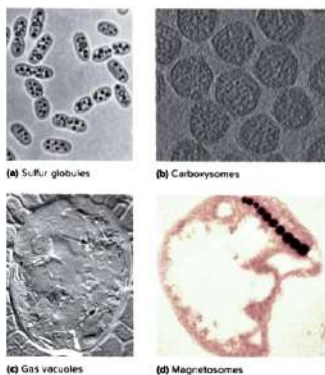


Figure 3.33 Bacterial Cell Inclusions. (a) Chromatium vinosum, a purple sulfur bacterium, with intracellular sulfur globules. (b) Carboxysomes in the bacterium *Halothiobacillus neapolitanus*. Each carboxysome is approximately 100 nm in diameter. (c) A freeze-fracture preparation of *Anaerobaculum fissosporum* showing gas vesicles and gas vacuoles. Both longitudinal and cross-section views of gas vesicles are indicated by arrows. (d) *Magnetococcus marinus* cells are in roughly spherical contact with a single chain of magnetic crystals.

are formed by bacteria that use reduced sulfur-containing compounds as a source of electrons during their energy-conserving metabolic processes (figure 3.33a). For example, some photosynthetic bacteria use hydrogen sulfide (rather than water) as an electron donor and accumulate the resulting sulfur either externally or internally. ▶ **Light reactions in anoxygenic photosynthesis** (section 11.12): Purple sulfur bacteria perform anoxygenic photosynthesis (section 22.1).

Microcompartments

Some bacterial inclusions serve functions other than simply storing substances for later use. These inclusions, called microcompartments, share several characteristics. They are relatively large polyhedrons formed by one or more different proteins. Enclosed within the protein shell are one or more enzymes. Microcompartments include the ethanolamine utilization (Eut) microcompartment, the propioniol utilization (Pdu) microcompartment, and carboxysomes. We focus here on carboxysomes as they are the best studied.

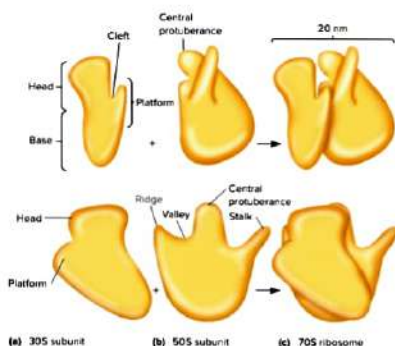
Carboxysomes are present in many cyanobacteria and other CO₂-fixing bacteria (figure 3.33b). Their polyhedral coat is composed of three different proteins and is about 100 nm in diameter. Associated with the shell is the enzyme carbonic anhydrase that converts carbonic acid and bicarbonate into CO₂. Recall that biological membranes allow the free diffusion of CO₂. However, the carboxysome shell prevents CO₂ from escaping so it can accumulate. Enclosed within the polyhedron is the enzyme ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO). RuBisCO is the critical enzyme for CO₂ fixation, the process of converting CO₂ into sugar. Thus the carboxysome serves as a site for CO₂ fixation. As such, it is critical that carboxysomes be distributed to both daughter cells during cell division. *ParA*, a cytoskeletal protein (table 3.2), helps ensure appropriate segregation of carboxysomes. **►**

Other Inclusions

Inclusions can be used for functions other than storage or as micro-compartments. Two of the most remarkable inclusions are gas vacuoles and magnetosomes. Both are involved in bacterial movement.

The **gas vacuole** provides buoyancy to some aquatic bacteria, many of which are photosynthetic. Gas vacuoles are aggregates of enormous numbers of small, hollow, cylindrical structures called **gas vesicles** (figure 3.33c). Gas vesicle walls are composed of many copies of a single small protein. These protein subunits assemble to form a rigid cylinder that is impermeable to water but freely permeable to atmospheric gases. Cells with gas vacuoles can regulate their buoyancy to float at the depth necessary for proper light intensity, oxygen concentration, and nutrient levels. They descend by simply collapsing vesicles and float upward when new ones are constructed.

Aquatic magnetotactic bacteria use **magnetosomes** to orient themselves in Earth's magnetic field. Northern Hemisphere bacteria use their magnetosome chain to determine northward and downward directions, and swim down to nutrient-rich sediments.



to locate the optimum depth in freshwater and marine habitats. Magnetotactic bacteria in the Southern Hemisphere generally orient southward and downward, with the same result. Magnetosomes are intracellular chains of magnetite (Fe_3O_4) or greigite (Fe_7S_8) particles (figure 3.33d). They are around 35 to 125 nm in diameter and enclosed within invaginations of the plasma membrane. The invaginations contain distinctive proteins that are not found elsewhere in the plasma membrane. For the cell to move properly within a magnetic field, magnetosomes must be arranged in a chain. A cytoskeletal protein called MammK is responsible for establishing a framework upon which the chain can form (table 3.2; see figure 22.14).

Bacterial Ribosomes

Ribosomes are the site of protein synthesis, and large numbers (10,000 to 20,000) are found in nearly all cells. The cytoplasm of rapidly growing bacterial cells is often packed with ribosomes, and additional ribosomes may be loosely attached to the plasma membrane. The cytoplasmic ribosomes synthesize proteins destined to remain within the cell, whereas plasma membrane-associated ribosomes make proteins that will reside in the cell envelope or be translocated to the outside.

Translation, the process of protein synthesis, is amazingly complex and is discussed in detail in chapter 13. This complexity is evidenced in part by the structure of ribosomes, which are made of dozens of proteins and several ribonucleic acid (RNA) molecules. The ribosomes of all three domains of life share similarities. However, there are important differences. Here we focus strictly on bacterial ribosomes. We compare bacterial and archaeal ribosomes in chapter 4 and compare all three types of ribosomes in chapter 5.

Bacterial ribosomes are called 70S ribosomes and are constructed of a 50S and a 30S subunit (figure 3.34). The S in these values stands for **Svedberg unit**. This is the unit of the sedimentation coefficient, a measure of sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater its Svedberg value. The sedimentation coefficient is a function of a particle's molecular weight, volume, and shape. Heavier and more compact particles normally have larger Svedberg numbers.

Bacterial ribosomes are composed primarily of ribosomal RNA (rRNA) molecules. The small subunit contains 16S rRNA, whereas the large subunit consists of 23S and 5S rRNA molecules. Approximately 55 proteins make up the rest of the mass of the ribosome: 21 in the small subunit, and 34 in the large subunit.

Nucleoid

The **nucleoid** is an ellipsoidal region that contains the cell's chromosome and numerous proteins (Figure 3.35). Although the nucleoid is not separated from the cytoplasm by a membrane, it is a distinct region of the cell, as observed by microscopy. Ribosomes are excluded from the nucleoid. The chromosome itself has an orientation, and particular regions occupy discrete locations within the nucleoid.

The chromosomes of most bacteria are a circle of double-stranded **deoxyribonucleic acid** (DNA), but some bacteria have a linear chromosome. Most bacteria have a single chromosome, but some bacteria, such as *Vibrio cholerae* and *Borrelia burgdorferi* (causative agents of cholera and Lyme disease, respectively), have more than one chromosome. Many bacteria carry a single copy of their chromosome (monoploid); but others are

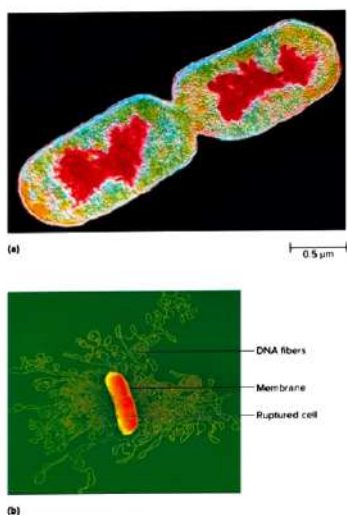


Figure 3.35 E. coli Nucleoids and Chromosomes. Bacterial chromosomes are located in the nucleoid, an area in the cytoplasm. (a) A color-enhanced transmission electron micrograph of a thin section of a dividing *E. coli* cell. The red areas are the nucleoids present in the two daughter cells. (b) Chromosome released from a gently lysed *E. coli* cell. Note how tightly packaged the DNA must be to fit inside the cell.

(a) © CNAS/Science Source. (b) © CD, Gupta/MP/Science Source.

known to be polyploid, often with more than 10 copies per cell. In *Deinococcus radiodurans*, this is believed to be necessary to allow the repair of damaged DNA under adverse environmental conditions, and the giant bacterium *Epulopiscium fishelsoni* (figure 3.4) appears to require tens of thousands of chromosome copies.

Bacterial chromosomes are longer than the length of the cell. Therefore, an important and unanswered question is how these microbes manage to fit their chromosomes into the relatively small space occupied by the nucleoid. For instance, *E. coli*'s circular chromosome measures approximately 1,400 μm , or about 230–700 times longer than the cell (figure 3.35b). Thus the chromosome must be organized and packaged in a manner that decreases its overall size yet retains easy

accessibility to the genetic information. Physical factors like macromolecular crowding and supercoiling contribute to nucleoid organization. Supercoiling produces a dense, central core of DNA with loops of DNA extending out from the core. Besides physical factors, architectural proteins also contribute to nucleoid structure. Nucleoid-associated proteins (NAPs) are small, abundant DNA binding proteins that cause the chromosome to bend and fold. For example, the HU protein, a NAP first identified in *E. coli*, is widely distributed in bacteria, and is abundant enough to cover about 10% of the chromosome. NAPs have multiple functions: They form bridges between one section of the chromosome and another and they participate in the many processes that occur on the chromosome. Cells have several different NAPs, but HU is the only one found in almost all bacteria. NAPs are particularly important during cell division, when they further compact the chromosomes. This extra level of packing is important for proper segregation of daughter chromosomes during cell division. ▶ *DNA is a polymer of deoxyribonucleotides (section 13.2)*

Plasmids

In addition to the genetic material present in the nucleoid, many bacteria contain extrachromosomal DNA molecules called plasmids. The genome of an organism includes all DNA, including plasmids. Indeed, most of the bacterial genomes sequenced thus far include plasmids. In some cases, numerous different plasmids within a single species have been identified. For instance, *B. burgdorferi* carries 12 linear and 9 circular plasmids. Plasmids play many important roles in the lives of the organisms that have them. They also have proved invaluable to microbiologists and molecular geneticists in constructing and transferring new genetic combinations and in cloning genes, as described in chapter 17.

Plasmids are double-stranded DNA molecules that can exist independently of the chromosome. Both circular and linear plasmids have been documented, but most known plasmids are circular. Plasmids have relatively few genes, generally less than 30. Their genetic information is not essential to the bacterium, and cells that lack them usually function normally. However, many plasmids carry genes that confer a selective advantage to the bacterium in certain environments.

Plasmids use the cell's DNA-synthesizing machinery to replicate, but their replication is not linked to any particular stage of the cell cycle. Thus regulation of plasmid and chromosomal replication are independent. However, some plasmids are able to integrate into the chromosome. Such plasmids are called **episomes** and when integrated are replicated as part of the chromosome. Plasmids are inherited stably during cell division, but they are not always equally apportioned into daughter cells and sometimes are lost. The loss of a plasmid is called **curing**. It can occur spontaneously or be induced by treatments that inhibit plasmid replication but not host cell reproduction. Some commonly used curing treatments are acridine mutagens, ultraviolet and ionizing

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Table 3.3 Major Types of Bacterial Plasmids

Type	Function	Example	Size (kbp)	Hosts	Phenotypic Features ¹
Conjugative Plasmids²	Transfer of DNA from one cell to another	F factor	95–100	<i>E. coli</i> , <i>Salmonella</i> , <i>Citrobacter</i>	Sex pilus, conjugation
R Plasmids	Carry antibiotic-resistance genes	RP4	54	<i>Pseudomonas</i> and many other Gram-negative bacteria	Sex pilus, conjugation, resistance to Amp, Km, Nm, Tet
Col Plasmids	Produce bacteriocins, substances that destroy closely related species	ColE1	9	<i>E. coli</i>	Colicin ET production
Virulence Plasmids	Carry virulence genes	Ti	200	<i>Agrobacterium tumefaciens</i>	Tumor induction in plants

¹ Abbreviations used for antibiotics: Amp, ampicillin; Km, kanamycin; Nm, neomycin; Tet, tetracycline.

² Many R plasmids and others are also conjugative.

radiation, thymine starvation, antibiotics, and growth above optimal temperatures.

Plasmids may be classified in terms of their mode of existence, spread, and function. A brief summary of the types of bacterial plasmids and their properties is given in table 3.3. These various types of plasmids can also differ in terms of the number of copies found within the cell. Single-copy plasmids produce only one copy per host cell. Multicopy plasmids may be present at concentrations of 100 or more per cell.

Comprehension Check

- Briefly describe the nature and function of the cytoplasm, and the regions and structures within it. How is the cytosol different from the cytoplasm?
- List the most common kinds of inclusions. How are they similar to eukaryotic organelles such as mitochondria and chloroplasts? How do they differ?
- How do plasmids differ from chromosomes? What is an episome?
- Explain the importance of each of the following plasmids: conjugative plasmid, R plasmid, Col plasmid, and virulence plasmid.

3.7 Many Bacteria Have External Structures Used for Attachment and Motility

After reading this section, you should be able to:

- Distinguish pili (fimbriae) and flagella.
- Illustrate the various patterns of flagella distribution.

Bacteria must constantly respond to their changing environments. Sometimes it is advantageous to attach to a surface. Other times it is better to move toward or away from something in the environment. Many bacteria have structures that extend beyond the cell

envelope and are involved in either attachment to surfaces or motility. In addition, these external structures can function in protection and horizontal gene transfer. Several are discussed in this section.

Bacterial Pili and Fimbriae

Many bacteria have fine, hairlike appendages that are thinner and typically shorter than flagella. These are called **fimbriae** (s., **fimbria**) or **pili** (s., **pilus**). The terms are synonymous, although certain structures are historically called pilus (e.g., sex pilus), while others are called fimbriae. We will use the terms interchangeably, except in those instances. A cell may be covered with up to 1,000 fimbriae, but they are only visible in an electron microscope due to their small size (figure 3.36). They are slender tubes composed of helically arranged protein subunits and are about 3 to 10 nm in diameter and up to several

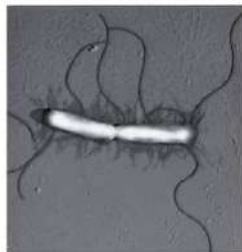


Figure 3.36 Flagella and Fimbriae. The long flagella and numerous shorter fimbriae are evident in this SEM of the bacterium *Proteus vulgaris*. © Thomas Derencik, NMR/Science Source

micrometers long. Pili grow by adding protein subunits to their base. Several different types have been identified and most function to attach cells to solid surfaces such as rocks in streams and host tissues. Type IV pili are involved in motility (section 3.8) and two gene transfer mechanisms: bacterial transformation and bacterial conjugation. **Bacterial conjugation requires cell-cell contact (section 16.6); Bacterial transformation is the uptake of free DNA from the environment (section 16.7).**

Many bacteria have up to 10 sex pili (s. *sex pilus*) per cell. These hairlike structures differ from other pili in the following ways. Sex pili often are larger than other pili (around 9 to 10 nm in diameter). They are genetically determined by conjugative plasmids and are required for conjugation. Some bacterial viruses attach specifically to sex pili at the start of their infection cycle.

Bacterial Flagella

Many motile bacteria move by means of **flagella** (s., **flagellum**), threadlike locomotor appendages extending outward from the plasma membrane and cell wall. Although the main function of flagella is motility, they can have other roles. They can be involved in attachment to surfaces, and in some bacteria, they are virulence factors, that is, they contribute to the ability of the bacterium to cause disease.

Bacterial flagella are slender, rigid structures about 20 nm across and up to 20 μm long. Flagella are so thin they cannot be observed directly with a light microscope but must be stained with techniques designed to increase their thickness. The detailed structure of a flagellum can only be seen in the electron microscope.

Bacterial species often differ in their patterns of flagella distribution, and these patterns are useful in identifying bacteria. **Monotrichous** bacteria (Greek *trichos*, hair) have one flagellum; if it is located at an end, it is said to be a **polar flagellum** (figure 3.37a). **Amphitrichous** bacteria (Greek *amphi*, on both sides) have a single flagellum at each pole. In contrast, **lophotrichous** bacteria (Greek *lophos*, crest or tuft) have a cluster of flagella at one or both ends (figure 3.37b). Flagella are spread

evenly over the whole surface of **peritrichous** (Greek *peri*, around) bacteria (figure 3.37c).

Transmission electron microscope studies have shown that the bacterial flagellum is composed of three parts (figure 3.38). (1) The **filament** is the longest and most obvious portion. It extends from the cell surface to the tip. (2) The **basal body** is embedded in the cell envelope; and (3) a short, curved segment, the **hook**, links the filament to its basal body and acts as a flexible coupling.

The filament is a hollow, rigid cylinder constructed of subunits of the protein **flagellin**, which ranges in molecular mass from 30,000 to 60,000 daltons, depending on the bacterial species. The filament ends with a capping protein. Some bacteria have sheaths surrounding their flagella. For example, *Vibrio cholerae* flagella have lipopolysaccharide sheaths.

The hook and basal body are quite different from the filament (figure 3.38). Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex part of a flagellum. The basal bodies of other typical Gram-negative bacteria have L, P, and MS rings embedded in the cell envelope. The C ring is on the cytoplasmic side of the MS, and positive bacteria have only two rings: an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan (figure 3.38b).

The synthesis of bacterial flagella is complex and involves at least 20 to 30 genes. Besides the gene for flagellin, 10 or more genes code for hook and basal body proteins; other genes are concerned with control of flagellar construction or function. Many components of the flagellum lie outside the cell envelope and must be transported out of the cell for assembly. Interestingly, the basal body is a specialized version of the type III protein secretion system observed in typical Gram-negative bacteria. Type III secretion systems have a needlelike structure through which proteins are secreted. In the flagellar type III secretion system, the filament replaces



Figure 3.37 Flagellar Distribution. Examples of various patterns of flagellation as seen in the light microscope. (a) Flagella of *Pseudomonas mendocina*. (b) *Spirillum volutans* ($\times 1,000$). Only the tufts of flagella at one end are clearly visible. (c) *Bacillus subtilis*.
 (a): © Source: CDC/Dr. William A. Clark. (b): © McGraw-Hill Education/James Beetham/photographer. (c): © Source: CDC/Dr. William A. Clark.

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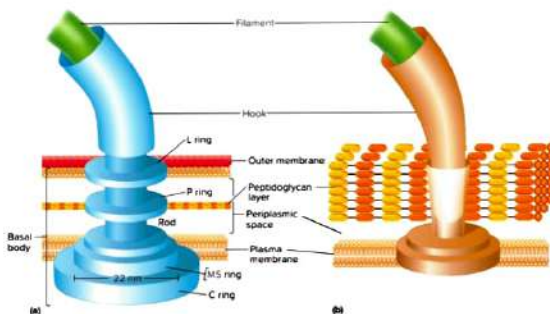


Figure 3.38 The Ultrastructure of Bacterial Flagella. Flagellar basal bodies and hooks in (a) typical Gram-negative and (b) typical Gram-positive bacteria.

the needle. Individual flagellin subunits are transported through the hollow filament. When the subunits reach the tip, they spontaneously aggregate under the direction of a protein called the filament cap; thus the filament grows at its tip rather than at the base (figure 3.39). Filament synthesis, like S-layer formation, is an example of **self-assembly**. **Protein maturation and secretion (section 13.8)**

Comprehension Check

1. What are the functions of fimbriae (pili) and sex pili?
2. What terms are used to describe the different flagella distribution patterns observed among bacteria?
3. What is self-assembly? Why does it make sense that the filament of a flagellum is assembled in this way?

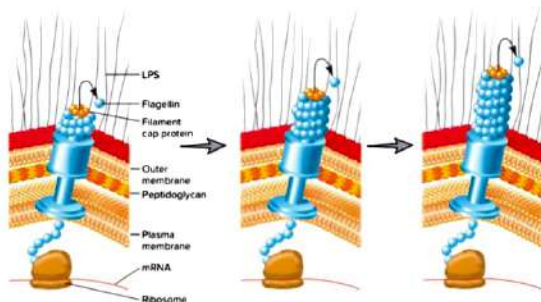


Figure 3.39 Flagellar Filaments Grow at the Tip. Flagellin subunits travel through the flagellar core and attach to the growing tip. Their attachment is directed by the filament cap protein.

MICRO INQUIRY How does flagellum growth compare to pilus growth?

micrometers long. Pili grow by adding protein subunits to their base. Several different types have been identified and most function to attach cells to solid surfaces such as rocks in streams and host tissues. Type IV pili are involved in motility (section 3.8) and two gene transfer mechanisms: bacterial transformation and bacterial conjugation. **Bacterial conjugation requires cell-cell contact (section 16.6); Bacterial transformation is the uptake of free DNA from the environment (section 16.7).**

Many bacteria have up to 10 **sex pili** (i.e., **sex pilus**) per cell. These hairlike structures differ from other pili in the following ways. Sex pili often are larger than other pili (around 9 to 10 nm in diameter). They are genetically determined by conjugative plasmids and are required for conjugation. Some bacterial viruses attach specifically to sex pili at the start of their infection cycle.

Bacterial Flagella

Many motile bacteria move by means of **flagella** (s., **flagellum**), threadlike locomotor appendages extending outward from the plasma membrane and cell wall. Although the main function of flagella is motility, they can have other roles. They can be involved in attachment to surfaces, and in some bacteria, they are virulence factors, that is, they contribute to the ability of the bacterium to cause disease.

Bacterial flagella are slender, rigid structures about 20 nm across and up to 20 μ m long. Flagella are so thin they cannot be observed directly with a light microscope but must be stained with techniques designed to increase their thickness. The detailed structure of a flagellum can only be seen in the electron microscope.

Bacterial species often differ in their patterns of flagella distribution, and these patterns are useful in identifying bacteria. **Monotrichous** bacteria (Greek *trichos*, hair) have one flagellum; if it is located at one end, it is said to be a **polar flagellum** (figure 3.37a). **Amphitrichous** bacteria (Greek *amphi*, on both sides) have a single flagellum at each pole. In contrast, **lophotrichous** bacteria (Greek *lophos*, crest or tuft) have a cluster of flagella at one or both ends (figure 3.37b). Flagella are spread

evenly over the whole surface of **peritrichous** (Greek *peri*, around) bacteria (figure 3.37c).

Transmission electron microscope studies have shown that the bacterial flagellum is composed of three parts (figure 3.38). (1) The **filament** is the longest and most obvious portion. It extends from the cell surface to the tip. (2) The **basal body** is embedded in the cell envelope; and (3) a short, curved segment, the **hook**, links the filament to its basal body and acts as a flexible coupling.

The filament is a hollow, rigid cylinder constructed of subunits of the protein **flagellin**, which ranges in molecular mass from 30,000 to 60,000 daltons, depending on the bacterial species. The filament ends with a capping protein. Some bacteria have sheaths surrounding their flagella. For example, *Vibrio cholerae* flagella have lipopolysaccharide sheaths.

The hook and basal body are quite different from the filament (figure 3.38). Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex part of a flagellum. The basal bodies of *E. coli* and most other typical Gram-negative bacteria have four rings: L, P, MS, and C, which are connected to a central rod (figure 3.38a). The L, P, and MS rings are embedded in the cell envelope, and the C ring is on the cytoplasmic side of the MS ring. Typical Gram-positive bacteria have only two rings: an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan (figure 3.38b).

The synthesis of bacterial flagella is complex and involves at least 20 to 30 genes. Besides the gene for flagellin, 10 or more genes code for hook and basal body proteins; other genes are concerned with control of flagellar construction or function. Many components of the flagellum lie outside the cell envelope and must be transported out of the cell for assembly. Interestingly, the basal body is a specialized version of the type III protein secretion system observed in typical Gram-negative bacteria. Type III secretion systems have a needlelike structure through which proteins are secreted. In the flagellar type III secretion system, the filament replaces



Figure 3.37 Flagellar Distribution. Examples of various patterns of flagellation as seen in the light microscope. (a) *Pseudomonas mendocina* (b) *Spirillum volutans* (c) *Bacillus oleris*. Only the tuft of flagella at one end is clearly visible. (c) *Bacillus oleris* as (c) Source: CDC/William B. Clark (b) CMO Group (c) Education James Beilstein, photography

3.7 Many Bacteria Have External Structures Used for Attachment and Motility 67

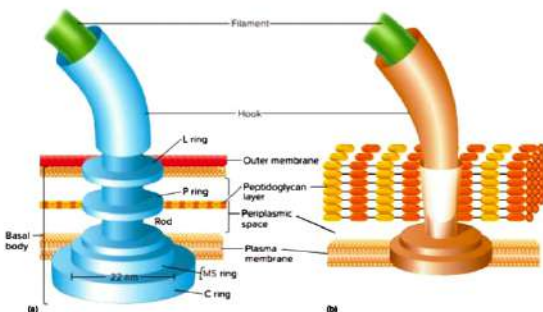


Figure 3.38 The Ultrastructure of Bacterial Flagella. Flagellar basal bodies and hooks in (a) typical Gram-negative and (b) typical Gram-positive bacteria.

the needle. Individual flagellin subunits are transported through the hollow filament. When the subunits reach the tip, they spontaneously aggregate under the direction of a protein called the filament cap; thus the filament grows at its tip rather than at the base (figure 3.39). Filament synthesis, like S-layer formation, is an example of **self-assembly**. **Protein maturation and secretion (section 13.8)**

Comprehension Check

1. What are the functions of fimbriae (pili) and sex pili?
2. What terms are used to describe the different flagella distribution patterns observed among bacteria?
3. What is self-assembly? Why does it make sense that the filament of a flagellum is assembled in this way?

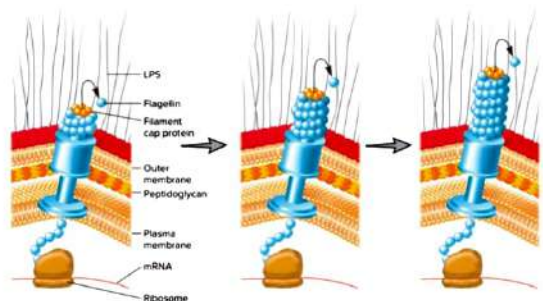


Figure 3.39 Flagellar Filaments Grow at the Tip. Flagellin subunits travel through the flagellar core and attach to the growing tip. Their attachment is directed by the filament cap protein.

MICRO INQUIRY How does flagellum growth compare to pilus growth?

3.8 Bacteria Move in Response to Environmental Conditions

After reading this section, you should be able to:

- Compare and contrast flagellar swimming motility, swarming, spirochete flagellar motility, and twitching and gliding motility
- State the source of energy that powers flagellar motility
- Explain why bacterial chemotaxis is referred to as a "biased random walk"

As we note in section 3.7, several structures extending beyond bacterial cell envelopes contribute to motility. Five major methods of movement have been observed: swimming movement conferred by flagella, flagella-mediated swarming, corkscrew movement of spirochetes, twitching motility associated with type IV pili, and gliding motility.

Motile bacteria do not move aimlessly. Rather, motility is used to move toward nutrients such as sugars and amino acids and away from many harmful substances and bacterial waste products. Movement toward chemical attractants and away from repellents is known as chemotaxis. Motile bacteria also can move in response to environmental cues such as temperature (thermotaxis), light (phototaxis), oxygen (aerotaxis), osmotic pressure (osmotaxis), and gravity.

Flagellar Movement

Swimming

The filament of a bacterial flagellum is in the shape of a rigid helix, and the cell moves when this helix rotates like a propeller on a boat. The flagellar motor can rotate very rapidly. The *E. coli* motor rotates 270 revolutions per second (rps); *Vibrio alginolyticus* averages 1,100 rps. For many bacteria in an aquatic environment, flagellar rotation results in two types of movement: a smooth swimming movement often called a **run**, which actually moves the cell from one spot to another, and a **tumble**, which serves to reorient the cell. As we shall see in our discussion of chemotaxis, alternating between runs and tumbles is important for responding to environmental conditions. Often, the direction of flagellar rotation determines whether a run or a tumble occurs. For example, many bacteria with monotrichous, polar flagella use a counterclockwise rotation for a run (figure 3.40). When rotation is reversed, the cell tumbles. Many peritrichously flagellated bacteria operate in a somewhat similar way. To move forward in a run, the flagella rotate counterclockwise. As they do so, the flagella bend at their hooks to form a rotating bundle that propels the cell forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles.

Not all bacteria use runs and tumbles for swimming motility. For instance, *Rhodospirillum rubrum* cells alternate between rotating their single flagellum in one direction (run) and no rotation, a so-called run-stop motility. When the cells are stopped, molecules in the environment bombard the cells and

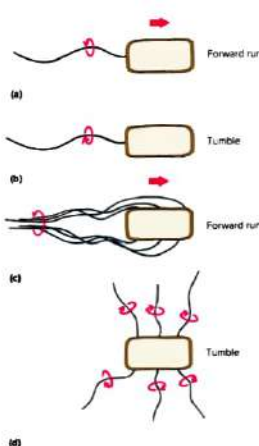


Figure 3.40 The Direction of Flagellar Rotation Often Determines the Way a Bacterium Moves. Parts (a) and (b) describe the motion of bacteria with monotrichous, polar flagella. Parts (c) and (d) illustrate the movements of bacteria with peritrichous flagella.

cause them to make small changes in orientation. When they resume a run, they move in a new direction. Another type of swimming motility is seen with the monotrichous bacterium *Vibrio alginolyticus*. It uses a run-reverse-flick pattern. It swims forward (run) when the flagellum rotates in one direction. When rotation reverses, the cell moves backward (reverse). Just as the rotation switches again for a run, the flagellum flicks, causing the cell to change its orientation and move in a new direction.

The motor that drives flagellar rotation is located at the base of the flagellum. Torque generated by the motor is transmitted to the hook and filament. The motor is composed of two components: the rotor (the moving parts) and the stator (the stationary components). It is thought to function like an electrical motor, where the rotor turns in the center of a ring of electromagnets, the stator. In typical Gram-negative bacteria, the rotor is composed of the central rod and all four rings (figure 3.41). The C ring protein, *FlgG*, is particularly important because it interacts with the stator. The stator is composed of the proteins *MotA* and *MotB*, which form a channel through the plasma membrane. *MotB* also anchors *MotA* to cell wall peptidoglycan.

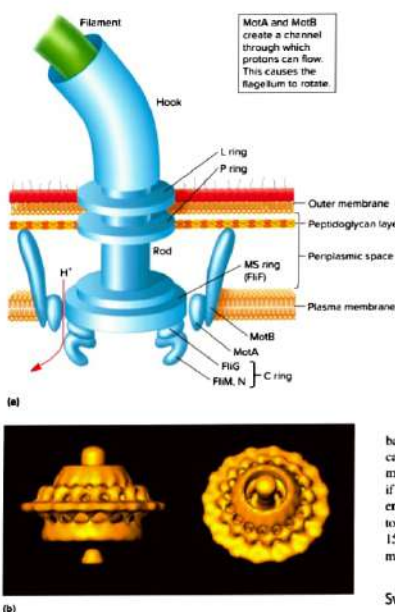


Figure 3.41 Mechanism of Flagellar Movement. (a) This diagram of a flagellum shows some of the more important components and the flow of protons that drives rotation. Some of the many flagellar proteins are labeled. (b) A three-dimensional electron cryotomographic reconstruction of the flagellar motor.

(a) © Green Murphy/Nature Science Source

MICRO INQUIRY Would this flagellum be found in a typical Gram-negative or Gram-positive bacterium?

As with all motors, the flagellar motor must have a power source that allows it to generate torque and cause flagellar rotation. The power used by most flagellar motors is a difference in charge and pH across the plasma membrane. This difference is called the proton motive force (PMF). PMF is largely created by the metabolic activities of organisms, as described in chapter 11. One important metabolic process is the transfer of

electrons from an electron donor to a terminal electron acceptor via a chain of electron carriers called the electron transport chain (ETC). In bacterial cells, most components of the ETC are located in the plasma membrane. As electrons are transported down the ETC, protons are transported from the cytoplasm to the outside of the cell. Because there are more protons outside the cell than inside, the outside has more positively charged ions (the protons) and a lower pH. PMF is a type of potential energy that can be used to do work: mechanical work, as in the case of flagellar rotation; transport work, the movement of materials into or out of the cell (section 3.3); or chemical work such as the synthesis of ATP, the cell's major energy currency.

How can PMF be used to power the flagellar motor? The channels created by the *MotA* and *MotB* proteins allow protons to move across the plasma membrane from the outside to the inside (figure 3.41). Thus the protons move down the charge and pH gradient. This movement releases energy that is used to rotate the flagellum. In essence, the entry of a proton into the channel is like the entry of a person into a revolving door. The "power" of the proton generates torque, rather like a person pushing the revolving door. Indeed, the speed of flagellar rotation is proportional to the magnitude of the PMF.

The flagellum is a very effective swimming device. From the bacterium's point of view, swimming is quite a difficult task because the surrounding water seems as viscous as molasses. The cell must bore through the water with its corkscrew-shaped flagella, and if flagellar rotation ceases, it stops almost instantly. Despite such environmental resistance to movement, bacteria can swim from 10 to 100 μm per second. This is equivalent to traveling from 2 to over 150 cell lengths per second. In contrast, an exceptionally fast human might be able to run around 5 to 6 body lengths per second.

Swarming

An increasing number of bacterial species has been found to exhibit an interesting type of motility called swarming. This motility occurs on moist surfaces and is a type of group behavior in which cells move in unison across the surface. Most bacteria that swarm have peritrichous flagella. Many also produce and secrete molecules that help them move across the substrate. When bacteria that swarm are cultured in the laboratory on appropriate solid media, they produce characteristic colony morphologies (figure 3.42).

Spirochete Motility

Spirochetes have flagella that work in a distinctive manner. In many spirochetes, multiple flagella arise from each end of the cell and wind around the cell (figure 3.43). The flagella do not extend outside the cell wall but rather remain in the periplasmic space and are covered by the outer membrane. They are called periplasmic flagella and rotate like the external flagella of other



Figure 3.42 Swarming Bacteria Often Produce Distinctive Patterns on a Solid Growth Medium. These bacterial cells swarmed from the center of the plate and produced a branching pattern called dendrites. (©iStockphoto.com)

bacteria, causing the corkscrew-shaped outer membrane to rotate and move the cell through the surrounding medium, even very viscous liquids. Flagellar rotation may also flex or bend the cell and account for the creeping or crawling movement observed when spirochetes are in contact with a solid surface. **Phylum Spirochaetes (section 21.6)**

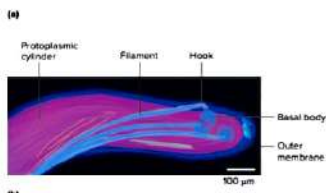
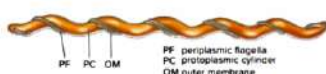


Figure 3.43 Spirochete Flagella. (a) Numerous flagella arise from each end of the spirochete. These intertwine and wind around the cell, usually overlapping in the middle. (b) Electron micrograph of the spirochete *Reptanspira dentata* showing three flagella arising from the tip of the cell. (© Jacques van Haelst/Scanned from Dental Medicine)

Twitching and Gliding Motility

Twitching and gliding motility occur when cells are on a solid surface. However, unlike swarming, neither involves flagella. Gliding motility varies greatly in rate (from 2 to over 600 μm per minute) and in the nature of the motion. Although first observed over 100 years ago, the mechanism by which many bacteria glide remains a mystery. We describe the gliding motility of *Flavobacterium* spp. and *Mycoplasma* spp. in detail in chapter 21. **Phylum Bacteroidetes includes important gut microbes (section 21.7). Class Mollicutes. Phylum Tenericutes: bacteria that lack cell walls (section 21.3)**

Here we focus on *Mycococcus* spp. because it exhibits both twitching and gliding motility. Its twitching motility is called social (S) motility because it occurs when large groups of cells move together in a coordinated fashion. **Twisting motility** is characterized by short, intermittent, jerky motions of up to several micrometers in length and is very moist surfaces. Type IV pili alternate to move cells during twitching motility. contacts the surface at a point some distance. When the pili retract, the cell is pulled. hydrolysis powers the extension/retraction process.

In contrast to the jerky movement of twitching motility, **gliding motility** is smooth. The gliding motility exhibited by *Mycococcus* spp. is called adventurous (A) motility; it is observed when single cells move independently. In *M. santhuss*, proteins similar to MotA and MotB of the flagellar motor function as the motors for gliding motility and these are also powered by PMF. They are located in the plasma membrane (like flagellar motors). Additional motility proteins are located in the periplasm and the outer membrane where they move independently. It is only when the full set of protein complexes aligns across the entire cell envelope that they can exert the mechanical force to propel the cell. Adhesion proteins on the cell surface are compressed and slide on the substrate, pushing the body of the cell forward. **Order Myxococcales: bacteria with morphological complexity and multicellularity (section 22.4)**

Chemotaxis

Imagine a motile bacterium in an ocean. Depending on where it is in the water column, light, oxygen, and nutrient levels will vary. These also vary over time due to the activities of all the organisms present. To position itself in the most beneficial location in the water column, this bacterium needs to sense changes in the environment and move accordingly. The movement toward or away from attractants or repellents is called taxis. As noted earlier, bacteria exhibit taxis to a variety of stimuli, including light and oxygen. The movement of cells toward chemical attractants or away from chemical repellents (**chemotaxis**) is the best-studied type of taxis. Chemotaxis is readily observed in Petri dish cultures. If bacteria are placed in the center of a dish of semisolid agar containing an attractant, the bacteria will exhaust the local supply of the nutrient and swim outward following the attractant gradient they have created. The result is an expanding ring of bacteria. When a disk of repellent is placed in a Petri dish

of semisolid agar and bacteria, the bacteria will swim away from the repellent, creating a clear zone around the disk.

Attractants and repellents are detected by **chemoreceptors**, proteins that bind chemicals and transmit signals to other components of the chemosensing system. Chemosensing systems, located in the plasma membrane, are sensitive and allow the cell to respond to low levels of attractants (about 10^{-4} M for some sugars). Some receptors also participate in the initial stages of sugar transport into the cell.

The chemotactic behavior of *E. coli* has been extensively studied and is our focus here. Its movements can be followed using a tracking microscope, a microscope with a moving stage that automatically keeps an individual bacterium in view. In the absence of a chemical gradient, *E. coli* cells move randomly, switching back and forth between runs and tumbles. During a run, the bacterium swims in a straight or slightly curved line. After a few seconds, the bacterium stops and tumbles. The tumble randomly reorients the cell so that it is facing in a different direction. Therefore when it begins the next run, it usually goes in a different direction (figure 3.44a). In contrast, when *E. coli* is exposed to an attractant, it tumbles less frequently (or has longer runs) when traveling toward the attractant. Although the tumbles can still orient the bacterium away from the attractant, over time the cell gets closer and closer to the attractant (figure 3.44b). The opposite response occurs with a repellent. Tumbling frequency decreases (the run time lengthens) when the bacterium moves away from the repellent.

E. coli must have some mechanism for sensing that it is getting closer to the attractant (or moving away from the repellent).

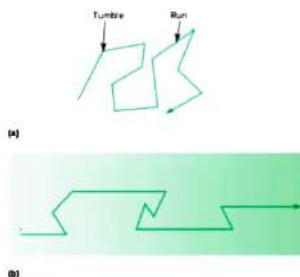


Figure 3.44 Tumbles and Runs Are Used to Direct the Movement of Many Bacteria Toward a Chemical Attractant. (a) Random movement of a bacterium in the absence of a concentration gradient. Tumbling frequency is fairly constant. (b) Movement in an attractant gradient. Tumbling frequency is reduced when the bacterium is moving up the gradient. Therefore runs in the direction of increasing attractant are longer.

The behavior of the bacterium is shaped by temporal changes in chemical concentration. The cell is able to compare the current concentration with the concentration a few seconds earlier. If the concentration of the attractant is increasing, tumbling is suppressed. Likewise, *E. coli* moves away from a repellent because it senses that the concentration of the repellent is decreasing. The bacterium's chemoreceptors play a critical role in this process. The molecular events that enable *E. coli* cells to sense a chemical gradient and respond appropriately are presented in chapter 14.

Comprehension Check

1. Describe the way many flagella operate to move a bacterium.
2. How does swimming differ from swarming?
3. Explain in a general way how bacteria move toward substances such as nutrients and away from toxic materials.
4. Suggest why chemotaxis is sometimes called a "biased random walk."

3.9 Bacterial Endospores Are a Survival Strategy

After reading this section, you should be able to:

- a. Describe the structure of a bacterial endospore
- b. Explain why bacterial endospores are of particular concern to the food industry and why endospore-forming bacteria are important model organisms
- c. Describe in general terms the process of sporulation
- d. Describe those properties of endospores that are thought to contribute to its resistance to environmental stresses
- e. Describe the three stages that transform an endospore into an active vegetative cell

Endospores, dormant cells formed within a so-called mother cell, are fascinating bacterial structures only produced by certain members of the genera *Bacillus* and *Clostridium* (rods), and *Sporosarcina* (cocci) within the phylum Firmicutes. It is important to distinguish bacterial endospores from spores formed by fungi and plants. Endospore formation is not a reproductive strategy as only a single endospore is produced by a bacterial cell. There are several reasons why endospores have long held the interest of microbiologists. The primary reason is that endospores are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants, and desiccation. In fact, some endospores have remained viable for around 100,000 years. Another important reason is that several species of endospore-forming bacteria are dangerous pathogens. For example, *Clostridium botulinum* causes botulism, a food-borne disease that results from ingestion of botulinum toxin, the deadliest toxin known. The extreme heat resistance of *C. botulinum*'s endospores is a major concern of the food industry. Other medically important endospore-forming bacteria include *B. anthracis* (causes anthrax), *C. tetani* (causes tetanus), and *C. perfringens* (causes gas gangrene and food poisoning). Endospores also are of considerable interest to scientists

because of their complex structures and the equally complex process that generates them. Over the decades, microbiologists have asked many questions about endospores and endospore formation. (1) How are endospores structurally different from vegetative cells? (2) What makes them so resistant to harsh environmental conditions? (3) What triggers endospore formation? (4) What are the steps in endospore formation and how is the process regulated? (5) How do endospores convert back to vegetative cells? In this section, we address these questions. We save the consideration of regulating endospore formation for chapter 14. ► *Sporulation in B. subtilis* (section 14.6)

Endospore Structure and Resistance

Electron microscopy has been an important tool for dissecting endospore structure. Electron micrographs show that the endospore consists of a core surrounded by several layers that vary significantly in composition. The core has normal cell structures such as ribosomes and a nucleoid but has very low water content (figure 3.45). The core is surrounded by an inner membrane, which is in turn covered by the core wall (also called the germ cell wall, as it contains the peptidoglycan that will form the wall of the vegetative cell that grows out of the endospore following germination). Next is the cortex, which may occupy as much as half the endospore volume. It is made of peptidoglycan that is less cross-linked than that in vegetative cells. The cortex is surrounded by a phospholipid bilayer called the outer membrane. Outside of the outer membrane is the coat, a complex structure composed of several layers. It is composed of more than 70 different proteins, which are highly cross-linked to each other. Finally, many endospore-forming bacteria produce endospores enclosed by a thin covering called the exosporium, which is made up of glycoproteins.

The ability of the endospore to survive heat, radiation, and damaging chemicals requires that its enzymes and DNA be protected. The various layers of the endospore contribute to this resistance in several ways. The coat protects the endospore from chemicals and various lytic enzymes such as lysozyme. The inner membrane is extremely impermeable to various chemicals, including those that cause DNA damage. The core has very low water content, high amounts of dipicolinic acid complexed with calcium ions (Ca-DPA), and a slightly lower pH, all of which contribute to the endospore's resistance to harsh conditions. The low water content seems to be especially important for endospore resistance. Evidence exists that the water content of the core is low enough to prevent rotation of enzymes and other proteins present. However, it is not low enough to prevent protein denaturation. It has been suggested that the immobilization prevents them from interacting with each other and becoming entangled. Thus, even though they may become denatured, they are able to refold to their proper active structure as the endospore germinates. The endospore's DNA is protected by two main mechanisms. Ca-DPA

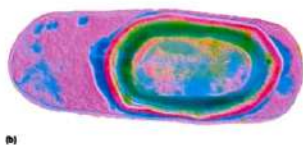
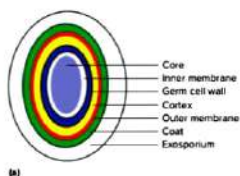


Figure 3.45 Bacterial Endospores. (a) A schematic showing the various layers of an endospore. They are not drawn to scale. (b) A colored cross section of a *Bacillus subtilis* cell undergoing sporulation. The oval in the center is an endospore that is almost mature; when it reaches maturity, the mother cell will lyse to release it.
art ©iStockphoto.com

complexes are inserted between the nitrogenous bases of DNA, which helps stabilize it. In addition, small, acid-soluble DNA-binding proteins (SASPs) saturate endospore DNA. SASPs alter the three-dimensional structure of DNA in the endospore core, converting it from the common B form to A form. The A form DNA is less susceptible to damage by ultraviolet (UV) light, which accounts for the ability of endospores to tolerate UV exposure. ► DNA is a polymer of deoxyribonucleotides (section 13.2)

Sporulation: Making Endospores

Endospore-forming bacteria are common in soil, where they must be able to withstand fluctuating levels of nutrients. **Sporulation** normally commences when growth slows due to nutrient limitation. Thus it is a survival mechanism that allows the bacterium to produce a dormant cell that can survive until nutrients are again available and vegetative growth can resume. These bacteria cycle between two states: vegetative growth and survival as an endospore. Vegetative growth is the normal, continuous cycle of growth and division. By contrast, sporulation is a complex process that occurs in a highly organized fashion over several hours. The mature endospore occupies a characteristic location in the mother cell (referred to as the sporangium), depending on the species of bacteria.

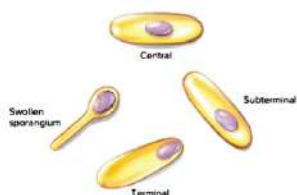


Figure 3.46 Examples of Endospore Location and Size.

Endospores may be centrally located, close to one end (subterminal), or terminal (figure 3.46). Sometimes an endospore is so large that it swells the sporangium. One of the best-studied endospore formers is *Bacillus subtilis*, which is an important model organism.

Sporulation may be divided into seven stages (figure 3.47). The cell's DNA is replicated (stage I), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum (stage II). The mother cell

membrane continues to grow and engulfs the immature endospore in a second membrane (stage III). Next, cortex is laid down in the space between the two membranes, and both calcium and dipicolinic acid are accumulated (stage IV). Protein coats are formed around the cortex (stage V), and maturation of the endospore occurs (stage VI). Finally, lytic enzymes destroy the sporangium, releasing the endospore (stage VII). Sporulation requires about 8 to 10 hours. ► *Bacterial Endospore Formation*

Endospore to Vegetative Cell

The transformation of dormant endospores into active vegetative cells is almost as complex as sporulation. It occurs in three stages: (1) activation, (2) germination, and (3) outgrowth. Activation is a process that prepares endospores for germination and can result from treatments such as heating. This is followed by **germination**, the breaking of the endospore's dormant state. It begins when proteins called germinant receptors, located in the inner membrane and the cortex, detect small molecules such as sugars and amino acids. Upon detection of these molecules by the germinant receptors, a series of events occur. These activated receptors trigger the release of the Ca-DPA complexes, breakdown of the peptidoglycan in the cortex, and water uptake. Eventually

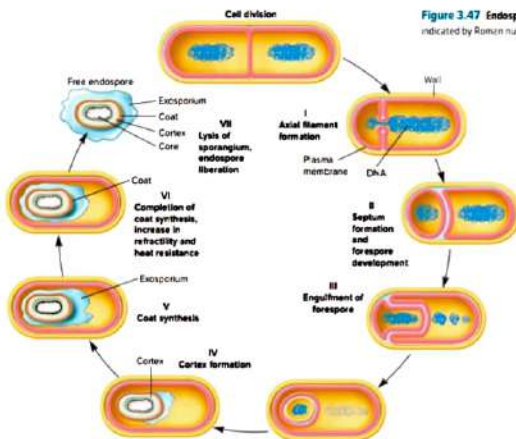


Figure 3.47 Endospore Formation. The stages are indicated by Roman numerals.

Archaeal Cell Structure

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Cows and Buffaloes and Sheep, Oh My!

The next time you drive or walk through the countryside, count the number of domesticated ruminants you see—dairy cows, beef cattle, sheep, and goats, among others. It is estimated that there are about 3 billion head worldwide. These animals are important because they are a substantial protein source. But they do something else that is noteworthy: Each year they exhale about 200 million metric tons of methane, a potent greenhouse gas (GHG). The manure these and other domesticated animals produce is thought to release another 18 million metric tons of methane. As detailed in chapter 28, carbon dioxide is an important GHG and is considered to be a significant contributor to global warming. Methane and nitrous oxide are also GHGs. Carbon dioxide is the dominant GHG in the atmosphere. However, methane has about 30 times the heat-retaining capacity of carbon dioxide. Thus, although the contribution of methane by domesticated ruminants and their manure represents only about 5% of the total GHG emissions worldwide each year, the impact of these emissions is of concern. But don't blame the animals; they are not directly responsible for generating methane. Rather, it is a unique group of archaea—the methanogens—that make methane from hydrogen gas, carbon dioxide, and acetate. Furthermore, these substrates arise in the animals as their food is digested with the help of a complex community of microbes living in their rumen. For these animals, methane is a waste product; for farmers and ranchers, methane represents a loss in production. There is considerable interest in figuring out how to decrease methane production so that GHG emissions are lowered and farmers and ranchers can earn more from each animal.

Methanogens are only one physiological group in domain Archaea. Other archaea have attracted attention because of their ability to live in extreme environments. All are of interest because of their chimeric natures: They have some features that are similar to bacteria and some that are similar to eukaryotes. Their cell structure is like that of bacteria; that is, they look like the canonical prokaryotic cell. However, the molecules used to construct their cell structures are often unique or are similar to those found in eukaryotes. As we describe in chapters 15 and 20, their physiologies exhibit this dichotomy. In general, the processes and molecules used to conserve energy are like those of bacteria, whereas the processes and molecules used to replicate and express their genomes are like those of eukaryotes. In this chapter, we describe some of the molecular and structural aspects of archaeal cells that set them apart.

Readiness Check:

Based on what you have learned previously, you should be able to:

- ✓ Summarize the structures observed in bacterial cells and their molecular makeup (chapter 3)
- ✓ Describe the mechanisms used by bacteria to obtain nutrients (section 3.3)
- ✓ Describe the mechanisms used by bacteria to move in response to stimuli (section 3.8)

4.1 Archaea Are Diverse but Share Some Common Features

After reading this section, you should be able to:

- a. Describe a typical archaeal cell
- b. Discuss key differences between bacteria and archaea

Recall that for many years, archaea and bacteria had been lumped together and referred to as prokaryotes. Today *Archaea* and *Bacteria* are recognized as distinct taxa. The separation of bacteria and archaea into distinct taxa correlates with the observation that they each have unique and distinguishing characteristics. Some of these are summarized in table 4.1.

Keep in mind that only a small subset of the archaea can be grown in the laboratory. Many archaea inhabit niches that constitute extreme environments from a human perspective. Their requirements for high temperature, low pH, or high salt present challenges for culturing them. Historically, environments dominated by archaea were not believed to support life. Once microbiologists broadened their exploration of earth's microbial habitats, archaea have been found to be ubiquitous, but in small numbers. Surveys of nonextreme environments have established that archaea reside in most habitats—again, in small numbers. Most of the studies described in this chapter were performed on archaea that are readily grown in the lab. Methanogens (microbes that generate methane gas) are killed by exposure to oxygen, and must be handled in sealed containers. The names of these organisms begin with *Methano-*. Halophiles (salt-lovers) require high salt conditions and are named *Halo-*. (Another commonly studied archaeon is *Sulfolobus*, which requires both high temperature and low pH for growth.)

Many archaea are known only in the sense that their 16S rRNA sequence or a partial genome sequence has been determined. Archaeal taxonomy is currently in a state of flux because of the vast

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Table 4.1 Comparison of Bacterial and Archaeal Cells

Property	Bacteria	Archaea
Plasma membrane lipids	Ester-linked phospholipids form a lipid bilayer	Glycerol diethers form lipid bilayers; glycerol tetraethers form lipid monolayers
Cell wall constituents	Peptidoglycan is present in nearly all; some lack cell walls	Very diverse but peptidoglycan is always absent; some consist of S-layer only; others combine S-layer with polysaccharides or proteins or both; some lack cell walls
Inclusions present	Yes, including gas vacuoles	Yes, including gas vacuoles
Ribosome size	70S	70S
Chromosome structure	Most are circular, double-stranded (ds) DNA	All known are circular, dsDNA
Plasmids present	Yes; circular and linear dsDNA	Yes; circular dsDNA
External structures	Flagella, fimbriae (pili) common	Archaeella, pili common
Capsules or slime layers	Common	Rare

amount of genomic data that has identified new archaea. There is controversy over the taxonomic designations used for these new organisms, but three phyla are well established: *Crenarchaeota*, *Euryarchaeota*, and *Thaumarchaeota*. We begin our discussion of archaeal cells by considering overall cell morphology and organization and then specific cell structures.

Shape, Arrangement, and Size

Archaeal cells, like bacterial cells, exhibit a variety of shapes. Cocci and rods are common (figure 4.1a). Both usually exist singly, but some cocci form clusters and some rods form chains. Curved rods, spiral shapes, and pleomorphic (many shaped) archaea have also been observed. To date, no spirochete-like and mycelial archaea have been discovered. However, some archaea exhibit unique shapes, such as the branched form of *Thermoproteus tenax* (fig-

ure 4.1b) and the flat, postage-stamp-shaped *Haloquadratum walsbyi*, an archaeon that lives in salt ponds and measures about 2 μm by 2 to 4 μm and only 0.25 μm thick. This shape has the advantage of greatly increasing the surface area-to-volume (SA/V) ratio (see figure 3.5), which in turn increases efficiency of nutrient uptake, diffusion of molecules, and growth rate. **4** Bacteria are diverse but share some common features (section 3.2)

Archaeal cells also vary in size as much as in shape. Typical rods are 1 to 2 μm wide by 1 to 5 μm long; cocci are typically 1 to 3 μm in diameter. However, extremely small and extremely large archaea have been identified. Several free-living, acid-loving (acidophilic), mine-dwelling microbes measure a mere 0.2 to 0.4 μm in diameter. Also at the small end of the size continuum is the parasitic archaeon *Nanoarchaeum equitans* (0.4 μm in diameter). At the other extreme are two recently observed giant archaea that form long



(a) *Methanosarcina mazei*—a coccus that forms clusters



(b) *Thermoproteus tenax*—a branched archaeal cell

Figure 4.1 Archaeal Cell Morphology. (a) Scanning electron micrograph. Bar = 5 μm . (b) Transmission electron micrograph.

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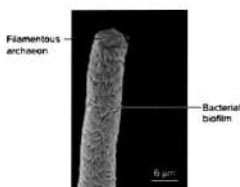


Figure 4.2 A Giant Archaeon. *Candidatus Gigantothaum karukerense* forms long filaments that are covered with a bacterial biofilm. The bacteria may be symbionts of the host archaeon. (Credit: Overlies)

filaments up to 30 mm in length. For one archaeon, the filament is composed of numerous cells, each measuring 8 to 10 μm wide by 20 to 24 μm long. An interesting characteristic of this archaeon is that its filaments are coated with a biofilm (formed by bacteria of a single species) (figure 4.2). The nature of this interaction is not known, but it is thought that the archaeon is the host and the bacteria are symbionts.

Cell Organization

Structures observed in members of both *Bacteria* and *Archaea* are summarized and their differences noted in table 4.1. The archaeal plasma membrane is composed of strikingly different lipids than those found in bacterial membranes; in fact, the unusual lipids were one of the first pieces of evidence to suggest that these microbes are phylogenetically distinct from bacteria. Most archaea have a cell wall, but their walls are considerably more diverse than bacterial walls. Notably, archaeal cell walls lack peptidoglycan. Capsules are not widespread among archaea examined thus far. Within the archaeal cytoplasm, a nucleoid, ribosomes, and inclusions can be found. Finally, many archaea use flagella for locomotion and attachment, but these structures differ sufficiently from bacterial flagella so they have been named *archaella* (s., *archaellum*).

In the remaining sections of this chapter, we describe the major structures observed in archaea in more detail, noting the aspects that distinguish them from the analogous bacterial structures. Understanding archaeal structures at the molecular level is an area of intense research because many archaea are found in extreme habitats. We note those structural adaptations that contribute to this lifestyle. However, the topic of survival in extreme habitats is considered more thoroughly in chapter 7.

► *Environmental factors affect microbial growth (section 7.5)*

Comprehension Check

- Which cell shapes are observed in members of both *Bacteria* and *Archaea*? Which are unique to bacteria? Which to archaea?
- Archaea* was first defined as a distinct taxon by comparisons of ribosomal RNA sequences. Identify two other molecules that could be used to determine if a microbe having a typical prokaryotic architecture is a bacterium or an archaeon.

4.2 Archaeal Cell Envelopes Are Structurally Diverse

After reading this section, you should be able to:

- Draw an archaeal cell envelope and identify the component layers
- Compare and contrast archaeal and bacterial cell envelopes in terms of their structure, molecular makeup, and functions
- Compare and contrast nutrient uptake mechanisms observed in bacteria and archaea

We define the **cell envelope** as the plasma membrane and any layers external to it. For bacteria, these extra layers include cell walls, S-layers, capsules, and slime layers. One of the most distinctive features of archaeal cells is the nature of their cell envelopes. Their uniqueness begins with their plasma membranes. Recall from chapter 3 that the fluid mosaic model describes membranes as lipid bilayers within which proteins float (see figure 3.7). The model is based on studies of eukaryotic and bacterial membranes and is well established. Imagine the surprise of microbiologists when they found that some archaea have monolayer membranes that function like bilayers. This structural difference is due to the presence of unique lipids in archaeal plasma membranes. Archaeal cell envelopes also differ in terms of organization. For many archaea, an S-layer is the major, and sometimes only, component of the cell wall. Slime layers have been observed in some archaea where they appear to mediate cell-cell interactions, but little is known about their composition and regulation. Finally, capsules are relatively rare among archaea and thus are not discussed.

Archaeal Plasma Membranes Are Composed of Unique Lipids but Function Like Bacterial Membranes

Archaeal membranes are composed primarily of lipids that differ from bacterial and eukaryotic lipids in two ways. First, they contain hydrocarbons derived from isoprene units—five-carbon, branched molecules (figure 4.3). Thus the hydrocarbons are branched as shown in figure 4.4. This affects the way the lipids pack together, which in turn affects the fluidity and permeability of the membrane. This is especially important for extremophilic archaea for which membrane fluidity and permeability could be compromised by extreme conditions. Second, the hydrocarbons are attached to glycerol by ether links rather than ester links (figure 4.4a). Ether linkages are more resistant to chemical attack and heat than are ester links.

Two major types of archaeal lipids have been identified: glycerol diethers and diglycerol tetraethers. **Glycerol diether lipids** are



Figure 4.3 Isoprene. This five-carbon, branched molecule is the building block of archaeal lipids.

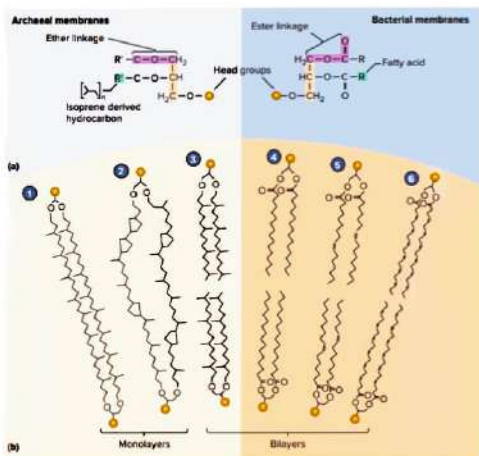


Figure 4.4 Comparison of Archaeal and Bacterial Membranes. (a) Archaeal membrane lipids are attached to glycerol by ether linkages instead of ester linkages, as found in bacteria and eukaryotes. The stereochemistry also differs: in archaeal lipids, the stereoisomer of glycerol is sn-glycerol-1-phosphate; in bacterial lipids, the stereoisomer is sn-glycerol-3-phosphate. Thus in archaeal lipids, the side chains are attached to carbons 2 and 3 of glycerol, and in bacterial lipids, the side chains are attached to carbons 1 and 2. (b) Examples of archaeal lipids are lipids 1, 2, and 3. Lipids 4, 5, and 6 are bacterial lipids. Note that some archaeal lipids form monolayers (figure 4.5), whereas all bacterial lipids form bilayers.

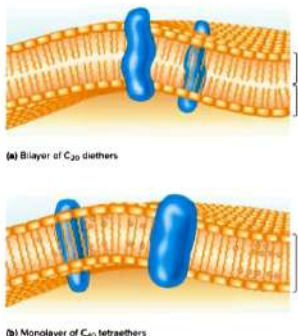


Figure 4.5 Examples of Archaeal Plasma Membranes.

formed when two hydrocarbons are attached to glycerol (figure 4.4b, lipid 3). Usually, the hydrocarbon chains in glycerol diethers are 20 carbons in length. **Diglycerol tetraether lipids** are formed when two glycerol residues are linked by two long hydrocarbons that are 40 carbons in length (figure 4.4b, lipids 1 and 2). Tetraethers are more rigid lipids than diethers. Cells can adjust the overall length of the tetraethers by cyclizing the chains to form pentacyclic rings (figure 4.4b, lipid 2). Phosphorus-, sulfur-, amino-, and sugar-containing groups can be attached to the glycerol moieties in the diethers and tetraethers, just like the phospholipids observed in bacterial and eukaryotic membranes.

Despite the significant differences in membrane lipids, the basic design of archaeal membranes is similar to that of bacterial and eukaryotic membranes: There are two hydrophilic surfaces and a hydrophobic core. When C_{20} diethers are used, a typical bilayer membrane is formed (figure 4.5a). When the membrane is constructed of C_{40} tetraethers, a monolayer membrane with much more rigidity is formed (figure 4.5b). The addition of pentacyclic rings further increases this rigidity. As might be expected from their need for stability, the membranes of extreme thermophiles such as *Sulfolobus* spp., which grow best at temperatures over 85°C, are almost completely tetraether monolayers. Archaea that live in moderately hot environments such as

Thermoplasma spp., have membranes containing some regions with monolayers and some with bilayers.

Archaeal membranes are believed to include microdomains, as described in chapter 3 for bacterial membranes. Flotillin-like proteins have been identified in *Pyrococcus* membranes where they cluster into complexes as observed in both bacterial and eukaryotic membranes.

As we discuss in chapter 3, organisms have several options for obtaining the nutrients they need from their environment. Importantly, microbes often find themselves in nutrient-poor environments and therefore must be able to accumulate nutrients in their cytoplasm at concentrations higher than the external milieu. Thus, although passive and facilitated diffusion have been observed, archaea primarily use active transport for nutrient uptake. Both primary (e.g., ABC transport) and secondary active transport systems (e.g., symport and antiport) have been identified in archaeal cells, often by searching an archaeon's genome for the genes encoding components of these systems (i.e., genomic analysis). These systems tend to be similar to those seen in bacteria. The group translocation system phosphoenolpyruvate:sugar phosphotransferase system (PTS) also functions in some archaea. **▶ Bacteria use many mechanisms to bring nutrients into the cell (section 3.3)**

▶ Microbial genomics (chapter 18)

There Are Many Different Types of Archaeal Cell Walls

Before they were distinguished as a unique domain of life, archaeal species were characterized as being either Gram positive or Gram negative based on their response to Gram staining. Thus, like all cells (even eukaryotic cells), they will stain either purple or pink when Gram stained. However, their staining reaction does not correlate reliably with a particular cell wall structure as it does for bacteria. Archaeal cell walls exhibit considerable variety in terms of their chemical makeup. Furthermore, their cell walls lack peptidoglycan.

The most common type of archaeal cell wall is an S-layer composed of either glycoprotein or protein (figure 4.6a). The S-layer may be as thick as 70 nm. Some methanogens (*Methanobacterium* and *Methanococcus* spp.), salt-loving archaea (*Halobacterium* spp.), and extreme thermophiles (*Sulfolobus*, *Thermoproteus*, and *Pyrodicticum* spp.) have S-layer cell walls.

Other archaea have additional layers of material outside the S-layer. For instance, *Methanospirillum* spp. have a protein sheath external to the S-layer (figure 4.6b). Other methanogens (*Methanosaeta* spp.) have a polysaccharide layer covering the S-layer (figure 4.6c). This material, called methanochondroitin, is similar to the chondroitin sulfate of animal connective tissue.

In some archaea, the S-layer is the outermost layer and is separated from the plasma membrane by a peptidoglycan-like molecule called pseudomurein (figure 4.6d). Pseudomurein differs from peptidoglycan in that it has *N*-amino acids instead of *D*-amino acids in its cross-links, *N*-acetylglucosamine instead of *N*-acetylmuramic acid, and $\beta(1\rightarrow3)$ glycosidic bonds instead of $\beta(1\rightarrow4)$ glycosidic bonds (figure 4.7). These differences mean that lysozyme, penicillin, and other chemicals that

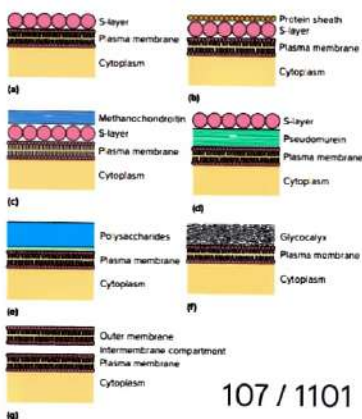


Figure 4.6 Archaeal Cell Envelopes. (a) *Methanococcus*, *Halobacterium*, *Pyrodicticum*, *Sulfolobus*, and *Thermoproteus* spp. cell envelopes. (b) *Methanospirillum* spp. cell envelope. (c) *Methanosaeta* spp. cell envelope. (d) *Methanobacterium* and *Methanopyrus* spp. cell envelopes. (e) *Methanohalobacter*, *Methanohalobium*, *Methanohalobium*, and *Halobacterium* spp. cell envelopes. (f) *Methanospirillum* and *Methanospirillum* spp. cell envelopes. (g) *Thermoplasma* cell envelope. (h) *Ignicoccus hospitalis* cell envelope.

affect peptidoglycan structure and synthesis in bacterial cell walls have no effect on pseudomurein-containing archaeal cell walls. **▶ Cell walls and osmotic protection (section 3.4)**

Another type of archaeal cell wall consists of a single, thick homogeneous layer resembling that in Gram-positive bacteria (figure 4.6e). These archaea lack an S-layer and often stain Gram positive. Their wall chemistry varies from species to species but usually consists of complex polysaccharides such as pseudomurein.

Some archaea lack any layer resembling a cell wall. For instance, members of the acidophilic genera *Ferroplasma* and *Thermoplasma* have envelopes consisting only of a plasma membrane covered by a layer of slime. The slime, which is referred to as a glycocalyx, may provide some of the protection needed for these archaea to survive in their acidic habitats (figure 4.6f). The most unique wall-less archaeon is *Ignicoccus hospitalis*. Its envelope consists only of the plasma membrane and an outer membrane, with an intermembrane compartment between them (figure 4.6g and figure 4.8). The outer membrane contains protein complexes that form pores, much like bacterial porin proteins create pores in the outer membrane of typical Gram-negative bacteria.

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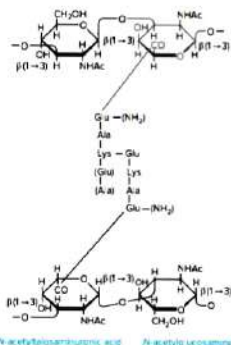


Figure 4.7 Pseudomurein. The amino acids and amino groups in parentheses are not always present. Ac, acetyl group.

MICRO INQUIRY How is pseudomurein similar to peptidoglycan? How does it differ?

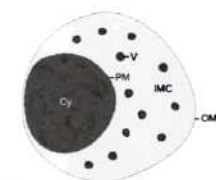


Figure 4.8 Cell Envelope of the Wall-less Archaeon *Ignicoccus hospitalis*. Cy, cytoplasm; IMC, intermembrane compartment; OM, outer membrane; PM, plasma membrane; V, periplasmic vesicles.

Many archaeal energy conservation reactions occur in the space between the plasma membrane and the S-layer. This region of the cell is sometimes referred to as the pseudo-periplasm, by analogy to the structure in Gram-negative bacteria.

Comprehension Check

1. Identify three features that distinguish archaeal plasma membranes from those of bacteria.
2. Both bacteria and archaea can have S-layers. How does their use within the cells differ?

4.3 Archaeal Cytoplasm Is Similar to Bacterial Cytoplasm

After reading this section, you should be able to:

- a. Compare and contrast the cytoplasm of bacterial and archaeal cells
- b. Discuss the organization of archaeal DNA and similarities to both bacteria and eukaryotes

Overall, the cytoplasm of archaeal cells is similar to that of bacteria. Within it can be found inclusions—polyhydroxyalkanoates, polyphosphate granules, glycogen granules, and gas vacuoles; ribosomes; a nucleoid; and plasmids. Proteins that might form a **cytoskeleton** have also been identified, including FtsZ (tubulin homologue), MreB (actin homologue), and crenactin, an actin homologue unique to certain members of the *Crenarchaeota*. In these archaea having FtsZ, the protein participates in cell division, as it does in bacteria. Evidence suggests that the actin homologues MreB and crenactin are involved in conferring a rod shape. Interestingly, a tubulin homologue called CelZ is observed in some members of the *Euryarchaeota*. CelZ confers a rod shape to cells rather than functioning in cytokinesis as FtsZ does. Some structures found in the cytoplasm of archaeal cells have distinctive molecular makeup. In this section, we focus on those structures. **▶ Phylum Crenarchaeota (section 20.2); Phylum Euryarchaeota: methanogens, halobacteria, and others (section 20.3)**

Archaeal Ribosomes Are the Same Size as Bacterial Ribosomes but Are Composed of Different Molecules

Like bacterial cells, the ribosomes of archaeal cells are 70S in size and are constructed of a 50S and a 30S subunit. However, their shape is somewhat different and their component molecules are not the same. Both have ribosomal RNA (rRNA) molecules of similar size: 16S in the small subunit, and 23S and 5S in the large subunit. However, as we discuss in chapter 1, the differences in nucleotide sequences of these molecules were the initial basis for establishing the taxon Archaea. Furthermore, at least one archaeon has an additional rRNA, a 5.8S rRNA, in the large subunit. This is of interest because the large subunit of eukaryotic ribosomes contains both 5S and 5.8S rRNA molecules.

The protein composition of bacterial and archaeal ribosomes also differs. Archaeal ribosomes have more proteins: about 68 rather than about 55. Archaeal ribosomal proteins can be sorted into three groups based on their similarity to proteins found in bacterial and eukaryotic ribosomes: (1) those observed in all three domains of life, (2) those unique to archaea, (3) those observed in both archaea and eukaryotes. There are only a few ribosomal proteins unique to archaea; the rest are about evenly divided between the other two groups. Importantly, all the ribosomal proteins present in both archaeal and bacterial ribosomes are also seen in eukaryotic ribosomes. Thus there are no ribosomal proteins that are present only in the ribosomes of archaea and bacteria. The difference in bacterial and archaeal ribosomes correlates with the fact that archaeal ribosomes are unaffected

by antibiotics that bind and inhibit bacterial ribosomes. **Protein synthesis inhibitors** (section 9.4)

Nucleoid

The nucleoid provides another example of the difference between archaea and bacteria. This region in the cytoplasm contains the cell's **chromosome** and numerous proteins. The chromosomes of all known archaea are circular, double-stranded **deoxyribonucleic acid (DNA)**. Members of the *Euryarchaeota* are polyploid; that is, they have multiple copies of their chromosomes throughout their life cycles. In contrast, members of the *Crenarchaeota* are monoploid, with a single copy of the chromosome. It is believed that this difference reflects the mechanism for chromosome partitioning to daughter cells during cell division. *Crenarchaeota* have a highly regulated chromosome segregation mechanism, but the process appears to be random in the *Euryarchaeota*. Maintaining multiple copies of the genetic information is good insurance for an intact copy to end up in each daughter cell. **Chromosome replication and partitioning** (section 7.2)

The problem of compacting a genome to fit into a cell is common to archaea and bacteria, and the solution is similar. Both supercoiling and the presence of nucleoid-associated proteins (NAPs) on the genomic DNA contribute to chromosome organization. As in bacteria, there is a variety of NAPs, but they bear little resemblance to bacterial NAPs. The common features of these proteins are small size and positive charge. Each archaeon contains at least two different NAPs that may act at different scales of chromosome structure. On short segments of DNA, certain NAPs bend or stiffen the chromosome; on a global level, other NAPs bridge nonadjacent regions of the chromosome.

Many members of the *Euryarchaeota* have histones associated with their chromosomes. There is a strong correlation between the presence of histones and multiple chromosome copies, although it is not clear how the two features are related. These **histones** form **nucleosomes** that are related to the nucleosomes observed in eukaryotes (see figure 5.10). In *Haloferax volcanii*, the nucleosomes differ from eukaryotic nucleosomes in that they consist of four histones rather than eight and they organize a shorter length of DNA. In *Thermococcus kodakarensis*, however, histones assemble into various-sized complexes containing from two histones to more than a dozen. It is hypothesized that in thermophilic archaea, histones help prevent denaturation of the chromosomes. **Nucleoid** (section 3.6); **Nucleus** (section 5.5)

Comprehension Check

- Thus far, homologues of intermediate filaments have not been identified in archaea. Is it likely that they will be identified eventually? Explain your answer.
- Archaea are often described as chimeric organisms having some features that are similar to bacteria and some that are similar to eukaryotes. Based on what you have just read in this section, provide two examples that illustrate the similarity of archaea to bacteria; list two examples of their similarity to eukaryotes.

4.4 Many Archaea Have External Structures Used for Attachment and Motility

After reading this section, you should be able to:

- Compare and contrast bacterial and archaeal pili
- Compare and contrast bacterial and archaeal flagella in terms of their structure and function

Like bacteria, many archaea have structures that extend beyond the cell envelope, namely pili and flagella. These external structures and their functions are discussed in this section.

Pili

Many archaea have **pili**, and these appendages are found in a range of lengths and diameters, some solid and others hollow. Pili are composed of many copies of single protein subunits termed **pilins**, but pilins are not related to one another, thus explaining the diversity in the structures observed. *Methanococcus maripaludis* makes a major pili that comprises the bulk of its pili, and several other less abundant pili. Its genome encodes at least six other pili, and changing the relative proportions of pili may be a means of responding to environmental stimuli.

Archaeal pili are **type IV pili**, which describes their mechanism of assembly within the cell envelope. Pilins are synthesized on ribosomes in the cytoplasm and then anchored to a protein complex in the plasma membrane. Pili proteins are secreted through the complex to assemble the mature pilus structure.

Pili perform a range of functions. *Sulfolobus solfataricus* can display at least two distinct types of pili. The archaeal adhesive pilus (Aap) is responsible for cell adhesion to surfaces under certain growth conditions. The ultraviolet (UV)-inducible pilus (Ups) is only produced following exposure of the cells to UV light. As UV light can damage DNA, the Ups mediates the aggregation of cells and promotes the transfer of DNA between cells. **Bacterial pili and fimbriae** (section 3.7)

Two particular pili are noteworthy: **canalae** and **hami**. **Canalae** are hollow, tubelike structures observed on the surface of thermophilic archaea belonging to the genus *Pyrodicticum* (figure 4.9). The function of canalae is unknown, but it is known that daughter cells arising from a single round of cell division remain connected to each other by canalae. After many rounds of cell division, a network of cells is formed. **Hami** are of particular interest because of their shape (figure 4.10). They look like tiny grappling hooks, which suggests they might function to attach cells to surfaces. Indeed, archaeal cells that produce hami are members of biofilm communities generally consisting of a hami-producing archaeon and a bacterium.

Archaea and Motility

Archaeal flagella, or **archaella**, have been studied in detail in only a few model archaea. They are superficially similar to

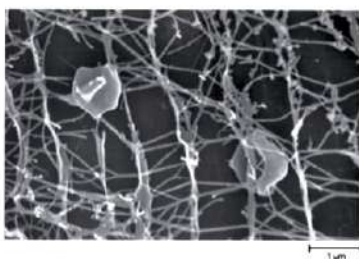
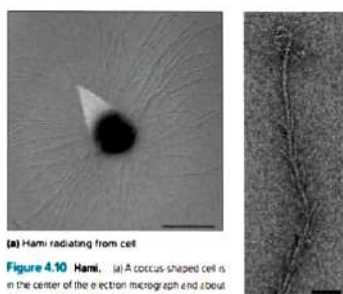


Figure 4.9 Canalae. Canalae are tubular structures about 25 nm in diameter. They have only been observed on *Pyrodicticum* spp. They connect daughter cells, ultimately forming a dense network of cells. SEM, bar = 1 μm. © Karl O. Sletten

their bacterial counterparts, but important differences have been identified. Archaella are thinner than bacterial flagella (10 to 14 nm rather than 18 to 22 nm) and some are composed of more than one type of protein subunit (figure 4.11). The filament is not hollow. Assembly of the archaellum also differs from bacterial flagellum assembly. In a manner similar to the assembly of bacterial type IV pili, the archaellin proteins are exported through the motor complex (figure 4.11) and added to the base of the filament. Additional proteins anchor the archaellum to the S-layer. Cryotomography has revealed bundles of archaella at one pole of the cell, organized by a proteinaceous structure in the membrane. **Bacterial flagella** (section 3.7)

Archaella from the two major phyla, *Euryarchaeota* and *Crenarchaeota*, have characteristic structural variations. Crenarchaeal filaments are composed of a single archaellin subunit. In contrast, euryarchaeal filaments have multiple archaellins, one of which is the primary subunit with others in lower abundance. Halophiles have been demonstrated to synthesize archaellins in different proportions under different culture conditions. It is not known how the filament properties vary with subunit composition. The motor complex also differs between these phyla, and it is believed that this variation is related to the presence of a bacterial-like chemotaxis system in *Euryarchaeota*.

Archaella work in a manner similar to bacterial flagella. Rotation propels the cell. Again, there are some important differences. First, rotation is powered by ATP hydrolysis rather than by proton motive force. Second, when the direction of rotation switches, it causes the cell to move in either the for-



(a) Hami radiating from cell

Figure 4.10 Hami. (a) A coccus-shaped cell is in the center of the electron micrograph and about 100 hami are radiating out from the plasma membrane. TEM, bar = 500 nm. (b) At the ends of the hami are grappling hook-like structures that are thought to allow cells to adhere to surfaces, including other cells. TEM, bar = 50 nm.

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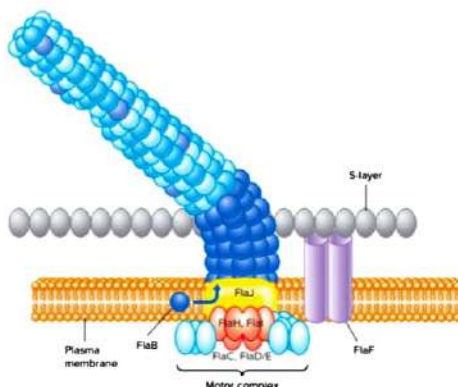


Figure 4.11 Structure of the Archaeal Flagellar Motor Complex. The central component is the

cell. ► **Chromosome replication and partitioning** (section 7.2) The problem of compacting a genome to fit into a cell is common to archaea and bacteria, and the solution is similar. Both supercoiling and the presence of nucleoid-associated proteins (NAPs) on the genomic DNA contribute to chromosome organization. As in bacteria, there is a variety of NAPs, but they bear little resemblance to bacterial NAPs. The common features of these proteins are small size and positive charge. Each archaeon contains at least two different NAPs that may act at different scales of chromosome structure. On short segments of DNA, certain NAPs bend or stiffen the chromosome; on a global level, other NAPs bridge nonadjacent regions of the chromosome.

Many members of the *Euryarchaeota* have histones associated with their chromosomes. There is a strong correlation between the presence of histones and multiple chromosome copies, although it is not clear how the two features are related. These histones form nucleosomes that are related to the nucleosomes observed in eukaryotes (see figure 5.10). In *Haloferax volcanii*, the nucleosomes differ from eukaryotic nucleosomes in that they consist of four histones rather than eight and they organize a shorter length of DNA. In *Thermococcus kodakarensis*, however, histones assemble into various-sized complexes containing from two histones to more than a dozen. It is hypothesized that in thermophilic archaea, histones help prevent denaturation of the chromosomes. ► **Nucleoid** (section 3.6); ► **Nucleus** (section 5.5)

Comprehension Check

- Thus far, homologues of intermediate filaments have not been identified in archaea. Is it likely that they will be identified eventually? Explain your answer.
- Archaea are often described as chimeric organisms having some features that are similar to bacteria and some that are similar to eukaryotes. Based on what you have just read in this section, provide two examples that illustrate the similarity of archaea to bacteria; list two examples of their similarity to eukaryotes.

Pili are composed of many copies of single protein subunits termed **pilins**, but pilins are not related to one another, thus explaining the diversity in the structures observed. *Methanococcus maripaludis* makes a major pilin that comprises the bulk of its pili, and several other less abundant pilins. Its genome encodes at least six other pilins, and changing the relative proportions of pilins may be a means of responding to environmental stimuli.

Archaeal pili are **type IV pili**, which describes their mechanism of assembly within the cell envelope. Pili are synthesized on ribosomes in the cytoplasm and then anchored to a protein complex in the plasma membrane. Pilin proteins are secreted through the complex to assemble the mature pilus structure.

Pili perform a range of functions. *Sulfolobus solfataricus* can display at least two distinct types of pili. The archaeal adhesive pilus (Aap) is responsible for cell adhesion to surfaces under certain growth conditions. The ultraviolet (UV)-inducible pilus (Ups) is only produced following exposure of the cells to UV light. As UV light can damage DNA, the Ups mediates the aggregation of cells and promotes the transfer of DNA between cells. ► **Bacterial pili and fimbriae** (section 3.7)

Two particular pili are noteworthy: **canalae** and **hami**. **Canalae** are hollow, tubelike structures observed on the surface of thermophilic archaea belonging to the genus *Pyrodicticum* (figure 4.9). The function of canalae is unknown, but it is known that daughter cells arising from a single round of cell division remain connected to each other by canalae. After many rounds of cell division, a network of cells is formed. **Hami** are of particular interest because of their shape (figure 4.10). They look like tiny grappling hooks, which suggests they might function to attach cells to surfaces. Indeed, archaeal cells that produce hami are members of biofilm communities generally consisting of a hami-producing archaeon and

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Archaea and Motility

Archaeal flagella, or archaeella, are only a few model archaea. They are superficially similar to

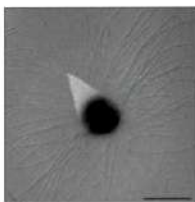


Figure 4.9 Canalae. Canalae are tubular structures about 25 nm in diameter. They have only been observed on *Pyrodicticum* spp. They connect daughter cells, ultimately forming a dense network of cells. SEM, bar = 1 μ m. ©Karl D. Ström

their bacterial counterparts, but important differences have been identified. Archaeella are thinner than bacterial flagella (10 to 14 nm rather than 18 to 22 nm) and some are composed of more than one type of protein subunit (figure 4.11). The filament is not hollow. Assembly of the archaeellum also differs from bacterial flagellum assembly. In a manner similar to the assembly of bacterial type IV pili, the archaeellin proteins are exported through the motor complex (figure 4.11) and added to the base of the filament. Additional proteins anchor the archaeellum to the S-layer. Cryo-electron tomography has revealed bundles of archaeella at one pole of the cell, organized by a proteinaceous structure in the membrane. ► **Bacterial flagella** (section 3.7)

Archaea from the two major phyla, *Euryarchaeota* and *Crenarchaeota*, have characteristic structural variations. Crenarchaeal filaments are composed of a single archaeellin subunit. In contrast, euryarchaeal filaments have multiple archaeellins, one of which is the primary subunit with others in lower abundance. Halophiles have been demonstrated to synthesize archaeellins in different proportions under different culture conditions. It is not known how the filament properties vary with subunit composition. The motor complex also differs between these phyla, and it is believed that this variation is related to the presence of a bacterial-like chemotaxis system in *Euryarchaeota*.

Archaea work in a manner similar to bacterial flagella: Rotation propels the cell. Again, there are some important differences. First, rotation is powered by ATP hydrolysis rather than by proton motive force. Second, when the direction of rotation switches, it causes the cell to move in either the forward or reverse direction, just as is seen for some bacteria having polar flagella. Thus far, alternation between runs and tumbles has not been observed.



(a) Hamus radiating from cell

Figure 4.10 Hami. (a) A coccus-shaped cell is in the center of the electron micrograph and about 100 hamus are radiating out from the plasma membrane. TEM, bar = 500 nm. (b) At the ends of the hamus are grappling hook-like structures that are thought to allow cells to adhere to surfaces, including other cells. TEM, bar = 50 nm. ©Dr. Christine Mores Eberhard



(b) "Grappling hooks" at cellular ends of hamus

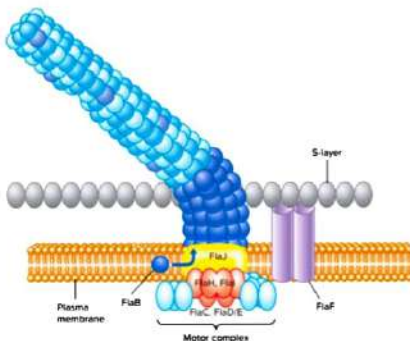


Figure 4.11 Structure of the Archaeellum, Based on Pyrococcus. The archaeellin protein FlaB comprises the filament, and monomers are added through FlaI. The motor complex is composed of multiple Fla proteins, and the FlaF protein anchors the rotating archaeellum in the cell envelope.

Halobacterium salinarum flagellar movement is the best studied. In this archaeon, clockwise rotation of the flagella pushes the cell forward, and counterclockwise rotation pulls the cell (i.e., the flagella are in front of the cell as it moves). *H. salinarum* exhibits both chemotaxis and phototaxis. Phototaxis is used to position the archaeon properly to absorb light when carrying out rhodospin-based phototrophy. Surprisingly, considering the difference in flagellar architecture, much of the machinery controlling chemotaxis and phototaxis in *H. salinarum* is homologous to that found in bacterial systems. The archaeellum in *Sulfolobus* also switches rotational direction, but the switch appears to be a random event. ► **Rhodospin-based phototrophy** (section 11.12)

Archaea have been observed to engage in two types of swimming behaviors, termed a relocate-and-seek strategy. Fast speeds in a straight line (up to ~500 μ m/sec) result in a rapid change of location. A slower zigzag motion (~50 to 100 μ m/sec) constitutes the seek, a scan for favorable conditions. The fastest known

swimmers, *Methanocaldococcus villosus* and *M. jannaschii*, can travel at about 500 body lengths per second (bps), compared to 20 bps for *E. coli* and 11 bps for humans. This extreme swimming behavior is an advantage in their deep sea hydrothermal vent environment. Temperature gradients force these microbes to inhabit a narrow space between the 400°C vent fluid and the surrounding 2°C seawater. When fast swimming identifies a suitable niche (50° to 90°C), the microbes use the archaeella for attachment.

In addition to their role in motility, archaeella are involved in adhesion to substrates, the formation of biofilms, and cell-cell interactions.

Comprehension Check

- What observations about canalae and hami suggest that they allow archaeal cells to adhere to surfaces, including other cells?
- List three aspects of archaeella and their motility that are similar to bacterial flagella and their motility.
- Defend the proposal that the archaeellum is a type of pilus.

Key Concepts

4.1 Archaea Are Diverse but Share Some Common Features

► Members of Bacteria and Archaea share a common cell.

4.3 Archaeal Cytoplasm Is Similar to Bacterial Cytoplasm

► Cytoskeletal proteins have been identified in archaeal cells. They include Ezf (tubulin homolog), MreB (actin homolog), and

Staining

the plane of focus, and a much sharper image is formed.

To generate a confocal image, a computer interfaced with the confocal microscope receives digitized information from each plane in the specimen. This information can be used to create a composite image that is very clear and detailed (figure 2.15c) or a three-dimensional reconstruction of the specimen (figure 2.15d). Images of x-z plane cross sections of the specimen can also be generated, giving the observer views of the specimen from three perspectives (figure 2.15e). Confocal microscopy has numerous applications. One is the study of biofilms, which can form on many different types of surfaces, including indwelling medical devices such as hip joint replacements. As shown in figure 2.15f, it is difficult to kill all cells in a biofilm. This makes biofilms a particular concern because their formation on medical devices can result in infections that are difficult to treat.

► *Biofilms are common in nature (section 7.6)*

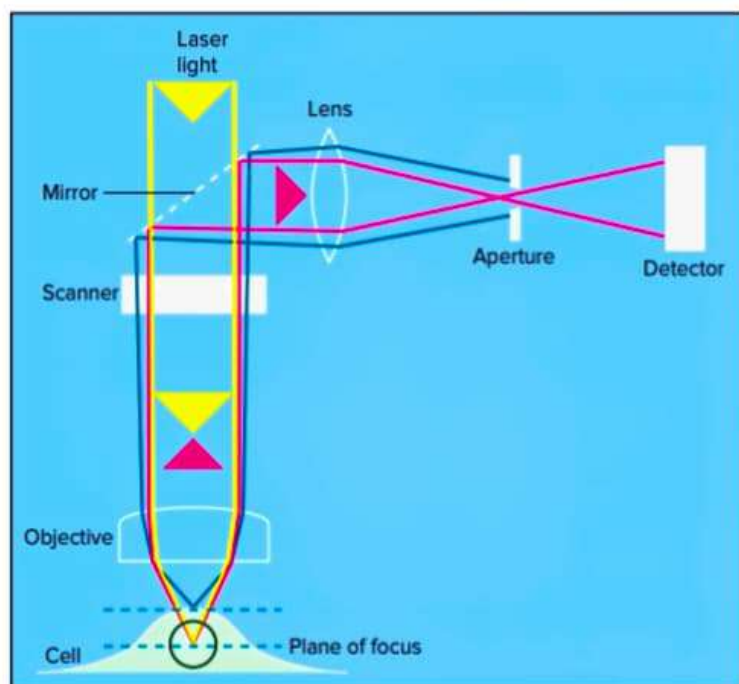


Figure 2.16 Ray Diagram of a Confocal Microscope. The yellow lines represent laser light used for illumination. Red lines symbolize the light arising from the plane of focus, and the blue lines stand for light from parts of the specimen above and below the focal plane.

MICRO INQUIRY How does the light source differ between a confocal light microscope and other light microscopes?

3. What might a microscopist see through an oil immersion objective if no immersion oil is present?
4. Why don't most light microscopes use 30× ocular lenses for greater magnification?
5. Compare and contrast how dark-field, phase-contrast, differential interference contrast, epifluorescence, and confocal microscopes work and the kind of image or images provided by each. Give a specific use for each type.
6. Which type of microscope(s) would be used to examine the following specimens? Photosynthetic bacteria, swimming bacteria, GFP-labeled microorganisms, unstained microbes, and pond scum; explain each of your answers.

2.3 Staining Specimens Helps to Visualize and Identify Microbes

After reading this section, you should be able to:

- a. Recommend a fixation process to use when the microbe is a bacterium or archaeon and when the microbe is a protist
- b. Plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible
- c. Compare what happens to Gram-positive and Gram-negative bacterial cells during each step of the Gram-staining procedure

As noted in section 2.2, specimens examined by bright-field microscopy are often fixed and stained before being examined. Such preparation serves to increase the visibility of the microorganisms, accentuate specific morphological features, and preserve them for future study. Importantly, some staining procedures help microbiologists identify the organism being examined.

Fixation

Stained cells seen in a microscope should resemble living cells as closely as possible. **Fixation** is the process by which the internal and external structures of specimens are preserved and fixed in position. It inactivates enzymes that might disrupt cell morphology and toughens cell structures so that they do not change during staining and observation. A microorganism usually is killed and attached firmly to the microscope slide during fixation.

There are two fundamentally different types of fixation: heat fixation and chemical fixation. **Heat fixation** is routinely used to observe bacteria and archaea. Typically, a film of cells (a smear) is

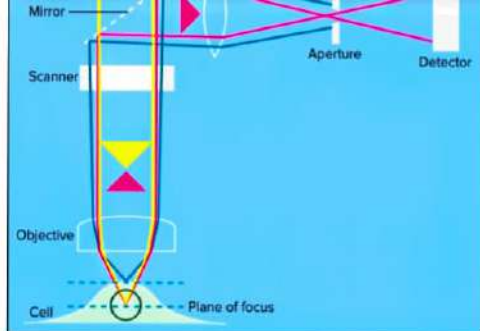


Figure 2.16 Ray Diagram of a Confocal Microscope. The yellow lines represent laser light used for illumination. Red lines symbolize the light arising from the plane of focus, and the blue lines stand for light from parts of the specimen above and below the focal plane.

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There are two fixation procedures: **heat fixation** and **chemical fixation**. Heat fixation is a procedure used to observe bacteria and archaea. It involves a brief exposure of a

gently heated. Heat fixation preserves overall morphology and inactivates enzymes. However, it also destroys proteins in subcellular structures, which may distort their appearance. **Chemical fixation** is used to protect fine cellular substructure as well as morphology. It is used when examining microorganisms by many electron microscopy techniques. Chemical fixatives penetrate cells and react with cellular components, usually proteins and lipids, to render them inactive, insoluble, and immobile. Common fixative mixtures contain ethanol, acetic acid, mercuric chloride, formaldehyde, and glutaraldehyde.

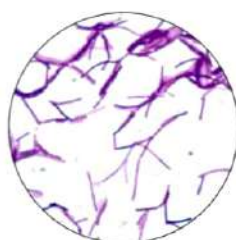
Dyes and Simple Staining

The many types of dyes used to stain microorganisms have two features in common. First, they have **chromophore groups**—chemical moieties with conjugated double bonds that give the dye its color. Second, they bind cells by ionic, covalent, or hydrophobic bonds.

Dyes that bind cells by ionic interactions are probably the most commonly used dyes. These ionizable dyes may be divided into two general classes based on the nature of their charged group: basic dyes and acidic dyes (table 2.4). The staining effectiveness of ionizable dyes may be altered by pH, since the nature and number of the charged moieties on cell components change with pH. Thus acidic dyes stain best under acidic conditions when proteins and many other molecules carry a positive charge; basic dyes are most effective at higher pH values.

Dyes that bind through covalent bonds or that have certain solubility characteristics are also useful. For instance, DNA can be stained by the Feulgen procedure in which the staining compound (Schiff's reagent) is covalently attached to the deoxyribose sugars of DNA. Sudan III (Sudan Black) selectively stains lipids because it is lipid soluble but does not dissolve in aqueous portions of the cell.

Microorganisms can be stained by **simple staining**, in which a single dye is used (figure 2.17). Simple staining's value lies in its ease of use. The fixed smear is covered with stain for a short time, excess stain is washed off with water, and the slide is blotted dry. Basic dyes such as crystal violet, methylene blue, and carbol-fuchsin are frequently used in simple staining to determine the size, shape, and arrangement of bacterial and archaeal cells.



Crystal violet stain of slender, rod-shaped bacteria.

Figure 2.17 Simple Staining Illustrates Cell Size and Morphology.

© Dr. Rita B. Mayes

While most dyes directly stain the cell or object of interest, some dyes (e.g., India ink and nigrosin) are used in **negative staining**. In negative staining, the background is stained, not the cell; instead, the unstained cells appear as bright objects against a dark background.

Differential Staining

The **Gram stain**, developed in 1884 by the Danish physician Christian Gram, is the most widely employed staining method in bacteriology. The Gram stain is an example of **differential staining**—procedures that distinguish organisms based on their staining properties. Use of the Gram stain classifies most bacteria into one of two groups—Gram negative or Gram positive, based on the composition of their cell walls.

The Gram-staining procedure is illustrated in figure 2.18. In the first step, the smear is stained with a primary stain (crystal violet). This is followed by treatment with an iodine solution. Iodine functions as a **mordant**, a substance that helps bind the dye tightly to the cell wall. The smear is then decolorized by washing with alcohol or acetone. This step generates the differential aspect of the Gram stain; the thick cell walls of Gram-positive bacteria retain the crystal violet, whereas Gram-negative bacteria lose the crystal violet and become colorless. Finally, the smear is counterstained, usually

Table 2.4 Ionizable Dyes

Type of Dye	Examples	Chromophore Characteristics
Basic dyes	Methylene blue, basic fuchsin, crystal violet, safranin, malachite green	Have positively charged groups; bind to negatively charged molecules such as nucleic acids, many proteins, and the surfaces of bacterial and archaeal cells
Acidic dyes	Eosin, rose bengal, and acid fuchsin—possess groups such as carboxyls (—COOH) and phenolic hydroxyls (—OH).	Have negatively charged groups; bind to positively charged cell structures

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2.3 Staining Specimens Helps to Visualize and Identify Microbes 31

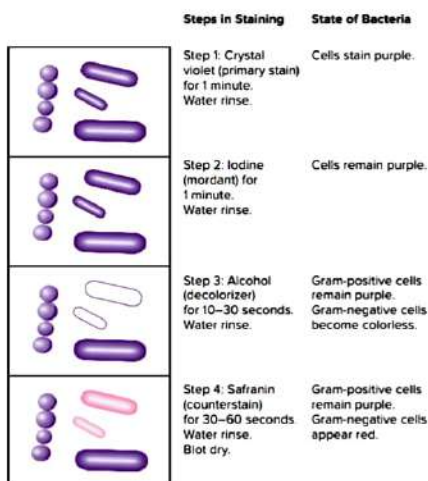


Figure 2.18 Gram Stain. Steps in the Gram-staining procedure.

MICRO INQUIRY Why is the decolorization step considered the most critical in the Gram-staining procedure?

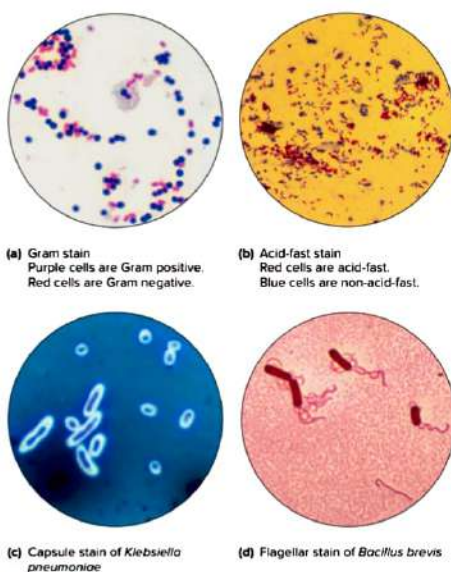


Figure 2.19 Differential Stains.

(a, c) © McGraw-Hill Education/Lisa Burgess, photographer. (b) © McGraw-Hill Education/James Redfearn, photographer. (d) Source: CDC/Di. William A. Clark

with safranin, which colors Gram-negative bacteria pink to red and leaves Gram-positive bacteria dark purple (figure 2.19a). **There are two main types of bacterial cell walls (section 3.4) • Gram staining**

Acid-fast staining is another important differential staining procedure. It can be used to identify *Mycobacterium tuberculosis* and *M. leprae* (figure 2.19b), the pathogens responsible for tuberculosis and leprosy, respectively. These bacteria, as well as other mycobacteria, have cell walls containing lipids constructed from mycolic acids, a group of branched-chain hydroxy fatty acids, which prevent dyes from readily binding to the cells (see figure 23.7). A commonly used staining procedure, the cold Ziehl-Neelsen method, uses high concentrations of phenol and carbol fuchsin, as well as a wetting agent, to drive the stain carbol fuchsin into mycobacterial cells. Once this dye has penetrated, the cells are not easily decolorized by acidified alcohol (acid-alcohol) and thus are said to be acid-fast. Non-acid-fast bacteria are easily decolorized by acid-alcohol and thus are stained another color by a second dye called a counterstain.

One of the simplest staining procedures is **capsule staining** (figure 2.19c), a technique that reveals the presence of capsules,

a network usually made of polysaccharides that surrounds many bacteria and some fungi. Cells are mixed with India ink or nigrosin dye and spread out in a thin film on a slide. After air-drying, cells appear as bodies surrounded by a halo of capsule in the midst of a blue-black background because ink and dye particles cannot penetrate either the cell or its capsule. Thus capsule staining is an example of negative staining. There is little distortion of cell shape, and the cell can be counterstained for even greater visibility. **Capsules and slime layers (section 3.5)**

Flagella staining provides taxonomically valuable information about the presence and distribution pattern of flagella on bacterial and archaeal cells (figure 2.19d; see also figure 3.37). Their flagella are fine, threadlike organelles of locomotion that are so slender (about 10 to 30 nm in diameter) they can only be seen directly using an electron microscope (although bundles of flagella can be visualized by dark-field microscopy). To observe bacterial flagella with a light microscope, their thickness is increased by coating them with mordants such as tannic acid and potassium alum and then staining with a dye such as basic fuchsin. **Bacterial flagella (section 3.7)**

Comprehension Check

Describe the two general types of fixation. Which would you use when Gram staining a bacterium? Which would you use before observing the organelles of a protist?

Why would basic dyes be more effective under alkaline conditions?

greatly compromises their usefulness for detailed studies of many microorganisms (figure 2.20). Viruses, for example, are too small to be seen with light microscopes (with the exception of some recently discovered giant viruses). Bacteria and archaea are visible, but because they are usually only 1 to 2 μm in diameter, only their general shape and major morphological features are visible. The detailed internal structure of microorganisms, therefore

Microscopy

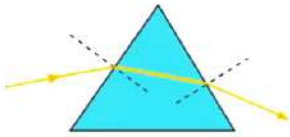


Figure 2.1 The Bending of Light by a Prism. Lines perpendicular to the surface of the prism are called normal, they are indicated by dashed lines. As light enters the glass, it is bent toward the first normal. When light leaves the glass and returns to air, it is bent away from the second normal.

strike the lens, a convex lens focuses the rays at a specific point, the focal point (F in **figure 2.2**). The distance between the center of the lens and the focal point is called the focal length (f in **figure 2.2**).

Our eyes cannot focus on objects nearer than about 25 cm (i.e., about 10 inches). This limitation may be overcome by using a convex lens as a simple magnifier (or microscope) and holding it close to an object. A magnifying glass provides a clear image at much closer range, and the object appears larger. Lens strength is related to focal length: a lens with a short focal length magnifies an object more than a lens having a longer focal length.

Comprehension Check

1. How do refraction, refractive index, focal point, and focal length differ?
2. Draw the path of a light ray through a thin and thick lens.
3. If the lenses in the above question were in corrective eyeglasses, which would be used for reading and which would improve distance vision?

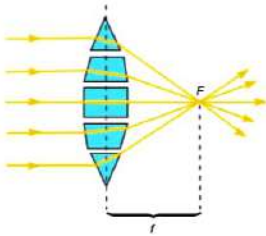


Figure 2.2 Lens Function. A lens functions somewhat like a collection of prisms. Light rays from a distant source are focused at the focal point F . The focal point lies a distance f , the focal length, from the lens center.

MICRO INQUIRY How would the focal length change if the lens shown here were thicker?

2.2 There Are Several Types of Light Microscopes

After reading this section, you should be able to:

- a. Evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope
- b. Predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen
- c. Create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced

Microbiologists currently employ a variety of light microscopes in their work, each designed for specific applications. Modern microscopes are compound microscopes; that is, they have two sets of lenses. The **objective lens** is the lens closest to the specimen. It forms a magnified image that is further enlarged by one or more additional lenses.

Bright-Field Microscope: Dark Object, Bright Background

The **bright-field microscope** is routinely used to examine both stained and unstained specimens. It forms a dark image against a lighter background, thus it has a “bright field.” It consists of a metal stand composed of a base and an arm to which the remaining parts are attached (**figure 2.3**). A light source, either a mirror or an electric illuminator, is located in the base. Two focusing knobs, the fine and coarse adjustment knobs, are located on the arm and move either the stage or the nosepiece vertically to focus the image.

The stage is positioned about halfway up the arm. Microscope slides are clipped to the stage, which can be moved during viewing by rotating control knobs. The substage **condenser** lens (or simply, condenser) is within or beneath the stage and focuses a cone of light on the slide. Its position may be fixed in simpler microscopes but can be adjusted vertically in more advanced models.

The curved upper part of the arm holds the body assembly, to which a nosepiece and one or more **ocular lenses** (also called **eyepieces**) are attached. Binocular microscopes have eyepieces for both eyes. The body assembly contains a series of mirrors and prisms so the barrel holding the eyepiece may be tilted for ease in viewing. The nosepiece holds three to five objective lenses of differing magnifying power and can be rotated to change magnification. Ideally a microscope should be **parfocal**; that is, the image should remain in focus when objective lenses are changed.

The image seen when viewing a specimen with a compound microscope is created by the objective and ocular lenses working together. Light from the illuminated specimen is focused by the objective lens, creating an enlarged image within the

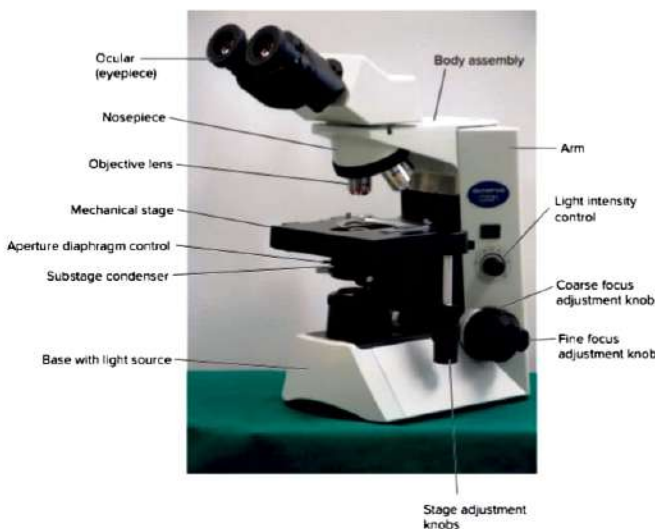


Figure 2.3 A Bright-Field Microscope.
©McGraw-Hill Education/James Redman, photographer

Figure 2.3 A Bright-Field Microscope.
© McGraw-Hill Education/James Redfern, photographer

microscope. The ocular lens further magnifies this primary image. The total magnification is calculated by multiplying the objective and eyepiece magnifications together. For example, if a 45x objective lens is used with a 10x eyepiece, the overall magnification of the specimen is 450x.

Better Microscope Resolution Means a Clearer Image

The most important part of the microscope is the objective lens, which must produce a clear image, not just a magnified one. **Resolution** is the ability of a lens to separate or distinguish between small objects that are close together. At best, the resolution of a bright-field microscope is 0.2 μm , which is about the size of a very small bacterium. Why is this the case? Resolution is in part dependent on the **numerical aperture** ($n \sin \theta$) of a lens. Numerical aperture is defined by two components: n is the refractive index of the medium in which the lens works (e.g., air = 1) and θ is 1/2 the angle of the cone of light entering an objective (figure 2.4). A cone with a narrow angle does not adequately separate the rays of light emanating from closely packed objects, and the images are not resolved. A cone of light with a very wide angle is able to separate the rays, and the closely packed objects appear widely separated and resolved. Recall that the refractive indices of all materials through which light

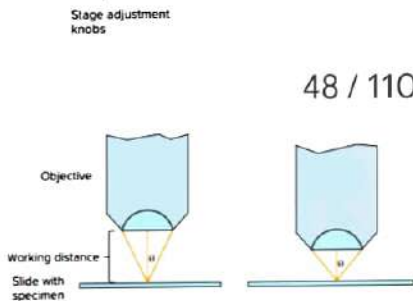


Figure 2.4 Numerical Aperture in Microscopy. The numerical aperture of a lens is related to a value called the angular aperture (symbolized by θ), which is 1/2 the angle of the cone of light that enters a lens from a specimen. The equation for numerical aperture is $n \sin \theta$. In the right-hand illustration, the lens has larger angular and numerical apertures; its resolution is greater and its working distance smaller.

waves pass determine the direction of the light rays emanating from the specimen. Some objective lenses work in air, since $\sin \theta$ cannot be greater than 1 (the maximum θ is 90° and $\sin 90^\circ$ is 1.00), no lens working in air can have a numerical aperture greater than 1.00. The only practical way to raise the numerical aperture above 1.00, and therefore achieve higher resolution, is

2.2 There Are Several Types of Light Microscopes 23

Table 2.2 Properties of Objective Lenses

Property	OBJECTIVE			
	Scanning	Low Power	High Power	Oil Immersion
Magnification	4x	10x	40–45x	90–100x
Numerical aperture	0.10	0.25	0.55–0.65	1.25–1.4
Approximate focal length (f)	40 mm	16 mm	4 mm	1.8–2.0 mm
Working distance	17–20 mm	4–8 mm	0.5–0.7 mm	0.1 mm
Approximate resolving power with light of 450 nm (blue light)	2.3 μm	0.9 μm	0.35 μm	0.18 μm

to increase the refractive index with immersion oil, a colorless liquid with the same refractive index as glass (table 2.2). If air is replaced with immersion oil, many light rays that would otherwise not enter the objective due to reflection and refraction at the surfaces of the objective lens and slide will now do so (figure 2.5). This results in an increase in numerical aperture and resolution.

Resolution is described mathematically by an equation developed in the 1870s by Ernst Abbé (1840–1905), a German physicist responsible for much of the optical theory underlying microscope design. The Abbé equation states that the minimal distance (d) between two objects that reveals them as separate entities depends on the wavelength of light (λ) used to illuminate the specimen and on the numerical aperture of the lens ($n \sin \theta$), which is the ability of the lens to gather light.

$$d = \frac{0.5 \lambda}{n \sin \theta}$$

The smaller d is, the better the resolution, and finer detail can be discerned in a specimen; d becomes smaller as the wavelength of light used decreases and as the numerical aperture increases. Thus the greatest resolution is obtained using a lens with the largest possible numerical aperture and light of the shortest

wavelength, at the blue end of the visible spectrum (in the range of 450 to 500 nm).

Examination of figure 2.4 shows that one objective is much closer to the specimen than the other. Specifically, the objective on the right has a higher numerical aperture and a shorter working distance. The working distance of an objective is the distance between the surface of the lens and the surface of the cover glass (if one is used) or the specimen when it is in sharp focus. As illustrated here, objectives with large numerical apertures and great resolving power have short working distances (table 2.2).

Numerical aperture is also an important feature of a microscope's condenser. The condenser is a large, light-gathering lens used to project a wide cone of light through the slide and into the objective lens. Although most condensers have a numerical aperture between 1.2 and 1.4, the numerical aperture will not exceed about 0.9 unless the top of the condenser is oiled to the bottom of the slide. During routine microscope operation, the condenser usually is not oiled, and this limits the overall resolution, even with an oil immersion objective.

The most accurate calculation of a microscope's resolving power considers both the numerical aperture of the objective lens and that of the condenser, as is evident from the following equation, where NA is the numerical aperture.

$$d_{\text{microscope}} = \frac{\lambda}{(NA_{\text{objective}} + NA_{\text{condenser}})}$$

However, in most cases the limit of resolution of a light microscope is calculated using the Abbé equation, which considers the objective lens only. We now see why the maximum theoretical resolving power of a microscope when viewing a specimen using an oil immersion objective (numerical aperture of 1.25) and blue-green light is approximately 0.2 μm .

$$d = \frac{(0.5)(530 \text{ nm})}{1.25} = 212 \text{ nm or } 0.2 \mu\text{m}$$

Given the limit of resolution of a light microscope, the largest useful magnification—the level of magnification needed to

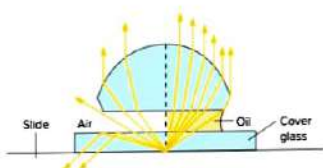


Figure 2.5 The Oil Immersion Objective. An oil immersion objective operating in air and with immersion oil.

microscope. The ocular lens further magnifies this primary image. The total magnification is calculated by multiplying the objective and eyepiece magnifications together. For example, if a 45 $\times$ objective lens is used with a 10 $\times$ eyepiece, the overall magnification of the specimen is 450 $\times$.

Better Microscope Resolution Means a Clearer Image

The most important part of the microscope is the objective lens, which must produce a clear image, not just a magnified one. **Resolution** is the ability of a lens to separate or distinguish between small objects that are close together. At best, the resolution of a bright-field microscope is 0.2 μm , which is about the size of a very small bacterium. Why is this the case? Resolution is in part dependent on the **numerical aperture** ($n \sin \theta$) of a lens. Numerical aperture is defined by two components: n is the refractive index of the medium in which the lens works (e.g., air = 1) and θ is 1/2 the angle of the cone of light entering an objective (figure 2.4). A cone with a narrow angle does not adequately separate the rays of light emanating from closely packed objects, and the images are not resolved. A cone of light with a very wide angle is able to separate the rays, and the closely packed objects appear widely separated and resolved. Recall that the refractive indices of all materials through which light

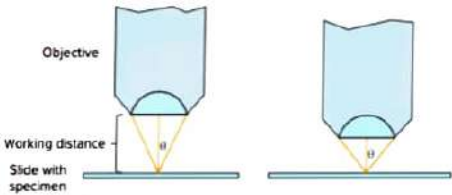


Figure 2.4 Numerical Aperture in Microscopy. The numerical aperture of a lens is related to a value called the angular aperture (symbolized by θ), which is 1/2 the angle of the cone of light that enters a lens from a specimen. The equation for numerical aperture is $n \sin \theta$. In the right-hand illustration, the lens has larger angular and numerical apertures; its resolution is greater and its working distance smaller.

waves pass determine the direction of the light rays emanating from the specimen. Some objective lenses work in air; since $\sin \theta$ cannot be greater than 1 (the maximum θ is 90° and $\sin 90^\circ$ is 1.00), no lens working in air can have a numerical aperture greater than 1.00. The only practical way to raise the numerical aperture above 1.00, and therefore achieve higher resolution, is

Table 2.2 Properties of Objective Lenses		OBJECTIVE			
Property	Scanning	Low Power	High Power	Oil Immersion	
Magnification	4 $\times$	10 $\times$	40–45 $\times$	90–100 $\times$	
Numerical aperture	0.10	0.25	0.55–0.65	1.25–1.4	
Approximate focal length (f)	40 mm	16 mm	4 mm	1.8–2.0 mm	
Working distance	17–20 mm	4–8 mm	0.5–0.7 mm	0.1 mm	
Approximate resolving power with light of 450 nm (blue light)	2.3 μm	0.9 μm	0.35 μm	0.18 μm	

to increase the refractive index with immersion oil, a colorless liquid with the same refractive index as glass (table 2.2). If air is replaced with immersion oil, many light rays that would otherwise not enter the objective due to reflection and refraction at the surfaces of the objective lens and slide will now do so (figure 2.5). This results in an increase in numerical aperture and resolution.

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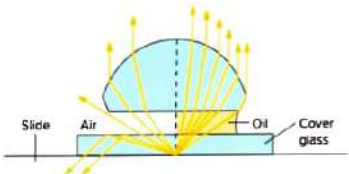


Figure 2.5 The Oil Immersion Objective. An oil immersion objective, operating in air and with immersion oil.

increase the size of the smallest resolvable object to be visible with the light microscope—can be determined. Our eye can just detect a speck 0.2 mm in diameter. When the acuity of the eye and the resolution of the microscope are considered together, it is calculated that the useful limit of magnification is about 1,000 times the numerical aperture of the objective lens. Most standard microscopes have 10x eyepieces and have an upper limit of about 1,000x with oil immersion. A 15x eyepiece may be used with good objective lenses to achieve a useful magnification of 1,500x. Any further magnification does not enable a person to see more detail. Indeed, a light microscope can be built to yield a final magnification of 10,000x, but it would simply be magnifying a blur. Only the electron microscope provides sufficient resolution to make higher magnifications useful.

Visualizing Living, Unstained Microbes

Bright-field microscopes are probably the most common microscope found in teaching, research, and clinical laboratories. However, many microbes are unpigmented so are not clearly visible because there is little difference in contrast between the cells, subcellular structures, and water. As we discuss in section 2.3, one solution to this problem is to stain cells before observation. Unfortunately staining procedures usually kill cells. But what if an investigator wishes to view living cells? Three types of light microscopes create detailed, clear images of living specimens: dark-field microscopes, phase-contrast microscopes, and differential interference contrast microscopes.

Dark-Field Microscope: Bright Object, Dark Background

The **dark-field microscope** produces detailed images of living, unstained cells and organisms by simply changing the way in which they are illuminated. A hollow cone of light is focused on the specimen in such a way that unreflected and unrefracted

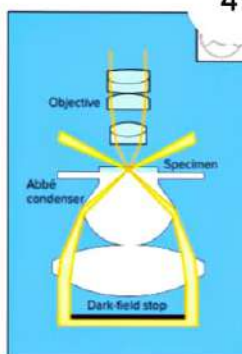
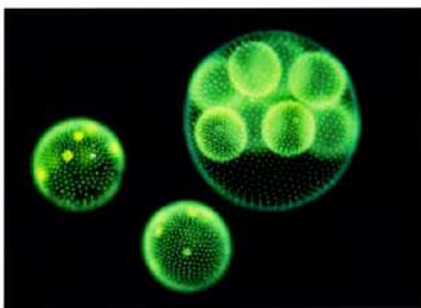


Figure 2.6 Dark-Field Microscopy. In dark-field microscopy, a dark-field stop (inset) is placed underneath the condenser lens system. The condenser then produces a hollow cone of light so that the only light entering the objective is reflected or refracted by the specimen.

rays do not enter the objective. Only light that has been reflected or refracted by the specimen forms an image (figure 2.6). The field surrounding a specimen appears black, while the object itself is brightly illuminated (figure 2.7). The dark-field microscope can reveal considerable internal structure in larger eukaryotic microorganisms (figure 2.7b). It also can be used to identify certain bacteria such as the thin and distinctively shaped *Treponema pallidum*, the causative agent of syphilis (figure 2.7a).



(a) *T. pallidum*



(b) Volvox

Figure 2.7 Examples of Dark-Field Microscopy. (a) *Treponema pallidum*, the spirochete that causes syphilis (x400). (b) The protist Volvox. Note daughter colonies within the mature Volvox colony.

(a) Source: CDC/Schwartz. (b) © McGraw-Hill Education/Stephen Durr, photographer

2.2 There Are Several Types of Light Microscopes 25

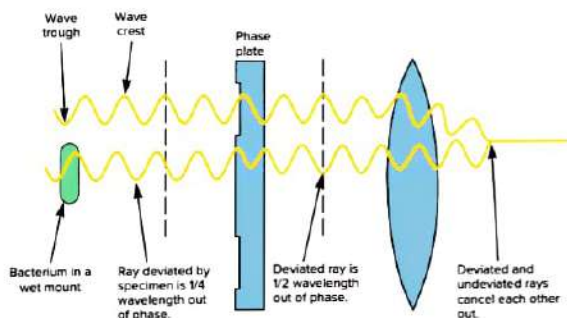


Figure 2.8 The Production of Contrast in Phase-Contrast Microscopy. The behavior of deviated and undeviated (i.e., undiffracted) light rays in the positive-phase-contrast microscope. Because the light rays tend to cancel each other out, the image of the specimen will be dark against a brighter background.

Phase-Contrast Microscope

To understand phase-contrast microscopy, consider a bacterium in a drop of water on a microscope slide (i.e., a wet mount) as illustrated in figure 2.8. The refractive indices of bacterial cell structures are greater than that of water. Therefore, light waves passing through a cell structure will be diffracted and slowed more than light waves passing through the water inside and

outside the cell. Thus both deviated light waves that interact with bacterial cell structures and undeviated light waves that pass around and through the cell are produced. Because the deviated light waves are slowed relative to the undeviated light waves, they are said to be out of phase. That is, the crests and troughs of the deviated and undeviated waves do not align. Typically the deviated light waves are slowed by about $\frac{1}{4}$ wavelength compared to the undeviated light (figure 2.8).

Phase-contrast microscopes take advantage of this phenomenon to create differences in light intensity that provide contrast to allow the viewer to see a clearer, more detailed image of the specimen (figure 2.9). They do so by separating the two types of light so that the undeviated light (primarily from the surroundings) can be manipulated and then recombined with the deviated light (from the bacterium) to form an image. Two components allow this to occur: a condenser annulus and a phase plate (figure 2.10). The condenser annulus is an opaque disk with a thin transparent ring. A ring of light is directed by the condenser annulus to the condenser, which focuses the light on the specimen as shown in figure 2.10. Deviated and undeviated light then pass through the objective toward the phase plate. The phase plate has a thin ring through which the undeviated light (i.e., from the surroundings) is focused (figure 2.8). In a common type of phase-contrast microscopy (positive phase contrast), the ring is coated with a substance that advances the phase of the undeviated light by $\frac{1}{4}$ wavelength. The deviated light is focused on the rest of the phase plate, which lets the deviated light pass through unchanged.



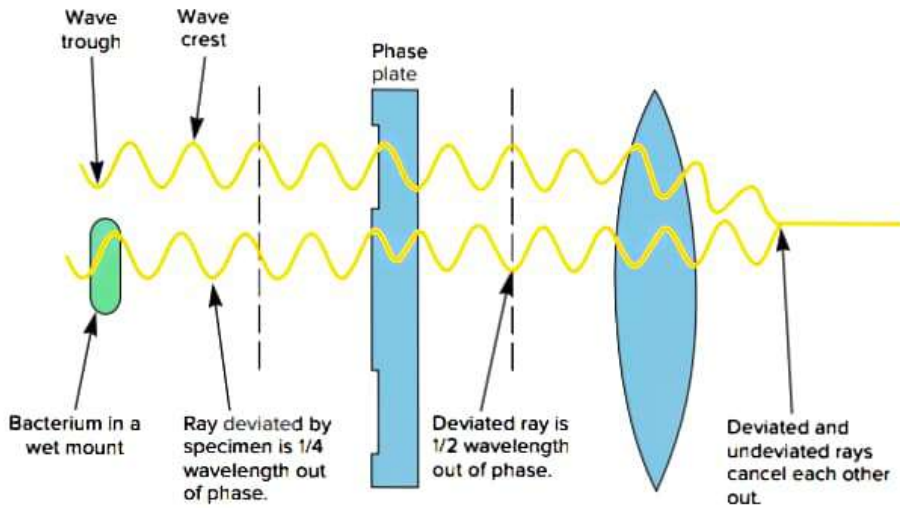


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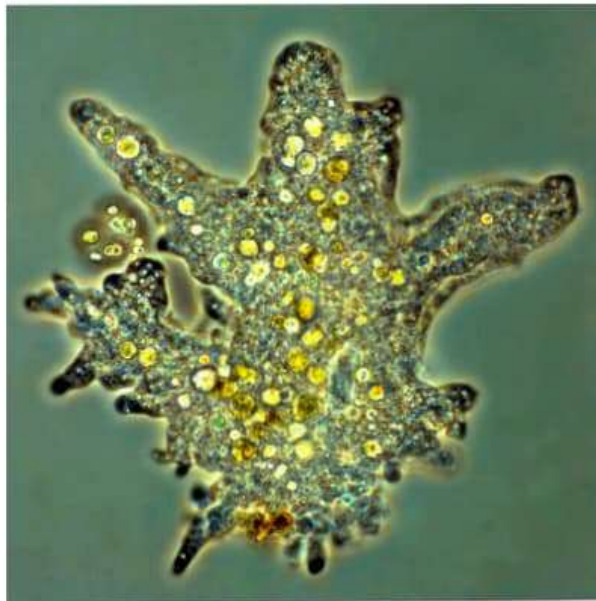
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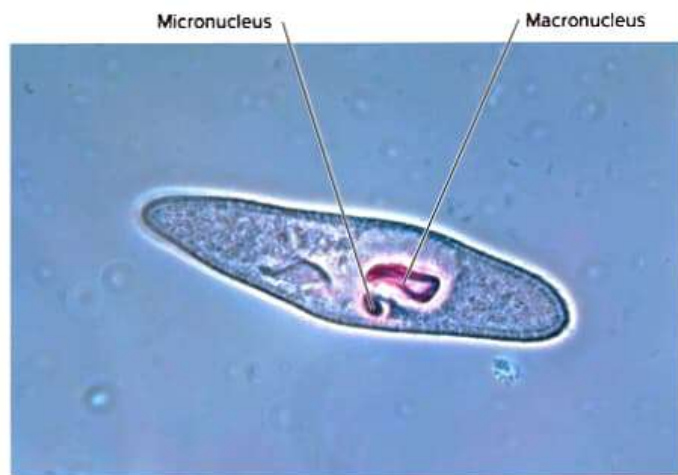
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(a) An amoeba



(b) *Paramecium* sp.

Figure 2.9 Examples of Phase-Contrast Microscopy. (a) An amoeba, a eukaryotic microbe that moves by means of pseudopodia, which extend out from the main part of the cell body. (b) *Paramecium* sp. stained to show a large central macronucleus with a small spherical micronucleus at its side ($\times 400$).

(a) © McGraw Hill Education/Stephen Durr, photographer. (b) © McGraw Hill Education/James Redfeern, photographer

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 (a) © McGraw-Hill Education/Stephen Durr, photographer. (b) © McGraw-Hill Education/James Redfern, photographer

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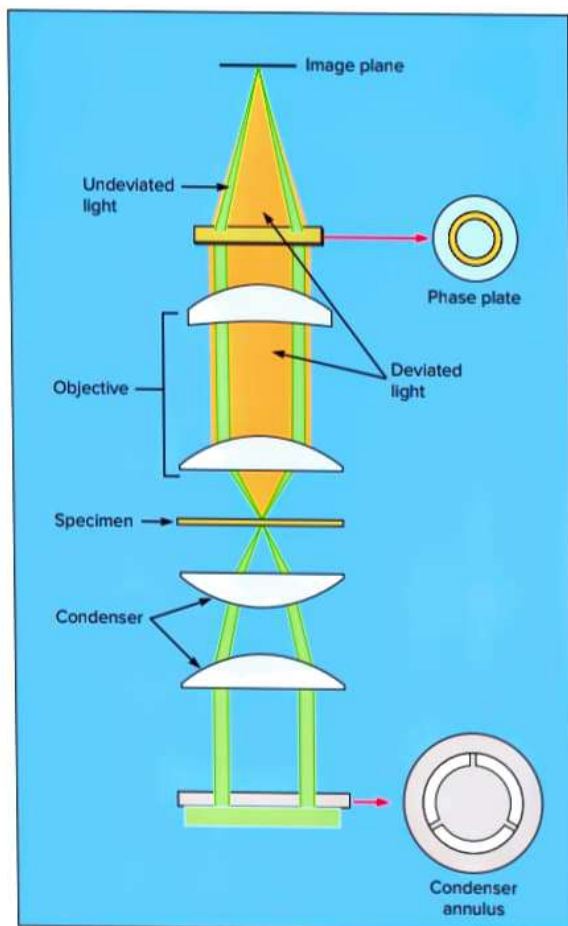


Figure 2.10 Phase-Contrast Microscopy. The optics of a positive-phase-contrast microscope.

MICRO INQUIRY What is the purpose of the annular stop in a phase-contrast microscope? Is it found in any other kinds of light microscopes?

After leaving the phase plate, the deviated and undeviated light are now out of phase by $\frac{1}{2}$ wavelength (figure 2.8). When the two rays of light recombine to form an image, they cancel each other out, a phenomenon called destructive interference. Destructive interference is also seen if the crest of a wave of water meets the trough of another wave—the two cancel each other out and the surface of the water remains calm at the point where they meet. In our example, the resulting image consists of a darker bacterium against a lighter background.

Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells, and detecting bacterial structures such as endospores and inclusions. These are clearly visible because they have refractive indices markedly different from that of water. Phase-contrast microscopes also are



Figure 2.11 Differential Interference Contrast Microscopy. This image of the protozoan *Amoeba proteus* appears three-dimensional and contains considerable detail.

© McGraw-Hill Education/Stephen Durr, photographer

widely used to study eukaryotic cells. ▶ *Bacterial endospores are a survival strategy* (section 3.9); *Inclusions* (section 3.6)

Differential Interference Contrast Microscope

The **differential interference contrast (DIC) microscope** is similar to the phase-contrast microscope in that it creates an image by detecting differences in refractive indices and thickness. Two beams of plane-polarized light at right angles to each other are generated by prisms. In one design, the object beam passes through the specimen, while the reference beam passes through a clear area of the slide. After passing through the specimen, the two beams combine and interfere with each other to form an image. A live, unstained specimen appears brightly colored and seems to pop out from the background, giving the viewer the sense that a three-dimensional image is being viewed (figure 2.11). Structures such as cell walls, endospores, granules, vacuoles, and nuclei are clearly visible.

Fluorescence Microscopes Use Emitted Light to Create Images

The light microscopes thus far considered produce an image from light that passes through a specimen. An object also can be seen because it emits light. This is the basis of fluorescence microscopy. When some molecules absorb radiant energy, they become excited and release much of their trapped energy as light. Any light emitted by an excited molecule has a longer wavelength (i.e., has lower energy) than the radiation originally absorbed. **Fluorescent light** is emitted very quickly by the excited molecule as it gives up its trapped energy and returns to a more stable state.

The **fluorescence microscope** excites a specimen with a specific wavelength of light that triggers the emission of fluorescent light by the object, which forms the image. The most

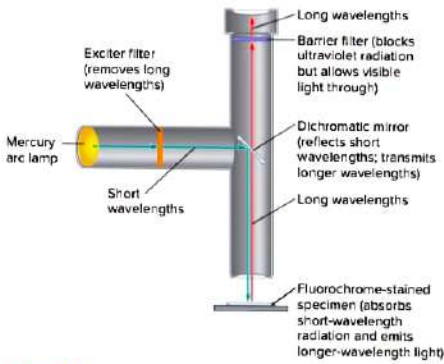


Figure 2.12 Epifluorescence Microscopy. The principles of operation of an epifluorescence microscope.

commonly used fluorescence microscopy is epifluorescence microscopy, also called incident light or reflected light fluorescence microscopy. Epifluorescence (Greek *epi*, upon) microscopes illuminate specimens from above. The objective lens also acts as a condenser (figure 2.12). A mercury vapor arc lamp or other source produces an intense beam of light that passes through an exciter filter. The exciter filter transmits only the desired wavelength of light. The excitation light is directed down the microscope by the dichromatic mirror. This mirror reflects light of shorter wavelengths (i.e., the excitation light) but allows light of longer wavelengths to pass through. The excitation light continues down, passing through the objective lens to the specimen, which is usually stained with molecules called **fluorochromes** (table 2.3). The fluorochrome absorbs light energy from the excitation light and emits fluorescent light that travels up through the objective lens into the microscope. Because the emitted fluorescent light has a longer wavelength, it passes through the dichromatic mirror to a barrier filter, which blocks out any residual excitation light. Finally, the emitted light passes through the barrier filter to the eyepieces.

The fluorescence microscope has become an essential tool in microbiology. Bacterial pathogens can be identified after staining with fluorochromes or specifically tagging them with fluorescently labeled antibodies using immunofluorescence procedures. In ecological studies, fluorescence microscopy is used to observe microorganisms stained with

Fluorochrome	Uses
Acridine orange	Stains DNA
Diamidino-2-phenyl indole (DAPI)	Stains DNA
Fluorescein isothiocyanate (FITC)	Often attached to DNA probes or to antibodies that bind specific cellular components
Tetramethyl rhodamine isothiocyanate (TRITC or rhodamine)	Often attached to antibodies that bind specific cellular components

fluorochrome-labeled probes or fluorochromes that bind specific cell constituents (table 2.3). In addition, microbial ecologists use epifluorescence microscopy to visualize photosynthetic microbes, as their pigments naturally fluoresce when excited by light of specific wavelengths. It is even possible to distinguish live bacteria from dead bacteria by the color they fluoresce after treatment with a specific mixture of stains (figure 2.13a).

► **Genetic methods are used to assess microbial diversity** (section 29.2): **Identification of microorganisms from specimens** (section 37.2)

Another important use of fluorescence microscopy is the localization of specific proteins within cells. One approach is to use genetic engineering techniques that fuse the gene for the protein of interest to a gene isolated from jellyfish belonging

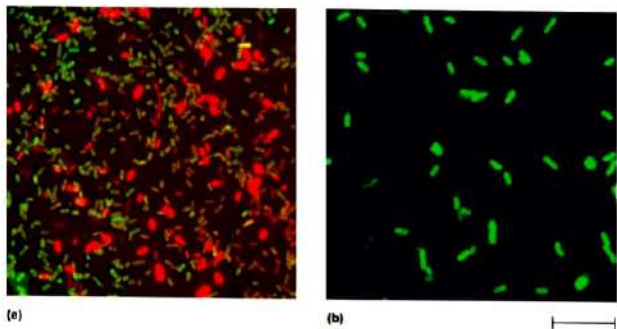


Figure 2.13 Fluorescent Dyes and Tags. (a) Dyes that cause live cells to fluoresce green and dead ones red. (b) Fluorescent antibodies tag specific molecules. In this case, the antibody binds to a molecule that is unique to *Yersinia pestis*, the bacterium that causes plague.

(a) CDC RIA B. Mayes; (b) Source: CDC/Courtesy of Larry Stauffer, Oregon State Public Health Laboratory

MICRO INQUIRY How might the fluorescently labeled antibody used in figure 2.13b be used to diagnose strep throat?



Figure 2.14 Green Fluorescent Protein. Visualization of Mbl, a cytoskeletal protein of *Bacillus subtilis*. The Mbl protein has been fused with green fluorescent protein and therefore fluoresces green.

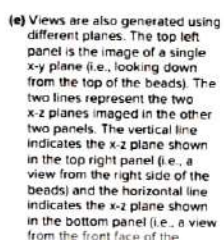
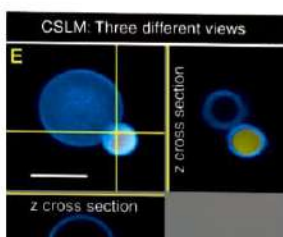
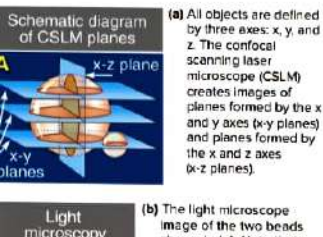
(Left: Emrington/Centre for Biocatalysis/Cell Biology/Neuroscience University)

to the genus *Aequorea*. This jellyfish gene encodes a protein that naturally fluoresces green when exposed to light of a particular wavelength and is called green fluorescent protein (GFP). Thus when the protein is made by the cell, it is

fluorescent. GFP has been used extensively in studies on bacterial cell division and related phenomena (figure 2.14). In fact, the 2008 Nobel Prize in Chemistry was awarded to Osamu Shimomura of Japan and Americans Martin Chalfie and Roger Tsien for their development of this important tool. ► **Fluorescence labeling** (section 17.4)

Confocal Microscopy

Like the large and small beads illustrated in figure 2.15a, biological specimens are three-dimensional. When three-dimensional objects are viewed with traditional light microscopes, light from all areas of the object, not just the plane of focus, enters the microscope and is used to create an image. The resulting image is murky and fuzzy (figure 2.15b). This problem has been solved by the confocal scanning laser microscope



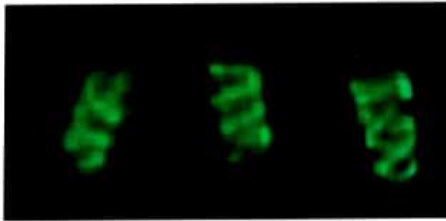


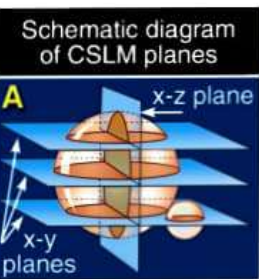
Figure 2.14 Green Fluorescent Protein. Visualization of Mbl, a cytoskeletal protein of *Bacillus subtilis*. The Mbl protein has been fused with green fluorescent protein and therefore fluoresces green.
©Jeff Errington/Centre for Bacterial Cell Biology/Newcastle University

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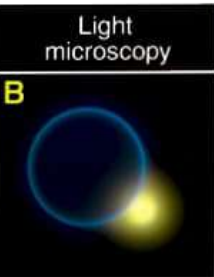
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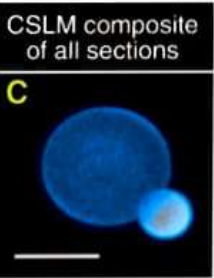
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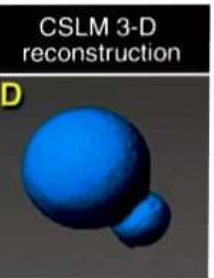
(a) All objects are defined by three axes: x, y, and z. The confocal scanning laser microscope (CSLM) creates images of planes formed by the x and y axes (x-y planes) and planes formed by the x and z axes (x-z planes).



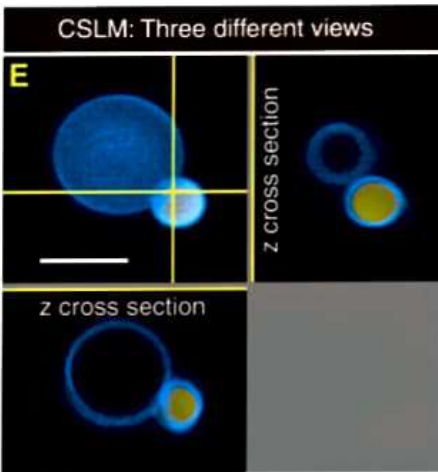
(b) The light microscope image of the two beads shown in (a). Note that neither bead is clear, because the image is generated from light emanating from multiple planes of focus.



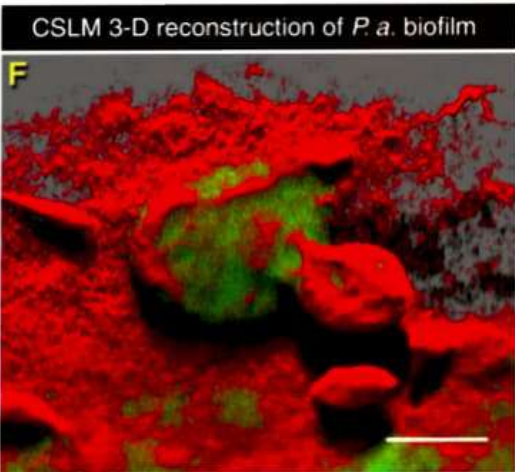
(c) A CSLM uses light from a single plane of focus to generate an image. A composite image of the two beads is generated using digitized information collected from multiple planes within the beads.



(d) Digitized information is collected from multiple planes within the beads to generate a three-dimensional reconstruction.



(e) Views are also generated using different planes. The top left panel is the image of a single x-y plane (i.e., looking down from the top of the beads). The two lines represent the two x-z planes imaged in the other two panels. The vertical line indicates the x-z plane shown in the top right panel (i.e., a view from the right side of the beads) and the horizontal line indicates the x-z plane shown in the bottom panel (i.e., a view from the front face of the beads).



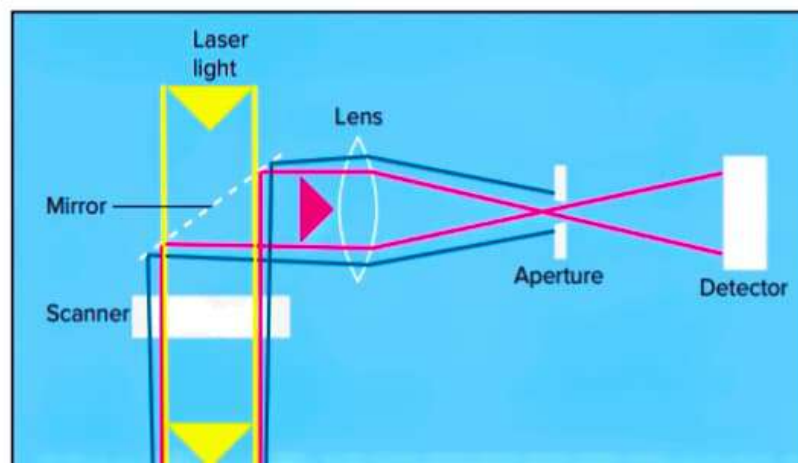
(f) A three-dimensional reconstruction of a *Pseudomonas aeruginosa* biofilm. The biofilm was exposed to an antibacterial agent and then stained with dyes that distinguish living (green) from dead (red) cells. The cells on the surface of the biofilm have been killed, but those in the lower layers are still alive. This image clearly demonstrates the difficulty of killing all the cells in a biofilm.

Figure 2.15 Light and Confocal Microscopy. (a–e) Two beads examined by light and confocal microscopy. (f) Three-dimensional reconstruction of a biofilm.
(b–f) ©P. Dirckx/Center for Biofilm Engineering/Montana State University

(CSLM), or simply, **confocal microscope**. The confocal microscope uses a laser beam to illuminate a specimen that has been fluorescently stained. A major component of the confocal microscope is an opening (that is, an aperture) placed above the objective lens. The aperture eliminates stray light from parts of the specimen that lie above and below the plane of focus (figure 2.16). Thus the only light used to create the image is from the plane of focus, and a much sharper image is formed.

To generate a confocal image, a computer interfaced with the confocal microscope receives digitized information from each plane in the specimen. This information can be used to create a composite image that is very clear and detailed (figure 2.15c) or a three-dimensional reconstruction of the specimen (figure 2.15d). Images of x-z plane cross sections of the specimen can also be generated, giving the observer views of the specimen from three perspectives (figure 2.15e). Confocal microscopy has numerous applications. One is the study of biofilms, which can form on many different types of surfaces, including indwelling medical devices such as hip joint replacements. As shown in figure 2.15f, it is difficult to kill all cells in a biofilm. This makes biofilms a particular concern because their formation on medical devices can result in infections that are difficult to treat.

► *Biofilms are common in nature (section 7.6)*



Comprehension Check

1. Compare and contrast the function of the condenser and eyepiece lenses.
2. What happens to resolution if each of the following factors is increased: wavelength of light, refractive index, and numerical aperture? How are these factors and magnification related?
3. What might a microscopist see through an oil immersion lens if no immersion oil is present?
4. Why don't most light microscopes use 30× magnification?
5. Compare and contrast how dark-field, phase-contrast, interference contrast, epifluorescence, and DIC microscopes work and the kind of image or images provided by each. What specific use for each type?
6. Which type of microscope(s) would be used to study each of the following specimens? Photosynthetic bacteria, swimming cells, labeled microorganisms, unstained microorganisms. Explain each of your answers.

2.3 Staining Specimens Helps to Visualize and Identify Microbes

After reading this section, you should be able to:

- a. Recommend a fixation process to use with a bacterium or archaeon and when to use a protist
- b. Plan a series of appropriate staining procedures for an unknown bacterium as fully as possible
- c. Compare what happens to Gram-positive and Gram-negative bacterial cells during each step of a staining procedure

As noted in section 2.2, specimens examined by light microscopy are often fixed and stained. Such preparation serves to increase the contrast of the organisms, accentuate specific morphological features, and serve them for future study. Im-

Acid-fast staining is another important differential staining procedure. It can be used to identify *Mycobacterium tuberculosis* and *M. leprae* (figure 2.19b), the pathogens responsible for tuberculosis and leprosy, respectively. These bacteria, as well as other mycobacteria, have cell walls containing lipids constructed from mycolic acids, a group of branched-chain hydroxy fatty acids, which prevent dyes from readily binding to the cells (see figure 23.7). A commonly used staining procedure, the cold Ziehl-Neelsen method, uses high concentrations of phenol and carbol fuchsin, as well as a wetting agent, to drive the stain carbol fuchsin into mycobacterial cells. Once this dye has penetrated, the cells are not easily decolorized by acidified alcohol (acid-alcohol) and thus are said to be acid-fast. Non-acid-fast bacteria are easily decolorized by acid-alcohol and thus are stained another color by a second dye called a counterstain.

One of the simplest staining procedures is **capsule staining** (figure 2.19c), a technique that reveals the presence of capsules.

in the midst of a blue-black background because ink particles cannot penetrate either the cell or its capsule. Capsule staining is an example of negative staining. There is little distortion of cell shape, and the cell can be counterstained for even greater visibility. ► *Capsules and slime layers* (section 3.5)

Flagella staining provides taxonomically valuable information about the presence and distribution pattern of flagella on bacterial and archaeal cells (figure 2.19d; see also figure 3.37). Their flagella are fine, threadlike organelles of locomotion that are so slender (about 10 to 30 nm in diameter) they can only be seen directly using an electron microscope (although bundles of flagella can be visualized by dark-field microscopy). To observe bacterial flagella with a light microscope, their thickness is increased by coating them with mordants such as tannic acid and potassium alum and staining with a dye such as basic fuchsin. ► *Bacterial flagella* (section 3.7)

Comprehension Check

1. Describe the two general types of fixation. Which would you use when Gram staining a bacterium? Which would you use before observing the organelles of a protist?
2. Why would basic dyes be more effective under alkaline conditions?
3. What procedural error(s) might be responsible if you Gram stained a mixture of bacteria known to be Gram-positive and Gram-negative and see only red cells?
4. Are capsular and flagellar staining differential staining procedures? Explain your answer.

2.4 Electron Microscopes Use Beams of Electrons to Create Highly Magnified Images

After reading this section, you should be able to:

- a. Create a concept map, illustration, or table that compares transmission electron microscopes (TEMs) to light microscopes
- b. Decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography

For centuries the light microscope has been the most important instrument for studying microorganisms. However, even the best light microscopes have a resolution limit of about 0.2 μm , which

greatly compromises their usefulness for detailed studies of many microorganisms (figure 2.20). Viruses, for example, are too small to be seen with light microscopes (with the exception of some recently discovered giant viruses). Bacteria and archaea can be observed, but because they are usually only 1 to 2 μm in diameter, only their general shape and major morphological features are visible. The detailed internal structure of microorganisms therefore cannot be effectively studied by light microscopy.

Recall that the resolution of a light microscope increases as the wavelength of the light it uses for illumination decreases. In electron microscopes, electrons replace light as the illuminating beam. The electron beam can be focused, much as light is in a light microscope, but its wavelength is about 100,000 times shorter than that of visible light. Therefore electron microscopes have a practical resolution roughly 1,000 times better than the light microscope; with many electron microscopes, points closer than 0.5 nm can be distinguished, and the useful magnification is well over 100,000 $\times$ (figure 2.20). The value of the electron microscope is evident on comparison of the photographs in figure 2.21. Microbial morphology can now be studied in great detail. We briefly review the most common types of electron microscopy.

Transmission Electron Microscope

A **transmission electron microscope (TEM)** uses a heated tungsten filament in the electron gun to generate a beam of

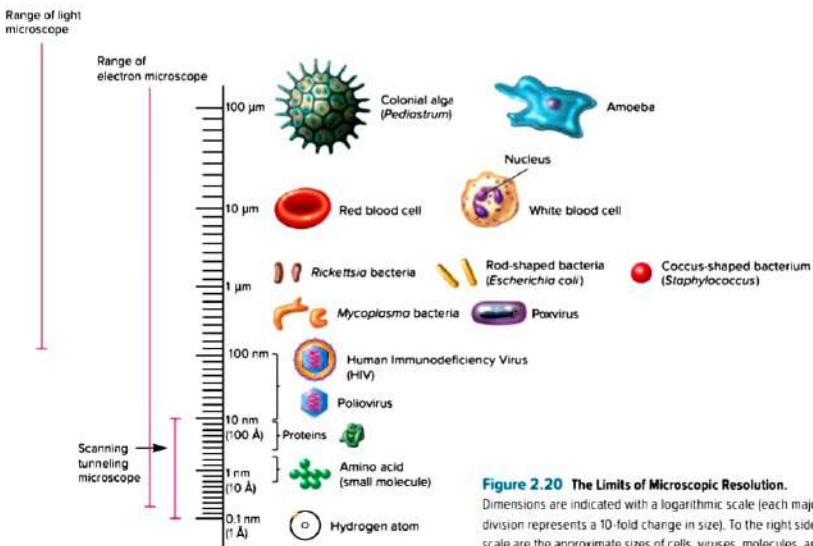


Figure 2.20 The Limits of Microscopic Resolution. Dimensions are indicated with a logarithmic scale (each major division represents a 10-fold change in size). To the right side of the scale are the approximate sizes of cells, viruses, molecules, and atoms.

2.4 Electron Microscopes Use Beams of Electrons to Create Highly Magnified Images



cut such a thin slice, specimens must be embedded in a supportive plastic matrix. To prepare specimens, they are fixed with chemicals such as glutaraldehyde and osmium tetroxide to stabilize cell structure. The specimen is then dehydrated with organic solvents (e.g., acetone or ethanol). Next the specimen is soaked in unpolymerized, liquid embedding plastic until it is completely permeated, and then the plastic is hardened to form a solid block. Thin sections are skillfully cut from the block with a glass or diamond knife using a

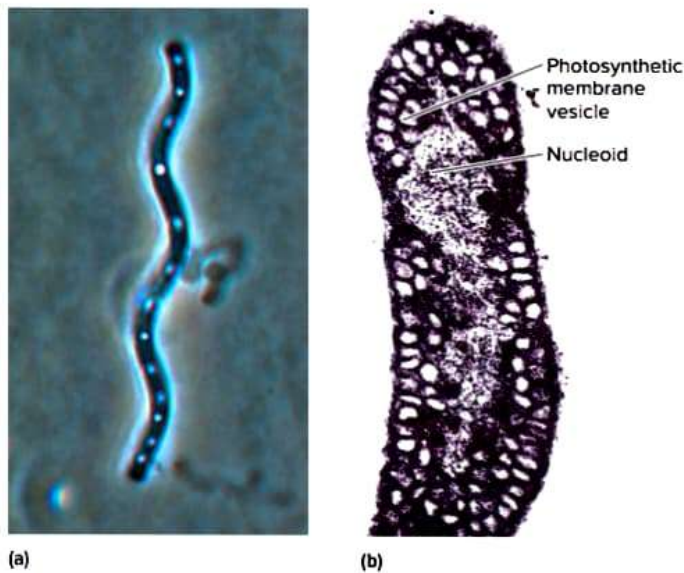


Figure 2.21 Light and Electron Microscopy. A comparison of light and electron microscopic resolution.

(a) The proteobacterium *Spirillum volutans* in phase-contrast light microscope ($\times 1,000$). (b) A thin section of another spiral-shaped proteobacterium *Rhodospirillum rubrum* in transmission electron microscope ($\times 100,000$).

(a) © McGraw-Hill Education/James Redfearn, photographer; (b) © Biology Media/Science Source

electrons that is focused on the specimen by the condenser (figure 2.22). Since electrons cannot pass through a glass lens, doughnut-shaped electromagnets called magnetic lenses focus the beam. The column containing the lenses and specimen must be under vacuum to obtain a clear image because electrons are deflected by collisions with air molecules. The specimen scatters some electrons, but those that pass through are used to form an enlarged image of the specimen on a fluorescent screen that interfaces with a computer monitor (figure 2.23). Denser regions in the specimen scatter more electrons and therefore appear darker because fewer electrons strike that area of the screen; these regions are said to be “electron dense.” In contrast, electron-transparent regions are brighter.

Table 2.5 compares some of the important features of light and transmission electron microscopes. The TEM has distinctive features that place harsh restrictions on the nature of samples that can be viewed and the means by which those samples must be prepared. Specimens must be viewed in a vacuum and only extremely thin slices (20 to 100 nm) of a specimen can be viewed because electron beams are easily absorbed and scattered by solid matter. To

cut such a thin slice, specimens must be embedded in a supportive plastic matrix. To prepare specimens, they are first fixed with chemicals such as glutaraldehyde and osmium tetroxide to stabilize cell structure. The specimen is then dehydrated with organic solvents (e.g., acetone or ethanol). Next the specimen is soaked in unpolymerized, liquid epoxy plastic until it is completely permeated, and then the plastic is hardened to form a solid block. Thin sections are skillfully cut from the block with a glass or diamond knife using a device called an ultramicrotome.

As with bright-field light microscopy, cells are usually stained so they can be seen clearly with a TEM. The probability of electron scattering is determined by the density (atomic number) of atoms in the specimen. Biological molecules are composed primarily of atoms with low atomic numbers (H, C, N, and O), and electron scattering is fairly constant throughout an unstained cell or virus. Therefore specimens are further

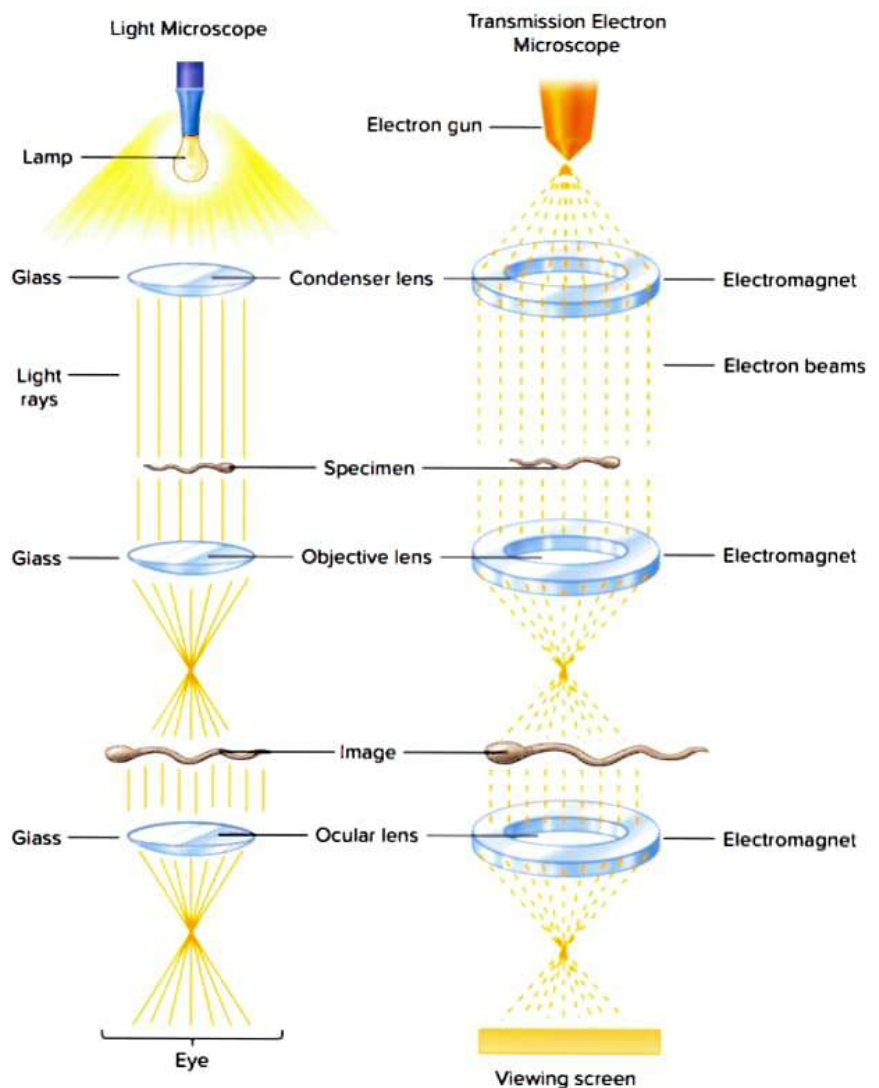


Figure 2.22 A Comparison of Light and Transmission Electron Microscopes.

faces with a computer monitor (figure 2.23). Denser regions in the specimen scatter more electrons and therefore appear darker because fewer electrons strike that area of the screen; these regions are said to be “electron dense.” In contrast, electron-transparent regions are brighter.

Table 2.5 compares some of the important features of light and transmission electron microscopes. The TEM has distinctive features that place harsh restrictions on the nature of samples that can be viewed and the means by which those samples must be prepared. Specimens must be viewed in a vacuum and only extremely thin slices (20 to 100 nm) of a specimen can be viewed because electron beams are easily absorbed and scattered by solid matter. To

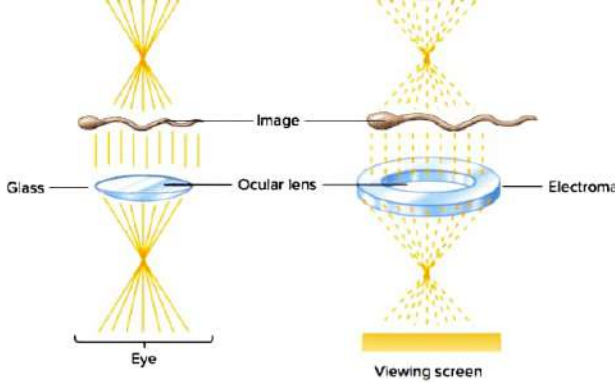


Figure 2.22 A Comparison of Light and Transmission Electron Microscopes.



Figure 2.23 A Transmission Electron Microscope. The electron gun is at the top of the central column, and the magnetic lenses are within the column. The image on the fluorescent screen is also viewed on a computer monitor.
© McGraw Hill Education

prepared by soaking thin sections with solutions of heavy metal salts such as lead citrate and uranyl acetate. The lead and uranium ions bind to structures in the specimen and make them more electron opaque, thus increasing contrast in the material. Heavy osmium atoms from the osmium tetroxide fixative also stain specimens and increase their contrast. The stained thin sections are then mounted on tiny copper grids and viewed.

Two other important techniques for preparing specimens for TEM include negative staining and shadowing. In negative staining, the specimen is spread out in a thin film with either phosphotungstic acid or uranyl acetate. Just as in negative staining for light microscopy, the specimen appears bright against a dark background, in this case because the heavy metals do not penetrate biological material. Negative staining enables visualization of viruses and cellular microbes, but unlike thin sections, internal structures cannot be discerned (figure 2.24a). In shadowing, a specimen is coated with a thin film of platinum or other heavy metal by evaporation at an angle of about 45 degrees from horizontal so that the metal strikes the microorganism on only one side. In one commonly used imaging method, the area coated with metal appears dark in photographs, whereas the uncoated side and the shadow region created by the object are light (figure 2.24b). This technique is particularly useful in studying virus particle morphology, bacterial and archaeal flagella, and DNA.

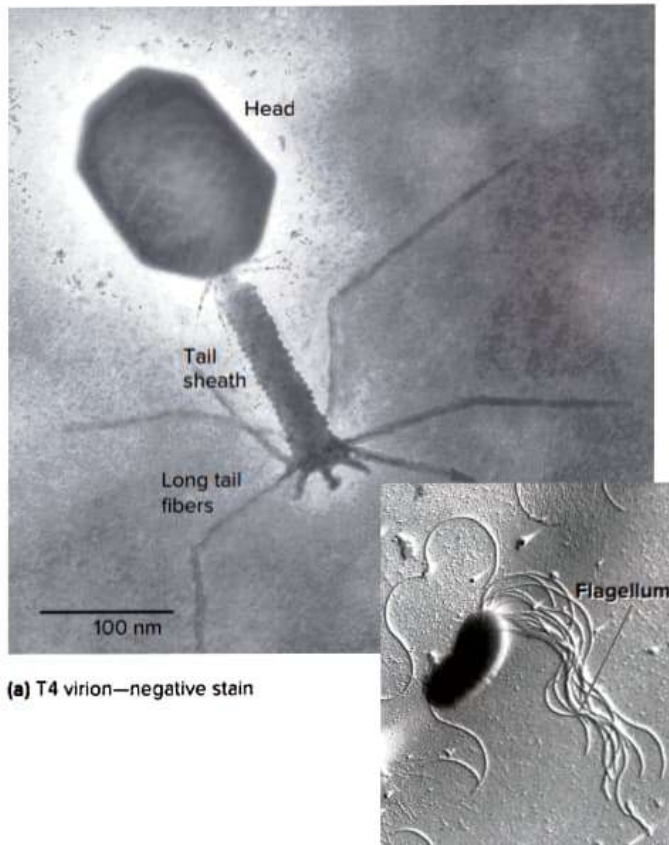
The process of chemical fixation and dehydration can introduce artifacts that can alter cellular morphology. This can be minimized or avoided by using a freeze-etching procedure. When cells are rapidly frozen in liquid nitrogen, they become very brittle and can be broken along lines of greatest weakness, usually down the middle of internal membranes (figure 2.25). The exposed surfaces are then shadowed and coated with layers of platinum and carbon to form a replica of the surface. After the specimen has been removed chemically, this replica is studied in the TEM, providing a detailed view of intracellular structure.

Scanning Electron Microscope

Transmission electron microscopes form an image from radiation that has passed through a specimen. The scanning electron microscope (SEM) produces an image from electrons released

Table 2.5 Characteristics of Light and Transmission Electron Microscopes		
Feature	Light Microscope	Transmission Electron Microscope
Highest practical magnification	About 1,000–1,500	Over 100,000
Best resolution ¹	0.2 μm	0.2 nm
Radiation source	Visible light	Electron beam
Medium of travel	Air	High vacuum
Type of lens	Glass	Electromagnet
Source of contrast	Differential light absorption	Scattering of electrons
Focusing mechanism	Adjust lens position mechanically	Adjust current to the magnetic lens
Method of changing magnification	Switch the objective lens or eyepiece	Adjust current to the magnetic lens
Specimen mount	Glass slide	Metal grid (usually copper)

¹ The resolution limit of a human eye is about 0.2 mm.



(a) T4 virion—negative stain



(b) *P. fluorescens*—after shadowing

Figure 2.24 Stained Microorganisms Visualized by TEM.

(a) T4 is a virus that infects *Escherichia coli*. (b) *Pseudomonas fluorescens* with its polar flagella.

(a) ©AMI Images/Science Source; (b) ©Dr. Tony Brain/SPL/Science Source

MICRO INQUIRY Why are all electron micrographs black and white (although they are sometimes artificially colorized after printing)?

from an object's surface. The surfaces of microorganisms are visualized in great detail; most SEMs have a resolution of about 10 nm.

Specimen preparation for SEM is relatively easy, and in some cases, air-dried material can be examined directly. However, microorganisms usually must first be fixed, dehydrated, and dried to preserve surface structure and prevent collapse of cells when they are exposed to the SEM's vacuum. Before viewing, dried samples are mounted and coated with a thin layer of metal to prevent the buildup of an electrical charge on the surface and to give a better image.

To create an image, the SEM scans a narrow, tapered electron beam back and forth over the specimen (figure 2.26). When the beam strikes a particular area, surface atoms discharge a tiny shower of electrons called secondary electrons, and these are trapped by a detector. Secondary electrons strike a material in the detector that emits light when struck by electrons (the material is called a scintillator). The flashes of light are converted to an electrical current and amplified by a photomultiplier. The signal is digitized and sent to a computer, where it can be viewed.

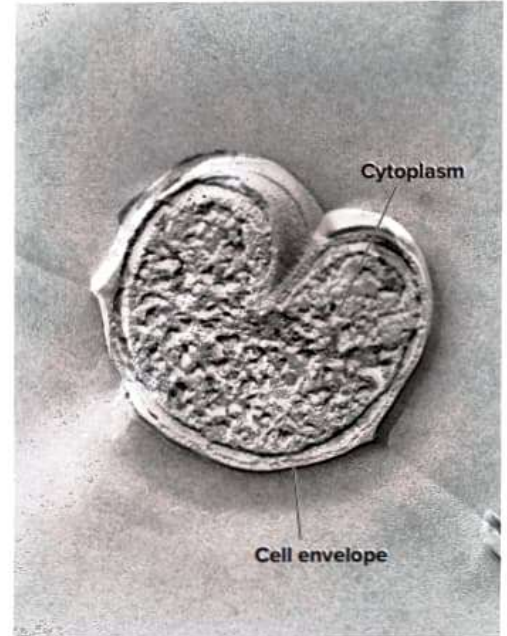


Figure 2.25 Example of Freeze-Etching. A freeze-etched preparation of the bacterium *Nitrospira* sp.

©Dr. Kari Launatmaa/SPL/Science Source

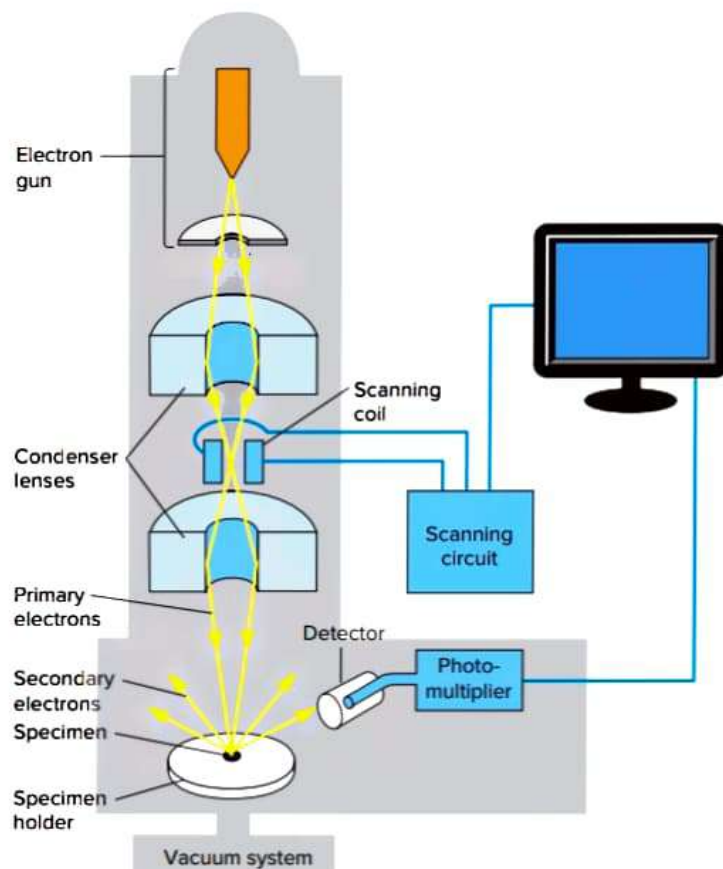


Figure 2.26 The Scanning Electron Microscope.

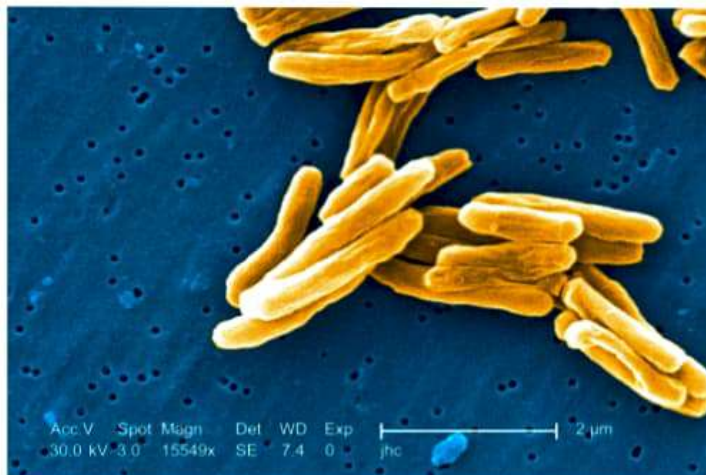


Figure 2.27 Scanning Electron Micrograph of *Mycobacterium tuberculosis*. Colorized image ($\times 15,549$).

Source: CDC/Janice Haney Carr

The number of secondary electrons reaching the detector depends on the nature of the specimen's surface. When the electron beam strikes a raised area, a large number of secondary electrons enter the detector; in contrast, fewer electrons escape a depression in the surface and reach the detector. Thus raised areas appear lighter on the screen and depressions are darker. A realistic three-dimensional image of the microorganism's surface results (**figure 2.27**). A single instrument can house both transmission and scanning electron microscopes (S/TEM).

Electron Cryotomography

Beginning approximately 40 years ago, a series of advances in electron microscopy paved the way for the development of **electron cryotomography**, a technique that since the 1990s has been providing exciting insights into the structure and function of cells and viruses. *Cryo-* refers to sample preparation and visualization. Samples are prepared by rapidly plunging the specimen into an extremely cold liquid (e.g., ethane) and the sample is kept frozen while being examined. Rapid freezing of the sample forms vitreous ice rather than ice crystals. Vitreous ice is a glasslike solid that preserves the native state of structures and immobilizes the specimen so that it can be viewed in the high vacuum of the electron microscope. *Tomography* refers to the method used to create images. The object is viewed from many directions, referred to as a tilt series. The individual images are recorded and processed by computer programs, and finally merged to form a three-dimensional reconstruction of the object. Three-dimensional views, slices, and other types of representations of the object can be derived from the reconstruction (**figure 2.28**). The ultrastructure of bacterial and archaeal cells has been the focus of numerous studies using electron cryotomography. Some of these studies have revealed new cytoskeletal elements, such as those associated with magnetosomes—the inclusions used by some bacteria to orient themselves in magnetic fields (see **figure 22.14**).

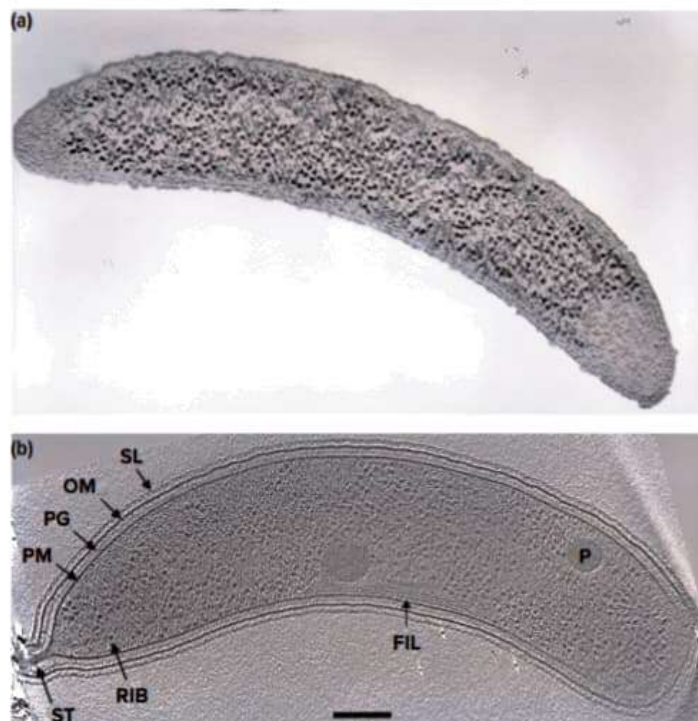


Figure 2.28 TEM and Electron Cryotomography. A comparison of (a) a thin section of a *Caulobacter crescentus* cell prepared using conventional TEM procedures and (b) a central slice from a three-dimensional reconstruction of an intact *C. crescentus* cell. FIL, a bundle of filaments; OM, outer membrane; P, a presumed inclusion; PG, peptidoglycan; PM, plasma membrane; RIB, ribosome; SL, S-layer; ST, stalk.

(a) © AMI Images/SPL/Science Source, (b) © Grant J. Jensen

Comprehension Check

1. Why does an electron microscope have much greater resolution than a light microscope?
2. Where is the electron gun located relative to the sample? Why must a TEM use high vacuum and very thin sections?
3. Under what circumstances would it be desirable to prepare specimens for a TEM by thin section? Negative staining? Shadowing? Freeze-etching?
4. How does a scanning electron microscope differ from a TEM? If you were shown a micrograph, how would you know if it were a TEM, SEM, or electron cryotomography?

2.5 Scanning Probe Microscopy Can Visualize Molecules and Atoms

After reading this section, you should be able to:

- a. Distinguish scanning tunneling from atomic force microscopes in terms of how they create images and their uses
- b. Compare and contrast light microscopy, electron microscopy, and scanning probe microscopy in terms of their uses, resolution, and the quality of the images created

Among the most powerful microscopes are **scanning probe microscopes (SPMs)**. These microscopes measure surface features of an object by moving a sharp probe over the object's surface. One type of

SPM is the **scanning tunneling microscope**. It can achieve magnifications of 100 million times, and it allows scientists to view atoms on the surface of a solid. The scanning tunneling microscope has a needlelike probe with a point so sharp that often there is only one atom at its tip. The probe is lowered toward the specimen surface until its electron cloud just touches that of the surface atoms. When a small voltage is applied between the tip and specimen, electrons flow through a narrow channel in the electron clouds. This tunneling current, as it is called, is extraordinarily sensitive to distance and will decrease about a thousandfold if the probe is moved away from the surface by a distance equivalent to the diameter of an atom.

The arrangement of atoms on the specimen surface is determined by scanning the probe tip back and forth over the surface while keeping the probe at a constant height above the specimen. As the tip follows the surface contours by moving up and down, its motion is recorded and analyzed by a computer to create an accurate three-dimensional image of the surface atoms. The surface map can be displayed on a computer screen or plotted on paper. The resolution is so great that individual atoms are observed easily. Even more exciting is that the microscope can examine objects when they are immersed in water. Therefore it can be used to study biological molecules such as DNA (figure 2.29). The microscope's inventors, Gerd Binnig and Heinrich Rohrer, shared the 1986 Nobel Prize in Physics for their work, together with Ernst Ruska, the designer of the first transmission electron microscope.

A second type of SPM is the **atomic force microscope**, which moves a sharp probe over the specimen surface while keeping the distance between the probe tip and the surface constant. It does this by exerting a very small amount of force on the tip, just enough to maintain a constant distance but not enough force to damage the surface. The vertical motion of the tip usually is followed by measuring the deflection of a laser beam that strikes the lever holding the probe (figure 2.30). Unlike the scanning tunneling microscope,

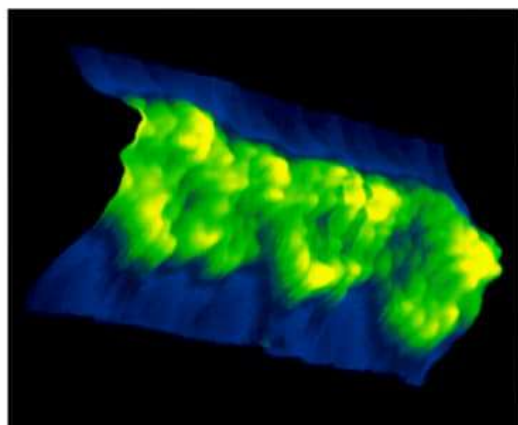


Figure 2.29 Scanning Tunneling Microscopy of DNA. The DNA double helix with approximately three turns shown (false color; $\times 2,000,000$). ©Driscoll, Youngquist & Bal deschwieler, Cal-tech/SPL/Science Source

MICRO INQUIRY Compare the magnification of this image with that shown in figure 2.27 (SEM). How many times greater is the magnification shown here?

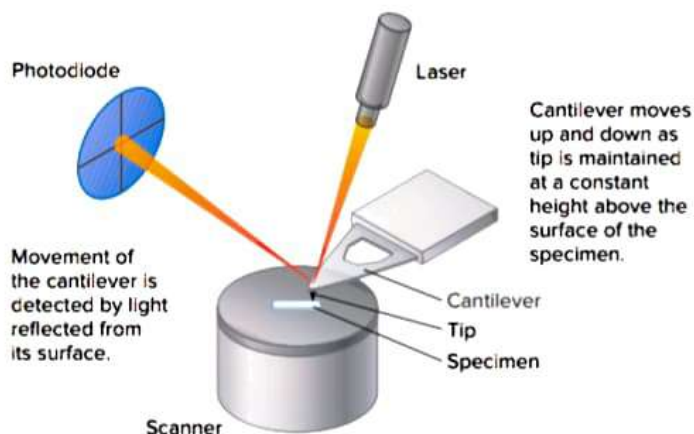


Figure 2.30 The Basic Elements of an Atomic Force Microscope.

The tip used to probe the specimen is attached to a cantilever. As the probe passes over the "hills and valleys" of the specimen's surface, the cantilever is deflected vertically. A laser beam directed at the cantilever is used to monitor these vertical movements. Light reflected from the cantilever is detected by the photodiode and used to generate an image of the specimen.

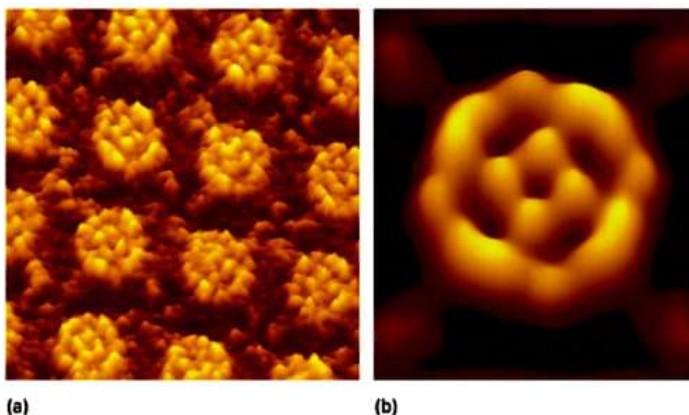


Figure 2.31 The Membrane Protein Aquaporin Visualized by Atomic Force Microscopy. Aquaporin is a membrane-spanning protein that allows water to move across the membrane. (a) Surface view of aquaporin proteins. (b) A single aquaporin protein observed in more detail and at higher magnification.

(a–b) ©Simon Scheuring

the atomic force microscope can be used to study surfaces that do not conduct electricity well. The atomic force microscope has been used to study the interactions of proteins, to follow the behavior of living bacteria and other cells, and to visualize membrane proteins such as aquaporins (figure 2.31).

Comprehension Check

- Briefly describe each of the most popular versions of the scanning probe microscope. Discuss a structure that might be visualized.
- Scanning probe microscopy can be applied to samples that have not been dehydrated, but electron microscopy cannot. Why is this an important advantage?